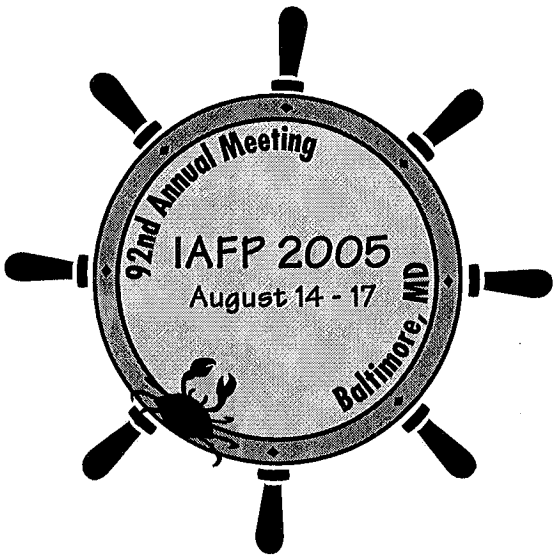

IAFP 2005 Abstracts

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Ivan Parkin Lecture Abstract

Food Safety 2005: Results Come Easy – Answers are Elusive

Presented by

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Under any circumstances and in any discipline, writing a scholarly review is a daunting task. In food microbiology and ecology, it becomes more difficult when one examines carefully the myriad of methods applied to a problem, the details omitted from the methods and materials section, the lack of culture standardization, and a host of other variables that make direct comparison of any two studies nearly impossible. Single studies, however, are relatively easy to conduct, and thus, the facts come easy. Taking the problem one step further, what if the basis for most microbiological studies, the colony forming unit, was found to be a flawed concept? Historically we have had faith in the tenet that if we plate on the correct medium, we can grow and count the bacteria we want to count. In the late 1970s, the viable but non-culturable theory challenged that belief. The nature of the viable but non-culturable phenomenon is still hotly debated, but not so much if it is real as what is the mechanistic basis for its occurrence. In either case, we have not examined food systems or the ecosystems within the entirety of the food chain with the fact in mind that we may not be seeing everything that is there. If we can't see it, should we still worry about it? Added to the other variables often omitted from the methods and materials section, what data can we trust, and should data be collected differently in the future? This question may become more important to regulatory agencies worldwide as they strive for mutual recognition of systems, and the regulatory questions become more complex. Without giving some consideration to standardization of studies on the ecology and microbiology of the food chain, the answers are sure to be elusive.

John H. Silliker Lecture Abstract

Managing the Safety of Food in International Trade

Presented by

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The safety of food in international trade is governed by the WTO/SPS agreement. This agreement has adopted the Standards, Codes and Guidelines of the Codex Alimentarius as reference documents. This means that foods in international trade must be produced, prepared, distributed, etc. in accordance with the Good Hygienic Practices and HACCP as described in the Codex documents. Whether this is the case has to be checked by the local food control (inspection) authority according to the Codex documents on inspection and certification of foods for export. When the exporting food control system is considered to be equivalent to the one of the importing country, border controls can be minimized.

Experience has learned that the application of HACCP varies widely and that GHP documents are not always sufficiently clear to prevent differences in interpretation. Expressions such as "when needed" and "where appropriate" are widely used in such texts. Even the SPS agreement uses a concept which may lead to dispute (i.e., that imported food should not endanger a country's "appropriate level of protection" [ALOP]). However, one notion in the SPS agreement is very clear: sanitary (control) measures should be scientifically based; risk assessment is in particular mentioned in this respect.

The concept of ALOP, which refers to the level of a particular foodborne disease in a country, is difficult to work with in an international food chain setting; the concept of a Food Safety Objective (FSO) offers an alternative. Risk ssessment allows converting ALOP into a FSO: the level of a hazard in a food at the time of consumption. For many foods, a Performance Objective (PO), the level of the hazard in a food at other points in the food chain, can be related to a FSO. The FSO/PO offers industry and governments a clear target which needs to be met in order to assure the undisputed safety or acceptability of a product.

The last draft of the Codex document on Microbiological Risk Management recognizes also the existence of a Performance Criterion (PC), i.e., the change in level of a hazard that will assure that a FSO of PO is met. Such PCs can be validated, guaranteeing the scientific basis of control measures. In this manner, auditing (inspecting) the application of Good Hygienic Practices and HACCP becomes a reliable tool to assure the safety of food in trade and provides the required level of consumer protection of imported foods.

Poster Abstracts

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DSC – Developing Scientist Competition

P1-01 DSC **Detection of *Listeria monocytogenes* Associated Febrile Gastroenteritis during Investigations of Foodborne Outbreaks**

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Listeria monocytogenes was identified in cheese products in two foodborne outbreaks in British Columbia. In both outbreaks, a total of 52 clinical fecal specimens were collected from individuals who had a history of eating the contaminated cheese and which met the case definition (consumption of cheese followed by symptoms of bacteraemia, meningitis or febrile gastroenteritis). Fecal specimens were enriched for *L. monocytogenes* in *Listeria* Enrichment Broth (LEB) for 24 to 48 h and plated onto *Listeria*-specific media (PALCAM and Oxford media), following the Health Canada MFHPB-30 method. Other methods included detection of *Listeria* antigen in LEB by use of a visual immunoprecipitate test, VIP (BioControl Systems), and rapid detection (less than 4 h) of suspect colonies on selective media by use of AccuPROBE *Listeria* (Gen-Probe). *Listeria* was also detected in fecal specimens held at 4°C in LEB before culture (once cheese consumption and symptoms were verified). In the first outbreak, 6 of 26 fecal specimens were positive for *L. monocytogenes*, as well as 2 cases of bacteraemia in pregnancy with miscarriage and 2 cases of meningitis. In the second outbreak, no invasive cases were reported; however, 14 of 26 fecal specimens were positive for *L. monocytogenes*. For positive fecal specimens associated with these outbreaks, the average time reported for the onset of illness after cheese consumption was 7.4 days (range 0.5 to 25 days), with specimens submitted an average of 7 days (range 1 to 27 days) after symptoms began. All clinical fecal specimens positive for *L. monocytogenes* matched the serotype (4b) and PFGE pattern detected in the suspect cheese vehicles. These investigations provide evidence that *Listeria* is an important cause of febrile gastroenteritis and that the application of food detection methods to clinical fecal specimens for the detection of *Listeria* during suspect foodborne illness investigations has merit.

P1-02 DSC **Modeling the Growth/No Growth Interface of *Listeria monocytogenes* in Ready-to-Eat Products as a Function of Lactic Acid Concentration and Dipping Time and Storage Temperature**

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The objective of this study was to model the growth/no growth boundaries of *Listeria monocytogenes* in ready-to-eat (RTE) products as a function of lactic acid (0, 1, 2, 3, or 4%) and dipping time (0, 1, 2, 3, or 4 min), and storage temperature (4, 7 or 10°C). A 10-strain composite of *L. monocytogenes* was inoculated (2 log CFU/cm^2) on frankfurters, turkey breast, and bologna slices. Samples were treated by dipping and were stored in vacuum packages for up to 60 days, depending on product and temperature. Bacterial populations were determined on tryptic soy agar plus 0.6% yeast extract and PALCAM agar at time-zero, and the middle and end point of storage. Significant ($P \leq 0.05$) increases (0.5 to 0.9 log CFU/cm^2) in bacterial counts during storage were designated as "growth," while non-significant changes in counts were designated as "no growth." The growth response data were modeled with logistic regression to determine growth/no growth interfaces. The model generated was used to develop a probability of growth prediction computer program with Java programming language. Growth potential and inhibitory activity varied with product type (increasing growth: frankfurters > turkey breast > bologna), while the minimum inhibitory concentration of lactic acid decreased with increasing dipping time. Storage at low temperatures (4 or 7°C) allowed inhibition at shorter dipping times. The study provides quantitative data on the antimicrobial effect of lactic acid, and the model and prediction computer program developed may be useful in selecting appropriate lactic acid concentrations and dipping times in RTE product processing for adequate control of *L. monocytogenes*.

P1-03 DSC Comparison of Growth Kinetics of *Listeria monocytogenes* Ribotypes Linked to Listeriosis at the Low-temperature Range

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Research was conducted to measure differences in growth kinetics of different *Listeria monocytogenes* strains in liquid growth media at refrigeration and mildly-abuse temperatures. A total of 19 *L. monocytogenes* food or animal isolates with distinct ribotype or serotype implicated in listeriosis cases were selected and grown in tryptic soy broth (TSB) with initial inoculum levels less than 1 CFU/ml. Growth of all the strains was determined at 4°C, 8°C, or 12°C in duplicate. Sample aliquots from TSB were spread plated onto tryptic soy agar supplemented with 1 g/l esculin monohydrate and 0.5 g/l ferric ammonium citrate (efTSA) and incubated at 37°C. Simple linear regression on the exponential part of the growth curve was performed to evaluate growth rate constants by use of the Monod model. The average growth rate constants of the 19 strains ranged from 0.021 to 0.043 h-1, 0.083 to 0.107 h-1, and 0.129 to 0.171 h-1 at 4°C, 8°C, and 12°C, respectively. Statistically significant differences ($P < 0.05$) were observed among several groups of strains, but no single strain was consistently slower or faster than the rest at the three temperatures. The range in growth rate was greater at 4°C (2-fold) than at 8°C (1.3-fold) and 12°C, but the variability was smaller at higher temperatures. Because of the growth rate differences at the studied temperatures, the current results stress the importance of using multiple strains to develop predictive food microbiology models and quantitative microbial risk assessments.

P1-04 DSC Growth and Stress Resistance Variation of *Listeria monocytogenes* Strains from Clinical, Food, Animal and Environmental Sources

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Listeriosis is generally associated with certain serotypes of *Listeria monocytogenes* and virulence variation among strains may be present. The objective was to compare 25 *L. monocytogenes* strains, including clinical, food, animal and environmental isolates, for differences in growth potential and in heat and acid resistance, as well as to assess potential correlation of these properties with origin and serotype of strains. Growth (4 and 30°C) in tryptic soy broth supplemented with 0.6% yeast extract was evaluated by plating on tryptic soy agar supplemented with 0.6% yeast extract (TSAYE). Broth cultures were exposed to 55°C or pH 3.0 (85% lactic acid) for up to 240 and 120 min, respectively, and survivors were determined on TSAYE supplemented with 0.1% sodium pyruvate. Growth differences among strains were less notable at 30 than at 4°C, where growth rates (d^{-1}) ranged from 0.28 ± 0.03 to 0.43 ± 0.01, and lag phase durations (LPD; d) from 0.48 ± 0.08 to 6.25 ± 1.41. No clear trends were evident within serotypes, as LPD for serotype 4b varied from 0.48 ± 0.08 to 4.92 ± 0.84, nor among strains of different origin, catalase reaction or source of isolation within a single outbreak. Strain variation was also observed in acid and heat resistance. Thermal death rates (min^{-1}) ranged from -0.023 ± 0.004 to -0.052 ± 0.003; outbreak-related isolates, however, showed no shoulder periods during heating. The findings of this study should be useful in strain selection for evaluation of antimicrobial alternatives, and for completion of risk assessments.

P1-05 Competitive Aerobic Growth between *Listeria monocytogenes* and *Pseudomonas aeruginosa* in Broth or Spoilage Flora in Milk

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The kinetics of *Listeria monocytogenes* (Lm) in the presence of competing microflora were investigated in two systems. For the broth experiments, nine combinations of initial concentrations of *L. monocytogenes* (6, 4, and 2 log CFU/ml) and *Pseudomos aeruginosa* (Pa) (6, 4, and 2 log CFU/g) were grown in Brain Heart Infusion broth (BHI) at 12°C. Lm were enumerated on Modified Oxford *Listeria* agar and Pa on MacConkey Agar. In the milk experiments, three different initial concentrations of Lm (2, 0, and -1 log CFU/g) were grown in fresh pasteurized milk (spoilage flora 2 log CFU/ml) at 7°C. Growth data were fitted to a three-phase linear function to determine lag phase duration (LPD), stationary phase (SP) and exponential growth rate (EGR). When initial concentrations were below enumerable levels, the EGR was extrapolated back to the known inoculum level to estimate the lag phase. No significant differences ($P > 0.05$) were observed in LPDs or EGR's for either microorganism at any inoculum in the broth system. The time to reach the Lm SP was not affected by Pa. Only the SP levels of Pa were notably suppressed by Lm reaching its SP. In milk, no significant differences were observed in the LP's or EGR's among the three Lm inocula. Lm continued to grow after the spoilage flora reached its maximum level; however, the SP reached by the low Lm inocula was reduced compared to the high inocula. This study suggests that for aerobic competition, the relative cell numbers between *L. monocytogenes* and a generic competitor have little relevance and any models of competition must be based on specific production of lactic acid, bacteriocins, siderophores or other inhibitors.

P1-06 The Behavior of *Listeria monocytogenes* in Ham Salad and Potato Salad as Affected by the Mayonnaise pH and Storage Temperature

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Deli salads have a relatively high incidence of contamination by *Listeria monocytogenes*. The objective of this study was to examine the behavior of this pathogen in ham salad and potato salad as affected by the pH of mayonnaise and storage temperature. Cooked ham and potato were inoculated with an 8-strain cocktail of *L. monocytogenes* and mixed with mayonnaise adjusted to pH 3.8, 4.2, or 4.6. The cell counts of *L. monocytogenes* in salads during storage at 4°, 8°, or 12°C were used to model the behavior of *L. monocytogenes* in ham salad and potato salad. At each storage temperature, *L. monocytogenes* was able to grow in ham salad but not in potato salad. The growth rates (GR) of *L. monocytogenes* in ham salad stored at 4°, 8°, and 12°C ranged from 0.0079 to 0.124, 0.0247 to 0.0339, and 0.0414 to 0.0458 log CFU/h, respectively. The GR of *L. monocytogenes* in potato salad stored at 4°, 8°, and 12°C ranged from -0.0115 to -0.210,-0.0138 to -0.0286, and -0.0265 to -0.0414 log CFU/h, respectively. A significantly longer lag phase duration (LPD, h) of *L. monocytogenes* in both ham salad and potato salad was observed at lower storage temperatures. Mathematical models that described the GR and LPD of *L. monocytogenes* in ham salad and potato salad as a function of mayonnaise pH and storage temperature were produced.

P1-07 Subtyping *Listeria monocytogenes* from Bulk Tank Milk Using Automated Repetitive Element-based PCR

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A total of 65 *Listeria monocytogenes* strains from raw milk were analyzed, using an automated repetitive element-based PCR system to examine the utility of this system for serotype grouping and to determine if regional relationships could be identified. Results of the similarity analysis revealed three primary clusters. Isolates in Cluster 1 represented serogroups 1/2a, 1/2b, 4b, 3b, and 4c. Cluster 2 exclusively contained serogroup 1/2a isolates, although two 1/2a isolates were also found in each of Clusters 1 and 3. Cluster 3 contained only four isolates from serogroups 1/2a (2), 1/2b (1), and 4c (1). Clusters 1 and 2 were separated at a relative similarity of 86% and these two clusters had < 65% similarity with Cluster 3. Cluster 3 isolates were shown to be more similar to Cluster 1 and 2 isolates than to *L. ivanovii* and *L. seeligeri*, and less similar to Cluster 1 and 2 isolates than *L. welshimeri*, *L. grayi*, and *L. innocua*. When rep-PCR fingerprints of the *L. monocytogenes* 1/2a isolates were compared, regional grouping was not apparent. However, discrimination between isolates suggests that this method might have utility in tracking *L. monocytogenes* 1/2a. It appears that the automated rep-PCR method used could not discriminate between serotypes 1/2b and 4b, but it has the potential for discriminating between *L. monocytogenes* 1/2a isolates and may be useful for differentiating 1/2a isolates from other serovars and for tracking isolates within this serotype.

P1-08 Characterization of *Listeria monocytogenes* Isolated from Deli Meats and Retail Chickens

DSC

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A total of 167 *Listeria monocytogenes* isolates were obtained from deli meats, conventional and organic chicken, and conventional and organic fresh produce samples. Strain characterization was carried out by serogroup identification and pulsed-field gel electrophoresis (PFGE). A broth microdilution method was followed to test the antimicrobial susceptibility profile of these strains. All isolates obtained belonged to 5 different serogroups. About 41% of the isolates were from serogroup 1/2b, 3b, followed by 32% from serogroup 4b, 4d, 4e, 26% from serogroup 1/2a, 3a, 1.2% from serogroup 1/2c, 3c and 0.6% from serogroup 4a, 4c. The identification of potential serotype 4b from all food categories, especially from organic chicken products, raised a public health concern, because serotype 4b has been the number one serotype associated with clinical isolates. One hundred and six PFGE patterns were identified among all 167 isolates, suggesting a very diverse population of *L. monocytogenes* genotypes. Percentages of resistance to ciprofloxacin, tetracycline, sulfonamide, and nalidixic acid were 1.8%, 9%, 73%, and 100%, respectively. Multiple resistances were observed in 82% of the isolates. These findings indicated that *L. monocytogenes* in food were diverse and might have originated from different sources, and that multiresistant *L. monocytogenes* were present in food products.

P1-09 Pulsed-field Gel Electrophoresis and Serotyping of *Listeria monocytogenes* Isolated from Ready-to-Eat Foods

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Pulsed-field gel electrophoresis (PFGE) and serotyping were performed on 544 isolates of *Listeria monocytogenes*: 502 isolates recovered from 577 contaminated products from among 31,705 ready-to-eat food (RTE) samples and 42 isolates recovered from human cases of listeriosis. Isolates were from Maryland (294)

and California (250) and were collected in 2000 and 2001. Based on the presence/absence and pattern of bands, there were 139 distinct *Asc*I fingerprint/pulsotypes (percent relatedness among strains ranged from ≥ 25%–100%). Analyses by Dice similarity coefficient and UPGMA associated these 139 pulsotypes into 15 *Asc*I pulsogroups (≥ 70% relatedness within each group). Approximately 46% of the isolates were associated into pulsogroup A (141 food plus 1 clinical) or pulsogroup B (102 food plus 4 clinical). The majority (86%) of the food isolates were either serotype 1/2a (293 isolates) or 1/2b (137 isolates), whereas the majority (71%) of the clinical isolates were either serotype 1/2a (16 isolates) or 4b (14 isolates). Additionally, 15 clinical isolates displayed pulsotypes found in food isolates. Of these 15, two isolates displayed the same pulsotype; however, the remaining 27 clinical isolates had unique pulsotypes. There was no appreciable difference in the pulsotype or serotype of the isolates based on geography or seasonality. These data indicate that the predominant *L. monocytogenes* isolates found in the RTE foods sampled displayed relatively few pulsotypes and only two serotypes and that these serotypes/pulsotypes were not found as often in clinical samples.

P1-10 Ultraviolet Light for the Inactivation of *Listeria monocytogenes* in Recycled Chill Brines

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Ready-to-eat meat products have been implicated in several foodborne listeriosis outbreaks. Microbial contamination of these products can occur after the product has been thermally processed and is rapidly chilled using salt brines. The objective of this study was to determine the effect of ultraviolet irradiation on the inactivation of *Listeria monocytogenes* and lactic acid bacteria in a model brine temperature-controlled chiller system. Commercially used brine was separately inoculated with a ~6.0 log CFU/ml of *L. monocytogenes* or lactic acid bacteria cocktail and passed through an ultraviolet (UV) treatment system for 18 h. Samples were taken aseptically every 15 min for the first h, every half h for the proceeding h and then every h until h eighteen. Brine temperatures within the chiller were maintained at 30°F +/- 1°F. Three replications of each bacteria were performed and resulted in a 5-log reduction in microbial numbers in all treated brines after exposure to ultraviolet light. Bacterial populations were significantly reduced after 60 min of exposure to UV light as compared to the control, which received no UV light exposure ($P < 0.05$). Overall, results indicate that inline treatment of chill brines with ultraviolet light (UVC) inactivates *L. monocytogenes* and lactic acid bacteria. The use of ultraviolet energy may prove to be beneficial for effectively controlling pathogens in recycled chill brines without interrupting the chilling operation. An inline ultraviolet system could be used in a hazard analysis and critical control points plan.

P1-11 Mutations in CRP/FNR Family of Regulatory Genes in *Listeria monocytogenes* Do Not Alter the Heat Resistance of the Pathogen

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The availability of whole genome sequencing data for *Listeria monocytogenes* has created opportunities for exploring the role that unique genomic features have in food safety and pathogenesis. A surprising feature of the *L. monocytogenes* genome is the presence of 15 regulatory genes in the Crp/Fnr family. By comparison, *Escherichia coli* has two regulators in this family and *Bacillus subtilis* has one. We constructed single transposon mutants for 12 of the 15 *L. monocytogenes* Crp/Fnr regulators, using site specific recombination. Since the gene networks controlled by these regulators is currently undetermined, each of the 12 Crp/Fnr mutants and the wild type strain were heat challenged at 60°C by use of an immersed-coil heating apparatus to determine if these *L. monocytogenes* Crp/Fnr mutants demonstrated altered heat tolerance. All the test strains of *L. monocytogenes* were independently grown to stationary phase in brain heart infusion broth and were heat challenged in four separate trials. The thermal inactivation curves were modeled to calculate the underlying mean and mode of the heat resistance distribution for each mutant strain, and each mutant strain was compared to the wild type strain by use of Dunnett's test to adjust for the multiple comparisons. All the Crp/Fnr mutant strains tested had heat resistance characteristics similar to the wild type strain (adjusted $P > 0.05$), indicating that mutations in these Crp/Fnr genes neither increased nor decreased the sensitivity of *L. monocytogenes* to mild heat. Additional tests are under way to further elucidate the regulatory role of these *L. monocytogenes* Crp/Fnr genes.

P1-12 Prevalence of *Listeria* Species in Street-vended, Ready-to-Eat Foods in Alexandria, Egypt

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A total of 576 street-vended, ready to eat foods, including sandwiches and dishes of traditional foods, were examined for the presence of *Listeria* spp. Overall, 24% of foods were found to contain the bacteria; however *L. monocytogenes* was isolated from only 14%. Most of the *Listeria* spp. isolated were *L. monocytogenes* (57%), followed by *L. innocua* (39%), with lower numbers of the other species. Potentially hazardous levels of *L. monocytogenes* (defined as ≥ 10^3 CFU/g) were found in 7% of the total examined food samples, including

meat, poultry, dairy products, seafood and foods of plant origin. Most samples contaminated with *L. monocytogenes* had high levels of total viable counts. Preventive actions and measures should be taken to prevent contamination by such organisms.

P1-13 Impact of Antibiotic Stress on Acid and Heat Tolerance and Virulence Factor Production of *Escherichia coli* O157:H7

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Antibiotics are used in livestock for therapy, disease prevention, or growth promotion. Studies have indicated that some antibiotics may increase virulence of *Escherichia coli* O157:H7. This study was conducted to determine the effect of antibiotic stress on virulence factor production, simulated gastric fluid (SGF, pH 1.5) survival, and heat tolerance (56°C) of *E. coli* O157:H7. The minimum inhibitory concentration (MIC) for three antibiotics (trimethoprim, ampicillin, and ofloxacin) was determined for two *E. coli* O157:H7 strains, using the dilution series method. Subsequently, cells were stressed at the MIC for each antibiotic for 4 h, and post stress tolerance and virulence factor production were evaluated. Heat tolerance (56°C) was determined by use of the capillary tube method, and SGF (pH 1.5) survival was used to assess acid tolerance. Virulence factor production (*Stx*, *Hly*, *Eae*A) was evaluated by the creation of *lacZ* gene fusions followed by use of the Miller assay (a â galactosidase assay). Stressed and control cells were evaluated in triplicate. MICs were: 0.26 mg/l for trimethoprim, 2.05 mg/l for ampicillin and 0.0256 and 0.045 mg/l for ofloxacin in each strain, respectively. Heat tolerance and SGF survival following antibiotic stress decreased compared to control cells ($P < 0.05$). Exposure to ofloxacin increased *Stx* and *Hly* production ($P < 0.05$) but had no effect on *Eae*A. Exposure to ampicillin or trimethoprim increased *Hly* production ($P < 0.05$). *Eae*A and *Stx* production increased when cells were stressed with trimethoprim ($P < 0.05$). Antibiotics can increase *E. coli* O157:H7 virulence factor production but do not produce a cross-protective response to heat or decreased pH.

P1-14 Survival of Lineage I and II Isolates of *Escherichia coli* O157:H7 in Acid

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Two distinct groups of *E. coli* O157: H7, lineage I consisting of mainly human isolates and lineage II consisting of bovine isolates, have been proposed. However, there is limited information available on the relative survivability of lineage I and II strains of *E. coli* O157: H7 in acid environments. Therefore, the survival of 5 randomly selected strains belonging to each of lineage I and lineage II were examined in acid media. Cells were incubated in tryptic soy broth (TSB) at pH 3.5 without glucose at either 4°C for up to 21 days, or room temperature for up to 7 days. Cells were also exposed to synthetic gastric fluid (SGF) at pH 2.5 and 37°C for 4 h. The effect of prior acid adaptation in TSB containing 1% (wt/vol) glucose for 18 h on survival in SGF at 37°C for up to 8 h was also investigated. The survival of non-acid adapted lineage II isolates was significantly higher ($P < 0.05$) than that of lineage I isolates under all conditions tested. However, following acid adaptation, the difference in the survival rate of the two lineages in SGF at 37°C was not significant ($P > 0.05$). Thus factors other than acid tolerance may account for the host specificity of lineages I and II strains of *E. coli* O157:H7.

P1-15 Blade-tenderization of Beef Subprimals Inoculated with *Escherichia coli* O157:H7

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We evaluated the penetration of *Escherichia coli* O157:H7 (ECOH) from the lean-side surface to the interior of a beef subprimal during a single pass (lean-side up), inoculated at ca. 0.6 to 3 log CFU/cm², through a mechanical blade-tenderizer. A second set of inoculated subprimals were not tenderized (positive controls). Ten core samples were removed from each tenderized subprimal and cut into six consecutive segments starting from the inoculated side: segments 1 to 4 represent the top four cm of the core and segments 5 and 6 represent the deepest four cm. Ten cores were obtained from control subprimals but only segment 1 was sampled. Each segment was weighed, diluted, and blended. The slurry was plated onto Sorbitol-MacConkey agar plates. Data for two trials at each inoculum level were averaged. The levels of ECOH recovered from the core surface of control subprimals were 0.6, 1.46, 2.44, and 3.15 log CFU/cm², respectively. The extent of penetration of the pathogen into segment 1 at these four inoculum levels was 0.22, 1.06, 2.04, and 2.7 log CFU/g, respectively. The levels recovered in segment 2 were at least seven-fold lower than levels in segment 1; however, depending on the inoculum level, it was possible to recover ECOH from all six segments. These results demonstrate that mechanical tenderization can transfer ECOH into the interior of a subprimal, most (ca. 40%) of which remains in the top segment. This information, coupled with proper cooking, can enhance the safety of blade-tenderized steaks that may become contaminated with ECOH.

P1-16 Characterization of Cytolethal Distending Toxin-producing *Escherichia coli*

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Cytolethal distending toxin is a toxic bacterial protein that targets the nucleus of eukaryotic cells, causing cell cycle arrest, cell distention, and eventually cell death. In this study, a total of 150 *Escherichia coli* strains, all from our laboratory collection, were examined for *cdt*A, one of the toxin genes, with PCR amplification analysis. *Cdt*A-positive strains were further screened for additional virulence genes such as *eae*A, *stx*1, and *stx*2, as well as for possible antibiotic resistance markers. Additionally, the plasmids isolated from a strain of *E. coli* O157:H7 and O15:H27 were analyzed with restriction enzymes, and the *cdt*A PCR product amplified from the strain of O15:H27 was sequenced. Of the 150 cultures, 14 tested positive for *cdt*A. They include *E. coli* strains with serotypes of O15:H27, O55:H7, O55:H9, O91:H21, O103:H2, O111:H-, O113:H21, O126:H7, O128:H, O157:H7, O157:H-, O163:H19, OR:H-, and O91 with an unknown H antigen. Each of the *cdt*A-positive strains carried *stx*1 and/or *stx*2, and the strains with serotypes O55:H7, O103:H2, O157:H-, and OR:H- also tested positive for *eae*A. The antibiotics to which the *cdt*A-positive *E. coli* were most commonly resistant include novobiocin and streptomycin. The plasmid of the *E. coli* O157:H7 strain appeared to have a size greater than 10 kb, while the plasmid of the *E. coli* O15:H27 strain had a predicted size of 5-6 kb. The *cdt*A PCR product amplified from the strain of *E. coli* O15:H27 was found to share 99% homology with the sequence of *cdt*-III on plasmid pVIR carried by *E. coli* O15:H21 strain S5.

P1-17 Identification of Shiga Toxin 2d Variants in *Escherichia coli* Isolated from Animals, Food and Humans

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Shiga toxin-producing *Escherichia coli* (STEC) are important foodborne pathogens and can cause life-threatening complications, such as hemolytic-uremic syndrome (HUS) or thrombocytopenic purpura (TTP). Shiga toxins (*Stx*) are major virulence factors identified in STEC, and *Stx*1 and *Stx*2. Different *Stx*2 variants have been identified, such as *Stx*2c, *Stx*2d, *Stx*2e and *Stx*2f. To determine the presence of the most virulent *stx*2 variant *stx*2d in *E. coli*, we screened 146 *stx*2 positive STEC isolates by PCR. Isolates containing *stx*2d variants were further confirmed by sequencing the amplified *stx*2 gene products. We identified six *stx*2d positive STEC that belonged to five different non-O157 serotypes. The isolates contained two point mutations (F938S and K955E) in subunit A and three point mutations in subunit B (A1009V, D1074N, and D1099A) which were identical to the point mutation in the *stx*2d sequence deposited in the GenBank (accession number AF479829). Our results indicate the presence of *stx*2d variant in non-O157 STEC isolates in food and animals and may have potential impact on public health.

P1-18 Fecal Shedding of Non-O157 Shiga Toxin-producing *Escherichia coli* (STEC) and *Escherichia coli* O157 in Fattening Pigs at Slaughter in Switzerland

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Since a high degree of genetic relatedness between O101 strains harboring *stx*2e of human and porcine origin was demonstrated, the role of pigs as asymptomatic carriers of STEC needs further research. Fecal samples from slaughtered fattening pigs were examined by polymerase chain reaction for *stx* (STEC, n = 458) and *rfb*E (O157, n = 400). Strains were isolated by colony hybridization and were further characterized. The proportion of positive samples was 10% for *stx* and 7% for *rfb*E. The 32 isolated STEC strains (31 sorbitol-positive) belonged to non-O157 STEC and comprised ten serotypes (O8:H9; O9:H-; O65:H-; O100:H-; O103:H2; O141:H17; O159:H-; ONT:H-; ONT:H10; ONT:H19), three of them (O9:H-; O100:H-; ONT:H-) accounting for 69% of strains. *Stx*1, *stx*2, and both toxin genes were detected in 3%, 97%, and 0% of strains. Among *stx*2-positive strains, 29 were positive for *stx*2e, one for *stx*2/*stx*2e, and one for *stx*2c/*stx*2e. One strain of serotype O141 harboring *stx*2e and *fed*A (fimbria F18) contained a virulence pattern typically associated with diarrhea or oedemic disease in pigs. None of the strains belonged to serogroup O101, and in only one strain harboring *stx*1, *eae* (intimin) and *ehx*A (Enterohemolysin), which are strongly correlated with human disease, were identified. Moreover, among the 18 isolated *Escherichia coli* O157 strains, 17 were positive for sorbitol fermentation, all were negative for *stx*, and one (sorbitol-negative) was positive for *eae* and *ehx*A. Therefore, the fact is emphasized that *E. coli* with the O157 antigen are not always STEC.

P1-19 Prevalence of Shiga Toxin-producing *Escherichia coli* in Dairy Cattle

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The safety concern with foods of bovine origin emerged two decades ago and increased in recent years because of the growing number of human infections with Shiga toxin-producing *Escherichia coli* (STEC). These infections result in illnesses such as diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome.

Dairy cattle are considered reservoirs of O157 and non-O157 STEC. Because contamination of raw milk, cheese, and ground beef from dairy cattle poses a significant risk to humans, this study was designed to determine effects of season and animal factors on STEC prevalence in dairy cattle. In four large dairy farms (averaging 713 cows and heifers) in California, 614 fecal samples were collected from Holstein cows (n = 465) and heifers (n = 149) in the summer and fall of 2004. The prevalence rates of STEC ranged from 0.7 to 2.7%. No effects on prevalence rates of STEC were found for the season, age (cows vs. heifers), or days in milk (1 to 60, 61 to 150, or ≥ 151 days). However, an increase in prevalence rate of STEC was detected for the second lactation vs. other lactations (4.6 vs. 0.8%). The STEC isolates belonged to 12 STEC serotypes (O1:H2, O125:HUT, O136:HUT, O146:H51, O158:HUT, O166:H6, O166:H28, OUT:H5, OUT:H19, OUT:H28, OUT:H41, and OUT:HUT). Of these, 3 (*E. coli* O1:H2, O166:H28, and OUT:H19) are known to cause human illnesses and 6 (*E. coli* O136:HUT, O146:H51, O158:HUT, O166:H6, OUT:H5, and OUT:H41) have not been reported previously in dairy cattle. Interestingly, *E. coli* O157 isolates were not found in the cattle tested.

P1-20 Attachment of Shiga Toxigenic *Escherichia coli* to Beef Muscle and Fat Tissue

DSC

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Shiga toxigenic *Escherichia coli* (STEC) are important foodborne pathogens causing gastrointestinal disease worldwide. Successful bacterial attachment to food surfaces may lead to persistence and possible foodborne disease. A variety of STEC isolates, including *E. coli* O157:H7/H- strains, were grown in planktonic (broth) and sessile (agar) culture and the initial attachment to beef muscle and fat tissue was determined. Viable counts were used to determine loosely and strongly attached cells and the strength of attachment (*Sr*) was calculated using these counts. Attachment counts were greater on fat tissue than on muscle tissue for all STEC isolates. On muscle tissue, viable counts obtained for strongly attached cells (planktonic and sessile) differed significantly ($P \leq 0.05$) among STEC isolates, while counts for loosely attached cells varied significantly ($P \leq 0.05$) among STEC isolates for planktonic culture only. On fat tissue, in contrast, viable counts obtained for strongly attached cells (planktonic and sessile) were not significantly different ($P \geq 0.05$) among STEC isolates, while counts for loosely attached cells were significantly different ($P \leq 0.05$) among STEC isolates. *Sr* values were not significantly different ($P \geq 0.05$) between STEC isolates for all assays. In addition, all bacterial isolates grown in sessile culture attached in greater numbers to muscle and fat tissue than those in planktonic cultures. Our study suggests that STEC, grown in planktonic and sessile culture, behave differently with respect to attachment to muscle and fat tissue. Cells in sessile culture may have a greater potential to strongly attach to meat surfaces.

P1-21 Simultaneous Examination of Oxidative Stress Response and Virulence Gene Expression in *Escherichia coli* O157:H7 Exposed to Hydrogen Peroxide

DSC

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Despite considerable efforts by the food industry, *Escherichia coli* O157:H7 continues to pose a significant threat to consumers. Exposure to sublethal stresses in foods and processing environments may have preparatory effects, resulting in increased survival and subsequent transmission through the food chain to humans. To determine if oxidative stress elicits a preparatory response, *E. coli* O157:H7 was subjected to 1 and 2.5 mM hydrogen peroxide (H_2O_2). cDNA microarrays were used to generate a transcriptional profile. A total of 124 genes (stress response, drug resistance, and virulence) were included. Exposure to 1 mM H_2O_2 resulted in significant upregulation of eight genes. Five were associated with acid resistance, one with oxidative stress, and two with virulence (*stx*AB). At 2.5 mM H_2O_2, 28 genes were significantly upregulated. Genes related to acid resistance, drug resistance, general stress response, oxidative stress, osmolarity adaptation, and starvation were induced. Also, five virulence genes (ler, lpfA, *stx*AB, and a putative enterotoxin {(ShET2)} were upregulated. ELISA-based shiga-toxin (*Stx*2) assays revealed significantly more *Stx*2 in cultures subjected to H_2O_2. The results of this study demonstrate a genomically diverse transcriptional response to H_2O_2 exposure that may better prepare *E. coli* O157:H7 for adverse conditions. Further, the induction of *Stx*2 production, coupled with the upregulation of the locus of enterocyte effacement regulator, ler, may significantly increase the virulence of *E. coli* O157:H7 post-exposure to H_2O_2.

P1-22 Production of Autoinducer-2 in *Escherichia coli* O157:H7 Inoculated Fresh Beef or Purge and Interaction with Level of Natural Flora

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This study examined factors that may affect production of autoinducer-2 (furanosyl borate diester; AI-2) by *Escherichia coli* O157:H7 in fresh beef or purge. Beef strips (4×4×1 cm) were prepared to contain low (LNB; 0.7 log CFU/cm^2; cut after dipping inside rounds in 85°C water) or high (HNB; 3.0 log CFU/ cm^2; no dipping) levels of natural flora, while meat purge samples were prepared by filtering (0.45 μm; LNP) or without filtering (HNP). Two levels (2 or 6 log CFU/cm^2 [beef strips] or ml [purge]) of *E. coli* O157:H7 ATCC43895 were inoculated in samples. Inoculated beef strips were stored aerobically or in vacuum packages and purge

samples were stored statically at 4, 10 or 25°C for 21, 18 and 9 days, respectively. Relative AI-2 activity, as a potential indicator of quorum sensing, was determined using the luminescence-based reporter strain *Vibrio harveyi* BB170, and bacterial populations were determined on tryptic soy agar and sorbitol McConkey agar supplemented with cefixime and potassium tellurite during storage. AI-2 activity was produced earlier and to higher levels in inoculated purge than in beef. In general, *E. coli* O157:H7 showed higher relative AI-2 activity in LNP than in HNP at 10 and 25°C. Also, *E. coli* O157:H7 showed higher relative AI-2 activity in LNB than in HNB, but only at 25°C. Aerobically stored beef slices had higher relative AI-2 activity than those stored anaerobically at 25°C. The results of this study indicated that AI-2 production by *E. coli* O157:H7 depends on level of natural flora, presence of oxygen, substrate composition, and storage temperature.

P1-23 The Most Common Non-beef Foods Linked to *Escherichia coli* O157:H7 Outbreaks

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Escherichia coli O157:H7 (*E. coli* O157:H7) causes an estimated 73,000 foodborne illnesses and 2,100 hospitalizations per year (CDC). The Center for Science in the Public Interest (CSPI) maintains a foodborne illness outbreak database, categorized by food vehicle. CSPI's database was compiled from sources such as the Centers for Disease Control and Prevention (CDC), state and local health departments, and medical and scientific journals. The database is updated regularly, and contains only outbreaks with known or suspected etiology and food vehicles. While the majority of *E. coli* O157:H7 outbreaks are linked to undercooked beef, we identified the most common non-beef foods linked to *E. coli* O157:H7 outbreaks. Between 1998 and 2002, there were 130 *E. coli* O157:H7 outbreaks, involving 3,548 cases. Forty percent (n = 55) of these were linked to non-beef products. The most common non-beef food sources were produce items (n = 19) including romaine and iceberg lettuce, other salads, grapes, pears and fruit salad. Produce outbreaks constituted thirty-five percent of the non-beef *E. coli* O157:H7 outbreaks, and twenty-eight percent of the cases. As the principal reservoir for *E. coli* O157:H7 is cattle, produce outbreaks are likely due to cross-contamination by beef or cattle by-products (such as manure) somewhere in the food production chain. However, unlike beef, produce is often not subject to cooking or another final kill step prior to consumption. While cooking improvements might reduce the outbreaks linked to ground beef, they have effect on those caused by cross-contamination, either on the farm or in food production.

P1-24 DSC The Effects of Anaerobic Incubation and Organic Acids on the Survival of *Escherichia coli* O157:H7 at pH 3.2

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Escherichia coli O157:H7 has caused disease outbreaks in acid foods that are typically stored under anaerobic conditions. Our objectives were to investigate the effects of anaerobic incubation and selected organic acids on the survival of *E. coli* O157:H7. *E. coli* O157:H7 was grown in TSB supplemented with 1% glucose to induce acid resistance. Acid challenge was carried out in 96-well micro-plates or test tubes at pH 3.2. During acid challenge, cells were incubated aerobically, with the dissolved oxygen naturally present in solution, or in an anaerobic hood, at 25°C with 5 mM D- or L-lactic acid, and/or 40 mM acetic acid (based on fully protonated species). Under aerobic conditions, acetic acid reduced *E. coli* O157:H7 viable cell counts by 5.5 logs or more in 6 h. When D-lactic acid was added to the acetic acid solution, only a 0.6 log reduction occurred. We also found that *E. coli* O157:H7 survived better at pH 3.2 under anaerobic conditions than aerobic conditions. When cells were challenged aerobically with L-lactic acid, a 5.5 or greater log reduction was observed in less than 3 h. Only a 2 log reduction in viable counts was observed when similar solutions were incubated for 24 h in the anaerobic hood. Results indicate that both anaerobic conditions and D-lactic acid can increase the survival of *E. coli* O157:H7 at pH 3.2. Understanding the effects of organic acids on the survival of microbial food pathogens will aid in the safe production of acid foods and acidified foods.

P1-25 DSC Sources of Beef Contamination with *Escherichia coli* O157:H7 from Feedlot to Harvest Floor

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The objective of this study was to examine sources and routes of cattle contamination with *Escherichia coli* O157:H7 from feedlot to slaughter. Hide samples and associated colon fecal samples were collected from market-ready steers/heifers at three feedlots which were sent to one of two corresponding packing facilities in the Midwestern region or from three feedlots and one corresponding packing facility in the Southwestern region of the U.S., until a total of 25 hide samples were confirmed positive for *E. coli* O157:H7 in each region. Companion samples were also collected from pen feces, water tanks, feed bunks, loading chutes, and truck trailers. At the corresponding packing plant, samples were collected from holding pens, pen water tanks, restrainers, hides, and colons. Sample groups were designated as either companion samples, hide samples or colon samples, and were analyzed in order: hides, followed by colons that were associated with *E. coli* O157:H7 positive hides, and companion samples derived from hide positive cattle lots. Samples were screened

for *E. coli* O157 using enrichment, immunomagnetic bead separation and plating on Sorbitol MacConkey agar supplemented with cefexime and potassium tellurite, and on Rainbow-plus agar. Presumptive *E. coli* O157:H7 colonies were confirmed by agglutination as well as biochemically. As an example, 25 positive hide samples from the Midwestern region were associated with eight positive colons, while companion positive samples included eight pen feces, two loading chutes, one feedlot water tank, one feedbunk, three truck trailers, two plant pen water tanks, and one plant pen sample. Potential origin of contamination and routes of transmission will be determined through molecular characterization of isolates by multiplex PCR and PFGE.

P1-26 Molecular Characterization of *Escherichia coli* O157:H7 Isolates from Dairy Farms in the Northwestern United States

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Automated ribotyping offers rapid and sensitive molecular characterization of pathogens, which is very useful for epidemiological investigation. The objective of this experiment was to identify and characterize *Escherichia coli* O157:H7 isolates obtained from dairy farms across the Northwestern United States to determine if isolates were transitory or persistent and to determine the genetic relatedness of isolates. Isolates of *E. coli* O157:H7 (n = 69) were obtained from samples collected from 1991 to 1995. Isolates were biochemically and serologically characterized and were ribotyped in the DuPont Qualicon Automated RiboPrinter, using EcoRI restriction enzyme. Cluster analysis was performed based on degree of similarity of band patterns, using molecular analyst software to produce a dendrogram. *E. coli* O157:H7 isolates were classified into 23 different ribogroups. The majority of isolates (n = 51) were 85 to 98% similar to the DuPont Identification Number in the Qualicon Riboprinter database. Cluster analysis produced 25 genetically distinct groups with 90% similarity cutoff. Molecular subtyping of isolates showed persistence of some isolates on farms over several years. There was also significant similarity of isolates regionally, indicating the potential for a common source of *E. coli* O157:H7 in the region.

P1-27 Phenotypic and Genotypic Traits of Non-O157 Shiga Toxin-producing *Escherichia coli* (STEC) Isolated from Ruminants in Switzerland

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In view of the increasing importance of non-O157 STEC as emerging foodborne pathogens in Europe and their high prevalence in ruminants in Switzerland, 42 bovine and 60 ovine STEC strains isolated from fecal samples of slaughtered healthy animals were characterized by phenotypic and genotypic traits. All strains were sorbitol-positive and belonged to non-O157 STEC. Bovine strains comprised 26 O:H serotypes, four of them (O103:H2, O113:H4, O116:H-, ONT:H-) accounting for 38% of strains. Ovine strains comprised 19 O:H serotypes, five of them (O87:H16, O91:H-, O103:H2, O128:H2, O176:H4) accounting for 68% of strains. Nine bovine (38% of strains) and eight ovine (52% of strains) serotypes have previously been associated with HUS. *Stx*1, *stx*2, and both toxin genes were detected in 43%, 48%, and 9% of bovine, and in 25%, 22%, and 53% of ovine strains. Among *stx*2 variants, *stx*2 (63%) and *stx*2c (33%) dominated in bovine, and *stx*2d (80%) in ovine strains. Enterohemolysin (*ehx*A), intimin (*eae*), and autoagglutinating adhesin (saa) were present in 21%, 17%, and 19% of bovine, and in 60%, 13%, and 67% of ovine strains. In strains from cattle, four (1, 1, 2/,), and in strains from sheep, two (1,) *eae* variants were identified. Only one bovine strain of serotype O145:H-, and four ovine strains of serotype O103:H2 and O121:H10 showed virulence patterns of highly virulent human strains. Therefore, to assess the potential pathogenicity of strains for humans, determination of virulence factors is important. Nevertheless, STEC isolates with less virulent patterns cannot be ruled out as a possible source of milder disease.

P1-28 Comparison of Ribotype Patterns with the Presence of Pathogenic Genes from a Diverse *Escherichia coli* O157:H7 Population

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The objective of this research was to molecularly characterize a diverse population of *Escherichia coli* O157:H7 obtained from eighteen farms (beef cattle, dairy cattle, poultry, and swine) across five states (CA, WA, NC, TN, AL). Sixty-four isolates of *E. coli* O157:H7 were obtained from rectal swabs, animal feed, and bedding materials over a period of twenty-four months. *E. coli* O157:H7 isolates were ribotyped and grouped by degree of pattern similarity. Real-time PCR was then used to determine the presence of three key pathogenicity genes (*stx*1, *stx* 2, and *eae*). The results from the ribotyping and rtPCR were compared. Isolates from TN, NC, and AL were more diverse than those from WA and CA. Most of the *E. coli* O157:H7 isolates did not

contain the pathogenic genes. The majority of isolates were clustered into three major groups. Two of these groups were predominantly found on the west coast (one group isolated from swine, the other from beef cattle). The third major group was recovered from four of the five states. In the third group, eleven of twelve isolates (92%) contained genes associated with pathogenicity and seven isolates had the potential to cause infection (contained eae and at least one *stx* gene). Isolates of potentially pathogenic *E. coli* O157:H7 with a high degree of molecular relatedness were widely distributed geographically in the United States in a variety of animal species and farm environments.

P1-29 DSC **Inactivation of *Escherichia coli* O157:H7 in Apple Cider with Elevated Temperature Storage and Dimethyl Dicarbonate**

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Elevated temperature storage and dimethyl dicarbonate (DMDC) were evaluated for reducing *Escherichia coli* O157:H7 and natural microflora populations in unpasteurized apple cider. *E. coli* O157:H7 (4-strain mixture) was inoculated into apple cider (7 log CFU/ml) containing 0 (control) or 250 ppm DMDC. Cider was held at 4 (control), 45, 50, or 55°C for up to 72 h, then moved to 4°C when microflora were no longer detected. Apple cider was plated in duplicate on media and incubated as follows: tryptic soy agar + 25 ppm natamycin (TSAN; 37°C, 48 h); yeast and mold agar + 10 ppm chloramphenicol (YMC; 25°C, 48 h); and modified eosin methylene blue agar (MEMB; 37°C, 48 h) for enumeration of aerobic bacteria, yeasts, and *E. coli* O157:H7, respectively. Holding cider (0 and 250 ppm DMDC) at 50 and 55°C reduced bacterial, yeast, and *E. coli* O157:H7 populations to non-detectable levels after 2 h; these populations were not detectable in cider held for 4 h at 45°C. Bacteria in cider containing DMDC held at 4°C had a > 3-log decrease after 72 h, while control samples had populations of > 6 CFU/ml. At 4°C, yeasts were reduced to non-detectable levels in cider containing DMDC after 4 h, but were recovered at > 4-log CFU/ml after 72 h in control cider. At 4°C, yeasts were reduced to non-detectable levels in cider containing DMDC after 4 h, but were recovered at > 4-log CFU/ml after 72 h in control cider. *E. coli* O157:H7 populations were reduced to non-detectable levels after 4 h in cider containing DMDC at 4°C, but persisted at > 6 log CFU/ml in control cider. Holding cider at 45 to 55°C provides greater than a 5-log reduction of *E. coli* O157:H7 within 4 h. Since indigenous bacteria and yeasts are also inactivated, use of preservatives such as DMDC may not be necessary to ensure subsequent stability during refrigerated storage.

P1-30 DSC **Antibiotic Resistance Profiles and Cell Surface Components of Salmonellae of Poultry Origin**

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Salmonellae were isolated from raw, chilled, retail poultry meats using the procedures outlined in the Bacteriological Analytical Manual and Microbiology Laboratory Guidebook. The isolates were tested for their resistance to 12 different antibiotics, as well as their ability to produce thin aggregative fimbriae and/or cellulose, two of the important surface components associated with cell attachment and biofilm formation. The sensitivity of the salmonellae to the antibiotics was determined with a disc diffusion assay by following the NCCLS M31-A2 guidelines. Of 52 *Salmonella* isolates, 48.0% were resistant to one antibiotic, 9.6% to two, 7.7% to three, 11.5% to four, and 9.6% to five antibiotics. Additionally, 3.8% of the tested isolates were resistant to up to nine of the antibiotics. The antibiotics to which the isolates were most commonly resistant include novobiocin (98.0%), streptomycin (34.6%), oxytetracycline (26.9%), and tetracycline (26.9%). The isolates were grown on LB no salt agar supplemented with Congo red/Calcofluor in order to determine whether they produced thin aggregative fimbriae and/or cellulose. Cells producing thin aggregative fimbriae formed brown colonies (bdar), while those producing cellulose formed pink colonies (pdar). Cells expressing both surface components formed red, dry, and rough colonies (rdar), whereas, those producing neither thin aggregative fimbriae nor cellulose formed smooth and colorless colonies (saw). Of the total 52 *Salmonella* cultures, 25 were bdar, 16 saw, 10 rdar, and 1 was pdar morphotype. The findings of this study suggest that salmonellae of poultry origin have the abilities to resist multiple antibiotics and synthesize thin aggregative fimbriae and/or cellulose.

P1-31 **Cross-protection of *Salmonella* Typhimurium Heat Shocked by Acid and Oxidative Stressors**

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Microbes are injured by stressors such as heat, acid, freezing, oxidation, and osmotic pressure. However, non-appropriate treatments result in survival of some microbes such as *Salmonella* under stressful conditions. The present study evaluated the effects of acid and oxidative stress on cross-protection of heat-shocked *S.* Typhimurium. Either acid or oxidative stress affected the survival of *S.* Typhimurium heat-shocked at 56°C for 1 h and non heat-shocked. Both non and heat shocked strains were eliminated at pH 3, while only non heat-shocked were eliminated at pH 4. *S.* Typhimurium non heat-shocked resulted in reduction of detectable level of 4~6 log CFU/ml while heat-shocked showed detectable level of 1~2 log CFU/ml under the presence of BHP

and H_2O_2 of 18ul/ml. Two dimensional gel electrophoresis resulted in 11 proteins from *S.* Typhimurium heat-shocked which were also maintained or expressed from non heat-shocked. Both acidic and oxidative conditions also resulted in identical patterns of protein expressed by 2D gel electrophoresis. Proteins from *S.* Typhimurium heat-shocked were identified as DnaK, ClpB and small heat shock protein by MALDI-TOF MS. The higher adaptation of *S.* Typhimurium heat-shocked resulted in more adaptation to other stresses. The present results indicate that the combination of heat shock and acid or oxidative stress did not eliminate *S.* Typhimurium; therefore it is suggested that cross-protection of *S.* Typhimurium in stressful conditions should be controlled by hurdle technology appropriately designed in food processing.

P1-32 Survival of *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* in Cranberry Juice Concentrates

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Survival of *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* was studied in cranberry juice concentrates to determine if a 5-log reduction might be achieved without any other treatment. Inactivation at 0°C in concentrates with different Brix levels was determined for a five-strain composite of the individual pathogens in two physiological states. In concentrates at 18 to 46 Brix (pH 2.2-2.4), all three pathogens (stationary phase or acid-adapted cells) showed at least 5-log reduction after 6 or 24 h incubation. At 14 Brix (pH 2.5), a greater than 5-log reduction was obtained for *L. monocytogenes* and *Salmonella* spp, but not for *E. coli* O157:H7 after up to 24 h incubation. All three pathogens survived longer in stationary phase than in acid-adapted phase under the same conditions. The most resistant was stationary phase *E. coli* O157:H7 and the most sensitive was acid-adapted *L. monocytogenes*. The highest log reduction occurred in the most concentrated product evaluated, which suggests that the Brix level and/or some intrinsic compounds may play an important role in the bactericidal properties of cranberry juice concentrates.

P1-33 Nalidixic Acid Resistance Increases Sensitivity of *Salmonella* to Ionizing Radiation in Buffer and in Orange Juice

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Resistance to the antibiotic nalidixic acid has been used as a selective marker for studies of pathogen-inoculated fruits and vegetables. However, resistance to this antibiotic has been shown to influence the sensitivity of *Escherichia coli* O157:H7 to ionizing radiation. To determine the ubiquity of this phenomenon, a collection of 24 foodborne-illness related isolates of *Salmonella* were screened for native resistance to nalidixic acid. The sensitivity to ionizing radiation was determined in a neutral buffer for three nalidixic acid-sensitive (Nal-S) isolates and three nalidixic acid-resistant (Nal-R) isolates. The radiation D10 values (the amount of ionizing radiation necessary to reduce the population by one log, or 90%) were significantly ($P < 0.05$) different among the isolates. As a group, Nal-R isolates of *Salmonella* were found to be significantly more sensitive to irradiation (D10 = 0.210 kGy) than Nal-S isolates (D10 = 0.257kGy). Through selective reculturing in nalidixic acid-amended liquid media (up to 50 μg/ml), the Nal-S isolates were induced to become resistant (Nal-Ri). The radiation sensitivity of Nal-Ri isolates was significantly increased relative to the respective Nal-S parent for two of the three isolates tested. As a group, Nal-Ri isolates had D10 value of 0.234 kGy, intermediate between Nal-S and Nal-R isolates. The study was repeated in reconstituted orange juice, using the same Nal-S, Nal-R and Nal-Ri isolates. Overall D10 values were higher than those observed in neutral buffer. The pattern of response was similar; as a group, Nal-R isolates were significantly more sensitive to ionizing radiation (D10 = 0.581 kGy) than Nal-S isolates (D10 = 0.764 kGy), with Nal-Ri intermediate between the two (D10 = 0.637 kGy). Similarly, the radiation sensitivity of Nal-Ri isolates in orange juice was significantly increased relative to the respective Nal-S parent for two of the three isolates tested.

P1-34 Death of *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* in Salad Dressings

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Salad dressings distributed and stored at ambient temperatures are formulated to be microbiologically stable. The low pH prohibits the growth of foodborne pathogens that may contaminate salad dressings after containers are opened. Results from this study identified the time required for elimination of *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* in three commercially manufactured regular ranch salad dressings, three light ranch salad dressings, two regular blue cheese salad dressings, and two light blue cheese salad dressings. The initial pH values of the four types of pourable dressings were 2.87 to 3.72, 2.82 to 3.19, 3.08 to 3.87, and 2.83 to 3.49, respectively. Dressings were separately inoculated with low (2.4 to 2.5 log CFU/g) and high (5.3 to 5.9 log CFU/g) populations of five-strain mixtures of pathogens and stored at 25°C. Regardless of the initial inoculum level, *Salmonella* was undetectable by enrichment (< 1 CFU/25-g sample in three replicate trials) in all samples within 1 day, and *E. coli* O157:H7 and *L. monocytogenes* were reduced to

undetectable levels by enrichment between 1 and 8 days and 2 and 8 days, respectively. *E. coli* O157:H7 was not detected in six of the ten dressings stored for 2 or more days after inoculation. Counts for yeasts/molds, lactic acid bacteria, and total aerobic mesophilic microorganisms (total plate count) remained low or undetectable throughout the 15-day period during which inoculated dressings were analyzed. Based on these observations, regular and light ranch and blue cheese salad dressings that may become contaminated during or after processing and subsequently stored unrefrigerated are not potentially hazardous foods by FDA Food Code definition.

P1-35 Distribution of *Salmonella enterica* from Aquatic Reservoirs in Florida

DSC

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Salmonella enterica infections are spread through consumption of contaminated food and water. Although > 2000 serotypes are described, only limited subsets are associated frequently with human disease, and their environmental distribution is relatively unknown. We examined *S. enterica* from two geographically distinct regions, the Suwannee River in North Florida and lakes in Central Florida, which are dominated by chicken and citrus agriculture, respectively. Presumptive *Salmonella* were obtained by standard methods and confirmed by DNA probes. Recovery of isolates was much greater from Suwannee River samples (96%) compared to samples from the Central Florida aquifer (11%). Repetitive-element PCR (Rep-PCR) typing was used to examine strain diversity in a database of clinical and environmental isolates (n = 266). This highly reproducible method uses primers derived from conserved repetitive elements that are dispersed throughout the chromosome to generate variable PCR products for molecular typing. Rep-PCR subdivided strains into 15 clusters at > 85% DNA similarity. Subspecies I strains clustered independently (< 65% similarity) from subspecies III isolates. Most serotypes (92%) clustered by Rep-PCR, but these clusters were frequently distributed between two genotypes. Clinical isolates were mostly (92%) distributed among predominantly clinical clusters, while environmental strains (75%) also grouped into predominantly environmental clusters. Very few Suwannee River isolates (11%) were associated with clinical genotypes; however, 95% of Central Florida isolates grouped with clinical strains. Results clearly demonstrated the discriminatory power of rep-PCR at or below subspecies and serotype levels, and these data support the use of rep-PCR for evaluation of isolate source and geographic distribution.

P1-36 Competitive Exclusion Bacteria to Reduce *Salmonella* Colonization of Poultry

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Poultry and poultry products are major sources of foodborne *Salmonella* and *Campylobacter*, the leading causes of foodborne bacterial gastroenteritis diseases. Our main objective was to develop a defined competitive exclusion (CE) culture that will substantially reduce/eliminate *Salmonella* and/or *Campylobacter* colonization in poultry. By screening millions of bacterial colonies from 9 chickens for antimicrobial activity to *Salmonella* (5 serotypes) and *Campylobacter* (6 strains) in vitro, isolates Salm-9 (*Lactobacillus salivarius*), List40-18 (*L. salivarius*), List40-41(*L. salivarius*), and List40-13 (*Streptococcus cristatus*) were selected as CE bacteria because of their strong antagonistic activity to these pathogens. The CE bacteria were perorally administered to day-of-hatch chicks at 10^6-10^8 CFU/chick and *Salmonella* were subsequently administered 2-days later at 5.5 x 10^3 to 5.0 x 10^4 CFU/chick by gavage. Feeding chickens an overnight culture of Salm-9, List40-18 or List40-41 reduced *Salmonella* carriage in cecal contents by 2.46, 2.49, and 2.03 log CFU/g (average of three trials), respectively. The percentage of *Salmonella*-positive chickens in these treatments was 46% (control was 79%), 31% (control was 84%), and 35% (control was 84%), respectively. Feeding chicks a mixture of the three CE isolates had a similar effect against *Salmonella* colonization as when they were used individually. Feeding mixture of List40-13 and List40-41 reduced *Salmonella* carriage in chickens from 90% to 65% and 88% to 31% in two trials, and by 2.2 and 4.0 log CFU/g of cecal contents of chickens. In conclusion, isolates Salm-9, List40-18, List40-41 and List40-13 could be used as effective CE bacteria against *Salmonella* colonization in chickens, either individually or in combination.

P1-37 Prevalence and Persistence of Select Foodborne Pathogens in the Turkey Processing Environment

DSC

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Raw poultry has been identified as the source of a variety of foodborne pathogens. However, most surveys to monitor the microbiological load of poultry have focused on broilers, with relatively less attention given to turkey processing. The purpose of this study was to investigate the prevalence of select pathogens (*Listeria monocytogenes*, *Salmonella* spp., and *Campylobacter* spp.) and microbiological indicators (*Enterococcus* spp.) in the turkey processing environment. Environmental samples were collected in one Southeastern processing facility using swab methods at two month intervals over a period of 18 months. Samples were taken from conveyors, drains, walls and various food contact surfaces. Bacteria were isolated and identified using USDA-FSIS protocols. *Salmonella* isolates were typed using pulsed-field gel electrophoresis (PFGE) with the restric-

tion enzyme *Xba*I. *Enterococcus* isolates were speciated by PCR and their susceptibility to 17 antibiotics of agricultural and human/animal clinical relevance was characterized in accordance with NARMS methods. The prevalence of contamination was 11.5%, 10.2%, and 0.4% for *L. monocytogenes*, *Salmonella*, and *Campylobacter*, respectively. *Enterococcus* spp., an environmental indicator of fecal contamination, was isolated from over 94% of samples. A diverse set of relatively non-persistent *Salmonella* strains were obtained from the processing environment, as evaluated by PFGE banding patterns. *E. faecalis* and *E. faecium* strains were susceptible to most antibiotics of human clinical relevance but frequently resistant to tetracycline, quinupristin/dalfopristin, and erythromycin. Studies such as these can help processors identify contamination frequency and sites in an effort to control resident pathogenic bacteria in the processing environment.

P1-38 DSC **Distribution of *Escherichia coli* O157 and *Salmonella* spp. on Hide Surfaces, the Oral Cavity, and Feces of Feedlot Cattle**

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To determine the distribution of pathogens on cattle hides, samples were taken from six hide surface locations (back, flank, hock, neck, perineum and ventrum), the oral cavity, and feces (rectal-anal junction swab and fecal grab), of feedlot cattle and subjected to *Escherichia coli* O157 and *Salmonella* spp. detection. *E. coli* O157 was isolated from one or more of the sampling locations from 13 animals. Location-specific prevalence of *E. coli* O157 among animals positive at one site or more was 15% for back samples, 15% for flank samples, 38% for hock samples, 23% for neck samples, 38% for perineum samples, 23% for ventrum samples, 8% for oral cavity samples, 15% for rectal-anal junction swabs, and 46% for fecal grab samples. *Salmonella* spp. was isolated from one or more of these sample locations from 50 animals. Location-specific prevalence of *Salmonella* spp. among animals positive at one site or more was 76% for back samples, 74% for flank samples, 94% for hock samples, 76% for neck samples, 88% for perineum samples, 86% for ventrum samples, 94% for oral cavity samples, 64% for rectal-anal junction swabs, and 50% for fecal grab samples. Both *E. coli* O157 and *Salmonella* spp. were isolated from one or more of these sample locations from 8 animals. The sampling locations that maximized prevalence of finding *E. coli* O157, *Salmonella* spp. or both (84%, 96% and 100%, respectively) were hock, perineum and fecal grab. These data suggest that using multiple sample locations is useful when isolating pathogens from feedlot cattle.

P1-39 DSC **Use of Commercial Household Steam and Steam/Vacuum Cleaning Systems to Control Microbial Quality of Meats**

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The feasibility of household steam and vacuum/steam cleaners was evaluated in reducing pathogenic and spoilage bacterial populations on the surfaces of pork skin contaminated with a five-strain mixture of *Listeria monocytogenes* at 10^7 or 10^5 CFU/10 cm^2 area. The contaminated pork skin was treated by three commercial household steam cleaners for 30, 60, 90, 120, 150, and 180 s. A sampling area of 5 × 2 cm^2 was excised from each test specimen and transferred into a sampling bag with 10 ml of Maximum Recovery Diluent, which was subjected to microbiological analysis to enumerate the populations of *L. monocytogenes*, total plate counts, and thermoduric bacteria counts. To determine the population of heat-injured bacteria, an enrichment procedure was employed. The application of steam and steam/vacuum yielded a reduction in populations of *L. monocytogenes* up to 7.68, 6.99, and 5.84 log CFU/10 cm^2 ($P < 0.05$) with a treatment time of 120, 150, and 180 s, respectively, at 10^7 CFU/10 cm^2 inoculation level, whereas a reduction of 5.75, 3.93, and 3.19 log CFU/10 cm^2 ($P < 0.05$) was observed with a treatment time of 120, 150, and 180 s, respectively, at 10^5 CFU/10 cm^2. A similar reduction trend was observed with total plate counts and thermoduric bacteria counts at both inoculation levels. Significant differences were observed between different inoculation levels, treatment times, and type of cleaning systems used ($P < 0.05$). The study infers that the commercial household cleaning systems could be effectively used by meat-processing facilities, especially small and very small meat-processing facilities, as an essential part of the HACCP system.

P1-40 Effect of Refrigerating Delayed Shipments of Raw Ground Beef on the Detection of *Salmonella* Typhimurium

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In eight separate trials, four groups of raw ground beef samples were inoculated with 0.04 to 0.3 CFU/g of *Salmonella* Typhimurium DT 104. Each group consisted of four samples, three inoculated and one uninoculated. After inoculation, raw ground beef samples were placed in shipping containers along with ice packs and transported by overnight courier from the USDA Eastern Regional Research Center in Wyndmoor, PA to the FSIS Eastern Lab in Athens, GA. A temperature data logger was placed inside each shipping con-

tainer to record the temperature during shipping and storage. The first group of ground beef samples was analyzed within approximately 1 h of arrival. The second group of samples was left in the original unopened containers for 24 h before processing. The third and fourth groups of samples were removed from the original shipping containers and stored at room temperature (21 ± 2°C) for 6 h, then stored in a refrigerator at 4 ± 2°C for 24 and 48 h, respectively, before analysis. The samples were analyzed for the presence of *S.* Typhimurium according to USDA/FSIS Laboratory Guidebook MLG, Chapter 4.02. There was no significant difference in the presence and levels of S. Typhimurium in ground beef samples among the four test groups. These data show that it is acceptable to process late-arriving ground beef samples for the detection of *Salmonella* if samples are stored at 4 ± 2°C for 24 to 48 h, or when the samples remain in the original shipping containers for an additional 24 h.

P1-41 Survival of *Salmonella* in Hog Manure during Storage

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The use of manure as an organic fertilizer may be responsible for foodborne illness associated with fruits and vegetables. The objective of this research was to evaluate the potential for *Salmonella* present in hog feces to survive during storage and to serve as a potential source of environmental contamination. One liter samples of 3 types of manure (nursery, sow and feeder barns) were inoculated using a *Salmonella* cocktail (*S.* Oranienburg, *S.* Hadar, *S.* Typhimurium and *S.* Agona) at 4 log CFU/ml and incubated for 90 days at three temperatures (4, 25, 37°C). Survival of *Salmonella* was evaluated using xylosine-lysine-tergitol-4 agar (XLT4) and by enrichment. Temperature was shown to have a great influence on *Salmonella* survival in manure. Lower temperatures provided better survival. After 7 days of storage, *Salmonella* was not recovered from any of the manure types stored at 37°C. At 25°C, *Salmonella* survived up to 37 days. At 4°C, *Salmonella* survived more than 91 days in all manure types. Survival was greatest in manure obtained from the nursery barn. In addition, temperature at 3 depths (10 cm, 2 m and bottom) in manure storage facilities was monitored for a full year using Tidbit® data loggers. The lowest temperature recorded was -11°C at the 10 cm depth during the winter. The highest temperature was 26°C at the 10 cm depth during summer. Results indicated that *Salmonella* present in contaminated manure may survive over winter storage and become a potential source of these pathogens when manure is applied to soil.

P1-42 Microbiological Validation of a Prosciutto Ham Process for *Salmonella* spp. and *Listeria monocytogenes*

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Prosciutto is a term broadly used to describe a ham that has been seasoned, salt-cured (but not smoked) and air-dried. The objective of this experiment was to validate a commercial manufacturing process for its ability to control *Salmonella* spp. and *Listeria monocytogenes* contamination on this product. The objective was to meet the USDA guidelines for lethality of the Prosciutto ham process. A cocktail of five *Salmonella* or five *Listeria monocytogenes* strains was used. Whole boneless hams were inoculated on the exterior and interior surfaces (voids where bone was removed) by misting. Three replications were performed. A non-inoculated ham was used for each replication. The control ham was used to measure pH of the hams throughout the experiment. Three excised cores (ca. 3 mm depth) from the outside (21.2 cm^2) surface and two cores from the inside (14.14 cm^2) were taken to enumerate residual pathogen populations on days 0, 32 (end of curing), and 43 (end of drying). The curing and heat treatment reduced the *Salmonella* spp. population by an average of 7.7 log CFU/cm^2 and *Listeria monocytogenes* by 6.8 log CFU/cm^2, thereby meeting USDA reduction standards.

P1-43 Controlling *Salmonella* Typhimurium, *Escherichia coli* O157:H7, *Yersinia enterocolitica*, and *Listeria monocytogenes* in Vacuum-packed Nham, a Thai Style Fermented Pork Sausage, by Lactacel 115 Alone or in a Combination with Dried Plum Mixtures

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Nham is a fermented sausage made from ground pork and other ingredients and is very popular in Thailand. Several harmful microorganisms such as *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7 have been reported to contaminate Nham. In this study, effects of Lactacel 115 (a commercial lactic starter), alone or in combination with dried plum mixtures, for the control of *Salmonella* Typhimurium, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Yersinia enterocolitica* during Nham production were determined. The pathogen cocktail was applied into Nham ingredients at ca. 10^4 CFU/g each. Four dried plum mixtures (fresh plum juice concentrate, lighter bake puree, dried plum puree, and prune pear powder) were separately added at 0%, 3% or 6%, with Lactacel 115 added at ca. 10^7 CFU/g. Natural fermentation without Lactacel 115 or dried plum mixtures was performed as a control treatment. The sausage was vacuum packaged and incubated at 37°C. The use of dried plum puree at 3% with Lactacel 115 provided 3.42 log reduction of *S.* Typhimurium at 12 h. The use of Lactacel 115 and fresh plum juice concentrate at 3% provided 3.04 and 2.22 log reduction of *E. coli* O157:H7 and *Y. enterocolitica*, respectively, at 24 h. For the control of *L. monocytogenes*, Lactacel 115 and fresh plum juice concentrate at 6% provided 2.99 log reduction at 24 h. This

research is significant as it shows that foodborne pathogens in Nham were greatly inhibited by the use of Lactacel 115 and dried plum mixtures.

P1-44 Measuring the Forces of Detachment of *Listeria monocytogenes* on Stainless Steel

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Foods may become unsafe to eat through contact with a microbially contaminated surface. An understanding of the processes of microbial adhesion to surfaces could eventually lead to the rigorous design of cleaning regimes in food processing facilities. As a first step towards this objective some estimate is required of the forces necessary to detach adherent microorganisms from the surfaces of commonly used materials. We report here on experiments conducted using dairy isolates of *Listeria monocytogenes* in which the forces necessary to detach strains from stainless steel were measured using a radial flow chamber. We contacted stainless steel discs with suspensions of *L. monocytogenes*, for periods ranging from 2 min to 24 h, then inserted them into the flow chamber which was operated at fixed conditions. Adherent cells were fixed using formaldehyde and then treated with a fluorescent stain. Cell coverage was determined as a function of radial distance using epiflorescence microscopy. Treatment of the discs in this way resulted in a central clearance zone from which all cells had become detached. Maintenance of fixed flow conditions enabled us to equate the diameter of these zones directly to the shear forces generated. For short contact times (below 30 min) there were large variations in the diameters of the clearance zones displayed by our dairy isolates. In contrast, for long contact times (2 h and above) there were no statistically significant differences in the diameters even though they were greater. We discuss the implications of these findings for the rigorous design of surface cleaning regimes.

P1-45 Characterization of *Listeria innocua* Biofilm Formation Using Tn917 Transposon Mutagenesis and Arbitrary PCR

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Listeria sp. can grow on surfaces and be a member of multi-species biofilms within food processing plants, rendering them more resistant to sanitizers and other antimicrobial agents. Little is known of the cellular physiological processes involved in *Listeria* sp. growth on surfaces. *Listeria* inoocua ATCC 51742 serotype 4b transposon mutagenesis was performed using plasmid pLTV3 (Tn917) with a temperature sensitive origin of replication. Mutants were selected by growth in the presence of erythromycin and lincomysin at 41°C. Five libraries of 2500 mutants were further screened using a PVC microtiter plate biofilm assay at 32°C at low nutrient levels. Reduced surface growth (RSG) mutants were defined as having a mean stained biofilm at 2 std dev below the mean of the wild-type. 36 RSG mutants were isolated and further characterized by testing cellular motility and initial adhesion phenotypes. RSG 24 exhibited a wild-type cellular motility with reduced initial adhesion, which indicated a surface associated genetic determinant. RSG 24 showed a reduced agglutination reaction with *Listeria antisera* type 4 and reduced PVC surface coverage using epiflorescence microscopy and scanning electron microscopy as compared to wild-type. A two step arbitrary PCR with subsequent PCR fragment TOPO® cloning and DNA sequencing was utilized to reveal transposon interruption site having homology to lin2619 (mutarotase) gene of *L. innocua* with enzyme utilized in sugar substitution of teichoic acid in *Listeria* serotype 4b strains. Knowledge of essential biofilm formation genes may eventually lead to unique strategies for biofilm prevention and removal in food processing environments.

P1-46 A Comparison of Biofilm Formation by *Listeria monocytogenes* and *Listeria innocua* on Stainless Steel

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Several studies have been published that deal with growth rates of *Listeria innocua* and *L. monocytogenes* in enrichment broths, food and environmental samples. To date little data has been published comparing growth and biofilm formation of *L. innocua* and *L. monocytogenes* on food contact surfaces such as stainless steel. A study was conducted to determine whether any differences exist between these two organisms' ability to form biofilms in a mixed culture. Preliminary studies to determine an effective means for harvesting biofilm cells from stainless steel coupons led to the conclusion that vortexing with glass beads followed by sonication provided the most effective method for biofilm recovery. An evaluation of *L. monocytogenes* and *L. innocua*'s ability to form biofilms at 4°C was performed using the CDC Biofilm reactor. Three strains of each species isolated from food processing environments were used. Biofilms were propagated in 10% Tryptic Soy Broth + 0.6% Yeast Extract + 1.0% Glucose (TSB-YEG) on stainless steel coupons. Coupons were sampled after 6, 10, 15 and 30 days and examined for the predominant *Listeria* species. The changes in population of *L. innocua* and *L. monocytogenes* when grown in mixed culture were 0.11 and 0.66 log CFU/cm^2, respectively, over the 30-day incubation period. The results of this study show that *L. monocytogenes* and *L. innocua* grow and form biofilms on stainless steel equally in a refrigerated environment.

P1-47 Inactivation of *Escherichia coli* O157:H7 in Biofilm on Stainless Steel

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A study was done to determine the effectiveness of an alkaline cleaner and a lytic bacteriophage specific for *Escherichia coli* O157:H7 in killing wild type and *rpo*S-deficient cells of the pathogen in a biofilm. Cells were attached to stainless steel coupons (ca. 7 to 8 log CFU/coupon) on which biofilms were subsequently developed at 22°C. Coupons were treated with 100% and 25% of working concentrations of a commercial alkaline cleaner (pH 11.9, with 100 μg/ml free chlorine) used in the food industry, chlorine solutions (50 and 100 μg/ml free chlorine), or sterile water at 4°C for 1 and 3 min. Treatment with 100% alkaline cleaner reduced populations by 5 to 6 log CFU/coupon, a significant ($P \leq 0.05$) reduction compared to treatment with water. The initial population (2.6 to 2.8 log CFU/coupon) of attached cells of both strains was reduced by 1.1 to 1.2 log CFU/coupon when treated with bacteriophage KH1 (7.7 log PFU ml). Biofilms on coupons containing 2.7 log of wild type or *rpo*S-deficient cells were not decreased by more than 1 log CFU/coupon when treated with KH1 (7.5 log PFU/ml). Higher numbers of cells of *E. coli* O157:H7 in biofilms were killed by treating with alkaline cleaner than with chlorine solution (100 μg/ml). Results show that the number of cells attached on coupons was reduced by treating with bacteriophage but cells enmeshed in biofilms were protected. It is concluded that the alkaline pH, in combination with hypochlorite, in a commercial cleaner is responsible for killing *E. coli* O157:H7 in biofilms.

P1-48 Comparison of Biofilm Formation by *Salmonella* spp. Originating from Produce, Animal, and Clinical Sources

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Cantaloupe melons have been implicated in six outbreaks of salmonellosis since 1990. Research in our laboratory has documented the formation of biofilms by *Salmonella* on cantaloupe surfaces. The entrapment of cells of *Salmonella* within a biofilm is likely responsible for enhanced sanitizer resistance. Two major components of *Salmonella* biofilms have recently been discovered: curli and cellulose. Our objective was to investigate whether *Salmonella* isolates from produce sources exhibited increased biofilm formation compared to those originating from animal and clinical sources, and to screen our collection for cellulose and curli production. Biofilm formation was determined using a crystal violet staining method. Curli and cellulose production were monitored by assessing morphotypes on LB agar without salt containing Congo Red and fluorescence on LB agar containing calcofluor, respectively. The crystal violet binding assay demonstrated no significant differences in biofilm formation by isolates from any source when tested in either LB broth supplemented with 2% glucose, tryptic soy broth, or 1/20th-strength tryptic soy broth. One hundred percent of clinical isolates exhibited curli biosynthesis and 73% demonstrated cellulose production. All meat-related isolates formed curli and 84% produced cellulose. Eighty percent of produce-related isolates produced curli but only 52% produced cellulose. Crystal violet binding was not statistically higher in isolates of any morphotype, but was related to the media in which the strains were tested. Results indicate that biofilm formation was not dependent on the source of the test isolate and suggest a correlation between curli and cellulose production and biofilm formation.

P1-49 A New Chromogenic Plating Medium for the Isolation and Identification of *Enterobacter sakazakii*

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A proprietary chromogenic plating medium, R & F® *Enterobacter sakazakii* Chromogenic Plating Medium (ESPM), has been developed for isolating presumptive colonies of *Enterobacter sakazakii*. ESPM contains not only chromogenic substrates that are relatively specific for *E. sakazakii*, but also sugars, a pH indicator, and inhibitors for both Gram-negative and Gram-positive contaminants. By use of ESPM, 47 pure culture strains of *E. sakazakii* yielded blue-black domed colonies 1-2 mm in diameter with or without clear halos. Other enterics tested included: *Enterobacter aerogenes*, *Enterobacter gergoviae*, *Pantoea* spp., *Escherichia coli* spp. *Citrobacter*, *Klebsiella*, *Morganella*, *Providencia*, *Salmonella* spp., *Shigella* (4 species), *Hafnia*, and the non-enteric *Pseudomonas*. All strains yielded either white or clear colonies +/- clear halos with the exceptions of *Shigella sonnei* (blue-black or blue-gray colonies), *E. aerogenes* (yellow colonies) and *Klebsiella pneumoniae* (yellow or green colonies). *E. sakazakii* from powdered infant formula, powdered and dried foods and ingredients, and environmental samples was isolated by picking blue-black colonies from ESPM, and false positives were eliminated by observing acid production in 6 h on a screening medium. ESPM is more selective and differential than the Violet Red Bile Glucose Agar/Tryptic Soy Agar (TSA) plating system currently used to presumptively identify *E. sakazakii* as yellow colonies on TSA. All presumptive isolates from foods and environmental sources were confirmed using both API 20E and Biolog Microlog3 4.20.

P1-50 Isolation of *Enterobacter sakazakii* Using Combined Selective and Differential Enrichment Broth and Agar

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Enterobacter sakazakii is an emergent pathogen causing meningitis, necrotising enterocolitis and sepsis. Outbreaks in neonates have been linked to ingestion of contaminated infant formula and it is essential that reliable detection methods are established to ensure the safety of infant foods. The Druggan-Forsythe-Iversen (DFI) method includes a broth incorporating a high carbohydrate concentration which promotes the growth of *E. sakazakii* over that of competing bacteria. This is followed by a selective and differential agar incorporating the chromogenic substrate 5-bromo-4-chloro-3-indolyl--D-glucopyranoside that allows presumptive identification of *E. sakazakii* in the presence of mixed cultures. The DFI method and other proposed *E. sakazakii* isolation media were assessed using over 250 *Enterobacteriaceae*, including over 160 *E. sakazakii* isolates. No desiccated *E. sakazakii* were recovered using direct plating of rehydrated strains onto non-selective agar, indicating the need for an enrichment step. Growth was detected for 100% of *E. sakazakii* strains tested in DFI broth compared to the persistence of only 88 to 97% of strains in other enrichment broths (mLST, EE broth and ESSB from AES). Incorporation of a chromogenic-glucosidase substrate into isolation agars improved the speed and accuracy of presumptive *E. sakazakii* identification. All methods failed to distinguish presumptive *E. sakazakii* from other -glucosidase positive colonies without further biochemical tests. The DFI method showed 100% sensitivity for growth of *E. sakazakii* and promoted the growth of *E. sakazakii* over that of competing bacteria, increasing the likelihood of detecting this pathogen in mixed cultures.

P1-51 DSC Cloning and Sequencing of the Gene Encoding Outer Membrane Protein A (*ompA*) in *Enterobacter sakazakii* and Development of an *E. sakazakii*-specific PCR for Its Rapid Detection in Infant Formula

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Enterobacter sakazakii is an emerging foodborne pathogen that causes severe meningitis, meningoencephalitis, sepsis, and necrotizing enterocolitis in neonates and infants, with a high case fatality rate. The disease has been primarily associated with the ingestion of contaminated, powdered infant formula. During the past two years, more than 1.5 million cans of *E. sakazakii*-contaminated dry infant formula powder have been recalled in the United States. Traditional detection methods take more than four days for identifying *E. sakazakii*. Outer membrane protein A (*ompA*) is a major virulence factor in gram-negative pathogenic bacteria. In this study, the gene encoding *ompA* in *E. sakazakii* (ATCC 51329) was cloned in pGEM-T Easy vector and sequenced by fluorescent dye-terminator sequencing. The nucleic acid sequence of the *ompA* gene was compared with other available gene sequences present in the National Center for Biotechnology Information Genbank database. Based on regions of the *ompA* gene unique to *E. sakazakii*, two primers were synthesized to develop and optimize an *E. sakazakii*-specific PCR. The PCR amplified a 469-bp DNA product from 17 isolates of *E. sakazakii* tested, but not from any of the 40 non-*E. sakazakii* strains. Experiments to determine the sensitivity of the PCR indicated that it can detect as low as 1000 CFU/ml of *E. sakazakii* in infant formula without any enrichment, and 0.1 CFU/ml after an 8 h enrichment step. This PCR could potentially be used as a rapid tool for screening infant formula for the presence of *E. sakazakii*.

P1-52 Survival and Growth of *Enterobacter sakazakii* in Infant Rice Cereal Reconstituted with Water, Milk, Infant Formula, or Apple Juice

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Enterobacter sakazakii infections in preterm neonates and infants have been associated with reconstituted infant formula. Children up to 4 years old and elderly adults also have been diagnosed with *E. sakazakii* infection. We conducted a study to determine survival and growth characteristics of *E. sakazakii* in infant rice cereal as affected by the type of liquid used for reconstitution and storage temperature after reconstitution. A commercially manufactured dry infant rice cereal was reconstituted with water, apple juice, milk, or liquid infant formula, inoculated with a ten-strain mixture of *E. sakazakii* at populations of 0.27, 0.92, and 9.2 log CFU/ml, and incubated at 4, 12, 21, or 30°C for up to 72 h. Growth did not occur in cereal reconstituted with apple juice, regardless of storage temperature, or in cereal reconstituted with water, milk, or formula and stored at 4°C. The lag time for growth in cereal reconstituted with water, milk, or formula was decreased as the incubation temperature (12, 21, and 30°C) was increased. Upon reaching maximum populations of 7 to 8 log CFU/ml, in some instances populations decreased to undetectable levels during subsequent storage, which was concurrent with decreases in pH. It is concluded that *E. sakazakii* initially at a low population can rapidly grow in infant rice cereal reconstituted with water, milk, or infant formula. Reconstituted cereal that is not immediately consumed should be discarded or stored at a temperature at which *E. sakazakii* and other foodborne pathogens cannot grow.

P1-53 The Survival and Growth of *Enterobacter sakazakii* in Human Breast Milk and Powdered Infant Formula

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Enterobacter sakazakii (Es) has been linked to outbreaks involving debilitated neonates consuming powdered infant formula (PIF). The only documented report of human milk and its effect on pathogens indicated that human breast milk may contain antimicrobial activity against Es. Considered a sterile environment, human breast milk was used to evaluate the growth and/or survival of Es, in order to assess its potential as a source of illness. Frozen (n = 10) and fresh human milk (n = 2) were inoculated with 10 CFU/ml Es and growth was monitored at 10°C, 23°C and 37°C. Generation times for 10°C, 23°C and 37°C, respectively, were obtained for clinical (3.3 days, 81 min, 67 min), environmental (4.5 days, 95 min, 65 min), and food (2.2 days, 77 min, 59 min) isolates. Whereas there were no significant differences at 23°C and 37°C, growth at 10°C was food>clinical>environmental isolates. A generation time of 56 min was obtained using a single fresh human breast milk sample (37°C). To date, Es has been able to survive in frozen breast milk for over 3 months with no decrease in numbers. Growth of Es (37°C) did not differ between reconstituted PIFs and human milk. To our knowledge, this is the first report demonstrating the ability of Es to grow in human breast milk. While all milk samples tested negative for Es, putative antimicrobial properties of breast milk do not appear to be effective against this foodborne pathogen. Current experiments are focussing on the effects of human milk fortifiers on the survival and/or growth of Es.

P1-54 Prior Exposure to An Acidic Environment Increases the Acid Resistance of *Enterobacter sakazakii*

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Previous studies with 12 strains of *Enterobacter sakazakii* showed BHI-grown strains were moderately acid resistant compared to other *Enterobacteriaceae* such as *Escherichia coli* O157:H7, decreasing by ≥ 5 log cycles when resuspended in TSB adjusted to pH 3.0, but generally decreasing by < 1.0 log cycle at pH 3.5. In the current study, the same 12 strains were evaluated to determine if they have an inducible pH-dependent stationary phase acid resistance that would enhance survival when cultures were subsequently exposed to an acidified environment. The strains were grown individually in TSB with (+G) or without (-G) 1% glucose for 18 h. The final pH of the 18-h cultures was 4.7-5.4 for the +G grown cells and 6.7 to 7.0 for the -G grown cells. The 18-h cultures were then suspended in the acidified TSB (pH 3.0) for 5 h at 36°C, with samples being taken hourly and viable counts determined. All 12 strains to varying degrees had increased survival if previously grown in the presence of glucose. At times during the acid challenge, the differential in counts between the +G and -G samples was a much as 4 log cycles. The results indicate that the acid resistance of *E. sakazakii* can be increased by prior growth in a moderately acidic environment and that the microorganism possesses one or more systems for inducible pH-dependent stationary phase acid tolerance.

P1-55 Development of a High Pressure Processing Inactivation Model for Hepatitis A Virus

DSC

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Hepatitis A virus (HAV) is transmitted by the fecal-oral route and has been commonly linked with enteric illness associated with the consumption of contaminated shellfish. Oysters are commonly consumed raw or lightly cooked, and pathogenic microorganisms taken up during filter feeding may not be completely removed by depuration prior to human consumption. High pressure processing (HPP) can extend the shelf life of various food products while preserving their organoleptic qualities, but there is little information on the ability of HPP to inactivate HAV in whole oysters. The aim of this study is to create a HPP inactivation model for HAV and to validate the model in artificially contaminated oysters. Exposure of HAV in cell culture supernatant to 600 MPa reduced HAV titer from 5.00 log TCID50/ml to an undetectable limit (< 2.17 log TCID50/ml) within 90 s, while a 300 s exposure at 500 MPa achieved a similar outcome. Exposure at 300 and 400 MPa reduced HAV titer by 2.16 (within 480 s) and 2.90 log TCID50/ml (within 600 s), respectively. A HPP kinetic inactivation model is under development to predict the effects of pressure on HAV in a defined environment, including salt concentrations and temperatures. These preliminary results demonstrate the effectiveness of HPP for the inactivation of HAV, and the potential for its future use in the seafood industry.

P1-56 Evaluation of Repair Potential of Sublethally Injured *Listeria monocytogenes* in Six Enrichment Broths

DSC

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Detection methods using enrichment broths to support growth of *Listeria* to detectable levels have historically lacked the sensitivity to detect injured subpopulations. The ability of enrichment media to promote repair of sublethal injury is a critical step in overall *Listeria* recovery. Six enrichment broths—UVM (used by

FSIS-USDA), *Listeria* Repair Broth (LRB), Peptone water, *Listeria* Enrichment Broth (M52, used by FDA), *Listeria* Enrichment Broth w/ Supplement (LEBS) and Trypticase Soy Broth (TSB) — were compared for their ability to recover heat-injured cells. *Listeria* was injured in a 56°C water bath for the appropriate time resulting in an injury level of >98%. Cells were resuspended in six broths and incubated at 35°C. Percent repair was evaluated using Tryptose Phosphate Agar (TPA) and TPA w/4% NaCl (TPAN) by plating at time 0 and once per h for 4 h. Results indicated that percent repair ranged from 8.1 to 22.9%. LEBS was the lowest at 8.1% indicating that adding selective ingredients inhibits the recovery of injured cells. The media recommended by the FDA had a lower repair rate at <14% than other selective media with recovery rates >19%. Results indicate that despite development of methods specifically designed to recover injured *Listeria* from foods, the efficacy of specific media to promote recovery of heat injured *Listeria* is poor, indicating that routine regulatory procedures underestimate the numbers of *Listeria* detected. Interventions used to reduce the presence of *Listeria* in RTE products mandates recovery of injured *Listeria* as a critical process to ensure food safety.

P1-57 DSC Use of the Feline Calicivirus Model in Evaluating Methods to Extract and Detect Noroviruses in Foods

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Foodborne transmission of noroviruses is common and occurs as a consequence of contamination with human fecal material. Because these viruses cannot be cultivated in vitro, their detection in contaminated foods is challenging. The purpose of this research was to determine the suitability of feline calicivirus (FCV) as a surrogate for noroviruses in evaluating extraction and detection methods in foods. Using the culturable FCV strain F9, representative at-risk foods (lettuce, deli meats) were inoculated and the virus was extracted by sequential steps of elution, precipitation, and organic solvent extraction. Initial studies focused on optimization of various extraction parameters, including elution buffer composition and pH, concentration of polyethylene glycol (PEG) for precipitation, and choice of solvent. Virus recovery was evaluated by cell culture infectivity assay using Crandall Reese feline kidney (CRFK) cells. Use of a glycine saline elution buffer, 8% PEG, and the organic solvent Vertrel® provided the best virus recoveries; optimal pH during elution and reconstitution was dependent upon product type. Recovery efficiency after elution ranged from 54 to 65%. The efficiency of PEG precipitation was virtually 100%, but substantial virus (> 50%) was lost during solvent extraction. Using primers corresponding to the nonstructural protein region of the FCV genome, an RT-PCR assay was developed that produced a 187 bp amplicon and detected FCV at levels of 10^2 to 10^3 PFU/reaction. Future work will focus on combining the extraction and amplification method, identifying detection limits in foods, and comparing these to RT-PCR detection limits achieved in foods artificially contaminated with various norovirus strains.

P1-58 DSC Development of a Common Rapid Method for Norovirus Concentration and Detection in Various Food Matrices

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Noroviruses (NoV) are the leading cause of viral gastroenteritis outbreaks in humans. The virus is highly infectious and easily spreads through foods and direct contact. Most current methods are geared towards detecting noroviruses in shellfish. With fruits, vegetables and ready-to-eat-foods becoming increasingly implicated in norovirus outbreaks the aim of this project is to develop an efficient and rapid common method to detect these viruses in these different food matrices. Feline calicivirus (FCV) was used as a surrogate for the noroviruses in initial studies. FCV was concentrated and its RNA was extracted from artificially-seeded ham samples by TRIzol™ reagent. The extracted RNA was purified by Instagene Matrix™ prior to the RT-PCR and subsequent visualization by gel electrophoresis. After standardization, this methodology was successfully used to detect noroviruses from deli meat and salad samples implicated in a recent norovirus outbreak. However, Instagene Matrix™ was not capable of complete removal of inhibitors present in foods which may hamper our detection limits. To overcome this limitation, subsequent trials using Dynabeads Oligo d(T)25™ were done on FCV and demonstrated an improved sensitivity of detection to limits <100 RT-PCR units of FCV inoculated onto ham, bread and lettuce samples. This methodology is currently being used to detect noroviruses from food samples artificially-inoculated with norovirus-positive stool. Once standardized, this methodology would be ideal in an outbreak setting due to its common application to various types of foods, increased sensitivity of detection, and its rapidity (whereby results can be obtained within a day).

P1-59 Rapid Extraction and Detection of Hepatitis A Virus from Food Samples

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Fruits, vegetables and ready-to-eat foods are increasingly implicated in Hepatitis A virus (HAV) outbreaks. Since only limited research has been done on virus detection in these foods as compared to virus detection in shellfish, this study aimed at 1) developing a rapid method for the extraction and detection of HAV from food of plant and animal origin, and 2) standardizing the sampling size for virus detection. The Pathatrix™ system, which relies on pumping and forceful circulation of immunomagnetic beads throughout the entire food sample,

has been successfully used for the capture and extraction of bacteria from foods. In this study, this system was adapted for HAV extraction from foods. Twenty five grams each of lettuce, green onion and ham were inoculated with HAV and placed in a stomacher bag containing 225 mL of EBSS buffer and 100 μL of anti-HAV coated magnetic beads. After one h of processing in the Pathatrix™, HAV-conjugated beads were separated from the food and suspended in 100 μL RNase-free water. This suspension was heated at 95°C for 5 min. to release viral RNA, and 10 μL volumes of this suspension were run through RT-PCR cycles. Our data indicated that the sensitivity of detection was 10 CFU/25g of food samples tested, which suggests that the Pathatrix™ system can be used for rapid virus extraction and detection (within 5 h) in large sample volumes of foods. This, we believe, is the first report demonstrating the use of the Pathatrix™ System for the detection of viruses in foods.

P2-01 Restaurant Inspection Practices and Beliefs of Environmental Health Specialists

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The public relies on environmental health specialists (EHSs) employed in public health agencies to ensure that restaurant food is safe. To that end, EHSs conduct restaurant inspections. To gain a better understanding of inspection practices and beliefs, we conducted six focus groups with randomly selected EHSs working in public health agencies in eight states (n = 42). Participants were asked to describe their most and least common and important inspection activities. Participants said that documenting and correcting critical violations (i.e., violations associated with foodborne illness [FBI] risk) of food safety regulations and educating restaurant managers about FBI risks were two of their most common and important inspection activities. They also said that documenting and correcting non-critical violations (i.e., violations not associated with FBI risk) and determining inspection scores were two of their more common, but least important, activities. Participants believed that non-critical violations were less important because they were not associated with FBI risk, and that inspection scores were less important because they often did not accurately reflect food safety (e.g., critical and non-critical violations often receive equal weight in scoring, even though non-critical violations are not associated with FBI risk). These findings indicate that many EHSs engage in FDA recommended activities for improving restaurant food safety, including focusing on FBI risks and establishing a dialog with restaurant operators in which FBI risks are identified and discussed. However, findings also indicate that EHSs engage in activities that are not in line with FDA recommendations, such as focusing on items that are not associated with FBI risk (non-critical violations).

P2-02 Prevalence and Genetic Diversity of *Arcobacter* and *Campylobacter* on Broiler Carcasses during Processing

DSC

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Campylobacter is the most common cause of acute bacterial gastroenteritis in humans worldwide, and poultry and poultry products are a major source of infection. *Arcobacter* is closely related to *Campylobacter* and has recently gained attention as an emerging foodborne pathogen. However, few reports have been published on the prevalence of *Arcobacter* in US poultry plants. The purpose of this study was to optimize methods of *Arcobacter* isolation and culture and to compare the prevalence of *Arcobacter* and *Campylobacter* on broiler carcasses from a commercial poultry processing plant. Carcasses were sampled at three sites along the processing line: 1) pre-scalding, 2) pre-chilling, and 3) post-chilling. Samples were collected during five plant visits from September to October of 2004. For *Arcobacter* isolation, Houf broth followed by plating on Brucella agar supplemented with 5% lysed horse blood and cefoperazone, vancomycin, and amphotericin B (CVA) produced the best results. For *Campylobacter*, Bolton's broth was used in conjunction with plating on CVA medium. CVA medium was very efficient for direct plating of *Campylobacter*. Overall, *Campylobacter* was isolated from 78.5% (255 of 325) of the carcasses from the three collection sites, while *Arcobacter* was isolated from 55.1% (179 of 325). Our results demonstrate significant contamination of broiler carcasses by *Arcobacter* although less than that found for *Campylobacter*.

P2-03 Microbial Analysis of Meals Served in Foodservice

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This study was performed to develop a basic model of predictive microbiology for microbial risk assessment by analyzing microorganisms of raw materials and cooking processes known to be the main causes of food poisoning in foodservices. Raw materials and cooking processes were classified by manufacturing and heating existence and non-existence, respectively, and their hazard and new types of hazard were investigated by experiment. To establish a basic model of predictive microbiology for MRA, Gompertz model was applied and the predictive growth models for *Escherichia coli* in MooSaengChae, and *Salmonella entertidis* in GalbiTang, which are the most common cause of food poisoning in foodservices, were suggested. Several suggestions on "HACCP Guidelines in Foodservices" in Korea was made through both literature review and experiments, in the part of thawing, heating temperatures and time fixing when cooking, cooling of cooked food, reheating, management before serving, management of hot-holding and temperature at serving. Therefore, this study has provided the basic data for guiding HACCP application to foodservices by providing the scientific sanitation standards for foodservices and confirming scientific foundation and objectiveness for assessment standards in aftercare and HACCP.

P2-04 Sequential Transfer of *Listeria monocytogenes* from a High-density Polyethylene Surface to Bologna

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Conveyor belts have been frequently targeted as a source for *Listeria* transfer to finished products in the processed meat industry. To assess this problem, duplicate high-density polyethylene (HDPE) coupons measuring 10 × 10 cm were inoculated (3 replicates) with a 6-strain *L. monocytogenes* cocktail to yield ~10^4 CFU/coupon. After drying 30 min, one bologna slice (20 g) was placed on the inoculated surface and subjected to a 5-pound deadweight for 1 min. This slice was then removed and replaced with an uninoculated bologna slice with this process repeated until 25 slices consecutively contacted the originally inoculated surface. Each slice was diluted 1:5 in phosphate buffer solution (pH 7.4) and stomached for 2 min. Five ml aliquots were pour-plated in duplicate into150-mm diameter Petri plates containing 20 ml of Modified Oxford agar and then incubated 48 h at 37°C, giving a detectable limit of 0.5 CFU/g (10 CFU/slice). Beginning with the tenth slice, samples were also enriched in University of Vermont Medium. *Listeria* populations transferred from the HDPE coupon to bologna decreased linearly (R^2 = 0.9783) during the first 15 contacts (y = -0.1859× + 3.3053) with a tailing effect seen thereafter by direct count and/or positive enrichment results. Slices 1 to 5, 6 to 10 and 11 to 15 accounted for 84.3, 11.1% and 1.9% of the *Listeria* cells transferred, respectively. However, the tailing effect seen for contacts 16 to 25 indicates continued sporadic transfer of *L. monocytogenes* with these low populations, posing a potential consumer risk if allowed to grow.

P2-05 Optimization of Recovery Methods for *Listeria monocytogenes* from Conveyor Belt Surfaces

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Current recovery methods lack the ability to accurately quantify bacterial transfer between foods and food contact surfaces. Consequently, three different methods — (1) mechanical vortexing (3000 rpm) for 2 min, (2) mechanical vortexing (3000 rpm) with sterile glass beads (3 to 4 mm dia.) for 2 min, and (3) sonication in a waterbath (40 kHz) for 5 min — were assessed for quantitative recovery of *Listeria monocytogenes* from interlocking conveyor belt pieces made of high-density polyethylene (HDPE), acetyl resin (A), and polypropylene (PP). The top surface of the conveyer belt piece (4 × 5 cm) was inoculated with a 6-strain *L. monocytogenes* cocktail (100 µl) to contain ~10^5 CFU/piece and then dried for 30 min. Each piece was then placed in a sterile plastic jar containing 100 ml of phosphate buffer solution (PBS, pH 7.0) and processed using the three methods. One hundred µl was plated on trypticase soy agar containing 0.6% yeast extract (TSAYE) and incubated 24 h at 37°C. Conveyor belt pieces were also pressed sequentially onto 5 or 6 TSAYE plates to quantify remaining listeriae. All three methods recovered similar numbers of *Listeria* from HDPE ($P < 0.05$). However, sonication and vortexing with beads removed more *Listeria* cells ($P < 0.05$) from PP compared to vortexing alone. Overall, recovery of *Listeria* was significantly greater ($P < 0.05$) using sonication compared to vortexing with or without glass beads. Thus, sonication can be used to more accurately assess bacterial transfer rates between foods and conveyor belt materials, with this information useful in refining current microbial risk assessments.

P2-06 Use of a Sensitive Adenosine Triphosphate Method to Quickly Verify Wet Cleaning Effectiveness at Removing Food Soils and Allergens from Food Contact Surfaces

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Rapid methods are needed to verify that food soils are removed during equipment cleaning and thus prevent cross contamination of foods with unlabeled allergens. The objectives of this work were to evaluate and compare an adenosine triphosphate (ATP) swab (AllerGiene™), that is more sensitive than conventional ATP swabs, to protein-specific allergen (ELISA) kits for verifying wet cleaning effectiveness. ATP sensitivity and detection of several NIST food solutions (peanut butter, whole egg, milk) were measured using the sensitive ATP swab. Preliminary work showed sensitivity to 0.01 femtomole (fmole) ATP and detection range 1 to 25 ppm to the food solutions tested. The ATP/ppm ratio varied with each food type. The sensitive ATP test and protein-specific ELISA kits demonstrated parallel sensitivity curves and a correlation ($r^2 > 0.95$) for each food soil. The sensitive ATP test and ELISA-based kits were then used to study the effects of wet cleaning protocols on food soils removal from a stainless steel surface. Wet cleaning parameters studied included cleaning solution temperature, cleaning time, and the type and detergent and/or sanitizer concentration. The sensitive ATP kit and the ELISA tests were useful for verifying the effectiveness of each of the wet cleaning parameters. Sensitive ATP and allergen-specific ELISA tests were also used to validate Sanitation Standard Operating Procedures (SSOPs) in a dairy plant using common equipment to process both milk, juice and water products. Sensitive ATP may be used to quickly verify food soil removal to ELISA test detection levels with certain food soils, during wet cleaning of food processing equipment.

P2-07 The Proteomic Response of *Escherichia coli* Filaments at Temperatures Near the Minimum for Growth

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Substantial fractions of *Escherichia coli* cells elongate to form filaments in broth cultures at temperatures experienced by chilled meat during storage and retail display. The mechanism for filamentation at low temperatures is not known. Therefore, the proteomic response of *E. coli* at temperatures just below and above the minimum for growth was examined. Proteins extracted from *E. coli* at different incubation times were separated using 2-dimensional gel electrophoresis and differentially expressed proteins were identified using quadropole-time of flight mass spectrometry. Levels of OsmY, a positive indicator of *Rpo*S, were approximately 100-fold or 10-fold higher in cells after 1 day of incubation at 6°C or 8°C, respectively, compared to cells incubated at 15°C. A number of proteins that were upregulated at temperatures near the minimum for growth are involved in protein folding and degradation, carbohydrate metabolism, electron transport, and the TCA cycle. Proteins that were down regulated are involved in protein synthesis or anaerobic carbon metabolism and energy generation. Cells appear to invoke the stringent response due to increased energy demands to sustain growth. DNA repli-cation generally ceases under unfavorable conditions but glucose inhibited division protein B (GidB) was upregulated, which may allow DNA replication to continue. These studies provide insight into the physiological response of *E. coli* at temperatures near the minimum for growth.

P2-08 Fitness Problems in *Escherichia coli* K-12 Transformed with a High Copy Plasmid Encoding the Green Fluorescent Protein

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The green fluorescent protein (GFP) of the jellyfish *Aequorea victoria* has been widely used as a biomarker in eucaryotic and prokaryotic cells and has potential for developing predictive models for behavior of single strains of bacteria in naturally contaminated food and environmental systems. However, constitutive production of GFP in bacteria can result in reduced fitness in the form of slower growth. Consequently, a high copy plasmid with gfp under the control of a tetracycline inducible promoter (pTIPgfp) was introduced into *Escherichia coli* K-12. To validate the GFP strain of *E. coli* K-12 for predictive modeling studies, growth kinetics of the parent and GFP strain were compared at 10, 25 and 40°C on sterilized cooked chicken breast meat (sterilized chicken). Although GFP expression was not induced during growth on sterilized chicken, maximum specific growth rate (umax) of the GFP strain was reduced ($P < 0.05$), regardless of incubation temperature. When growth kinetics were compared in BHI broth at 40°C, umax and maximum population density were reduced ($P < 0.05$) to the same extent in the absence and presence of tetracycline. These results indicated that the presence of the high copy plasmid introduced a fitness problem in *E. coli* K-12 in the form of slower growth and reduced cell yield that was independent of GFP production. Thus, use of a low copy plasmid or insertion of a single copy of GFP into the chromosome may be required to avoid fitness problems in GFP bacteria.

P2-09 DSC Quantitative Analysis of the Growth of *Salmonella* Stanley during Alfalfa Sprouting and Evaluation of *Enterobacter aerogenes* as Its Surrogate

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Raw seed sprouts have been implicated in several food poisoning outbreaks in the last 10 years. The US FDA recommends that sprouters use interventions (including testing of spent irrigation water) as means to control the presence of pathogens in the finished product. Few studies have looked at factors influencing effectiveness of testing of spent irrigation water. Alfalfa seeds were inoculated with *Salmonella* Stanley or its presumptive surrogate (Nalidixic acid resistant *Enterobacter aerogenes*) at three inoculum levels (3 CFU/g, 30 CFU/g, and 300 CFU/g), then transferred into either flasks or a bench top-scale chamber. Microbial concentrations on seeds, sprouts or in irrigation water at different times in four-day sprouting process were determined. Data were fit to logistic regression models. Growth rates and maximum concentrations were compared using generalized linear model procedure of SAS statistical software. No statistically significant differences in growth rates were observed between samples taken from flasks or chamber. Microbial concentrations in irrigation water showed no statistically significant difference from samples taken at the same time from sprouts. Data analysis verified that *E. aerogenes* concentrations were similar to *S.* Stanley concentrations at corresponding time points, for all three inoculum levels. Growth rates were also constant irrespective of inoculum level or strain, except that smaller inoculum levels reached lower final levels proportional to their initial levels. This research demonstrates that a non-pathogenic, easy to isolate surrogate (Nalidixic acid resistant *Enterobacter aerogenes*) gives similar results to those obtained with *S.* Stanley, supporting its use in future, larger scale experiments.

P2-10 **Fecal Pat Sampling and Homogenation for Detection of *Escherichia coli* O157**

DSC

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Human illnesses caused by *Escherichia coli* O157 have been associated with the consumption of ground beef and other products of animal origin. In order to evaluate and compare epidemiological studies and the efficacy of pre-harvest interventions to reduce this pathogen, standardization and accuracy in sampling and detection methods are required. In this ongoing experiment, 48 bovine fecal pats were sampled. Five samples were collected systematically from each pat, moving west to east (positions A to E, respectively) and analyzed for *E. coli* O157. Additionally, a homogenate was made by combining approximately 2 g from each of the five positions. All samples were pre-enriched and subjected to immunomagnetic separation with final confirmation using an agglutination test and PCR. Of the 48 fecal pats, *E. coli* O157 was not recovered from 36 (75%) pats in either the individual positions or the homogenate. A total of 6 (12.5%), 5 (10.42%), 8 (16.67%), 6 (12.5%) and 7 (14.58%) samples were positive in positions A, B, C, D, and E, respectively, while 7 homogenates (14.58%) were positive. *E. coli* O157 was recovered from 41.66% of the 12 positive pats in at least one of the A-E positions while not being recovered from the homogenate. One of the 12 pats had a homogenate test positive while none of the pat's A-E positions tested positive. Due to uneven distribution of the pathogen in fecal material, the testing of several samples from the same fecal pat could play a vital role in the detection of *E. coli* O157:H7.

P2-11 **Development of Rapid Immuno-diaganostic Methods for the Detection of Atrazine in Water Samples**

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The objective of this study was development of a rapid and simple analytical method for the detection of atrazine (AT) in surface and drinking water. For the production of monoclonal antibodies (MAbs), hybridoma cells were produced by the fusion of myeloma cell (V653) and spleen cells from immunized mice with AT conjugated to keyhole limpet hemocyanin or bovine serum albumin. The highly sensitive Mab (AT-1-M3) was selected and used to develop a direct competitive enzyme linked immunosorbent assay (DC-ELISA) in the microtiter plate and a one step test trip based on the immunochromatographic assay. AT-1-M3 MAb reacted with atrazine but it did not interact with other pesticides in both methods. The sensitivity of DC-ELISA was higher than the one step test strips as in 0.1 ng/ml and 20 ng/ml, respectively. However, the one step test strip method required only 10 min to get a result and it needed only one step as a drop of sample solution in a sample pad. On the other hand, the DC-ELISA method required more than 1 h as well as complex and multiple steps. Both methods were sufficiently accurate to be useful for the rapid detection of AT in various water samples.

P2-12 **Prediction of Pathogen Growth on Iceberg Lettuce under Real Temperatures History during Farm-to-Table Distribution**

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The growth of pathogenic bacteria *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* on iceberg lettuce under constant and fluctuating temperatures was modelled in order to estimate the microbial safety of this vegetable during distribution from the farm to the table. We examined pathogen growth on lettuce at constant temperatures, ranging from 5 to 25°C, and then we obtained the growth kinetic parameters (lag time, maximum growth rate and maximum population density (MPD)) using Baranyi's primary model and determined the Ratkowsky's secondary models of square root growth rate. The parameters were similar to those predicted by the Pathogen Modeling Program (PMP), with the exception of MPD. The MPD of each pathogen on lettuce was 2 to 4 log CFU/g lower than that predicted by PMP. Furthermore, the MPD of pathogens decreased with a fall in temperature. The relationship between the MPD and temperature was described by a linear equation. Predictions of pathogen growth under fluctuating temperature used the Baranyi primary microbial growth model along with the Ratkowsky secondary model and MPD equation. The fluctuating temperature profile used in this study was the real temperature history during distribution. Overall predictions for each pathogen agreed well with observed viable counts in most cases. However, the prediction concerning *E. coli* O157:H7 and *Salmonella* spp. on lettuce greatly overestimated growth in the case of a temperature history starting relatively high, such as 25°C for 5 h. In contrast, the overall prediction of *L. monocytogenes* under same circumstances agreed with the observed data.

P2-13 **Predictive Modeling for the Growth of *Listeria monocytogenes* as a Function of Temperature, pH, and NaCl**

DSC

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To implement scientific management of food safety, predictive microbiology can estimate the growth, survival, and inactivation responses of foodborne pathogens to various environmental conditions by the use

of mathematical models. The current study developed the predictive model on the separate effect of temperature, pH, and sodium chloride (NaCl) against of the growth of *Listeria monocytogenes* in tryptic soy broth (TSB). The TSB containing three different concentrations of NaCl (2, 4, and 5%) was adjusted to six different initial pH levels (pH 4, 5, 6, 7, 8, and 9) and incubated at either 4, 10, 25 or 37°C. The growth of *L. monocytogenes* in TSB was significantly ($P < 0.05$) inhibited by acidic or basic pH levels, stepwise increase concentrations of NaCl or stepwise decrease of temperature. The growth curves were fitted to a Gompertz model to obtain lag time (LT) and specific growth rate (SGR) using GraphPad prism Software. All R^2 values as one of the goodness of fit to the data obtained from the primary Gompertz model were over 0.98. In each model variables, natural logarithm transformations of LT and SGR from growth curve fits, were subjected to regression analysis using a response surface model to develop a secondary model. Correlation coefficient values between observed and predicted SGR and observed and predicted LT were 0.97 and 0.91, respectively. Therefore, these results indicated that the developed secondary model may be applied to predict the growth of *L. monocytogenes* exposed to various temperatures, pH values, and NaCl concentrations.

P2-14 Exposure Assessment of *Staphylococcus aureus* Inoculated into Potentially Hazardous Foods in School Foodservice Operation

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The objective of this study was to investigate major risk factors affecting the degree of *Staphylococcus aureus* contamination to meet the scientific criteria of HACCP plan for potentially hazardous foods served in school foodservice. The effects of competitive microorganisms, initial contamination level (10^2, 10^5), and storage temperatures (10, 25, 35°C) on the growth of *S. aureus* ATCC 25923 inoculated into cooked, potentially hazardous foods (seasoned bean sprouts, glass noodles with beef and vegetables, fried-seasoned tofu, and meat ball) were determined. The growth curves were fitted into two-phase linear model using a GraphPad Prism and @Risk's simulation was used to analyze the risk factor caused high contamination level of *S. aureus* before serving. Meat ball has highest potential to become a hazardous food among the prepared foods tested in this study. The growth of *S. aureus* was accelerated with higher (10^5 CFU/g) contamination levels and temperature (35°C), while storage at 10°C resulted in no growth of *S. aureus* in all foods. In general, *S. aureus* proliferated well without competitive organisms. The highest risk factor associated with contamination level of *S. aureus* in meat ball was the prevalence of *S. aureus*, followed by its growth before serving and initial contamination level. These results indicate that the prevalence of *S. aureus* in potentially hazardous ingredients and cooked foods via cross-contamination is the critical risk factor and its growth must be controlled by keeping the cooked foods over 57°C to secure the safety of foods even under the worst situations in school foodservice.

P2-15 Microbial Risk Assessment of *Staphylococcus aureus* in Ready-to-Eat Kimbab in Korea

DSC

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This study was conducted to address specific risk management questions with *Staphylococcus aureus* in ready-to-eat Kimbab (rice rolled in laver) in Korea using microbial risk assessment (MRA). MRA model was conducted by constructing four nodes from retail to table pathway. Predictive microbial growth model and survey data were combined with probabilistic modeling to simulate the level of *S. aureus* in retail Kimbab at the time of consumption. Due to the lack of dose-response models, the final level of *S. aureus* in retail Kimbab was used as a proxy for potential for causing illness. Therefore, we assumed the potential hazard level as 5 log CFU/g and estimated the possibility of contamination over this level as 30.7%. The consumption level of *S. aureus* through Kimbab was estimated as 3.67 log CFU/g. The regression sensitivity results showed that time-temperature during storage at selling was the most significant factor. These results suggested that temperature control under 10°C was a critical control point in Kimbab establishment to prevent the growth of *S. aureus* and showed that MRA was useful to evaluate several factors that influence the potential risk and to draw inferences with relevance to risk management.

P2-16 Results from a Pilot Survey on Domestic Handling of Meat and Poultry in Christchurch, New Zealand

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To improve quantitative risk assessments for foodborne pathogens in New Zealand, preliminary data on domestic handling of meat and poultry has been gathered during a pilot survey in Christchurch. Meat and poultry were usually bought fresh, rather than frozen; 49/52 respondents (94%) bought 80% or more of their meat fresh, and 33/52 (63%) respondents bought 80% or more of their poultry fresh. Most respondents transported their food home by car (50/53; 94%) and 47/53 (89%) took less than an h to do so. Just two respondents (2/52; 4%) used an insulated container for transporting meat and poultry home. However, 25/52 (48%) usually packed chilled/frozen meat and poultry with other frozen foods or wrapped the meat/poultry in thick newspaper to carry home. Approximately 70% of the meat or poultry was reported to be frozen at home following

transport. Thawing preferences included: refrigerator (20%); room temperature (44%); and microwaving (30%). A total of 22/40 (55%) respondents usually used an automated dishwasher to wash up. Questions about food safety behaviors (handwashing before and after food preparation, use of utensils and surfaces for different foods, reheating leftovers, etc.) elicited a high proportion of "correct" answers in this survey, although other studies have demonstrated a difference between reported and observed behaviours. The majority of respondents reported that they always prepared the main meal (63%) and most were female (75%). Although this survey of predominantly urban respondents may not be representative of New Zealand as a whole, information about purchasing and storage behaviours will be useful.

P2-17 Results from a Pilot Survey of Domestic Refrigerators in Christchurch, New Zealand

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To improve quantitative risk assessments for foodborne pathogens in New Zealand, preliminary data on food handling associated with refrigerators has been gathered during a pilot survey of 53 respondents in Christchurch. Respondents placed two temperature data loggers in their refrigerators (top and bottom shelves) for at least 72 h (one weekday and a weekend; temperatures recorded every 10 min), as well as completing a questionnaire about food handling in relation to refrigerators. Respondents also provided a photograph of the open refrigerator, so that layout could be assessed. Twenty three (43%) refrigerators had average air temperatures between 1°C and 5°C, while 26 (49%) had average air temperatures between 5°C and 7°C, and 4 (8%) had average air temperatures over 7°C. The refrigerator with the warmest average air temperature recorded 9.9°C. The average difference between the maximum and minimum temperature recorded inside the refrigerators was 6°C. The highest temperature recorded was 16.5°C. Most refrigerators (72%) recorded higher temperatures on the top shelf than on the bottom shelf. From the questionnaire, out of the 52 people who answered the questions, 42 (80%) correctly identified that food should be kept between 1°C and 5°C, but 50 (96%) did not have a refrigerator thermometer. None of the refrigerators surveyed had an inbuilt thermometer; although many had graduated setting scales. Just over half of the respondents (52%) stated they never changed their thermostat settings. The temperature profiles provided by the data loggers will allow the assessment of potential growth by pathogens, if present.

P2-18 Acute and Subacute Toxicity and Antimicrobial Activity of Nano Au

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This study is performed to investigate toxicity and antimicrobial activity of Nano Au (nanosize metallic gold particles which have potential as antimicrobial agents) both in vivo and in vitro. 5 week-old ICR mice and Sprague Dawley (SD) were used and raised for 14 days for the acute toxicity test and were divided into three subgroups (n = 10); mice received oral administration doses of 0, 3000, 4000 ppm/kg b.w., and rats of 0, 5000, 6000 ppm/kg b.w. In subacute toxicity test, rats were divided into four groups (n = 10) and received oral doses of 0, 50, 500, 5000 ppm/kg b.w. for 28 days. In both acute toxicity and subacute toxicity test, the body weight, consumption of feed and drinking water did not differ in ICR mice and SD rats. In subacute toxicity test, blood biochemistry test showed no difference; however, glucose and TG level in experimental groups were significantly lower than in control group ($P < 0.05$). It fell into the normal range. Nano Au had strong antimicrobial activity against gram-negative microorganisms, gram-positive microorganisms, and yeast. Especially, in case of *Salmonella* Enteritidis, *Streptococcus faecalis*, *Candida albicans*, all died in 5 ppm of Nano Au at one-h incubation. This result demonstrated that Nano Au has no toxicity in 0, 2,000, 3,000 ppm/kg b.w. in ICR mice and 0, 50, 500, 5,000, 6,000 ppm/kg b.w. in rats. Also, it has strong antimicrobial activity.

P2-19 Adsorption of Cadmium and Lead by Various Cereals from Korea

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The occurrence of heavy metal contaminants in crops has been mainly an issue in countries that consume cereals as the primary source of nutrition. The content of heavy metals (Hg, Pb, Cd, and As) of cereals consumed in Korea and their binding capacity to Pb and Cd in an in vitro digestion model were analyzed. Heavy metal contents of 18 kinds of cereals were measured by atomic absorption spectrophotometer for Pb, Cd, and As and by a mercury analyzer for Hg. The cereals were also prepared in both the soluble and the insoluble fraction to examine the extent of their binding capacity to Pb and Cd under a simulated gastric solution. The average concentrations of heavy metals for this study were 1.31, 10.7, 0.71, 48.6 μg/kg for Hg, Pb, Cd, and As, respectively. The insoluble fraction showed higher binding capacity to Pb and Cd than the soluble fraction in all cereals tested. For Pb adsorption, the insoluble fraction of prosomillet (84.1%) was the most effective, followed by black rice (83.4%), job's tears (83.6%), wheat (83%), and buckwheat (81.8%). Likewise, Cd adsorption by the insoluble fraction of prosomillet (99.7%) was the highest, followed by brown glutinous rice (99.5%), wheat (97.7%), black rice (97.1%), brown rice (96.5%), and job's tears (94.5%). In conclusion, heavy metals in cereals from Korea were at low levels (< 100 ppb). This study suggests that such cereals as prosomillet can be used as an adsorbent of Pb and Cd for enhancing the excretion of Pb and Cd from the human body.

P2-20 DSC Antibiotic Resistance Patterns in *Escherichia coli* O157 Isolates Collected from 2001 to 2004

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Using antimicrobial drugs in animals destined for human consumption has become a great public concern due to the potential development and dissemination of antibiotic resistance through the food chain. *E. coli* O157 isolates recovered from cattle fed typical finishing rations containing monensin and tylosin were examined for susceptibility to 16 antimicrobial drugs and antimicrobial combinations. Of banked isolates recovered from 2001–2004, 25 isolates representing each year were evaluated for a total of 100 isolates. Minimum inhibitory concentrations (MICs) were determined using standards set by the National Antimicrobial Resistance Monitoring System. Thirty-six percent of the isolates were resistant to at least one antimicrobial drug. Nine percent of the isolates were resistant to two antimicrobial drugs, i.e., sulfamethoxazole and tetracycline. Resistance to tetracycline was only identified in isolates from 2001. Two isolates were resistant to three different antimicrobial drugs; one isolate was resistant to amoxicillin/clavulanic acid, cephalothin, and cefoxitin while the other was resistant to streptomycin, sulfamethoxazole, and tetracycline. Thirty percent were resistant to sulfamethoxazole. Substantial variation in the proportion of isolates resistant to sulphamethoxazole was observed between years (10 isolates in 2001, 0 in 2002, 1 in 2003, and 19 in 2004). The drugs to which resistance was observed most commonly after sulfamethoxazole were tetracycline and cephalothin (10% and 6% resistant, respectively). Overall, 2004 and 2001 had the greatest number of isolates resistant to at least one antimicrobial drug (19 and 11, respectively) compared to 2003 and 2002 (4 and 2, respectively).

P2-21 Antimicrobial and Genetic Characterization of *Salmonella* spp. Isolated from Broiler Washes from Four Different Sampling Points

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The objectives of this study were to determine the antimicrobial resistance profile of *Salmonella* spp. isolated from whole broiler rinses at four sampling points (prechill, post chill, rehang and reprocessing). One-hundred and sixty whole broiler carcasses selected from four different sampling points (rehang; reprocessing; prechill and post chill) were analyzed for *Salmonella* spp. using tetrathionate as an enrichment broth and bismuth sulfite and brilliant green agar (BSA and BGA) as selective media. The isolates were characterized for production of sugars, reaction to O and H antisera, and use of primers ST 11 and ST 15, which are specific for *Salmonella* by pcr assay. To determine antibiotic resistance profile, 10 different antimicrobial agents (ampicillin, cefafzolin, cefixime, clindamycin, gentamicin, novobiocin, rifampin, spectomycin, tetracycline and trimethoprim) were used by the disk diffusion method. Forty-eight (30.0%) of broiler samples were positive for *Salmonella*. A total of 360 isolates were obtained, of which 75 (20.8%) were positive for *Salmonella*, using O and H antisera. Of the 75 *Salmonella* positive isolates, 38 (50.7%) gave positive pcr for detection of *Salmonella* and these isolates were tested for their antimicrobial susceptibility. Of the 10 antimicrobial agents tested, 53% of pre-chill; 50% post chill; 88.2% reprocessing and 69.2% of rehang isolates were resistant to the antimicrobial agents. The findings indicate that high levels of antimicrobial resistant strains *Salmonella* can be isolated from broiler carcasses and use of pcr and antimicrobial profiles may be a useful epidemiological tool.

P2-22 Characterization of Multi-Antimicrobial Resistant Enterohemorrhagic *Escherichia coli* Isolated from Whole Broiler Carcass Rinses

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The objectives of this study were to determine the antimicrobial resistance profile and the presence of the verotoxin (VT) and attachment and effacement (*Eae*) genes of isolates from whole broiler rinses from four different sampling points. One-hundred and sixty whole broiler carcasses selected from four different sampling points (rehang; reprocessing; prechill and post chill) were analyzed for *E. coli* O157:H7 using modified tryptic soy broth supplemented with novobiocin as an pre-enrichment broth and cefixime-tellurite sorbitol MacConkey agar (CT-SMAC) as selective media. The isolates were characterized for glucoronidase activity, reaction to O157 and H7 antisera, and the presence of VT 1 or 2 and *E. coli* attachment and effacement genes by pcr assay. To determine antibiotic resistance profile, 10 antimicrobial agents were used by the disk diffusion method. Sixty (37.5%) of broiler samples were positive for *E. coli*. A total of 235 isolates were obtained, of which 47 (20%) were O157:H7; 40 (17%) O157:non H7; and 148 (63%) non O157:H7. Of the 47 O157:H7 positive isolates, 35 (74.5%) gave positive pcr for both VT and *Eae* genes and these isolates were tested for their antimicrobial susceptibility. Of the 10 antimicrobial agents tested, a total of 78.6% of the isolates from rehang, reprocessing and pre-chill were resistant, while 3.7% were sensitive to the antimicrobial agents. This antimicrobial resistant profile indicates that there is a possible increase in antibiotic resistance among EHEC strains in poultry. This trend could potentially pose a serious threat to controlling EHEC infections in susceptible populations.

P2-23 Diversity of Plasmid Mediated Multidrug Resistance in *Proteus mirabilis* Isolates from Retail Meat Products

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The extensive use of antibiotics in medical and agricultural practices has resulted in widespread horizontal transfer of mobile genetic elements carrying resistance genes between bacterial species. Emergence of multidrug-resistant bacteria is of particular concern because of their potential for widespread dissemination and acquisition of additional resistance genetic elements. In this study, antibiotic-resistant *Enterobacteriaceae* were isolated from retail meat products. Among the tested strains, *Proteus mirabilis* was prevalently detected as a multidrug-resistant species. Therefore, diversity of integron-mediated antibiotic resistance genes in these *Proteus mirabilis* isolates were evaluated for better understanding of dissemination of the resistance genes between species in meat products. A total of 64 multidrug-resistant isolates of *Proteus mirabilis* were obtained, and their antibiotic resistances were examined by Kirby-Bauer disc diffusion test. The types of resistance genes were determined by polymerase chain reaction assay and DNA sequencing. Most of these isolates were resistant to ampicillin, tetracycline, and gentamycin, and kanamycin. Class 1 integrons were detected as a common carrier of the antibiotic-resistant genes, such as *aadA1, aadB*, and *aadA2*. A few isolates (9%) contained class 2 integrons with three gene cassettes included, the *dhfr1*, *sat1*, and *aadA1*. These isolates were even resistant to nalidixic acid due to mutations in the *gyrA* and *parC*. All ampicillin-resistant isolates contained *bla*TEM-1. Plasmids containing class 1 or 2 integrons and *bla*TEM-1were able to be transferred from *P. mirabilis* isolates into *E. coli* by conjugation, indicating that conjugal transfer could contribute to the dissemination of antibiotic-resistance genes between the *Enterobacteriaceae* species.

P2-24 Subtyping of Antibiotic Resistant Generic *Escherichia coli* by Manual and Automated HindIII Ribotyping

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Escherichia coli is both the most common commensal bacterium and the most frequent community-acquired pathogen in humans. A method for ribotyping of this organism, which uses HindIII for restriction of DNA, has proved useful for source tracking of a number of isolates of *E. coli*. However, it has not been extensively studied as a method of subtyping antibiotic resistant *E. coli* isolates. In the present study the HindIII ribotyping method was used to investigate strains of antibiotic resistant generic *E. coli* and provided subtyping data that were consistent with information obtained from antibiotic susceptibility typing investigations. The method was able to distinguish between multi drug resistant isolates of *E. coli* (defined in this study as resistant to 3 or more antibiotics) and isolates of *E. coli* that were resistant to two or less antibiotics. Several distinct ribotypes were found within the multidrug isolates, which agreed with pulsed field gel electrophoresis typing of the isolates. Although the ribotyping method proved to have a superior discriminatory ability in resolving clusters, it does not have high enough throughput for use in epidemiological investigations of antibiotic resistant bacteria. Therefore the method was adapted for use in automated ribotyping with a RiboPrinter, and the results were compared with those obtained by manual ribotyping. Both methods produce equivalent results and are useful for obtaining epidemiologically relevant subtyping data on antibiotic resistant isolates of *E. coli*.

P2-25 Staphylococcal Cassette Chromosome mec (SCCmec) Characterization and Molecular Analysis for Methicillin-resistant *Staphylococcus aureus* Isolated from Bovine Milk in Korea

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Methicillin-resistant *Stapylococcus aureus* (MRSA) is one of the most prevalent pathogens that cause nosocomial infections. MRSA produces a specific penicillin-binding protein PBP2a that is responsible for the ß-lactam antibiotic resistance. PBP2a is encoded by the mecA gene carried by a large mobile genetic element, staphylococcal cassette chromosome mec (SCCmec). SCCmec element contains the mec gene complex composed of the mecA gene and its regulators, and the ccr gene complex that encodes site-specific recombinases responsible for the mobility of SCCmec. Fourteen MRSA and a silent mecA carrying methicillin susceptible *S. aureus* (smMSSA) were isolated from milk of cows suspected to be infected with *S. aureus* in Korea, for an isolation rate of 0.18%. On the basis of SCCmec analysis, SCCmec of 13 strains was classified as subtype IVd, and one smMSSA strain was not classified. Interestingly, one MRSA strain was found to harbor novel combination of class D mec gene complex and type 2 ccr gene complex, which was designated as new SCCmec type VI in this study. All the MRSA and smMSSA isolates showed no multi-drug resistance and had community-acquired MRSA (C-MRSA) characteristics. Pulsed-field gel electrophoresis revealed that all the MRSA isolates belonged to the same genetic lineage, and multilocus sequence typing analysis showed that MRSA strains had no genetic relatedness with C-MRSA which caused human infection in Korea. This study suggests that the emergence of C-MRSA in human infection might not be correlated with MRSA isolated from bovine milk in Korea.

P2-26 Difference of Molecular Characteristics of Vancomycin-resistant *Enterococci* between Poultry and Humans in Korea

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It has been suggested Vancomycin-resistant *Enterococci* (VRE), which cause serious illness and result in limited therapeutic options in hospitalized patients, were induced by the use of glycopeptide antibiotics in human medicine and animal husbandry. We investigated phenotypic and genetic characteristics of the VRE isolates from humans and animals in Korea. All the VRE isolates were highly resistant to vancomycin (MIC > 512 μg/ml) and teicoplanin (MIC 32 μg/ml), and showed multi-drug resistance. Phenotypically, VRE from humans (23) and poultry (20) presented different patterns of resistance to Am, Gm, and P and Te. Most human isolates (18/23), but only three poultry isolates, were able to transfer vancomycin resistance between *Enterococci*. Pulsed-field gel electrophoresis (PFGE), overlapping PCR of Tn1546, and sequencing analyses were performed to compare genetic characteristics between humans and poultry isolates. PFGE revealed extensive heterogeneity with no PFGE-deduced genetic overlap between VRE from different origin. Structural analyses of the Tn1546 showed that 20 of 23 VRE from humans had insertion sequences, IS1542, IS19, or IS1216V in orf2-vanR, vanS-vanH or vanX-vanY region, respectively, whereas only 3 out of 20 VRE from poultry had only one kind of insertion sequence, IS1216V, in vanX-vanY region. In conclusion, no evidence of clonal or horizontal dissemination of VRE between humans and poultry was determined in Korea. It suggests that the evolution of VRE of humans and poultry origin may be different and more likely related to the glycopeptide antibiotics use in human hospital and avoparcin use in animal, independently.

P2-27 Antimicrobial Resistance in Generic *Escherichia coli* Isolated from Commercial Beef Packing Plant

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This study was designed to gain information about the antimicrobial resistance (AMR) in generic *Escherichia coli* recovered from a commercial beef packing plant. *E. coli* isolates were recovered using a hydrophobic grid membrane filtration method by direct plating on SD-39 medium. A total of 129 isolates comprising 27 and 33 from hides and ground beef respectively and 23 isolates each from conveyers, beef trimmings and washed carcasses were analyzed. The antimicrobial resistance was evaluated using the Sensititre system and the genetic characterization of these isolates was performed using the RAPD method. Of the 129 isolates, 43% were sensitive to all 15 antimicrobials tested. Resistance to tetracycline was observed in 46% of *E. coli* isolates followed by resistance to amoxicillin/clavulanic acid (14% isolates) and streptomycin (12% isolates). A higher percentage of *E. coli* isolates recovered from conveyers (39%), beef trimmings (65%) and ground beef (55%) were resistant to ≥ 1 antimicrobials. The resistance to ≥ 1 antibiotics was observed in 33% and 13% of *E. coli* isolates recovered from the hides and washed carcasses, respectively. The RAPD analysis showed that the majority of *E. coli* isolates were genetically diverse. The results of this study suggest that AMR *E. coli* were prevalent in a commercial beef packing plant and *E. coli* recovered from conveyers, beef trimmings and ground beef somehow encountered a higher selection pressure. These findings are significant for the beef processing industry and to our knowledge AMR in *E. coli* recovered from various sources in a commercial beef packing plant have not been reported previously.

P2-28 Characterization of *Staphyloccocus aureus* Isolates from Milk by RAPD Analysis and Antibiotic Disk Diffusion Method

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The objectives of this study were to determine the antimicrobial profiles and genetic diversity of *Staphyloccocus aureus* isolates from clinical and milk samples. Twenty-two milk and twenty-eight clinical samples were analyzed for *S. aureus*, using tryptone soy broth supplemented with 10% sodium chloride and sodium pyruvate as an enrichment broth and Baird-Parker agar supplemented with egg yolk-tellurite as a selective agar. Plates exhibiting dark color colonies were characterized biochemically with mannitol salt agar, catalase activity by slide spot test, deoxyribonuclease enzyme production with Dnase agar and *S. aureus* specific (Clf A and Mec A) gene by PCR assay. To determine the antimicrobial susceptibility profile, 12 antimicrobial agents were tested by the disk diffusion method. The isolates were further characterized by random amplified polymorphic DNA analysis, using six universal primer pairs. Results indicated 100% of the samples were positive for the production of catalase, coagulase and deoxyribonuclease enzyme activity. Fifty (100%) isolates were positive for Clf A gene and 12 (24%) for Mec A gene, and these Clf A and Mec A positive isolates were tested for their antimicrobial susceptibility. Of the 50 strains tested, 67% of the strains were sensitive, while 63% were resistant to antibiotics. RAPD analysis indicated 20% of the isolates had similar profiles and strains resistant to multiple antibiotics exhibited higher number of polymorphic fragments (12) compared to sensitive (8) strains. Based on the antibiotic susceptibility and random amplified polymorphic DNA patterns, this study indicates that both methods are useful for the characterization of *S. aureus*.

P2-29 Mold Growth Response to Vapors of Cinnamon, Lemon Grass and Orange Peel Extracts

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A wide range of natural antimicrobial sources is being evaluated. However, data on the effect of vapors of natural antimicrobials are scarce. The effect of cinnamon (C), lemon grass (L) and orange peel (O) extract vapors (0.25, 0.5 or 1 ml/l of air) on *Aspergillus flavus*, *A. niger*, *A. parasiticus*, *Penicillium digitatum* and *P. italicum* growth response were evaluated. Cinnamon sticks, dried lemon grass and orange peel were milled with sufficient ethanol, filtered, vacuum concentrated to dryness and dissolved in 50 ml of ethanol. For every mold, PDA plates were inoculated (2 microl of a 10^6 spore/ml), incubated at 25°C in 5 liter hermetic chambers, and observed every third day for 14 days, measuring the colony diameter. The increase in C, L or O extract concentration in the air significantly ($P < 0.05$) affected mold colony diameter. Minimal inhibitory concentration for each mold depends on the extract concentration being higher for O followed by L and C vapors. An atmosphere with 0.25 ml vapor extract/l air had little effect on mold growth; similar colony diameters were obtained in these systems and in the controls (air). *A. flavus* colony diameter was 80% lower than in the control when 1 ml C vapor/l air was used, whereas the rest of evaluated molds were complete inhibited. In general, *A. flavus* was more resistant than the other molds. C, L and O vapors are promising antifungal agents for foods compatible with their flavor and odor, such as baked goods, dairy and certain fruit-based products.

P2-30 Antibacterial Effects of Vaporized Essential Oils from Spice against *Vibrio* Species

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Antibacterial effects of seven essential oils from four spices (ginger, mustard, garlic and clove) against three *Vibrio* species on BHI agar medium and sliced raw flatfish were examined by gaseous contact methods. Growth of *V. parahaemolyticus* ATCC 17802 on BHI agar medium was completely inhibited by treatment with 1,000 ppm of vaporized natural and synthetic mustard oil. *V. vulnificus* 02 isolated from a hospital patient was inhibited at the level of 75.2 and 80.3% by treatment with 500 and 1,000 ppm of clove leaf essential oil, respectively, and showed stronger tolerance than that of *V. parahaemolyticus* against the essential oils tested. However, the essential oils tested showed weak growth inhibitory effect upon *V. vulnificus* 01. In addition, synergistic effects by using a mixture of essential oils were shown in most of the microorganisms tested. Viable cell population of *Vibrio* spp. which were inoculated on the sliced raw flatfish were decreased at the level of 1–2 log CFU/g by treating 1,000 ppm of vaporized natural and synthetic essential oil during storage at 20°C but did not show significant difference at 5°C. From these results we estimated that the vaporized natural and synthetic essential oil of mustard have strong antibacterial effect and could be used potentiality as a pretreated modified atmosphere packing agent to extend the shelf life of food.

P2-31 Killing and Suppressive Effects of the Combination of Cranberries and Wild Blueberries on *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Staphylococcus aureus*

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We investigated the synergistic antimicrobial effects of cranberries and blueberries for the foodborne pathogens *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Staphylococcus aureus*, in both water and growth media. According to our preliminary results, a berry concentrate mixture [5% (v/v) blueberry and 5% cranberry concentrate] was prepared in distilled water (DW) for the study of the bactericidal effect, and in Brain Heart Infusion (BHI) broth for the suppressive effect. Pathogen cocktail was inoculated (4 log CFU/ml) in both DW and BHI and incubated at 7°C and 21°C. Pathogen counts were made for the DW at 0, 1, 5, 7, and 24 h, and for the BHI on day 0, 1, 3, and 5. A synergistic berry powder blend (10% w/v) was also evaluated. Results from the DW experiments showed that while no reduction of pathogens was observed in pure DW at 7°C or 21°C, bactericidal effects on four pathogens started at 1 h in berry concentrate mixture and berry powder blend. At 24 h, no *L. monocytogenes*, *S.* Typhimurium and *S. aureus* were recovered from the berry concentrate mixture treatments. The berry powder blend, which was developed for enhancing gastrointestinal health, showed 2 log CFU/ml reduction of *E. coli* O157:H7. BHI data indicated that the growth of all pathogens tested was reduced (4 to 9 log CFU/ml difference) compared to the negative control at both temperatures. The synergistic effects of cranberries and blueberries have not only heath benefits but significant antimicrobial effects. It may be considered for food applications.

P2-32 Inhibition of Enteric Foodborne Viruses by Natural Peptide and Protein

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It is well known that some dairy proteins or peptides are potent inhibitors towards several bacteria. For instance, lactoferricin, a peptide obtained from the enzymatic hydrolysis of lactoferrin, shows antibacterial activities against *Escherichia coli*, *Listeria monocytogenes* and *Salmonella*. Furthermore, natural peptides called

bacteriocins, produced by various bacterial species, have been isolated and studied for their antibacterial effects. However, very few studies have demonstrated the potential of those proteins and peptides against foodborne viruses. In this study, the inhibitory effects of bovine lactoferrin and nisin A against hepatitis A virus (HAV) and human rotavirus were evaluated. Different concentrations of these inhibitory substances were added at one or more of three time points during indirect immunofluorescence and plaque reduction assays. Cytotoxic effect of lactoferrin on host-cells (FRhK-4, MA-104) was not observed at any concentrations tested (0 to 3 mg/ml), with cell viability higher than 85%. An inhibition of 76% of HAV replication was observed when 2 mg/ml of lactoferrin were present with cells and viruses for the entire assay period. However, nisin A have shown a cytotoxic effect at concentrations higher than 500 μg/ml for MA-104 and 200 μg/ml for FRhK-4, with viability of 58% and 32%, respectively. The exposure of cell lines and viruses to 100 μg/ml of nisin A has resulted in 78% and 99% inhibition of HAV and rotavirus replications, respectively. This work showed the interesting potential of natural substances to inhibit foodborne enteric viruses. Further work remains necessary to elucidate the mechanisms involved in the antiviral activity of these inhibitory substances.

P2-33 Effect of Sodium Hexametaphosphate against *Carnobacterium viridans*

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Carnobacterium viridans causes green discoloration of cured meat products. The study objective was to evaluate the antimicrobial activity (AM) of sodium hexametaphosphate (SHMP) (a metal chelating agent added to cured meat products to enhance moisture) against *C. viridans* in Lauria broth (LB) or All Purpose Tween (APT) broth containing 0.5 or 2.5% NaCl during incubation at 30, 10, or 4°C. The AM of SHMP was found to be dependent on its concentration, temperature, type of growth medium and NaCl concentration. One or 3 mg/ml SHMP was bacteriostatic at tested temperatures in LB containing either 0.5 or 2.5% NaCl. No effect of 1 mg/ml SHMP was found in APT while the bacteriostatic effect of 3 mg/ml at 30°C in the presence of 0.5% NaCl was eliminated at lower temperatures or in the presence of 2.5% NaCl. The bactericidal effect (4 log reduction) of 5 mg/ml of SHMP after 16 h in both broths containing 0.5% NaCl at 30°C became bacteriostatic when NaCl was increased to 2.5% or when the temperature of LB was decreased. In APT containing 5 mg/ml SHMP, *C. viridans* increased ≤ 3.3 and ≤ 0.80 log CFU/ml at 10 and 4°C, respectively, regardless of NaCl level (0.5 or 2.5%). The reduced activity in APT may be explained by the greater concentration of divalent cations in APT and by the greater capacity of *C. viridans* to grow in the presence of NaCl.

P2-34 Reduction of *Escherichia coli* O157:H7 from Refrigerated Nitrogen Packed Ground Beef Using Microencapsulated Allyl Isothiocyanate (AIT)

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Allyl isothiocyanate (AIT) is an effective inhibitor of various pathogens but its use in the food industry has been curtailed by its volatility and pungency. To overcome these problems AIT was microencapsulated in gum acacia and tested for antimicrobial potency against *Escherichia coli* O157:H7 in refrigerated ground beef. Twenty-five grams of aseptically ground beef were inoculated with a five-strain cocktail of *E. coli* O157:H7 to yield 4 or 8 log CFU/g. AIT at four levels was microencapsulated in gum acacia and freeze dried at a ratio of 1:4 (wall: AIT). Microcapsules were mixed with the meat samples, nitrogen packed, and stored at 4°C for 18 d. Samples were analyzed for *E. coli* O157:H7 and the total aerobic count (TAC) every 3 d. AIT concentrations of 4980 ppm eliminated both low and high levels of inoculated *E. coli* O157:H7 after 15 and 18 d of storage, respectively. AIT at 2828 ppm reduced *E. coli* O157:H7 ≤ 2.7 log CFU/g after 18 d storage. AIT levels < 1000 ppm were not effective in reducing *E. coli* O157:H7 viability. AIT levels of 170 to 1480 ppm had negligible effects on TAC, while 4980 ppm kept the TAC < 3 log CFU/g during 18 d storage compared with 7.25 log CFU/g in control samples. Work showed that AIT microencapsulated in gum acacia could be used in refrigerated ground beef to eliminate large numbers of *E. coli* O157:H7.

P2-35 Antibacterial Effect of Nisin-adsorbed Silica and Corn Starch Powder against *Listeria monocytogenes*

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Nisin is an antimicrobial produced by *Lactococcus lactis* and is very effective against gram positive bacteria including *Listeria monocytogenes*. However, it is difficult to retain nisin activity in food products because food components, processing treatments and storage conditions can block or reduce activity rapidly. This has prompted research on retaining antimicrobial activity and applying new techniques to deliver a more controlled effect. This study tested the efficacy of silica and corn starch powders to adsorb nisin, using different nisin levels in the adsorbing solution. It was determined, using the agar diffusion method, that antimicrobial activity of nisin adsorbed silica powder increased continuously as nisin concentration of the adsorbing solution increased from 200 IU to 600 IU. No increase in inhibition of *L. monocytogenes* at nisin levels over 600 IU was found. Residual

nisin activity was found in the supernatant after centrifugation. At 600 IU, nisin activity of silica powder was 87% of original adsorbing solution. Antimicrobial activity of nisin adsorbed corn starch powder increased continuously as nisin solution increased from 200 IU to 700 IU. The use of adsorbing solutions containing over 700 IU of nisin showed no increase in *L. monocytogenes* inhibition. However, at 700 IU, nisin activity of corn starch was only 29.4% of original adsorbing solution. The optimal concentration of nisin adsorbed silica and corn starch powder against *L. monocytogenes* can be estimated by efficiency of adsorption and cost of ingredients. Further research will investigate the use of alternate antimicrobials to obtain a more efficient adsorption rates.

P2-36 DSC Inhibition of *Listeria monocytogenes* by Natural Plant Extracts in Brain Heart Infusion Broth at 4°C and 10°C

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The inhibition of *Listeria monocytogenes* by cranberry and grape seed extracts in brain heart infusion (BHI) broth was evaluated. Stationary-phase cells grown in BHI broth (35°C) were harvested by centrifugation and washed in 0.85% (w/v) saline. Cranberry extract (CE) and grape seed extract (GSE) were added separately to 100-ml portions of BHI broth to obtain the following concentrations of CE: 1%, 3%, 5%, and 10%, and of GSE: 1%, 5%, and 10%. Broth without added extract served as control. Erlenmeyer flasks (250-ml) containing BHI broth were inoculated with a 5-strain mixture of *L. monocytogenes* to obtain 10^5 CFU/ml and then incubated at 4°C and 10°C for 42 days. Populations of *L. monocytogenes* survivors were determined on day 0, 1, 7, 14, 28, and 42 by surface-plating samples on BHI agar and counting bacterial colonies after incubation (35°C, 24 h). CE (3% or 5%) was bacteriostatic to *L. monocytogenes* at 4°C and 10°C for 42 days. The highest concentration of CE (10%) was bactericidal, reducing initial numbers of *L. monocytogenes* by 3 log (4°C) and 5 log (10°C) at day 7. GSE (5% or 10%) was bactericidal to *L. monocytogenes* irrespective of temperature. At day 7, GSE reduced initial populations of *L. monocytogenes* by approximately 3 and 5 log at 4°C and 10°C, respectively. The results indicate that CE and GSE have good potential for use in food applications to control *L. monocytogenes*. Further studies are necessary to evaluate and optimize the antimicrobial efficacy of these natural antimicrobials in actual food systems.

P2-37 Effects of Oregano Essential Oil and Nisin Combinations on Growth of Gram Positive and Gram Negative Foodborne Pathogens

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Natural antimicrobials, like bacteriocins and essential oils, play an important role in biopreservation of foods. However, their antimicrobial activity depends on the structure of the target microbe cell wall. Bacteriocins are active against Gram positive bacteria, while phenolic compounds in essential oil may be effective over Gram positive and Gram negative bacteria as well. In this study, we evaluated the antimicrobial activity of oregano essential oil (Frutarom, UK), alone and in combination with the bacteriocin nisin (Nisaplin, US) on the inhibition of growth of *Listeria monocytogenes* ATCC 5069r and *Salmonella* Enteritidis ATCC13076. The inhibitory activity was tested by the well diffusion method, measuring the inhibition halos after 24 h / 30°C. The concentrations tested were 0.05%, 0.1%, 0.2%, 0.5%, 1.0%, and 2.0% for oregano essential oil and 6.25, 10, 12.5, 25, 50 and 100 ppm for nisin. Results were subjected to ANOVA and Duncan Test (alpha = 5%). When essential oil was tested alone, the minimal concentration required for inhibition of *L. monocytogenes* was 0.2%, while *S.* Enteritidis was always inhibited, regardless of the concentration of these antimicrobials. When the essential oil was used in combination with nisin, a synergistic effect was observed for *L. monocytogenes*, i.e, the oil enhanced the activity of the bacteriocin. However, when *S.* Enteritidis was tested, the presence of nisin caused a reduction of the antimicrobial activity of the essential oil ($P < 0.05$). These results indicate that these antimicrobials have potential applications to improve food safety, but their activity depends on the target pathogen.

P2-38 DSC Effect of Surfactants on Antimicrobial Activity of Nisin against Bacterial Pathogens

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It is widely recognized that bacterial pathogens are highly adaptive to stressful situations and have powerful resistance capabilities against a variety of antimicrobials. This study investigated potential interactions between the bacteriocin nisin, the surfactants Polysorbate 20 (Tween 20) and octyl phenol ethoxylate (Triton X-100), and subsequent inhibition of *Listeria monocytogenes* Scott A and 310 and *Escherichia coli* O157:H7 ATCC 43895 and 932. It was hypothesized the combination of bacteriocin and surfactant would exert an inhibitory effect on target pathogens greater then that of the antimicrobial or surfactant alone. Minimum inhibitory concentration (MIC) tests started with bacterial cultures, adjusted to a desired concentration, inoculated into microtiter plates with wells containing growth medium and an inoculum of antimicrobial, surfactant, or a combination thereof. Microbes were exposed to nisin between 1.0 and 25.0 µg/ml; surfactants were tested between 0.1 and 1.0%. At selected time points, optical density readings at 630 nm (OD630) were recorded.

Experiments were duplicated and statistically significant differences determined with Student's t-test ($P < 0.05$). Inhibition of *L. monocytogenes* by nisin or surfactant alone was approximately equivalent for both strains. Inhibition of *E. coli* O157:H7 by nisin or surfactant alone produced slight inhibitory effects against both strains. *L. monocytogenes* 310 was more sensitive then Scott A to both antimicrobial and surfactant combinations. *E. coli* O157:H7 43895 was more sensitive than strain 932 to nisin/surfactant combinations. Addition of low levels of surfactant was shown to amplify the inhibitory activity of nisin against both foodborne pathogens.

P2-39 Evaluation of Frankfurters Manufactured Using Cellulose Casings Dipped in Buffered Sodium Citrate on *Listeria monocytogenes* and Shelf Life

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The efficacy of soaking cellulose casings in a 30 percent solution of buffered sodium citrate before stuffing frankfurters was studied as a means of controlling *Listeria monocytogenes* and spoilage microflora. Frankfurters were manufactured using non-treated and BSC-treated cellulose casings, and were inoculated at a target *L. monocytogenes* level of 10^3 CFU/cm^2. The frankfurters were vacuum packaged with three frankfurters/package and stored at 4 and 10°C in boxes to simulate retail storage for nine weeks. The frankfurters were sampled weekly. The *L. monocytogenes* population during 4°C storage increased from 2.77 to 8.13 log CFU/cm^2 on the non-treated frankfurters and 2.17 to 7.91 log CFU/cm^2 on the BSC treated frankfurters. At 10°C, the non-treated samples increased from 2.16 to 7.03 log CFU/cm^2 and the BSC-treated frankfurters increased from 2.03 to 7.07 log CFU/cm^2. At 4°C, total aerobic bacterial populations for the non-treated frankfurters increased from 0.9 to 3.27 log CFU/cm^2 and for the BSC-treated frankfurters increased from 1.44 to 4.35 log CFU/cm^2. At 10°C the non-treated frankfurters increased from 1.02 to 6.92 log CFU/cm^2 and BSC-treated frankfurters increased from 1.43 to 5.87 log CFU/cm^2. Throughout the nine week study no significant difference was found between the non-treated and BSC-treated frankfurters, in *L. monocytogenes* populations.

P2-40 Cold Atmospheric Pressure Plasma Reduces *Listeria innocua* on the Surface of Apples

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Fresh produce can be contaminated with bacterial pathogens, leading to increased risk of foodborne illness. High energy plasma discharges effectively inactivate bacteria on inert surfaces, and have potential application to fragile surfaces such as fruits and vegetables. A novel technology, the gliding arc plasma discharge apparatus, allows the deposition of a cold atmospheric pressure plasma (CAPP) onto surfaces through the use of forced air, thereby reducing the potential for excessive heat or spot damage to the surface. Golden delicious apples were spot inoculated with approximately 8 log CFU of the non-pathogenic surrogate *Listeria innocua* in log growth phase. On separate sets of apples, the spot was left untreated (control) or treated with one of two levels of CAPP discharge. In the first ("Low"), the discharge amperage was set to 115 mA, placing the CAPP discharge height approximately 1 cm away from the apple surface, and the treatment lasted for 2 min. In the second ("High"), the discharge amperage was set to 150 mA, placing the plasma discharge in full contact with the apple surface, and the treatment lasted for 4 min. The voltage was held constant at 10.02 kV. Separate sets of inoculated apples were treated with either 2 or 4 min of forced air with no CAPP discharge. Apple surfaces were sampled with sterile swabs and phosphate buffer, serially diluted and plated on PALCAM agar. There were no significant ($P < .05$) differences in *L. innocua* recovery among the control, Air-2 or Air-4 treatments, which had 4.29, 4.36 and 4.42 log CFU, respectively. The CAPP treatments significantly reduced the recoverable *L. innocua*, by 0.39 log CFU in the case of "Low", and by 1.10 log CFU in the case of "High". The apples were examined for gross changes in color and appearance at the site of treatment immediately after treatment, and after 2 and 5 days in storage at 8°C. No changes were observed. These results suggest that CAPP may be an effective treatment for the reduction of microbial populations on fragile produce surfaces.

P2-41 Yeast Inhibition with Ternary Mixtures of Naturally Occurring Phenolic Compounds and Potassium Sorbate

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Phenolic compounds, naturally present in plants, can be used as antimicrobial agents. When considering ternary mixtures of antimicrobials, it is not easy to anticipate the effects. Individual and combined effects of eugenol (Eu), thymol (Th), or carvacrol (Cr) with potassium sorbate (KS) were evaluated as antifungal (*Zygosaccharomyces rouxii*, *Pichia membranaefaciens* and *Debaryomyces hansenii*) agent mixtures. Checker-board experimental designs were used to determine synergic mixtures by evaluating combinations that include two phenolics and KS. PDA was prepared with sucrose and hydrochloric acid to reach a_w (0.99) and pH (3.5), and the necessary amount of Th, Eu or Cr was added. Over plates of every binary combination, 50 µl of KS solution (1%) were exponentially deposited using a spiral plater, dried for 2 h, inoculated (10^6 cell/ml) using a swab making 6 cm long radial marks of each yeast, incubated at 25°C, and observed after 5 days.

KS inhibitory concentrations were determined by calculating concentration at growth end points. Minimal inhibitory concentrations (MIC) for phenolic compounds and KS were determined, as well as inhibitory concentrations for every ternary antimicrobial mixture. Fractional inhibitory concentrations (FIC) and FIC Index were calculated. MICs were 50 (Eu) and 100 ppm (Th, Cr or KS). In ternary mixtures of KS-Th-Eu or KS-Th-Cr yeast inhibition was observed when combining less than 10 ppm of phenolics with 16 ppm KS. Calculated FIC indexes show synergistic effects. Ternary mixtures of phenolic compounds and KS drastically reduced individual inhibitory concentrations.

P2-42 Inactivation of *Salmonella* spp. and *Escherichia coli* O157:H7 on Cantaloupe Skin Using Citric Acid, Hydrogen Peroxide and a Foodgrade Surfactant

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The study evaluated the efficacy of citric acid (CA) alone or combined with hydrogen peroxide (H_2O_2) and sodium lauryl sulfate (SLS) for destroying *Escherichia coli* O157:H7 and *Salmonella* spp. on the outer rind surface of cantaloupe. Cantaloupe rinds were cut to 2.5 cm^2 pieces and inoculated with either a five strain mixture of nalidixic acid resistant *Salmonella* spp. or *E. coli* to give ~10^8 CFU/ rind. Inoculated rinds were dipped for 2.0 min in 500 ml of water (control) or sanitizer solutions at 23°C and then homogenized in buffered peptone water (100 ml). Samples of homogenate were serially diluted and plated onto Bismuth sulfite agar and MacConkey sorbitol agar, both containing nalidixic acid. All inoculated agar plates were incubated at 35°C for 48 h. *Salmonella* and *E. coli* survivors were enumerated following incubation and expressed as log CFU/rind. Compared to the water dipped control, exposure of rinds to 5% H_2O_2 reduced populations of *Salmonella* and *E. coli* by 2.77 and 2.83 log CFU/rind, respectively. A combination of 5% H_2O_2 and CA (1%) decreased populations of *Salmonella* and *E. coli* by 3.16 and 3.72 log, respectively. Further reductions were achieved by combining 5% H_2O_2 and CA (1%) with 1% SLS; populations of *Salmonella* spp. and *E. coli* decreased by 3.9 and 4.0 log, respectively. Based on these results, the combined use of acidified H_2O_2 with an anionic surfactant has good potential for improving the microbial safety of cantaloupe melons.

P2-43 Decontamination of *Campylobacter*, *Salmonella* and *Escherichia coli* on Chicken Carcasses Using Ca-acidified Sodium Chlorite and SAS-acidified Sodium Chlorite Antimicrobial Solutions under Commercial Plant Conditions

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Poultry slaughter facilities require effective interventions to meet USDA FSIS *Escherichia coli* and *Salmonella* regulatory requirements and to decrease *Campylobacter* prevalence. The objective of this study was to assess the antimicrobial efficacy of Ca- and SAS-acidified sodium chlorite (ASC) against *Campylobacter, Salmonella* and *Escherichia coli* during chicken carcass washing. A total of 320 chicken carcass rinse samples (400 ml) were collected from a small and large bird line. Samples were collected pre- and post- Ca- and SAS-ASC treatments. Samples were analyzed by USDA methods for incidence of *Escherichia coli* Biotype I, incidence of *Salmonella* species, and incidence and counts of *Campylobacter* species. Incidence of *Escherichia coli* were reduced by 96% and 100% by Ca- and 94% and 100% by SAS-ASC treatments in the large and small carcass samples, respectively. Incidence of *Salmonella* spp. was reduced by 100% and 88% by Ca- and 93% and 73% by SAS-ASC treatments in the large and small carcass samples, respectively. *Campylobacter* spp. numbers were below 10 colonies pre- and post-ASC treatments in each line. Incidence of *Campylobacter* spp. was reduced by 100% and 92% by Ca- and 74% and 55% by SAS-ASC treatments in the large and small carcass samples, respectively. Incidence of *Campylobacter* spp., *E. coli* and *Salmonella* spp. in the rinse samples varied by the lines (small vs. large) and by the date of sample collection. Ca-ASC proved efficacious in reducing *E. coli, Salmonella*, and *Campylobacter* in chicken rinse samples under commercial plant conditions.

P2-44 DSC Efficacy of Ultraviolet Light in Combination with Chemical Preservatives for the Reduction of *Escherichia coli* in Apple Cider

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Inactivation of *Escherichia coli* in apple cider treated with ultraviolet light (UV) in combination with chemical preservatives was evaluated. *Escherichia coli* ATCC 25922 was suspended (~7 log CFU/ml) in apple cider. Inoculated cider (4°C) containing sodium benzoate (NaB; 1000 and 500 ppm), potassium sorbate (KS; 1000 and 500 ppm), hydrogen peroxide (HP; 150 and 75 ppm) or dimethyl dicarbonate (DMDC; 150 and 75 ppm) was treated with ultraviolet light (peak output = 254 nm) in a thin-film UV treatment unit. UV treatment was performed either before or after application of chemical preservatives. Cider that received UV treatment but contained no added preservatives served as a control. Surviving populations of *E. coli* were determined on

TSA prior to UV exposure and at 0, 24, 48 and 72 h after treatment. Greater reductions in *E. coli* populations were observed when preservatives were added to cider after UV treatment rather than before ($P < 0.05$). Combined treatments of UV and DMDC or HP were better than controls for reducing *E. coli* populations ($P < 0.05$). However, inactivation of *E. coli* was less in juices inoculated with KS before UV treatment than in controls ($P < 0.05$). This work demonstrates potential benefits of combining chemical preservatives with UV treatment for the inactivation of *E. coli* in juices.

P2-45 Evaluation of an Ozonated Water System and a Steam Pasteurization System for Controlling *Listeria monocytogenes* and *Salmonella* spp. on Raw Whole Shelled Almonds

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The objective of this study was to validate 20, 60 and 120 s exposure to 2 ppm ozonated water applied with an ozone system, and to validate 8, 20 and 30 s steam pasteurization applied using a commercial steam pasteurization system for reducing *Listeria monocytogenes* and *Salmonella* spp. populations on raw whole shelled almonds. After exposure to ozonated water, 1.1, 1.4 and 1.8 log CFU/g reductions of *Salmonella* spp. and 0.3, 0.3, and 0.2 log CFU/g reductions of *L. monocytogenes* were recorded after 20, 60 and 120 s, respectively. All of these reductions were similar to those obtained with a water-only control treatment; thus using 2 ppm ozonated water was not an effective antimicrobial process as applied in this study. An average of 2.2 and 2.6 log reductions for low inoculum levels and 3.2 and 3.6 log reductions for high inoculum levels were achieved for *L. monocytogenes* and *Salmonella* spp., respectively, after 8 s exposure to steam pasteurization. *L. monocytogenes* or *Salmonella* spp. populations were not recovered by direct plating procedures after 20 or 30 s steam exposure (detection limit: 0.5 log CFU/g). Steam pasteurization, as applied in this study, provided a high level of control for both pathogens and appears to be a very practical application for raw almonds. Initial appearances indicated little effect on raw nut quality; however, more formal evaluation of almonds for shelf life and organoleptic quality is suggested.

P2-46 Evaluation of a Commerical Steam Pasteurization System for Controlling *Listeria monocytogenes* and *Salmonella* spp. on Raw Whole Shelled Pistachios

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The objective of this study was to validate the effectiveness of 20, 30 and 40 s steam pasteurization applied with a commercial steam pasteurization system for reducing *Listeria monocytogenes* and *Salmonella* spp. populations on raw whole shelled pistachios. Pistachios were inoculated with each pathogen at ca. 6 (high) and 4 (low) log CFU/g using a misting procedure. Samples inoculated at high levels and exposed to the saturated steam environment for 20, 30 and 40 s demonstrated average *L. monocytogenes* reductions of 2.3, 2.6, and 4.5 log CFU/g, respectively. Population reductions were 3.3, 3.9, and 5.5 log cycle reductions for *Salmonella* spp. An average of 1.6, 2.4, and 3.4 log cycle reduction for *L. monocytogenes*, and 3.5, 3.5, ad 3.9 log cycle reductions for *Salmonella* spp. populations were observed when pistachios samples with low levels of inoculum were exposed to same treatments. Overall, steam pasteurization using a commercial unit provided a high level of control for both pathogens and appears to be a very practical application for raw pistachios. Further evaluation of pistachios for shelf life and sensory quality is suggested.

P2-47 DSC Antimicrobial Efficacy of Methanobactin against *Listeria monocytogenes* Scott A in Laboratory Medium

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Methanobactin is a novel extracellular peptide produced by *Methylosinus trichosporium* OB3b, a methane-oxidizing bacterium. This easily culturable organism is important in global carbon cycling and for single-cell protein production. This study investigated the antimicrobial efficacy of methanobactin against *Listeria monocytogenes* Scott A in brain heart infusion (BHI) broth at pH 5.5 to 7.3. Minimum inhibitory concentrations (MICs) were determined at 24 h for stationary-phase *L. monocytogenes* cultures in BHI broth (32°C) via use of a broth micro-dilution method. Growth was monitored via absorbance (595 nm) in 6-h increments and viability was determined at 24 h via surface-plating samples onto BHI agar and enumerating colonies after incubation (32°C, 72 h). There was a non-linear relationship between MIC of methanobactin and pH, with *L. monocytogenes* being most sensitive to methanobactin at pH 6.0. MICs were 5.70, 6.41, 3.56, and 6.41 mM at pH 7.3, 6.5, 6.0, and 5.5, respectively. MICs of methanobactin exhibited a bactericidal action and resulted in 3.34-, 3.96-, 4.87-, and 4.87-log reductions in populations of *L. monocytogenes* at pH 7.3, 6.5, 6.0, and 5.5, respectively. Overall, log reductions of the pathogen increased with increased concentration of the peptide. At pH 6.0, methanobactin concentrations greater than the MIC consistently resulted in undetectable levels

(> 5.00-log reductions) of *L. monocytogenes*. Based on the results of this study, methanobactin, a natural antimicrobial peptide, is bactericidal to *L. monocytogenes* and has good potential for use in food applications to destroy this pathogen.

P2-48 Response Surface Model for Effects of Temperature, pH, and Combined Potassium Lactate and Sodium Diacetate on Growth Kinetics of *Listeria monocytogenes* Scott A in Broth

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Antimicrobial effects of lactate and diacetate mixture against the growth and survival of *Listeria monocytogenes* Scott A have been observed. However, a mathematical model describing the combined effects of temperature, pH and potassium lactate and sodium diacetate (PL + SDA) on the behavior of *L. monocytogenes* has not been developed. In our present study, the effects and interactions of temperature (4 to 37°C), pH (5.5 to 7.0), and PL+SDA (0.0 to 4.5%) on the growth and survival of *L. monocytogenes* Scott A in broth were investigated. Ninety-six primary growth models were iteratively fitted to the Buchanan three-phase linear model. Response surface model was generated for effects of temperature, pH and PL + SDA on specific growth (or death) rate of *L. monocytogenes* in broth model by regression analysis. The growth (or death) kinetics of *L. monocytogenes* were significantly ($P < 0.005$) affected by the interactions of pH with temperature and PL + SDA. Specific growth (or death) rate was affected ($P < 0.005$) by linear and quadratic effect of PL+ SDA and linear effect of temperature. The model was evaluated for goodness of data. This verification step indicated that the models can provide prediction of specific growth (or death) rates of *L. monocytogenes* in the matrix of conditions described and can be used as a tool to estimate the impact of food formulation and storage conditions on the growth of the pathogen. In addition, this model can be incorporated into the pathogen model program for food industries and risk assessors.

P2-49 DSC Potential for Development of Resistance by *Listeria monocytogenes* and *Salmonella* Typhimurium to Potassium Sorbate or Sodium Benzoate following Repeated Exposure

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A focus on safer foods and longer shelf life has led to increased use of regulatory-approved food antimicrobials to inhibit or inactivate foodborne pathogens. However, there are few data on the possible emergence of pathogens resistant to food antimicrobials. The aim of this study was to investigate the potential development of resistance to potassium sorbate and sodium benzoate of *Listeria monocytogenes* and *Salmonella* Typhimurium. Four strains of *L. monocytogenes* (101, 108, 310, Scott A) and *S.* Typhimurium DT 104 (2380, 2576, 2582, 2486) were used. The minimum inhibitory concentrations (MIC) of potassium sorbate (PS) and sodium benzoate (SB) against the parent strains at pH 6.0 were determined using an agar dilution assay. Parent strains were exposed to increasing concentrations of PS or SB at pH 6.0 without any pre-stress. Adapted strains were stored in the presence of the highest concentrations of the antimicrobials for three months and re-grown with the same antimicrobial to determine the effect of storage on survival. The MIC of the parent strains of *L. monocytogenes* and *S.* Typhimurium was 0.2 to 0.3 % (w/v) and 0.3 to 0.4% for both compounds, respectively. *L. monocytogenes* adapted to grow at up to 0.4% PS or SB. *S.* Typhimurium grew at up to 0.5% SB and 0.6% PS. One strain, 2380, grew to 0.6% SB. Strains stored three months and re-exposed to the same antimicrobial did not lose viability. Results suggest the possibility for development of adaptation to food antimicrobials by *L. monocytogenes* or *S.* Typhimurium following exposure to sub-lethal concentrations.

P2-50 Effect of a Hydrogen Peroxide-producing Strain of Lactic Acid Bacteria on Aflatoxin B1 and Fumonisin B1 at pH 2 and 7

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Our current studies have demonstrated the potential use of lactic acid bacteria (LAB) to degrade aflatoxin and fumonisin through surface binding and production of hydrogen peroxide. Ninety seven strains of LAB were isolated from the gastrointestinal tract of healthy chickens and screened for production of hydrogen peroxide, using the ferrous oxidation xylenol orange 2 (FOX-2) test. Among these bacteria, 46 strains were found to produce hydrogen peroxide at various concentrations. Three highest hydrogen peroxide-producing strains have been identified by sugar fermentation, biochemical tests and 16s ribosomal RNA typing. *Lactobacillus fermentum* strain D115, *L. acidophilus* strain L314 and *L. acidophilus* strain L234 have been found to produce 7952, 6449, and 6153 uM, respectively of hydrogen peroxide after incubation at 37°C. The cell concentration of *L. fermentum* strain D115, *L. acidophilus* strain L314 and *L. acidophilus* strain L234 were 4.5×10^{10}, 3.6×10^{9} and 2.0×10^{9} CFU per ml, respectively. In addition, viable cells and ethanol-deactivated cells of *L. fermentum* strain D115 was tested against aflatoxin B1 (AFB1) and fumonisin B1 (FB1) at pH 2 and 7 for its surface-

binding ability and degradation effect of hydrogen peroxide. Results demonstrated that ethanol-deactivated cells of *L. fermentum* strain D115 bound and removed 52.2% of AFB1 at pH 2. However, the binding efficacy decreased to 22.9% when the pH was increased to 7. Viable cells of *L. fermentum* strain D115 have been shown to degrade an additional 10.5 and 13.1% of AFB1 in pH 2 and 7 solutions, respectively. In contrast, no significant removal and/or degradation of FB1 (less than 10%) by *L. fermentum* strain D115, either through surface binding or hydrogen peroxide, was observed.

P2-51 The Role of Efflux Pumps and Outer Membrane Protein in the Susceptibility of *Escherichia coli* and *Salmonella* Typhimurium to Biocides

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The objective of this study was to investigate the role of efflux pumps and outer membrane protein in the susceptibility of *Escherichia coli* and *Salmonella* Typhimurium to biocides. Efflux pump *Arc*AB and outer membrane protein *Tol*C of *E. coli* and *S.* Typhimurium were selected and each of the genes was deleted using one-step inactivation method. Mutant and wild type strains were tested for susceptibility to biocides including triclosan, sodium chlorine, chlorhexidine, and benzalkonium chloride. The concentrations of each biocide ranged from 0.0019 to 64 mg/L in Mueller Hinton agar (MHA). Test strains were inoculated onto biocide-containing MHA plates and incubated 16 to 18 h at 35°C. The growth of the strains was recorded to determine minimal inhibitory concentration (MIC). Δ*acr*AB mutants of *E. coli* 3008 and *E. coli* 4 were four times more susceptible to triclosan. The two mutants were also 16 and 64 times more susceptible respectively to benzalkonium chloride. Δ*tol*C mutant of *S.* Typhimurium PY1 was 64, 32, and 32 times more susceptible to triclosan, benzalkonium chloride and chlorhexidine, respectively, than the wild type strain. The MICs of sodium chlorine did not show significant differences between mutant and wild type strains. These data show that the deletion of efflux pump gene *arc*AB or outer membrane protein gene *tol*C resulted in the increased susceptibility of *E. coli* and *S.* Typhimurium to biocides, indicating they play an important role in resistance to biocides.

P2-52 The Sensitivity to Alpha-Amylase of Pediocin 34 Produced by *Pediococcus pentosaceus* 34 Isolated from Indian Cheddar Cheese was Due to Contaminating Serine Proteinases

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Pediocin 34 produced by *Pediococcus pentosaceus* 34 isolated from Indian Cheddar cheese lost its activity after treatment with various proteolytic enzymes. It was found insensitive to various non-proteolytic enzymes such as catalase, ribonuclease A, lipase, beta-amylase and lysozyme. However, the activity was adversely affected by alpha-amylase treatment indicating pediocin 34 to be a glycoprotein. We investigated whether the observed sensitivity of pediocin 34 to alpha-amylase was truly due to alpha-amylase activity or if it was due to the result of contaminating proteases present in the enzyme preparation. The alpha-amylase enzyme was treated with two types of protease inhibitors, namely polymethylsulfonylfluoride and iodoacetamide, individually as well as in combination prior to its use in pediocin 34 inactivation studies. The alpha-amylase treated with the combination of iodoacetamide and polymethylsulfonylfluoride was found ineffective against pediocin 34 revealing that the observed adverse effect of alpha-amylase against pediocin 34 was due to contaminating cysteine proteinases or serine proteinases or both. However, the enzyme treated with polymethylsulfonylfluoride alone, but not with iodoacetamide, remained ineffective against pediocin 34 thereby confirming the presence of serine proteinases in the alpha-amylase preparation. Thus pediocin 34 was confirmed to be a simple protein. It is concluded that the sensitivity to non-proteolytic enzymes such as lipases, amylases, etc. of bacteriocins should be reported cautiously as it could be due to contaminating proteases and the effect of such contaminating proteases should be nullified by treating the non-proteolytic enzymes with various types of protease inhibitors prior to their use in bacteriocin inactivation studies.

P2-53 Development of the Direct ELISA and Immunochromatographic Assay for the Determination of Aminoglycosides

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Monoclonal antibodies against gentamicin, kanamycin, and neomycin were prepared, and the competitive direct enzyme-linked immunosorbent assay (ELISA) and the immunochromatographic assay were developed using the monoclonal antibodies to detect those aminoglycosides in animal plasma or milk. No cross-reactivity of any monoclonal antibody was observed with other aminoglycosides, indicating that each antibody is highly specific. Calibration curves were constructed for each aminoglycoside in rabbit plasma and bovine milk, using a competitive direct ELISA. Based on these curves, the detection limits of gentamicin, kanamycin, and neomycin in rabbit plasma and bovine milk were estimated to be about 10, 20, and 50 ppb, respectively. Using competitive ELISA methods developed in this study, the concentration of each aminoglycoside was successfully

monitored in rabbit plasma after intramuscular administration. The concentration increased rapidly after intramuscular administration and decreased gradually up to 12 h. The half-lives of gentamicin, kanamycin, and neomycin in rabbit plasma have been estimated to be 1.8, 1.6, and 1.2 h respectively. Moreover, a rapid test kit based on the immunochromatographic assay was developed, using the monoclonal antibodies against gentamicin, kanamycin, and neomycin. The detection limits of aminoglycosides were estimated to be 10 ppb by use of the kit in both rabbit plasma and bovine milk. In conclusion, the competitive direct ELISA was developed using the monoclonal antibodies to detect the aminoglycosides in animal plasma or milk. Moreover, the immunochromatographic assay developed in this study could be applied to the detection of aminoglycosides in veterinary fields due to its rapid and simple procedure. Therefore, two assays developed in this study could be complementary with each other between the field and laboratory.

P2-54 *In Vitro* Inhibition of Adsorption of Foodborne Viruses by Human Bifidobacterial Isolates

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Among foodborne pathogens, viruses are responsible for an important part of estimated cases of infections. Noroviruses, rotavirus and hepatitis A virus (HAV) are three major etiologic agents in cause. The principal gateway for these viruses is the epithelial cells of the small intestine. Once in the intestinal lumen, they can cause damage to microvilli and disrupt intestinal mucosa. Promising treatment using probiotic bacteria such as *Bifidobacterium* are now proposed. However, *in vitro* and *in vivo* studies are still required to confirm antiviral activity. The aim of this study was to evaluate the potential of the probiotic bifidobacteria isolated from infant feces for *in vitro* inhibition of a norovirus-surrogate (feline calicivirus), human rotavirus (Wa) and HAV (HM-175). The adsorption of viruses to human colonic Caco-2 and HT-29 cells was measured by an indirect immunofluorescence method and plaque technique assay. The percentage of adsorption to cell lines was calculated for all viruses, alone (10^4 to 10^6 CFU/ml) and in the presence of bifidobacteria (10^8 CFU/ml). The results indicated that among studied viruses, rotavirus exhibits the highest capacity to adsorb to enteric cells (7% using 10^6 CFU/ml). Moreover, the presence of bifidobacterial strains RBL67, 69 or 70 (10^8 CFU/ml) on Caco-2 cells for 30 min prior to HAV (10^4 PFU/ml) addition have shown 100%, 39% and 76% inhibition of the viral adsorption, respectively. For HT-29 cells, 52%, 75% and 78% inhibition of the viral adsorption has been observed with the same probiotic strains. *In vivo* studies are in progress to evaluate the antiviral properties of the selected bifidobacteria.

P2-55 Antimicrobial Activity of Thai Rhizomatous Spices against *Listeria monocytogenes* and *Salmonella* Enteritidis Associated with Chicken Breast Meat

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It was previously shown that Thai rhizomatous spices and their essential oils (EO) had antimicrobial effects *in vitro* against *Salmonella* Typhimurium and *Listeria monocytogenes*; however, their effects in food system needs to be investigated. The objective of this study was to determine the antimicrobial effect of Thai rhizomatous spices including ginger (*Zingiber officinale*), fingerroot (*Boesenbergia pandurata*) and turmeric (*Curcuma longa*) against *L. monocytogenes* strain 101 and *S.* Enteritidis strain DMST 8536 associated with chicken breast meat. The chicken meat was surface-inoculated at low and high concentrations (3 and 5 log CFU/g). Paste of fresh spice (10 and 20%, w/w), either fresh juice or spice residue (10 to 20%, w/w) obtained by juice extractor, and commercial EO (0.5, 1, 2 and 5%, w/w) were marinated to the inoculated chicken meat and stored at 10°C for 4 h. No concentration of fresh ginger and its EO showed antibacterial activity. At 20%, turmeric and fingerroot paste reduced listerial and salmonellae by ca. 0.6 and 1.0 log CFU/g, respectively. Moreover, antibacterial effect of fresh juice was similar to the paste. Among the EO tested, 5% of fingerroot EO showed the best bactericidal effect against *S.* Enteritidis within 4 h, while it had slight antibacterial effect against *L. monocytogenes*. Between two organisms tested, *S.* Enteritidis was more sensitive to fingerroot EO than *L. monocytogenes*. Results showed that Thai rhizomatous spices, especially fingerroot EO, have potential to be used as natural antimicrobial against foodborne pathogenic bacteria in poultry products.

P2-56 Efficacy of Aerosolized Peroxyacetic Acid as a Sanitizer of Lettuce Leaves

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Aerosolized sanitizer was investigated as a potential alternative to aqueous and gaseous sanitizers. Peroxyacetic acid was aerosolized (5.42-11.42 μm particle diameter) by a commercially available nebulizer into a model cabinet. Iceberg lettuce leaves were inoculated with a three strain each cocktail of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Typhimurium and then treated with aerosolized peroxyacetic acid for 10, 30 or 60 min in a model aerosol cabinet at room temperature (22 ± 2°C). After treatment, surviving healthy and injured cells were enumerated on appropriate selective agar and with the overlay agar method, respectively. Inoculated iceberg lettuce leaves exposed to aerosolized peroxyacetic acid for 10 min exhibited a 0.8 log reduction in *E. coli* O157:H7, a 0.3 log reduction in *Salmonella* Typhimurium

and a 2.5 log reduction in *L. monocytogenes* when compared to the control. After 30 min treatment, the three pathogens were reduced by 2.2, 3.3, and 2.7 log and after 60 min, the reductions were 3.4, 4.5, and 3.8 log, respectively. Aerosolized peroxyacetic acid is an effective sanitizer of lettuce leaves, and has potential for application in the food industry. In particular, aerosolized sanitizers may be useful for preventing regrowth of pathogenic and spoilage microorganisms during shipping or extended storage of produce, when dipping or spraying with aqueous sanitizers is unfeasible.

P2-57 Detection and Identification of Animal Species in Raw and Processed Food Using a High-density DNA Probe Array

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Recent food crisis in Europe and globalization of food trade on a worldwide scale have raised interest in the identification of animal species in food for safety reasons. Meat and bone meals of two ruminant species, sheep and cow, were shown to be responsible for the transmission of BSE, whereas products of animal origin are known to be allergenic: eggs, dairy products, fish, crustaceans. Current immunological identification techniques methods lack both sensitivity and specificity. They are also less effective when applied to processed foods due to protein denaturation. There are few commercially-available DNA techniques, and they are limited in the number of species detected, cannot identify mixtures of species in the same sample and are tedious to perform or interpret. We developed a unique DNA test to detect and identify 33 species of commercial interest (12 mammals, including beef, sheep, and pork, 5 birds, and 16 fish species) together with the class status (mammal, bird and/or fish), in raw and processed food samples, alone or present in mixtures, at a level of less than 5% weight/weight in the final product, typically down to 1 to 2%. Using a more sensitive protocol allows detection of animal species in feed characterized by the reference microscopic technique, with a good correlation. Data on reference and commercial samples will be presented. This complete solution consists of a single protocol from sampling to interpretation, a kit with reagents and an Affymetrix DNA chip, as well as secured interpretation software. This semi-automated system offers a reliable and sensitive method which will allow such testing to be put into routine use.

P2-58 Influence of Growth Temperature on the Thermal Resistance of *Yersinia* Species

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Environmental growth conditions are known to influence the thermal resistance of bacteria. In this study, the effect of growth temperature on the subsequent heat resistances of *Y. enterocolitica* and *Y. pseudotuberculosis* were examined. In *Yersinia* strains carrying the 41-48 Mdal virulence plasmid associated with the production of a series of outer membrane proteins at 35-37°C was compared to a non-pathogenic isogenic strain lacking the plasmid. Strains were grown at 25 and 37°C in non-selective media under aerobic conditions. Thermal resistance was tested from 52 to 62°C in 2 degree increments using a submerged coil device. Both *Yersinia enterocolitica* and *Y. pseudotuberculosis* grown at 37°C were up to 10 fold more heat resistant than when grown at 25°C. Z-values for cultures grown at 25 and 37 were 4.3 and 3.6, respectively. No differences in heat resistance were found between paired strains with and without the virulence plasmid.

P3-01 Prevalence of *Listeria* in Pork Bulbogi Obtained from Restaurants and Retail Markets in Korea

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The prevalence of *Listeria* in pork bulgogi was studied in three locations in Kyunggi-do, Korea. Samples were collected from restaurants and retail markets. A total of 78 samples were collected from different sampling site in the cities of Anyang, Seongnam, and Suweon. The percent positive to *Listeria* for samples collected from Anyang, Sungnam, and Wuweon was 11, 38, and 52%, respectively. Overall, 25 of 35 (69%) samples collected from restaurants were positive to *Listeria*, whereas only 3 of 42 (7%) samples collected form retail markets were positive to *Listeria*. To isolate *Listeria* from pork bulgogi, enrichment of 25 g sample in *Listeria* enrichment broth, followed by incubating in fraser broth, was achieved. Then loopfuls of growth were streaked onto plates of oxford agar and blood agar. The six most frequent serovars isolated were *L. grayi* (19.2%), *L. ivanovii* (12.8%), *L. welshimeri* (11.5%), *L. seeligeri* (7.7%), *L. monocytogenes* (3.8%), and *L. innocua* (3.8%). The *Listeria* positive samples were were enumerated by most probable number (MPN). Numbers of Listeria were from below 0.12 MPN/g to 4.6 MPN/g.

P3-02 Incidence of *Listeria* in Pork Collected from School Foodservice Programs in Korea

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School foodservice programs are growing very fast in Korea, and the safety of raw materials of animal origin has become more important. The objective of this study was to observe the incidence and quantity of *Listeria* in pork from school foodservice programs. A total of 116 samples were collected from schools located in Kyunggi province. Isolation of *Listeria* from pork was achieved by enrichment of 25 g of sample in *Listeria*

enrichment broth, followed by incubating in fraser broth. Then loopfuls of growth were streaked onto plates of oxford agar and blood agar. Overall, 95 of 116 samples (81.9%) collected were positive for *Listeria*. Identification of species of *Listeria* was accomplished using API kit. The five most frequent serovars isolated were L. grayi (49.1%), *L. welshimeri* (19.8%), *L. ivanovii* (16.4%), *L. seeligeri* (16.4%), and *L. innocua* (12.1%). *Listeria* positive samples were enumerated by most probable number (MPN). Numbers of *Listeria* ranged from below 0.12 MPN/g to 1840 MPN/g. From the above results, the incidence of *Listeria* in pork collected from school foodservice program was very high (81.9%), but *L. monocytogenes* was not detected.

P3-03 Survival of *Listeria monocytogenes* on Ready-to-Eat Meat Products Stored at Freezer Temperatures under Vacuum and Non-vacuum Packaging

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Our study investigated the survival of *Listeria monocytogenes* (Lm) at freezer temperatures (-20°C) on the surface of RTE meat products. An 18-h culture of Lm was decimally diluted and inoculated (5.8 log CFU/g) onto 45 g cubes of RTE roast beef, ham, sausage, chicken breast, and turkey. The samples (vacuum or non-vacuum packaged) were stored at -20°C and bacterial counts determined at day 0, 7, 14, 21, 28, 30, 60, and 90 by spread plating onto modified Oxford media and then incubating plates at 37°C for 48 h and determining CFU/g. Our results showed that at day 7 there was no significant difference in Lm counts between the different RTE meats tested. However, by day 28 Lm counts were significantly higher for the vacuum and non-vacuum RTE roast beef samples (6.7 log CFU/g) than for the other meat samples (6.3 log CFU/g). Furthermore, at day 28 all the RTE meat samples, except the vacuum packaged turkey, had Lm counts greater than 0.5 log CFU/g compared to day 0. Conversely, by day 90 the Lm counts had reached or were lower than the initial day 0 bacterial levels for all RTE meats. Throughout the study the vacuum packaged roast beef, chicken and hot dogs Lm counts were significantly higher then the non-vacuum packaged whereas the non-vacuum packaged turkey Lm counts were significantly higher then the vacuum packaged. Our study has shown that the ability of Lm to survive at freezer temperatures on ready-to-eat meats was dependent on the meat product and packaging condition.

P3-04 Microbial and Environmental Hazards in Street Foods of Urban Vadodara, India

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In recent years, street foods in the developing countries have become a part of urban life as they provide a source of inexpensive and convenient food which in turn serves as a sole source of income for low income group families. A systematic study was undertaken to investigate the microbial and environmental hazards present in street foods sold by 100 vendors by means of in-depth spot observations, semi-structured questionnaire and estimation of some foodborne pathogens, using methods of FAO, 1979 in a sub-sample. The results showed that most vendors exhibited fair to poor practices of cleaning the stalls and utensils, personal hygiene, environmental sanitation and food safety knowledge. Handwashing and dishwashing practices exhibited by vendors were far from satisfactory. Microbial analysis of Bhelpuri (prepared from 8 moist and dry ingredients) and sandwich samples revealed Total Aerobic Mesophilic bacteria and *S. aureus* counts in range of log 6 to log 8 CFU/g and log 6 to log 7 CFU/g respectively, along with presence of *Escherichia coli*, *Salmonella* and *Shigella* in 60%, 55% and 65% of Bhelpuri samples and 35%, 65% and 10% of sandwich samples respectively. To identify the source of contamination, hazard analysis was conducted for all the ingredients and other samples of personal hygiene and contact surfaces involved in the making of Bhelpuri and sandwiches in the two selected stalls. Results showed presence of *E. coli* in almost all samples and *Salmonella* and *Shigella* in knife, hand rinse, dishwater, butter, tomato, ketchup and sevpuri samples. Thus street foods prepared under the prevalent conditions of hygiene, sanitation and inadequate water and garbage disposal facilities may pose a health hazard and calls for immediate control measures on part of government officials to reduce the risk of outbreaks of foodborne illnesses.

P3-05 Dynamic Assessment of the Microbial Quality of Fresh Broccoli in a Food Supply Chain

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Fresh produce is known to harbor pathogens associated with foodborne illness. However, little is known about the evolution of the microbial quality of fresh fruits and vegetables as they move through the food supply chain. This study aimed to assess the microbial quality of fresh broccoli at every step in a production and distribution system. Locally produced fresh broccoli was tracked through a supply chain by following 33 distinct lots of produce from harvest to storage, wholesale handling, and retail display over two seasons. A total of 201 randomly selected samples were collected and 804 microbial analyses were conducted on the florets, using standard methods, to determine the numbers of viable aerobic bacteria (aerobic colony count,

ACC), fecal coliforms, *Escherichia coli* and *Listeria monocytogenes*. The temperature of broccoli was also monitored. All the samples had low ACC, low or non-detectable levels of fecal coliforms and *E. coli*, and non-detectable levels of *L. monocytogenes*. A small number of samples with detectable levels of fecal coliforms and *E. coli* were found at each step of the production and distribution system during the first season, but none were found during the second season. A slight increase in ACC was observed between harvest and retail display during both sampling seasons. Broccoli temperature was relatively well controlled throughout the supply chain, both years. Overall, the change in its microbial quality in the supply chain considered seemed to be more influenced by the age of the broccoli (time since harvest) than by its calculated average temperature.

P3-06 Microbiological Hazard Assessment for the Construction GAP System in Strawberry Farms in the Gyeongnam Province of Korea

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The objectives of this study were the assessment of microbiological hazards and provision of the microbiological information for the introduction of Good Agricultural Practice (GAP) systems in strawberry farms located in the western Gyeongnam province of Korea. A total of 141 samples were collected from water, soil, protected houses, and package houses. These samples were examined for sanitary indication bacteria, such as aerobic plate count, coliforms, and *Escherichia coli*. Pathogenic bacteria were also investigated, such as *E. coli* O157:H7, *Salmonella* spp., *Staphyloccoccus aureus* and *Listeria monocytogenes*. As a result of microbial assessment, a significant difference between hydroponic cultivated farms and soil cultivated farms was not found. APC in the 6 farms studied varied from ≤ 1.5 log CFU/100 cm^2 to ≥ 7.0 log CFU/g. The isolation rate of coliform and *E. coli* were 78% and 19%, respectively. Specifically, over 4.5 log CFU/hand of *E. coli* was observed on employees' hands from F farm. According to the results of pathogenic bacteria detection, only the soil of F farm was contaminated with *Salmonella* spp. *Staphylococcus aureus* was detected in 14% of the collected samples. *Staphylococcus aureus* especially was found frequently on hands. However, *E. coli* O157:H7 and *L. monocytogenes* were not detected anywhere.

P3-07 Role of Post-harvest Processing Practices in Contamination of Lettuce

DSC

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During post-harvest processing foodborne pathogens can be transferred to a product through processing waters, equipment, or contaminated product. In the present study, a series of experiments were conducted using *Escherichia coli* O157:H7, fluorescent microspheres (beads), and *Enterobacter aerogenes* to determine efficacy of washing, impact of cross-contamination, and whether bacterial factors are required for pathogen transfer. The efficacy of washing to remove *E. coli* O157:H7, beads, and *E. aerogenes* from lettuce was investigated by exposing contaminated lettuce (ca. log 7 CFU or particles/g) to the following; wash, spin-dry, wash, and spin-dry. Population of *E. coli* O157:H7, beads, and *E. aerogenes* decreased, 2, 2.6, and 1.3, respectively, on lettuce. In experiment 2, pathogen and bead free lettuce was washed in contaminated wash water (5.5 log CFU or 4.1 beads/ml), spin dried and washed again in contaminated wash water (4.9 log CFU or 3.9 beads/ml) and spin dried. Populations of *E. coli* O157:H7, beads, and *E. aerogenes* on lettuce were 3.5, 2.4, and 4.1, respectively. In experiment 3, a single piece of contaminated lettuce (~2 g/piece; 7.6 log CFU/g *E. coli* O157:H7) was mingled with 200 g of pathogen free lettuce. The lettuce was subjected to two wash and spin dry cycles and then *E. coli* O157:H7 populations determined. Following the second spin-dry step, level of *E. coli* O157:H7 on lettuce was 5.2 log CFU/g. These series of experiments demonstrate the limited efficacy of washing in removing bacteria from lettuce and the cross-contamination of product during processing.

P3-08 Microflora on Georgia-grown Cantaloupes Related to Packaging and Handling Practices

DSC

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In recent years, there have been outbreaks associated with the consumption of cantaloupe. Contamination of cantaloupes with microorganisms could occur anywhere from the field to the packing line. Cantaloupes are handled and packed differently in various regions of the United States. Typically, California cantaloupes are field packaged while in Georgia they are brought to sheds, washed, and packaged. The objective of this study was to enumerate aerobic bacteria on cantaloupes from the field, after washing, and after packaging. Four Georgia growers with packing facilities using different variations in product handling were visited four times during the harvest season. For each visit, 20 cantaloupes were sampled after transport from the field, after washing and after packaging. The washing methods varied among the facilities with 2 using chlorinated water, 1 using heated water, and 1 using a combination of heat and chlorinated water. Exposing cantaloupes to water between 41 to 50°C for 5 to 10 min did not result in a significant change in the microbial population sizes. Microbial populations on cantaloupes from the two farms using chlorinated treatments were < 0.5 log lower than on those not chlorinated. However, aerobic populations after packing were approximately the same as that on the prewashed cantaloupes. Thus washing, chlorination and hot water treatments applied under actual field packing conditions in Georgia do not affect the total aerobic populations on cantaloupes.

P3-09 Levels of Microbial Contaminants in Highbush Blueberries before, during and after Processing

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Concerns regarding blueberry spoilage, safety and development of microbiological standards prompted a 2003 – 2004 survey in which highbush blueberries were collected from 18 different Michigan fields before harvest and quantitatively examined for mesophilic aerobic bacteria (MAB) coliforms, *Escherichia coli* and yeasts (Y) and molds (M). Thereafter, blueberries from these same fields were harvested and similarly assessed during processing (post-harvest, blower exit, after washing and at freezing) at four facilities along with environmental samples (blower and filler conveyor belts, chlorinated wash water). Duplicate blueberry (100 g), wash water (50 ml) and environmental swab samples (~10 × 10 cm) were analyzed for MAB, coliforms, *E. coli*, Y and M by plating on Trypyicase soy agar + 0.6% yeast extract, Petrifilm *E. coli*/coliform plates and potato dextrose agar containing streptomycin and ampicillin, respectively. Average counts on blueberries for MAB, Y and M were 3.53, 3.85 and 3.33 at pre-harvest, 5.01, 4.45, and 4.13 at post-harvest, and 4.23, 3.88 and 3.67 log CFU/g after washing, respectively. Coliform and *E. coli* counts increased 0.68 and 0.12 logs from pre-harvest to after washing, respectively. Microbial populations were highest on the blower and filler belts and lowest in the chlorinated wash water. Overall, microbial populations increased ~1.5 logs between harvest and processing (4 to 18 h) with the chlorinated wash water (~10 to 200 ppm chlorine) reducing populations <1 log. Thus, improved storage strategies before processing and more effective microbial reduction strategies during washing are needed to prolong the quality and shelf life of blueberries.

P3-10 Influence of Biosurfactant-producing *Pseudomonas fluorescens* on Growth Kinetics and Dynamics of Adherence of *Escherichia coli* O157:H7 on Greenhouse-grown Romaine Lettuce

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Characterizing the interactions of epiphytes on the fate of zoonotic bacterial pathogens arriving on the phyllosphere may provide management options in food safety systems for edible horticultural crops. In this study, the interactions and effects on growth kinetics and dynamics of adherence on greenhouse-grown Romaine lettuce leaves, pre-colonized by a biosurfactant producing *Pseudomonas fluorescens* 123 or *P. fluorescens* EG3 (Tn5 mutant that does not secrete biosurfactant) were conducted. *P. fluorescens* 123 or *P. fluorescens* EG3 were inoculated at approximately 0 and 5.0 log CFU/leaf and held at 25°C under lights (16D/8N) and high relative humidity (>95%). At 48 h, approximately 5.0 log CFU/leaf *Escherichia coli* O157:H7 was inoculated at the target site and returned to the same conditions. Recovery of pseudomonads and *E. coli* O157:H7 at inoculation (-0.5 h) and at 0, 24, 48, 72, 96, and 120 h was conducted on appropriate selective media. Individual populations from 6 replicate leaves were enumerated, correcting for carry-over of tightly adhering wash water. Recovery was conducted using a sequential rub-shake-rub (1°) followed by a separate stomaching (2°) procedure. In contrast to previous studies with *Salmonella* spp. on Romaine, although significant differences in recovery ($P < 0.05$) were observed for pre-colonized leaves, no significant difference could be ascribed to the role of the biosurfactant synthesis. Biosurfactant did not enhance removal or measurable internalization. An apparent differential increase in adherence over time was observed with *E. coli* O157:H7 recovered from leaves inoculated with *P. fluorescens* EG3 at 120h ($P < 0.05$) between the 1° and 2° recovery.

P3-11 Using Sanitizers and Heat Treatments to Enhance Microbial Safety of Fresh-cut Mangoes

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Inactivation of artificially contaminated on and in Numdokmai mangoes (*Mangifera indica*) either with single strain or five strain of salmonellae using sanitizers or heat treatments were investigated. Results showed salmonellae survived on and in mangoes at 30°C and 15°C, for up to 5 and 9 days until spoilage. Washing contaminated mangoes with sodium hypochlorite (200 ppm, pH 4 for 10 min) reduced salmonellae cells on surface by 2.0 to 3.0 log from initial population of 3.0 to 4.0 log CFU/fruit. Hydrogen peroxide at 1.5, 3 and 5% for 10 min or hot water (60°C, 15 min and 62°C, 4 min) also reduced cells by 2.0 to 4.0 log. However, chlorine dioxide (5, 10, 20 ppm for 30 min) and H_2O_2 (1, 5, 10% for 30 min) did not inactivate internalized cells in mangoes. Cross-contamination examination by cutting washed mangoes to get the half-piece (with rinds remaining) showed no growth after storage for 24 h at 15°C. However, cells revived after 48 to 72 h. While fresh mangoes prepared from fruit washed with 200 ppm sodium hypochlorite 200 ppm for 10 min and H_2O_2 5% for 10 min and stored at 30°C and 15°C for up to 72 h showed negative salmonellae detection, cells were found from hot water treated mangoes (60°C for 20 min) by tryptic soy broth enrichment. Use of sanitizers and heat treatments in appropriate conditions could enhance food safety in fresh-cut fruit preparation; however the shelf life of this product should be considered due to the possible recovery of injured cells.

P3-12 The Use of Peracetic Acid for Microbial Control of Minimally Processed Cheiro Verde

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Saving time and adopting healthy habits are some of the reasons to explain the increasing consumption of vegetables minimally processed. They must be free of pathogenic microorganisms and a disinfecting step is considered a critical point for the reduction of microbial load. Therefore, the main objective of this study was to evaluate the efficacy of peracetic acid as a chlorine substitute. A mixture of parsley and welsh onion (1:1) was used since it was the most contaminated vegetable analyzed in previous work. Tests were performed at 3 concentration levels (60, 80, 100 ppm) and 3 exposure times (5, 10, 15 min) with 3 repetitions. A 15 min treatment with tap water was used as control. The treatment efficiency was evaluated by the number of decimal reductions of microbial population (total and fecal coliforms, molds and yeast) and by *Salmonella* detection. The results showed that concentration less than 100 ppm and time less than 10 min was not effective. Therefore, logarithm cycle reductions of 2.7 and 3.0; 1.5 and 2.5; 1.0 and 1.5 were obtained for molds and yeasts; total coliforms; fecal coliforms at 10 and 15 min treatments. In relation to the use of sodium hypochloride (120 ppm for 15 min) the reductions were about 0.4, 1.0 and 1.0 logarithm cycles for molds and yeasts, total and fecal coliforms, respectively. Thus, it is necessary to use at least 10 min treatment at the concentration of 100 ppm in order to get good results. At the 15 min treatment peracetic acid was more effective than sodium hypochloride. *Salmonella* was not detected.

P3-13 Survival of *Escherichia coli* O157:H7, *Salmonella* spp. and *Shigella* spp. on Watermelon Surfaces

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The survival of *Escherichia coli* O157:H7, and multistrain mixtures of *Salmonella* spp. and *Shigella* spp., was evaluated on watermelon surfaces. Culture preparations (8 log CFU/ml) suspended in sterile 5% horse serum albumen were spot inoculated (20 µl) central to 9-cm² areas or the stem scar and allowed to dry at 22°C for 3 h. Watermelons were stored at 10, 20, or 29°C for up to 14 days. At each time point, a 9-cm² core was removed, the exocarp aseptically excised and stomached in 0.1% peptone with 0.05% Tween 80. Replicate aliquots were plated onto appropriate selective agar amended with 0.1% pyruvic acid. Applied populations declined by approximately 1 log CFU/disc following drying. When stored at 10°C for 14 days, *Salmonella* spp. was reduced 3.0 and 3.3 log CFU/disc, *E. coli* O157:H7 was reduced 4.9 and 4.8 log CFU/disc, and *Shigella* spp. was reduced 4.7 and 5.0 log CFU/disc on the green and yellow (ground spot) surface, respectively. When stored at 20°C for 7 days, *Salmonella* spp. was reduced 2.3 and 2.6 log CFU/disc, *E. coli* O157:H7 was reduced 4.4 and 4.8 log CFU/disc, and *Shigella* spp. was reduced 3.2 and 3.9 log CFU/disc on the green and yellow rind, respectively. *Salmonella* spp. was selected for further studies. No differences in survival were found among inoculations spotted on the smooth green rind, naturally-healed superficial wounds, or the stem end of watermelons stored at 20°C for 10 days. Post-drying survival of *Salmonella* on the green upper-rind surface of watermelon was 1.83 log CFU/disk greater when stored at 20°C compared to 29°C. In contrast, the log difference at the stem-end was less than 0.3 log CFU at these temperatures. While postharvest factors common to watermelon handling would likely limit survival, the capacity of bacterial pathogens to persist on the external surfaces of watermelon underscore the importance of prevention of initial contamination and further reduction interventions prior to processing for consumption.

P3-14 Survival of *Salmonella* on Wounded Orange Surfaces

DSC

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Oranges with minor surface wounds may go undetected at the packing house. The objective of this study was to determine the impact of puncture wounds on survival of *Salmonella* on orange surfaces. Oranges were wounded by puncturing the peel (2 mm diameter × 3 mm deep). After wounding, oranges were held at 24°C for 0 or 24 h before inoculating the wound or intact control site with 10 µl of a five-strain cocktail of *Salmonella* spp. suspended in 3% sodium bicarbonate (SB) or 5% horse serum. Oranges were incubated at 24°C for 1 h (to dry inoculum) or for 24 h before analysis. *Salmonella* were recovered by rubbing the whole orange for 60 sec in 10 ml of DE broth and/or by excising a 1-cm² area around the inoculation site and albedo below the wound. The excised peel was stomached for 1 min in 10 ml of DE broth. Samples were plated onto tryptic soy and bismuth sulfite agars and incubated at 37°C for 24 or 48 h, respectively. Reductions of 1 log (1 h) and 2 log (24 h) were observed on both wounded and unwounded sites regardless of inoculum carrier. However, 1-log greater reductions were observed after 24 h on intact sites when SB was used. Survival was similar on intact surfaces and 24-h old wounds. When *Salmonella* was recovered by rubbing, similar levels were recovered from intact and wounded sites. When inoculation sites were subsequently excised and stomached 3-log greater recovery of *Salmonella* was observed at the wound sites. *Salmonella* in liquid suspension can enter and survive in minor orange peel wounds emphasizing the need for postharvest systems that minimize fruit damage and maintain water sanitation.

P3-15 DSC **Survival and Growth of *Enterobacter sakazakii* on Fresh-cut Fruits and Vegetables**

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Enterobacter sakazakii is known to cause infections in infants and elderly immunocompromised individuals. Its documented presence in food processing plants, homes, and other environments raises concern about its ability to survive and grow in a wide range of foods. We conducted a study to determine the survival and growth characteristics of *E. sakazakii* on fresh-cut lettuce, cabbage, carrot, cucumber, tomato, apple, strawberry, watermelon, and cantaloupe and in juice prepared from these fruits and vegetables. Inoculated produce (2.6 log CFU/g) and juice (1.5 log CFU/ml) were stored at 4, 12, and 25°C and monitored for populations of *E. sakazakii* for up to 7 days. Populations steadily declined on produce and in juice but were detected throughout storage at 4°C. *E. sakazakii* grew on fresh-cut watermelon, cantaloupe, apple, cucumber, and tomato and in watermelon, carrot, cucumber, and lettuce juice at 12°C. Growth occurred at 25°C on all produce except strawberries and in all juices except apple, strawberry and cabbage. Fresh-cut watermelon, cantaloupe, cucumber, apple, and tomato supported population increases of 1 to 3 log CFU/g within 3 days at 12°C and 4 to 6 log CFU/g within 2 days at 25°C. Growth of *E. sakazakii* in juices at 25°C was followed by reductions to undetectable populations (< 1 CFU/ml) within 2 to 4 days post inoculation. Death was concurrent with decreases in pH, which were attributed in part to growth of lactic acid bacteria. Results show that *E. sakazakii* can grow on a wide range of temperature-abused fresh-cut produce and produce juice.

P3-16 DSC **Attachment of *Escherichia coli* O157:H7 to Lettuce and Carrot Surfaces and Possible Internalization**

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Potential attachment and internalization of *Escherichia coli* O157:H7 to fresh produce was investigated using scanning electron microscopy (SEM). Pieces (approx. 0.5 x 0.5 cm) of baby carrots and lettuce were inoculated by submerging them into a suspension of *E. coli* O157: H7 (8.3 log CFU/ml) for 4 h at ambient (20°C) and refrigeration temperature (4°C) and allowed to drain on filter paper in a laminar flow hood. Then, the pieces were fixed with cacodylate buffer, dehydrated in an ethanol series, dried in critical point dryer, and coated with gold using a sputter coater for SEM examination. The results indicated that *E. coli* O157:H7 cells could attach to both carrots and lettuce with greater numbers attached at ambient rather than at refrigeration temperature. *E. coli* O157:H7 attached to produce surfaces at different angles which may affect intensity of attachment. *E. coli* O157:H7 could internalize through stomata in lettuce. Due to processing, stomata were missing on the baby carrots. However, visible mechanical damage from processing was observed on the surface of baby carrots and played a role in bacteria internalization. Internalization may result in an underestimation of bacterial contamination and reduce sanitizing efficacy due to limited contact with sanitizing agents.

P3-17 DSC **Incidence and Distribution of *Salmonella* Serotypes Isolated from Tomato and Related Environmental Materials from Hydroponic Greenhouses**

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In the last decade, at least three outbreaks of salmonellosis have been associated with the consumption of raw tomatoes. Sources and mechanisms of *Salmonella* contamination have been widely studied for produce grown in open fields. The prevalence of *Salmonella* during three years averaged 3.0% on tomatoes grown in hydroponic greenhouses. During 2003 – 2004, modern hydroponic greenhouses were affected by flooding and later, by the presence of wild animals. The objective of this work was to evaluate the influence of such events on the incidence and distribution of *Salmonella* on hydroponic tomatoes. *Salmonella* serotypes were investigated in 910 samples of tomato and 382 of different environmental materials. *Salmonella* was present in 7.9% of tomatoes collected in the greenhouses. Also, it was recovered from personnel shoes (10.6%), puddles (11.7%), vehicle wheels (14.0%), working-shoes (16.3%), soil (22.6%), and feces of both wild and farm animals (57.9%). The identified serovars were Montevideo (58.0%), Newport (4.2%), Abaetetuba (2.8%), Oranienburg (2.8%), Midway (1.4%), and Muenchen (1.4%). Strains from serogroup F were 29.6%. *S.* Montevideo was identified in 55.0% of positive tomato samples and 48.8% of positive environmental samples, whereas serogroup F strains were present in 20.0% and 25.8%, respectively. Apparently, *S.* Montevideo showed an association among tomatoes, wild animals and puddles. Meanwhile, serogroup F was associated with contamination of tomatoes, shoes and farm animals. This work shows the importance of flooding and the presence of wild animals as sources of contamination to the greenhouses, thus affecting the microbial safety of tomatoes even before harvest

P3-18 Isolation of *Salmonella* Enteritidis PT 30 from a Single Almond Orchard Over a Four-year Period

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In 2000/2001 an outbreak of *Salmonella* Enteritidis Phage Type 30 was linked to consumption of raw almonds. Traceback investigations lead to isolation of the outbreak strain from almond orchard soils. The objective of this study was to evaluate the persistence of *S.* Enteritidis in a single 61-hectare almond orchard. Swabs (string tied to gauze and saturated with evaporated skim milk) were pulled along the orchard floor in a standardized manner for a distance of 55 m. At each sample time, two pooled samples (4 swabs each) were collected from each of the four orchard quandrants. Swabs were enriched for *Salmonella* by addition of tetrathionate broth (TTB) and incubation at 42°C for 18 to 24 h. The TTB was held for a further 5 to 7 days at room temperature, transferred to fresh TTB, and incubated at 37°C for 18 to 24 h. Primary and delayed enrichment broths were streaked onto selective agar. Suspect *Salmonella* isolates were selected, confirmed, serotyped, phage typed, and pulsed field gel electrophoresis (PFGE) patterns were determined (Xba I and Bln I). *Salmonella* was seldom recovered from December through July (3 of 48, 6%). During harvest when large amounts of dust were generated, isolation frequency increased: August (4 of 20, 20%), September (13 of 64, 20 %), October (22 of 49, 45%), and November (2 of 8, 25%). All isolates were identified as *S.* Enteritidis PT 30 with two PFGE patterns that differed by the presence of an approximately 40-kb fragment. These data demonstrate the potential for long-term environmental survival of *Salmonella* in almond orchards.

P3-19 Survival of *Salmonella* Enteritidis PT 30 on Almonds after Exposure to Hot Water

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Almond skins are removed by treatment with steam and/or hot water (blanching). The objective of this study was to evaluate survival of *Salmonella* on almonds exposed to hot water. Whole Nonpareil almonds (40 g) inoculated (10^8 CFU/g) with *S.* Enteritidis PT 30 were submerged in 24 l of water maintained at 70, 80, or 88°C (± 0.2°C). Almonds were heated for 0.5 to 4.0 min, drained for 4 sec, transferred to 80 ml of cold (4°C) tryptic soy broth, and stomached for 2 min. Appropriate dilutions were plated onto tryptic soy and bismuth sulfite agars, and incubated at 37°C for 24 and 48 h, respectively. Skins remained fully or partially attached to kernels and were included in the analysis. Reductions of 1.7, 3.0, and 4.6 log CFU/g were observed within 30 s at 70, 80, and 88°C, respectively. Thereafter, *S.* Enteritidis declined at a slower rate. Standard deviations were large particularly at the longer treatment times. This may have been due to partial detachment of skins from kernels. D values were calculated assuming that the outer surface of the almond uniformly and immediately reached the water temperature and that reduction of *Salmonella* followed first order thermal death kinetics. D values of 1.0, 0.6, and 0.3 min were determined for exposure to 70, 80, and 88°C, respectively. The z-value was calculated to be 29°C. Current industry practice of blanching at 88 to 96°C for 2 to 3 min for removal of almond skins should be adequate to achieve a 5-log reduction of *Salmonella*.

P3-20 Survival of *Salmonella* Enteritidis PT 30 on Almonds after Exposure to Hot Oil

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Almonds are roasted by exposure to hot air or immersion in hot oil. However, little data exists on the behavior of *Salmonella* under roasting conditions. Our objective was to evaluate *Salmonella* survival on almonds in hot oil. Whole Mission almonds (50 g) inoculated (5 or 8 log CFU/g) with *S.* Enteritidis PT 30 were submerged in 2.8 l of hot oil. Almonds (8 log CFU/g) were heated for 0.5 to 4.0 min, drained for 10 s, transferred to 100 ml of cold (4°C) TSB, and stomached for 2 min. Samples were plated onto tryptic soy and bismuth sulfite agars, and incubated at 37°C for 24 and 48 h, respectively. Reductions of 2.9, 3.0, or 3.6 log CFU/g were observed within 30 s of exposure to 116, 121, or 127°C, respectively. Thereafter, the reduction was linear but at a slower rate of decline. The time to achieve a 5-log reduction was estimated to be 3.0, 2.2, or 1.3 min at 116, 121 or 127°C, respectively. To confirm these data, almonds (3 × 50 g) inoculated at 5 log CFU/g were heated for 0.5 to 3.5 min. Each sample was blended in 450 ml of lactose broth and three 10-ml samples were enriched for *Salmonella* using standard methods. A 5 to 6-log reduction was achieved in 1.5 to 2.5 min or 1.0 to 1.5 min of exposure to 121 or 127°C oil, respectively. Current industry practice of roasting in oil heated to 138 to 150°C for 3 to 10 min should achieve a 5-log reduction of *Salmonella* on almonds.

P3-21 Inhibition of Bacterial Human Pathogens by Plant-Associated Pseudomonads Both *In Vitro* and on Sprouting Alfalfa Seed

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Foodborne illness due to the consumption of raw or lightly cooked sprouts contaminated with *Salmonella* or *Escherichia coli* O157:H7 continues to occur. Much research has been devoted to the study of chemical and physical methods for sanitizing sprout seed, but few studies have been undertaken on the use of biological approaches for pathogen control. In this study the ability of plant-associated Pseudomonads to inhibit growth

of *Salmonella*, *E. coli* O157:H7, *Listeria monocytogenes* and *Shigella* on agar and in broth media was determined. The ability of selected Pseudomonads to inhibit outgrowth of *Salmonella* and *E. coli* O157:H7 from artificially contaminated alfalfa seed during sprouting was also evaluated. Strong inhibition of *Salmonella*, *E. coli* O157:H7 and *Shigella* was demonstrated by *Pseudomonas fluorescens* strain 2-79 *in vitro*. In broth co-culture experiments *Salmonella* and *E. coli* O157:H7 populations were reduced by 5 to 6 log CFU/ml at 24 h. The pathogen *L. monocytogenes* was strongly inhibited in vitro by *P. fluorescens* strains Pf-5 and CHA0. After 6 days of growth, the population of a five strain cocktail of *Salmonella* on alfalfa sprouts propagated from inoculated seeds treated with strain 2–79 was reduced by 4 to 5 log CFU/g in comparison to growth on the control sprouts. Population reductions for *E. coli* O157:H7 were less, but still significant. The results demonstrate that the use of antagonistic bacteria for controlling the outgrowth of bacterial human pathogens on sprouting seed may be a viable intervention for use by conventional as well as organic sprout growers.x5

P3-22 DSC Microbial Profiles of Online-procured Sprouting Seeds and Potential Hazards Associated with Enterotoxigenic *Bacillus* spp. in Home-grown Sprouts

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We examined the microbiological quality of sprouting seeds sold through the Internet. Overall, five types of seeds each from six organic and six conventional sources were evaluated. Furthermore, the growth and toxin production of naturally occurring *Bacillus* spp. in sprouts produced using home-scale sprouting devices were investigated. Respectively, for alfalfa, broccoli, lentil, mungbean and radish seeds, the average microbial counts were 3.3, 4.0, 2.8, 3.5 and 3.6 log CFU/g, presumptive *B. cereus* counts were 0.7, 1.0, 0.8, 1.0 and 0.9 log CFU/g, and total coliform counts were -0.3, -0.4, -0.5, 0.0 and -0.4 log MPN/g. No *Salmonella*, *Escherichia coli* O157, other fecal coliform, or *Staphylococcus aureus* was found on seeds. Compared to conventional seeds, the organic seeds had less or equivalent counts of total microorganisms, presumptive *B. cereus*, and total coliforms. When sprouting by a glass jar, the growth of presumptive *B. cereus* was significant in radish and broccoli, but not alfalfa, lentil and mungbean sprouts; the counts exceeded 5.0 log CFU/g in radish sprouts. When sprouting by an automatic sprouting device, presumptive *B. cereus* showed slight growth (< 3.0 log cycles) in radish, broccoli, and mungbean sprouts, but no growth in alfalfa and lentil sprouts. Although the presumptive *B. cereus* isolates were enterotoxigenic, they did not produce or accumulate detectable levels of diarrhoeal toxins in freshly produced sprouts. In conclusion, the microbial quality of sprouting seeds could be influenced by seed type and source. The growth of Enterotoxigenic *Bacillus* spp. during sprouting is influenced by both seed type and sprouting method.

P3-23 Influence of Blanching Treatments on *Salmonella* during Home-type Dehydration and Storage of Potato Slices

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Traditional treatments for home-dried vegetables may not effectively inactivate pathogens that could be present on minimally processed fruits and vegetables. The objective of this study was to evaluate the influence of modified treatments on *Salmonella* during preparation, home-type dehydration (60°C, 6 h) and storage of Russet potato slices. Inoculated (five-strains, 8.4 log CFU/g) potato slices were left untreated or treated with steam blanching (88°C, 10 min), water blanching (88°C, 4 min), 0.105% citric acid blanching (88°C, 4 min), or 0.210% citric acid blanching (88°C, 4 min), dried for 6 h (60°C), and stored for up to 30 days. *Salmonella* populations were reduced by 4.5 to 4.8 and >5.4 log CFU/g immediately following steam or water blanching, respectively. Bacterial populations were below the detection limit (0.80 log CFU/g) immediately following acid blanching, except for samples blanched in 0.105% citric acid and recovered on TSAP. After 6 h dehydration at 60°C, *Salmonella* reductions on blanched potato slices (5.3-5.4 log CFU/g) were significantly ($P < 0.05$) greater than those on unblanched (control) samples (1.9 to 2.7 log CFU/g). Bacterial populations on all samples continued to decrease throughout 30 days of storage. After 6 h dehydration and 30 days of storage, populations on all blanched samples were undetectable by direct plating (regardless of blanching method), but ranged from 3.14 to 3.92 log CFU/g on unblanched (control) samples. It was concluded that the blanching methods used in this study promoted inactivation of *Salmonella* during drying and storage of potato slices and, therefore, may enhance the safety of the finished product.

P3-24 Effectiveness of Gaseous Chlorine Dioxide in Killing *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and Yeasts and Molds on Fresh and Fresh-cut Fruits and Vegetables

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An increased frequency in outbreaks of illness associated with the consumption of fresh produce in recent years has raised interest in developing more effective sanitization methods. Among the sanitizers of interest is gaseous chlorine dioxide (ClO_2). We previously observed that treatment of blueberries, strawberries, and

raspberries with gaseous ClO_2 was effective in killing large numbers of *Salmonella* without compromising shelf life. We expanded these studies to evaluate the efficacy of gaseous ClO_2 in killing *Salmonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* inoculated onto the surface of fresh-cut cabbage, carrot, and lettuce as well as fresh whole apples, peaches, onions, and tomatoes. Sachets containing reactant chemicals were formulated to produce gaseous ClO_2 at concentrations of 1.4, 2.7, and 4.1 mg/L of air in a sealed cabinet within 5.4 to 10.5, 10.5 to 20.5, and 20.5 to 30.8 min, respectively, at 22°C and high relative humidity (62 to 98%). Treatment with 1.4 mg/L ClO_2 significantly ($P \leq 0.05$) reduced populations of pathogens on all produce. Significant reductions in yeasts and molds on apples and peaches but not on tomatoes and onions were achieved by treatment with ClO_2. With the exceptions of apples, tomatoes, and onions, treatment of produce with gaseous ClO_2 at concentrations causing reductions in pathogens similar to those achieved using 100 to 200 μg/ml chlorine adversely affected the sensory quality during subsequent storage. The effect of a more rapid release of ClO_2 into the atmosphere surrounding produce on inactivation of pathogens and on sensory quality should be further studied.

P3-25 Disinfectant/Sanitizer Management for Food Utensils, Containers and Packages in Korea

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In Korea, by the amendment to Food Sanitation Act in Aug. 2002, disinfectant/sanitizer products have been managed as a part of food additives, which is indirectly transferable to food by being used for the purpose of disinfection or sterilization of utensils, containers and packages for foods. Under the law, the Temporary Standards and Specifications for disinfectant/sanitizer products has been enforced (Amended by KFDA notification No. 2004-87, Nov. 23. 2004). In conformity with this regulation, all disinfectant/sanitizer products should be proved to have bactericidal activity by an accredited method, for instance AOAC, CEN, etc., diluted to manufacturer's recommended in-use concentration. However the methods might show different disinfectant/sanitizer efficacy because of factors influencing a test method for antimicrobial activity of disinfectant/sanitizer. Therefore based on AOAC and CEN disinfectant/sanitizer efficacy testing methods and Microbial Test of Food Code of Korea, we established the National Standardization Test Method to ascertain whether a disinfectant/sanitizer product has bactericidal activity. Up to date, Jan. 2005, KFDA has permitted about 50 types of disinfectant/sanitizer products with this method.

P3-26 DSC Susceptibility of *Penicillium expansum* Spores to Sodium Hypochlorite, Electrolyzed Oxidizing Water, and Chlorine Dioxide on Wood and Plastic Surfaces in the Presence of Surfactants

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Penicillium expansum is a post-harvest apple pathogen that usually produces patulin, a potentially carcinogenic mycotoxin, in apple products contaminated with the organism. Storage bins are logical sanitation targets because they are a reservoir for *P. expansum* spores, which accumulate between seasons and increase the contamination rate of stored apples. In this study, Sodium hypochlorite, Electrolyzed oxidizing (EO) water, and Chlorine dioxide modified with 0.5% V/V of the non-ionic surfactants polyoxyethylene sorbitan monooleate (Tween 80™), polyoxyethylene sorbitan monolaurate (Tween 20™), and sorbitan monolaurate (Span 20™) were compared for their efficacy against spores of *P. expansum* on wooden and plastic bin materials. The surfaces were inoculated and dried, then dipped and gently agitated for 5 min in each solution. Surfaces were rinsed off and surviving spores enumerated by plating on Potato Dextrose Agar medium.Tween 20™ and Span 20™ significantly improved the efficacy of 200 ppm sodium hypochlorite, but Tween 80™ reduced its activity. The activities of EO water or chlorine dioxide on the wood surfaces were not improved by the surfactants. All the treatment solutions were generally more effective on plastic than on wood surfaces. The combination of surfactants with sanitizers on plastic surfaces improved activity more than two-fold, except for Tween 80, which reduced the activity of EO water significantly. Specific non-ionic surfactants could potentially be combined with some chlorine-based sanitizers to control *P. expansum* spores on storage bins. It would be prudent to use plastic rather than wood bins for storage of apples in order to better control *P. expansum*.

P3-27 Monitoring and Survey of Sanitizers and Disinfectants in Food Processing Plants in South Korea

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We investigated commonly used sanitizers and disinfectants (SD) in food manufacturing establishments and analyzed the problems in the usage to suggest the desirable use of SD in food processing plants in South Korea. We surveyed 76 SD products supplied in commercial market and 101 SD products practically used in

37 food processing plants in South Korea from May to July, 2004. The main active ingredients of 76 SD products supplied in market were QAC (quaternary ammonium compounds, 26%), chlorine compounds (22%), acid-alkali compounds (16%), iodophor compounds (12%), oxygen compounds (8%), alcoholic compounds (6%), and surfactants (4%). The main type of SD products was liquid (84%) and food contact sanitizers were more highly used (61%) than non-food contact sanitizers (39%). 101 SD products used in 37 food processing plants were classified as chlorine compounds (33%), alcoholic compounds (26%), acid-alkali compounds (12%), oxygen compounds (11%), surfactants (9%), QAC (2%), and iodophor compounds (1%). The materials to be used were mainly for stainless steel (54%) and hands (20%). As the results of this survey, chlorine and alcoholic compounds were mainly used in food processing plants in South Korea because of the past legal limit. Before Dec. 2003, every SD products sold in South Korea must be approved as a food additive by Food Sanitation Law. In the future, many and various SD containing various kinds of active ingredients should be supplied and used in food processing plants in South Korea.

P3-28 Evaluation of Effectiveness of Sanitizers and Disinfectants in Food Processing Plants in South Korea

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We evaluated both effectiveness of sanitizers and disinfectants (SD) practically used in food processing plants and stability of SD during 2 months of storage to suggest desirable standards for using SD in food processing plants in South Korea. Chlorine and hydrogen peroxide compounds produced 2 to 3 log reductions and effectiveness of these was higher than other SD products. Otherwise, alcohol produced less than 1 log reduction in many cases. Before treatment of SD in food processing plants, total aerobic plate count (APC) were 10^1 to10^5 CFU/cm^2, coliforms were 10^1 to 10^2 CFU/cm^2, and *Escherichia coli* was below 10 CFU/cm^2. APC were reduced to 10^1 to 10^2 CFU/cm^2 after SD treatment. *S. aureus* ATCC 6538 and *E. coli* ATCC 10536 were used to examine the stability of effectiveness of SD during storage. Alcohol compound, QAC (quaternary ammonium compounds) and hydrogen peroxide compound maintained their effectiveness constantly during 2 months at the recommended use concentration. But effectiveness of chlorine compound was reduced from 8 log to 4 log reduction within 2 weeks. Actually, legally approved products which showed 5 log reduction against *S. aureus* and *E. coli* in vitro were not effectively working in the field and the effectiveness was partially reduced during storage. In the future, the practical simulation test must be added to the evaluation method for effectiveness of SD in South Korea and development of a manual containing proper directions for use of SD depending on food type, processing step, object material, etc. is desirable.

P3-29 Microbial Hazard Analysis of Potentially Hazardous Foods and Food Contact Surfaces in School Foodservice Establishments

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A generic HACCP plan has been developed and implemented to assure school foodservice safety since 2000 in Korea. The purpose of the study was to assess microbiological hazards for the verification of the implemented HACCP plan. Samples from high-risk food materials and cooked foods, utensils, employee's hands and gloves, and drinking water from 10 school foodservice operations were collected and total plate counts, coliforms, *Escherichia coli*, *Staphyloccocus aureus* and *Enterobacteriaceae* analyses were performed using 3M Petrifilms. The samples of employees' hands and gloves were taken by glove juice method. Two samples out of twelve cooked foods were in satisfactory conditions and five samples were unsatisfactory for total plate counts test. Total plate counts of thirty-five food materials ranged from 1.0 to 8.2 log CFU/g, while employees' hands were 2.7 to 7.5 log CFU/hand, and *E. coli* was not detected. Surface microbial counts of total plate counts, coliforms, *E. coli* and *S. aureus* indicated that knives, cutting boards, vessels and mixing bowls were under poor hygienic control. *S. aureus* was detected from wiping cloths in two operations ranging from 2.4 to 3.6 log CFU/g. The levels of microbial contamination in drinking water were within the control limit. Results indicate that environmental cross contamination could be risk factor in controlling microbial hazard. In order to improve the microbial safety for school foodservice operations, more effective monitoring procedures and managerial control should be applied at critical control points.

P3-30 Efficacy of Sterilox Hypochlorous Acid to Disinfect Norovirus and Bacteriophage MS2 on Ceramic Tile and Stainless Steel Surfaces

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Noroviruses (NVs), a common cause of human gastroenteritis, persist for long periods on environmental surfaces, from which they can be transmitted to other people. Despite increased awareness of the inanimate surface-mediated spread of NV, effective methods of surface disinfection of the virus have not been docu-

mented. This study determined the activity of a hypochlorous acid (Sterilox) against NV and coliphage MS2 (a surrogate for NV) on ceramic tile (porous) and stainless steel (non-porous) carriers as model food production and preparation surfaces. Square, 1-inch carrier surfaces were inoculated with NV and MS2, which was allowed to dry before applying a thin layer of 188 mg/l, pH 6.3 hypochlorous acid (HOCl) solution for varying contact times at room temperature. Surfaces were treated with 0.1% thiosulfate-3% beef extract to neutralize HOCl and elute viruses. After 1 min. contact with undiluted Sterilox HOCl or 10 min contact with a 10-fold dilution, both viruses were reduced by at least 3 log on both carrier surfaces as detected by RT-PCR. MS2 infectivity reduction was 1 log greater than RT-PCR titer reduction, suggesting that NV infectivity reduction is also likely greater than the observed RT-PCR titer reduction. Both carriers were similar for NV or MS2 disinfection by Sterilox product. These studies indicate that ~200 mg/l HOCl (Sterilox) rapidly disinfects NV on environmental surfaces.

P3-31 DSC Potential for Application of Rapid Methods to Monitor Sanitation of Food Service Areas in Child Care Centers

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Young children are one of the most susceptible groups to foodborne illness. The objective of this study was to evaluate sanitation of food service areas in child care facilities by use of three rapid assessment tools and compare results to those of standard microbiological evaluation methods. The ultimate goal was to determine if one of the rapid assessment tools could possibly be used by centers to monitor sanitation. Samples were taken three times a day, twice per month for eight months in six facilities (n = 288). Three food contact surfaces and a diaper changing area were tested. A 50 square cm area was swabbed, using methods designed to detect ATP (bioluminescence), protein or carbohydrate. In addition, aerobic plate counts (APC) were done for each surface. Mean ATP counts ranged from 2,570 to 5,460 RLU per area for the six centers. Protein and carbohydrate swabs were designed to be presence/absence tests; however, results were scored on a 4 point scale with 1 = no residue and 4 = strong positive. Ranges of mean scores for areas in the six centers were 1.23 to 1.52 and 1.58 to 2.04 for the protein and carbohydrate tests, respectively. Mean log APCs for the areas ranged from 1.32 to 1.96 CFU/area. Correlations were found between the APC and the ATP and protein assays. The findings in this study indicate that rapid tests may be useful for the monitoring of sanitation in food contact surfaces in child care centers. These tests could assist in improving sanitation and preventing cross-contamination.

P3-32 DSC An Investigation into the Efficacy and Acceptability of Two Commercially Available Hand-cleansing Products to Determine Their Potential Suitablity as an Alternative to Traditional Hand-washing Methods

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The hands of food-service workers may become soiled throughout the working day with pathogenic microorganisms and to ensure that cross contamination does not occur it is essential that regular and effective hand cleansing is performed by food operatives. Hand wipes and gels may be employed as an alternative to the traditional hand washing procedure using soap and water. The aim of this investigation was to evaluate two commercially available hand cleansing products, determining their efficacy through microbiological testing and establishing their acceptability through a series of ranked questions. A range of soils were developed and the tests were undertaken under controlled conditions of level of soiling and consistant application and use of the gel and hand wipe. The results obtained show that the wipe was more effective both microbiologically and with regard to odor and residue level, but were percieved by the participants as less effective at cleansing than the gel. These results suggest that both the wipes and gel may have some potential as a substitute for traditional soap and water hand washing, particularly in areas where access to wash hand basins may be limited, for example in mobile food vending outlents. Both the gel and the wipes were effective against a wide range of soiling and showed a statistically significant reduction in the hand microflora of the participants. The results that will be discussed include more detail on the types of soil, the effect against specific microorganisms and the attitudes of the participants to these products as handwashing substitutes.

P3-33 Evaluation of Exterior Sanitary Garments for Meat Plant Employees

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The sanitary characteristics of three different frock materials were assessed for growth and absorption of generic *Escherichia coli* (EC), *E. coli* O157:H7, *Listeria monocytogenes* (LM) and *Salmonella* spp. Precise Systems SP2 disposable frocks made of spun-bond polypropylene and an outer polyethylene layer were tested against traditional cotton/polyester frocks widely used in meat processing facilities. The three materials [polypropylene (spun-bond), polyethylene (poly) and cotton/polyester] were inoculated with 2.5 to 3.0 log CFU/ml of each bacterial culture. Samples were collected at 0, 1, 2, 4, 6 and 8 h. Results indicated that the cotton/polyester material absorbed and maintained 2.6 to 2.8 log CFU/cm^2 generic EC, 3.1 log CFU/cm^2 EC O157:H7,

1.7 to 2.9 log CFU/cm² *Salmonella* spp., and 1.9 to 2.6 log CFU/cm² LM over the six sampling times. The spun-bond material absorbed and maintained 1.9 to 2.4 log CFU/cm² generic EC, 1.8 to 2.8 log CFU/cm² EC O157:H7, 2.2 to 2.7 log CFU/cm² *Salmonella* spp., and 1.0 to 2.1 log CFU/cm² LM. The poly material was not absorbent but maintained 1.0 to 1.9 log CFU/cm² generic EC, 1.0 to 2.1 log CFU/cm² EC O157:H7, 1.1 to 1.9 log CFU/cm² *Salmonella* spp., and 1.0 to 1.2 log CFU/cm² LM. In most cases, the cotton/polyester absorbed and maintained the initial inoculation level throughout the entire 8 h period. The spun-bond was somewhat absorbent but contamination levels were slightly lower than cotton/polyester. The polyethylene was non-absorbent and performed the best.

P3-34 Field Assessment of Sanitation Management Practices in School Foodservice Operations

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Effective and systematic sanitation management programs in school foodservice establishments are mostly required to prevent foodborne-disease outbreaks. The purposes of this study were to assess sanitation management practices implemented under HACCP plan and to provide baseline data for determining critical control points. Sanitary management practices were assessed using a food-safety auditing tool developed by Korea Ministry of Education & Human Resource Development for school foodservice operations. It consisted of seven dimensions and fifty items. The dimensions are sanitation control of facilities and equipment, personal hygiene, food materials management, sanitary management in food product flows, environmental sanitation and maintenance, HACCP system management, and accident prevention and crisis management. Field assessment was conducted at ten school foodservice establishments in Korea during the period of July in 2004. Descriptive statistics were used to analyze data. The average auditing score was 66.9 out of 100. The three highest percentages in compliance were for the dimensions of personal hygiene (80.0%), accident prevention and crisis management (77.8%), and sanitary management in food product flows (70.8%). The lowest three percentages in compliance were for the dimensions of food materials management (50%), HACCP system management (50%), and sanitation control of facilities and equipment (57.5%). Results can provide guidance for improvement of HACCP plan implementation in terms of prerequisite programs as well as HACCP system, including identification of potential barriers and needed resources for school foodservice operations.

P3-35 Evaluation of the DOX System for Hygiene Screening at Real Food Manufacturing Factories

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The DOX system is a rapid and easy bacterial monitoring system applied to an oxygen electrode in order to find out the activity of bacteria with a decreasing concentration of dissolved oxygen. Using the DOX system, we have done the fieldwork of total viable cells and the coliform test at real food manufacturing factories since 2000. By now, over 10,650 kinds of food samples have been tested at 225 factories. The kinds of factories and the number of food samples are meat products 1248, marine products 868, agricultural products 1531, ready to eat foods 3934, bread 371, flour 360, milk and dairy products 318, sauce and spice 458, cake 827, fat and oil 10, and beverage 65. With the results of this fieldwork, meat products, vegetables, marine products and ready-to-eat products achieved a satisfactory level of performance in correlation with conventional method. For example, the coefficient of correlation of meat products is 0.867 to 0.957 (total bacteria) and 0.852 to 0.969 (coliform). Over 85 calibration curves were prepared for each food sample. Some of them were categorized by foodstuff or process of the food. The conventional method has some problems such as taking up a lot of time and being a complicated operation. However, this system is easy to handle and is able to measure many samples at the same time with the fitting calibration curve. The DOX system is available for the first screening test at many food manufacturing factories.

P3-36 Characteristics of Swabbing Solutions and Their Influence upon the Recovery of Microorganisms

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The detection and enumeration of microorganisms remains an important means of assessing the hygienic status of a variety of food processing environments. Conventional hygiene swabbing is widely used, yet it has acknowledged shortcomings and no one universally accepted protocol. An important step in standardization is optimisation. However, in order to improve a system, there must first be a clear understanding as to why that system should fail; yet in general, information is currently lacking with regard to variables that affect the accuracy of the swabbing technique. The effect that eleven different swabbing solutions, used in isolation and in combination with five different swab types, had upon the recoverability of standard bacterial inocula was investigated. When stored at room temperature, swabbing solutions containing buffered peptone water, letheen broth or maximum recovery diluent significantly increased bacterial numbers over time, many within 6 h.

Minimal bacterial growth occurred when cultures were stored at 4°C and/or when nutrient availability was low. Regardless of solution, the number of bacteria recovered from either rayon or cotton swabs was significantly lower than from any of the other swab types, despite the latter absorbing a significantly larger volume of bacterial suspension. This suggests that in terms of sampling efficiency, the ability of a swab to effectively release bacteria into a diluent is more important than its ability to initially absorb or remove the cells from a surface. Depending on the swab type used, some solutions did appear to significantly improve bacterial recovery, yet some also exhibited anti-clumping properties, which, under certain circumstances, could adversely affect sampling efficiency. While an optimum swabbing solution was identified, the implications of these findings will be discussed in relation to the problems associated with microbiological surface sampling methods.

P3-37 Effects of Welding on Attachment of *Listeria monocytogenes* to Stainless Steel

DSC

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Austenitic stainless steels, widely used in food processing, undergo microstructural changes during welding, resulting in 3 distinctive zones: weld, heat affected (HAZ), and parent metal. This research was conducted to determine attachment of *Listeria monocytogenes* to weld vs HAZ vs parent metal, and the effect of corrosion of these 3 zones on bacterial attachment. All experiments were done with tungsten inert gas welding of type 304 stainless steel. Welding treatments (4) were big or small beads with high or low heat inputs. After welding, all surfaces were polished to an equivalent surface finish. A 10 µl droplet of *L. monocytogenes* (10^7 CFU/ml) in BHI was placed on tested surfaces (coupons), which were held in saturated humidity for 3 h at 23°C, then washed 3 times with 200 ml sterile water for 2 min at 100 rpm. After washing, coupons were prepared for scanning electron microscopy (SEM). Using SEM, attachment of *L. monocytogenes* was determined by counting cells remaining on each test surface. For each surface treatment, 6 coupons were tested, and bacteria were counted in 10 fields of view/coupon. There were no differences ($P > 0.05$) in numbers of bacteria on the 3 surface zones. However, numbers of bacteria on the 3 zones of welds exposed to corrosive media were higher ($P < 0.05$) than those on the corresponding zones of non-corroded surfaces. Among corroded surfaces, bacterial counts on parent metal were lower ($P < 0.05$) than those on HAZ and weld zones. Results indicate that polished stainless steel welds do not lead to differences in bacterial attachment; however, corrosion of the different weld zones leads to differential attachment of *L. monocytogenes* to stainless steel.

P3-38 Effect of Nutrients on the Antimicrobial Activity of Copper and Brass against *Listeria monocytogenes*

DSC

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Our study examined the effect of different nutrient content in modified Welshimer's broth (MWB) on the antimicrobial effects of copper and brass against *Listeria monocytogenes* (Lm). Glucose in MWB (10 g) was evaluated at concentrations of 1.0, 10, and 20 g/L. The glucose in MWB was replaced with cellobiose, fructose, or mannose, at a concentration of 10 g/L. A decimally diluted overnight culture of Lm (10 µl) was inoculated into MWB (10 ml), poured over the surface of copper, brass or Petri dishes (control) and incubated at 37°C. Bacterial counts were determined at 0, 24, or 48 h. During this study the Lm control counts ranged from 5.5 to 6.8 log CFU/ml. Our results showed that if the concentration of glucose in MWB (10 g) was reduced (1 g) or increased (20 g) the antimicrobial activity of the copper and brass against Lm was significantly reduced by 1.2 and 1.8 log CFU/g, respectively. At 24 h Lm grown on copper had bacterial counts of 2.4 log CFU/ml in MWB with glucose, 2.9 log CFU/ml in MWB with fructose, 4.1 log CFU/ml in MWB with mannose and 4.3 log CFU/ml in MWB with cellobiose, whereas by 48 h all copper treatments were at non-detectable levels. Conversely, by 48 h Lm grown on brass bacterial counts were at non-detectable levels in MWB with glucose, 2.1 log CFU/ml in MWB with mannose, 2.6 log CFU/ml in MWB with cellobiose and 2.9 log CFU/ml in MWB with fructose. Our study has shown that the availability of nutrients can affect the antimicrobial activity of copper ions against *Listeria monocytogenes*.

P3-39 Biofilm Formation by *Salmonella* spp. on Cantaloupe Surfaces

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In recent years *Salmonella* spp. have been implicated in numerous outbreaks linked to fresh produce, most notably, cantaloupe melons. Previous research in our laboratory has documented the inability of a variety of sanitizing rinses and other treatments to inactivate *Salmonella* inoculated onto cantaloupes. In addition, the efficacy of sanitizers decreased significantly when the organism was allowed to reside on the melon surface for more than 24 h. We speculated that increased contact time allowed for the formation of a bacterial biofilm prior to sanitation. The entrapment of cells of *Salmonella* within a biofilm is likely responsible for enhanced sanitizer resistance.Therefore, the goal of our research was to demonstrate that cells of *Salmonella* form

biofilms on cantaloupe surfaces. Two outbreak-related strains of *Salmonella* were utilized in our study. Ten microliters of bacteria were spot-inoculated onto melon rinds in pre-marked areas, and melons were held at either 10 or 22°C. Biofilm formation was monitored using scanning electron microscopy (SEM) on excised portions of the melon rind at 2, 24, 48, and 144 h post-inoculation. Micrographs indicated that biofilm formation occurred rapidly following introduction of cells onto the melon surface. Fibrillar material was visible after just two h of inoculation, and cells were embedded in extracellular polymeric material within 24 h of storage at either temperature. These results indicate that *Salmonella* spp. are capable of rapidly forming biofilms on cantaloupe tissue and that biofilm formation could be the reason for the increased recalcitrance of attached bacteria to aqueous sanitizers.

P3-40 In Situ Biofilms Associated with Baker's Yeast Processing Equipment

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This study assessed equipment surfaces associated with the commercial production of Baker's yeast for the presence of microbial biofilms. During three replicate surveys of a yeast manufacturing plant, eight sterile stainless steel 'mock' surfaces (4 cm^2) were aseptically attached to each of five equipment surfaces. Duplicate surfaces were sampled from each site after 7, 14, 21 and 28 days of production. One of these surfaces was prepared for bacteriological analysis, the other for scanning electron microscopy. After 7 days, all surfaces showed attachment of *Enterococcus* and aerobic bacteria (2 to 3 log CFU/cm^2) and *Saccharomyces cerevisiae* (4 to 5 log CFU/cm^2), with *Escherichia coli* and coliform counts below the lower detection limit (< 0.7 log CFU/cm^2). Counts of attached microorganisms on processing equipment which was easily accessible for cleaning remained fairly constant over 28 days, compared to processing equipment which was less accessible for cleaning, showing progressive increases in coliform counts after 14 days (2 log CFU/cm^2) and *Enterococcus* or aerobic bacterial counts after 21 days (3 to 4 log CFU/cm^2). During this study, the number of attached yeast cells did not exceed 5 log CFU/cm^2. Scanning electron micrographs confirmed the presence of attached rod- and coccoid-shaped bacteria, and early bacterial biofilm formation incorporating the production of extracellular polymeric substances. This study highlighted the need for efficient cleaning practices in inaccessible areas which could otherwise result in the build-up of yeast product harboring biofilms of spoilage or potentially pathogenic bacteria.

P3-41 DSC Biofilm Formation by *Enterococcus faecalis* and *Saccharomyces cerevisiae* on Stainless Steel and Polyurethane Surfaces

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This in vitro study investigated biofilm formation by *Enterococcus* (E.) *faecalis* ATCC 29212 and *Saccharomyces* (S.) *cerevisiae* on stainless steel (304 L) and/or polyurethane surfaces. Test surfaces (4 cm^2) were sampled from inoculated flasks containing Tryptone Soya Broth (30°C, 100 rpm) after 2, 4, 8, 24 and 48 h; attached cells were dislodged, plated and counted by standard methods. Further surfaces were prepared for Scanning Electron Microscopy (SEM) by standard methods or stained with the BacLight Bacterial Viability KitTM for Confocal Scanning Laser Microscopy (CSLM). Scanning electron micrographs and counts of single species *E. faecalis* biofilms showed cell attachment to both surface types within 2 h, microcolony formation within 8 h and complex biofilm formation within 24 to 48 h. Similarly counts showed *S. cerevisiae* cell attachment to both surface types within 2 h, reaching a maximum of ca 4 log after 48 h. Mature single species *S. cerevisiae* biofilms were not observed on both surface types by SEM. Higher counts of *S. cerevisiae* on polyurethane, compared to stainless steel surfaces, suggested that different surface types influenced *S. cerevisiae* attachment. Counts of *E. faecalis* and *S. cerevisiae* in binary species biofilms were generally not significantly different ($P > 0.01$) from counts of the corresponding single species biofilms on both surface types. SEM images confirmed the presence of binary species biofilms on both surface types and CSLM revealed that both species were viable. This is the first in vitro study showing mixed biofilm formation of *E. faecalis* and *S. cerevisiae*. Practically, biofilm formation of *Enterococcus* spp. on yeast processing equipment surfaces may create in situ contamination reservoirs in yeast processing plants.

P3-42 Gage R&R Study Comparison of Variability in Two Measurement Systems, 3M™ Petrifilm™ Plate Readers and Trained Technicians, to Enumerate Counts below Plate Count Ranges

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Accurate enumeration and reporting of microbial results is important in the food industry. The 3M™ Petrifilm™ Plate Reader is a compact computerized image analyzer designed and validated to accurately read and report microbial results within the counting ranges of three validated methods: 3M™ Petrifilm™ Aerobic, Coliform and E. coli/Coliform Count Plate methods. Increased demands for safe food have led many food processors to set product specifications at sensitivities below the validated counting ranges of approved methods. In this study, Petrifilm Coliform and E. coli/Coliform Count plates were inoculated with pure strains of coliform organisms, including *Escherichia coli*, at levels below the validated counting ranges of these

methods. Three Petrifilm Plate Readers and three trained technicians counted four replicates of 33 Petrifilm Coliform plates and four replicates of 33 Petrifilm E. coli/Coliform plates. A Gage R&R (Repeatability and Reproducibility) study was used to demonstrate variation due to each of the measurement systems used: enumeration of Petrifilm Plates by humans, and enumeration of Petrifilm Plates by the Petrifilm Plate Reader. Analysis of data following logarithmic transformation showed that counts enumerated by the Petrifilm Plate Reader were not statistically different ($P > 0.05$) from counts enumerated by the trained technicians. The difference between the percent of variability due to the measurement systems for enumeration of Petrifilm Coliform and E. coli/Coliform plates was no more than 2.03%. Results suggest enumeration of low levels of colonies on Petrifilm Coliform and E. coli/Coliform Count plates using the Petrifilm Plate Reader show similar variability to enumeration by trained technicians.

P3-43 DSC **Attachment and Biofilm Forming Abilities of Persistent and Non-persistent *Listeria monocytogenes* Isolates**

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Certain strains of *Listeria monocytogenes* were repeatedly isolated from a specific chicken further processing plant. The twenty seven isolates were obtained from environmental sites and raw product, of which 23 were persistent (representing 5 genotypes) and 4 were non-persistent (representing 4 genotypes). The objective of the present investigation was to study whether the persistent *Listeria monocytogenes* isolates showed different attachment and biofilm forming ability than the non-persistent isolates. Attachment and biofilm forming ability was determined using hydrophilic and hydrophobic microtiter plates. Cell attachment studies were carried out in phosphate buffer saline (pH: 7.2) and calcium chloride enriched phosphate buffer saline (2.2 mmol Ca, pH: 6.8) at 25°C for 2 h. Biofilm formation was determined using tryptic soy broth (TSB) and a 1:10 dilution of tryptic soy broth at 25°C for 24 h. The *Listeria* isolates showed similar attachment to the hydrophilic and hydrophobic microtiter plates when using both attachment media ($P < 0.05$). However, two persistent *Listeria* genotypes exhibited significantly greater ($P < 0.05$) biofilm formation than the non-persistent genotypes in both normal and low nutrient media and on hydrophobic and hydrophilic surfaces. Overall, maximum biofilm formation was observed when using 1:10 diluted TSB as the medium and a hydrophobic surface. The results indicate that higher attachment and biofilm formation may be associated with the cell persistence and that significant phenotypic variation occurs within persistent genotypes.

P3-44 DSC **The Effect of Select Lactic Acid Bacteria on *Listeria monocytogenes* Scott A Biofilm Formation on Stainless Steel as Detected by Impedance Measurement**

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The poultry environment has been shown to be a major source of contamination by *Listeria monocytogenes*. It often takes at least 24 h for the microbial status of a surface to be determined. Studies were undertaken to devise a more rapid method of detecting surface microbial status. Impedance is a measurement of low molecular charged particles that are the result of the breakdown of peptides, carbohydrates and protein. Impedance was used to detect the time (h) it takes to detect microbial growth, which is an indicator of microbial contamination. *Lactobacillus acidophilus* and *L. plantarum* (LAB) were used in conjunction with *L. monocytogenes* Scott A to determine if the presence of LAB has any effect on the growth of *L. monocytogenes*. All surfaces were inoculated and incubated at 4 or 21°C with the respective organisms and was detected by impedance measurement (< 10 h). The detection times for biofilms grown at 4° for *L. monocytogenes*, *L. monocytognes* and *L. acidophilus*, and *L. monocytogenes* and *L. plantarum* were 7.2, 8.2 and 6.4 h respectively. The detection times for biofilms grown at 21° for *L. monocytogenes*, and *L. acidophilus*, and *L. monocytogenes* and *L. plantarum* were 7.7, 4.4 and 7.3 (h), respectively. Detection by impedance in < 11 h is an indication that a surface is highly contaminated. The use of impedance as a rapid method by swab samples could be used by food processors for the rapid detection of the microbial status of processing equipment, thus eliminating the need for plate count methods, which take at least 24 h.

P3-45 **Comparison of Neutralizing Media for Recovery of *Listeria monocytogenes* from Environmental Swabs after Exposure to Sanitizers**

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Environmental monitoring is recognized as a key element in controlling *Listeria monocytogenes* in the food processing plant. Swabs taken from production surfaces should be treated to neutralize sanitizers commonly used in the plant so that cell viability is preserved. Neutralizers may be incorporated into transport media, which are used for shipping environmental swabs to the laboratory. Our objective was to compare different neutralizing media for recovery of *L. monocytogenes* after exposure to sanitizer. Cells were added to either hypochlorite or quaternary ammonium compound sanitizer, and after a 1-min exposure, transferred

to sponges that had been pre-soaked in one of the following neutralizers: buffered peptone water (BPW), DE Neutralizing Broth (DE), Neutralizing Buffer (NB), Letheen Broth (LB) or a proprietary formulation (PF). After the sponges had been stored at 4°C or 25°C for up to 72 h, the cells were released by stomaching and then plated. The media showed similar recoveries when the sponges were kept at 4°C; however, significant differences were observed at 25°C. Compared to initial counts, NB showed a decrease of approximately 1 log; BPW, DE and LB supported increases of up to 3 logs. PF allowed nearly 100% recovery. For all media, recoveries were stable between 24 h and 72 h of storage. The data indicate that neutralizing media may vary in recovery efficiency. For pathogen monitoring or detection, it may be appropriate to choose a medium that provides maximum recovery. For quantitative risk assessments, a medium that preserves environmental population levels may be most appropriate.

P3-46 DSC MPN of *Listeria monocytogenes* in Luncheon Meats by Use of Conventional and Modified Dilutions in UVM Broth

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The analysis of larger sample sizes improves recovery by increasing the chance of detection of *Listeria monocytogenes* in ready-to-eat meats compared to the typical 25 g samples. However, the expense and handling of the volume of enrichment broth involved with conventional dilutions poses problems when a large number of samples are being analyzed. The objective of our study was to compare the conventional method to a modified initial dilution in UVM enrichment broth in order to determine the effect on most probable number (MPN) of *L. monocytogenes* from ham. Ham samples were spiked with an inoculum of 1.0, 3.0, 4.0, and 10.0 CFU/125 g. Each 125 g sample was mixed with UVM enrichment broth using conventional (1:10) and modified (1:4 and 1:5) dilutions and then were stomached and blended. UVM was incubated at 30°C for 24 h and 0.1 ml was transferred to Fraser broth and incubated for 24 h at 35°C. The MPN was determined according to the USDA method. Samples were also screened using the Neogen GeneQuence™ DNA probe. Blackened Fraser broth was streaked onto ALOA™ chromogenic agar and MOX plates and incubated for 48 h at 37°C. No significant differences were detected in the MPN of the samples with use of either conventional or modified dilutions. Typical colonies of *L. monocytogenes* were recovered on all ALOA™ and MOX plates. Results from this study showed that modified dilutions in UVM enrichment broth did not significantly impact recovery of low levels of *L. monocytogenes*.

P3-47 Evaluation of the Warnex™ *Campylobacter* Real-time PCR Assay

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The objective was to evaluate the performance of a new rapid PCR test assay for the detection of *Campylobacter jejuni*, *C. coli* and *C. lari* in foods. A screening of naturally contaminated products as well as artificial inoculation experiments were included in the study. The test assay was compared to the ELISA-based Diffchamb Transia rapid detection assay. A total of 52 products were screened for natural contamination, including 37 samples of poultry and poultry products. The test assay detected 11 contaminated products whereas the Transia assay obtained 9 positive results. Artificial inoculation studies were conducted on chicken breasts and beef cubes. For each food matrix, two large lots of foods were prepared and inoculated at low (1 to 10 CFU/25 g) and high (10 to 50 CFU/25 g) levels. Following a 2-day refrigeration period, the inoculated food lots were divided into 20 individual 25-g portions and tested with both assays. For chicken breasts, the test assay detected 6 and 17 positive samples at the low and high levels, respectively. The Transia assay obtained 1 and 18 positive samples at the same levels. For beef cubes, the test assay found 6 and 20 positive samples at the low and high spiking levels, whereas 4 and 19 samples were found positive with the Transia assay. These results indicate that the test assy kit for *Campylobacter* is sensitive, specific and reliable for the detection of *C. jejuni*, *C. coli* and *C. lari* in raw meats when compared to current methods.

P3-48 Evaluation of 3M™ Petrifilm™ Staph Express Count System for Enumerating Coagulase Positive *Staphylococcus*

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Classical methodologies used for enumerating *Staphylococcus* in food are time consuming, taking up to 78 h. 3M™ Petrifilm™ Staph Express Count System (STX) is a rapid test that has recently been commercialized in order to shorten the time. When using the STX, the population of *S. aureus* in food samples can be determined in 22 h because the identification of *S. aureus* is not based on coagulase production. The objective of this study was to evaluate the efficiency of STX on enumerating coagulase positive *Staphylococcus* in naturally contaminated foods samples. 128 food samples were first screened for *Staphylococcus* by plating dilutions onto Baird Parker (BP) agar. Samples showing characteristic colonies were re-sampled and simultaneously plated

onto STX plate, according to manufacturer's instructions, and onto BP according to FDA-BAM procedure. The results were compared, submitted to variance analysis ($P > 0.05$) (Minitab™ Release14 Statistical Software) and linear regression (Log BP = -0,1545 + 1,00 Log STX). Amongst the 77 samples with characteristic colonies in the screening evaluation, 62 (80%) showed similar results by both methods ($P > 0.05$) indicating their equivalence (0.951). The number of coagulase positive *Staphylococcus* was higher in STX than BP for 9 (11.7%) samples and was higher in BP than STX for 6 (7.8%) samples. A higher proportion of coagulase negative colonies was observed amongst typical colonies isolated from BP (52%) than from STX (13%). No atypical colony was coagulase positive in STX. Therefore, STX may be an alternative to the FDA-BAM protocol for enumeration of coagulase positive *Staphylococcus*.

P3-49 Development of a Bioluminescent ATP Assay of Somatic Cells and Total Bacterial Contamination (TBC) in Milk and Other Food Samples

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Various methods are available for the specific identification of bacteria, virus and toxins. Nevertheless, there remains a need for an accurate and sensitive method that provides results within min. Adenosine Triphosphate (ATP) bioluminescence is widely used in rapid methods for assessment of cleanliness in food processing plants. Most methods, however, neither differentiate between bacterial and non-bacterial ATP, nor correlate with standard culture methods. Residual chemicals as well as other substances such as fat may cause a significant variation in the bioluminescence signal, thereby underestimating or overstating the actual ATP signal and consequently affecting the sample disposition. A filtration-based bioluminescence technique, which is able to separate bacteria from non-bacterial sources and remove interfering substances, and is thus able to detect bacterial ATP, has been developed. The addition of a unique enzyme – detergent (BPN) allowed for the processing of milk samples for total bacterial counts (TBC) as well as somatic cells in raw milk. A detection limit of 10^3 CFU/ml within 15 min was demonstrated for TBC in milk. With the addition of a different enzyme – detergent (BCN) and with a 35-min procedure, detection of 10^4 CFU/ml was seen for ground meat samples. Somatic cell detection limits were less than 10^5. Correlation between culture and ATP measurement has been demonstrated to be approximately 96%. The system is hand-held and the reagents are sufficiently stable for field analysis.

P3-50 DSC Performance of Media for Recovering Stressed *Enterobacter sakazakii* Using Spiral Plating and Ecometric Techniques

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Cells of two strains of *Enterobacter sakazakii* isolated from powdered infant formula (Pif) and two strains from infected neonates were exposed to five stress conditions: heat (55°C for 5 min), freezing (-20°C for 24 h, thawed, frozen again at -20°C for 2 h, thawed), acidic pH (3.55), alkaline pH (11.25), and desiccation in Pif (a_w 0.25, 25°C for 30 days). Control and stressed cells were spiral plated on tryptic soy agar supplemented with 0.1% pyruvate (TSAP), two new fluorogenic agars developed by Leuschner, Baird, Donald, and Cox (LBDC) and Oh and Kang (OK), fecal coliform agar (FC), Druggan-Forsythe-Iversen medium (DFI), violet red bile glucose agar (VRBG), and *Enterobacteriaceae* enrichment agar (EE). With the exception of desiccation-stressed cells, suspensions were also plated on these media and on R & F *Enterobacter sakazakii* chromogenic plating medium (RF), using the ecometric technique. The general order of performance of media for recovering control and heat-, freeze-, acid-, and alkaline-stressed cells by spiral plating was TSAP > LBDC > FC > OK > VRBG > DFI > EE; the order for recovering desiccated cells was TSAP > LBDC > FC > OK > DFI > VRBG > EE. With the ecometric technique, the order was TSAP > LBDC > FC > RF > VRBG > OK > EE > DFI. Results indicate that differential, selective media vary greatly in supporting resuscitation and colony formation by stressed cells of *E. sakazakii*. The general order of performance of media was similar using spiral plating and ecometric techniques.

P3-51 D-radiation Values of *Salmonella* DT104 Inoculated in Ground Beef and Pork and on Radish Sprouts

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Multi-resistant *Salmonella* Typhimurium DT104 infections have been associated with the consumption of ground beef. Since irradiation in the United States is an approved intervention for removal of bacteria from ground beef, the rational destruct values were determined for ground beef (83% lean) and the study also included determining the D-value with ground pork (90% lean) and radish sprouts. Prior to inoculation with a mixture of six *Salmonella* DT 104 strains, all samples were irradiated to remove the background microflora. The D-radiation values were determined by use of a gamma source at 4°C. The D-values for the *Salmonella* DT104 in the ground beef and pork and on the radish sprouts were 0.51, 0.56 and 0.55 kGy, respectively. The

D-radiation values for the DT 104 strains are within the reported range for irradiation destruction of other *Salmonella*-contaminated raw meat and produce products.

P3-52 Predictive Models for the Growth of *Bacillus cereus* and *Staphylococcus aureus* in Ready-to-Eat Kimbab in Korea

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This study determined the predictive model of the kinetic growth of *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus* in kimbab, which is a traditional ready-to-eat rice rolled in laver in Korea at different temperature. Results showed *B. cereus* and *S. aureus* in kimbab did not grow at below 60°C and grew slowly up to 8 to 100°C, but grew fast above these temperatures. The growth data was well fitted with Gompertz equation, and specific growth rate (SGR) and lag time (LT) were obtained using the GraphPad prism program; the R2 value obtained from the primary Gompertz model was 0.98. From the SGR and LT value using square root equation, a new secondary model was constructed using SAS program. The values of LT and SGR on the growth of *B. cereus* and *S. aureus* in kimbab were obtained from the developed secondary equation. The validation between experimental values and induced values from the new secondary model was conducted using statistical indicators, such as mean square error (MSE), bias factor (BF) and accuracy factor (AF). Results showed that MSE, BF and AF of SGR for *S. aureus* were 0.001, 0.995 and 1.06, whereas those of LT were 0.03, 0.96, and 1.12, the experimental and developed values were highly similar. Similar results was observed for *B. cereus*. These results indicate that the developed secondary model could be used to predict the growth of *B. cereus* and *S. aureus* in kimbab with enviromental temperature changes.

P3-53 Growth of *Clostridium perfringens* during Cooling of Bulk Pie Filling

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Some pie manufacturers pre-cook fillings in large volumes, allowing them to cool for many hours before filling pastry cases prior to final baking. This study was undertaken to evaluate the predicted growth of *Clostridium perfringens* given time and temperature profiles of cooling bulk pie filling from five bakeries located throughout New Zealand. The purpose was to provide information to the New Zealand Baking Industry to formulate guidelines for cooling cooked bulk pie fillings. Experimental work was carried out in two parts: (1) microbiological testing to assess the numbers of *C. perfringens* in cooked and cooled bulk pie fillings and to compare these with the data for the same filling after final pie baking and cooling; and (2) monitoring the time and temperature of cooling to estimate the potential growth of *C. perfringens* using a pathogen-modelling program. Current cooling methods can allow *C. perfringens* to grow (three samples tested prior to baking showed *C. perfringens* at 76,000, 9,200 and 24,000 CFU/g). However, no *C. perfringens* was detected after final baking and cooling. Results of temperature monitoring showed that only one bakery achieved the stringent cooling standards set by FDA. Given the findings of this study, it is recommended that cooling of bulk pie filling should be an effectively managed control point (i.e., cool to 54.5°C, store under continuous refrigeration until used, ensuring product temperature reaches 10°C within 24 h) and the critical control point is the final baking of the pie (must achieve core temperature of 71 to 82°C).

P3-54 Manage Risks of *Clostridium botulinum* in Process Cheese Using Machine Learning

DSC

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Current modeling strategies in predictive microbiology apply statistical methods to determine model parameters. Few studies have investigated machine learning (a computational approach) for making predictions. In machine learning, computational strategies are applied by computer to detect patterns in experimental data. These patterns are used for predictions.Our goal was to develop novel methods to characterize the responses of microorganisms to environmental conditions. Specifically, we investigated toxin production of *Clostridium botulinum* in response to formulation adjustment in process cheese, by use of machine learning. Machine learning methods developed here included Bayesian networks, decision tree and artificial neural networks. The formulation factors considered included moisture level, pH, cheese type, water activity and others. These approaches were compared with statistical models built by logistic regression, and validated by experimental data. The results show that the Bayesian network was able to estimate probabilities of toxin production for risk analysis. The decision tree effectively evaluated antibotulinal properties of formulation factors. The artificial neural network provided necessary accuracy to separate potential hazardous formulations from safe formulations. Graphical model interpretations were generated. The coordination of these computational strategies provided toxin production predictions quickly and economically. The statistical logistic regression models failed when large number of formulation factors and their combinations were considered. These results suggest that computational methods can assist manufacturers in developing safe process cheese formulations. Machine learning can separate safe environmental conditions from potentially hazardous

conditions and predict microorganism responses. It can be a new tool in predictive microbiology to assist in food microbial safety and quality control.

P3-55 Identification of Yeasts by Use of Automated Ribosomal Intergenic Spacer Analysis

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Identification of yeasts has historically been achieved using biochemical methods. These are still popular, but can be supplemented by ribosomal sequencing: this is more time consuming but useful when there is doubt over identification, or where absolute confirmation is required. Here we describe a quick yet accurate method that uses DNA fragment analysis to identify yeasts at genus or species level. Intergenic fragment analysis on standard agarose gels has been used to discriminate between yeast strains for some years, but we propose the use of this technique as an identification tool. DNA from yeasts of known identity isolated from food factories or products (soft drink/yogurt) was amplified using PCR primers spanning the variable region between the 5.8S and 28S ribosomal genes, i.e., the ITS2. After restriction enzyme digestion with HaeIII, a fluorescent reporter on the reverse primer then allowed the fragment lengths to be analysed with a Beckman Coulter CEQ8000. The resulting fragment lengths vary because of the variability of the region and were compared to a predictive database of fragment sizes, generated via sequences downloaded from the NCBI website. Proof of principle has been achieved and of the 70 yeasts processed, the majority gave fragment sizes very similar to those predicted. Preliminary indications are that as the database expands this technique will provide a useful identification tool for yeasts relevant to the food industry.

P3-56 Effect of the Activation Temperature of Zeolite and Bentonite for the Adsorption of Fumonisin B1

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Mycotoxins are toxic chemicals produced by molds growing on food grains and feed. They are a worldwide problem. Fusaria are common field fungi that infect most cereal grains. They produce a number of mycotoxins which are known to be toxic. Fumonisins are a group of naturally occurring mycotoxins produced by Fusarium verticilloides and others and occur worldwide in corn. They cause toxic effects in animals (hepatotoxicity in rats, leucoencephalomalacia in equines and pulmonary edema in swine). Several detoxification techniques have been used such as nixtamalization, amoniation, and reductive sugars, and recently mycotoxin binders have been developed to render them unavailable for absorption by animals. For this reason, in our study natural zeolite and bentonite were heat activated (25, 100, 200, 225, 250, 275, 300, 500, 650, and 700°C). Acetonitrile-water (1:1) absortion rate was determined. Particle size was evaluated by using a Coulter LS230 system. A 10 µg fumonisin B1 (FB1) solution was passed through the adsorbents and the percentage of adsorbed FB1 was quantified according to the HPLC procedure described by Sydenham et al (1996). Temperature affected the absortion rate for both adsorbents and the particle size was affected only for zeolite. FB1 adsorption was not affected by heat treatment for both adsorbents. FB1 trapped by zeolite and bentonite were higher than 90% in all the treatments. These findings indicate that natural zeolite and bentonites can probably be used to reduce fumonisin B1 in animal feed.

P3-57 Determination of Residual Pesticides in Produce According to Distribution Channel and Storage

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The appearance of residual pesticide in the perilla leaf, hot pepper, and leek, which are generally eaten raw with minimal processing in Korean menu, were monitored in conventional- and modernized markets during Spring season in 2004 and their losses following storage at different temperatures (4 and 15°C) were analyzed. The pesticides which have been detected and/or exceeded MRL (minimum residual level) most often from the subject samples in recent years in Korea, such as endosulfan, fenitrothion, cypermetrin, dichlofluanid, chlorpyrifos, and procymidon (perilla leaf); chlorpyrifos, endosulfan, procymidone, and dichlofluanid (hot pepper); and diazinon and endosulfan (leek), were subjected to analysis. In all samples, the residues of pesticides were below the MRL as established by the Korean government. No significant differences in the contents of residual pesticides were noted according to the market type. The residual pesticides in the leeks disappeared rather easily during storage for seven days showing 15.8~40.6% (endosulfan α+ß, 15 and 4°C) to 19.8~33.1% (chlorpyrifos, 15 and 4°C) of the residual rate by indicating the faster disappearance of the chemicals at higher temperature. However, even the same pesticide, endosulfan α+ß, showed very wide range of residual rates from 15.8% and (leek, 15°C) to 100.0% (perilla leaf, 15°C) depending upon the physiological condition of the produces. The most destruction of the pesticide took place during initial three days of the storage regardless of temperatures, while the changes have stagnated thereafter.

P4-01 Prevalence of Foodborne Pathogens in Human Feces, Retail Meat, and Animal Intestines in Mexico

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A network for foodborne pathogens was established in Mexico in collaboration with the U.S. FDA Center for Veterinary Medicine in 2002. Human feces, retail meat and food-animal intestines were obtained through active statewide surveillance. Laboratory methods for isolation, identification, antimicrobial susceptibility testing, and serotyping of *Salmonella* were performed according to internationally accepted methods. During 2004, the prevalence of *Salmonella* in chicken, swine and bovine intestine and retail meat samples was 21.5% and 16.8%; 44.4% and 28.2%; and 19.3% and 28.2%, respectively. For *Campylobacter*, recovery from these sources was 87% and 43.8%; 67.3% and 6.8%; and 22.8% and 1.6%, respectively. *Salmonella* and *Campylobacter* were recovered in 9.6% and 4.1%, respectively, of humans with diarrhea, and in 3.8% and 3.2%, respectively, of asymptomatic humans. Ciprofloxacin-resistant *Escherichia coli* was highest in chicken (39.2%), and lowest in bovine intestines (3.5%); prevalence in humans was 20%. In *Salmonella* isolates, ceftriaxone resistance was highest in humans (15.9%), followed by swine (5.2%), chicken (3.8%) and bovines (2.8%). Nalidixic acid resistance in chicken (29.3%), swine (21.7%), bovines (16.5%) and humans (15.9%) has increased, and ciprofloxacin resistance emerged in chicken (0.6%) and swine (1.6%). *Campylobacter jejuni* presented high resistance to ciprofloxacin in humans (43.2%) and chicken (94.3%). Conclusions: There is a high prevalence of foodborne pathogens in food animals and retail meats from Mexico. In humans, both symptomatic and asymptomatic infections are common. Antimicrobial resistance to clinically valuable antibiotics is progressively increasing. Urgent measures are needed to stop this trend.

P4-02 Rapid Detection of *Salmonella* Enteritidis by Quantum Dot Biolabeling Coupled with Immunomagnetic Separation

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Colloidal semiconductor CdSe-ZnS core-shell nanocrystal quantum dots (Qdot) are luminescent inorganic fluorophores that have the potential to overcome some of the functional limitations encountered by organic dyes in fluorescence labeling application. *Salmonella* Enteritidis emerged as a major cause of human salmonellosis worldwide from the 1980s through 1990s and remains an important serovar. A rapid, specific, and sensitive method for the detection of *Salmonella* Enteritidis was developed using Qdot as a fluorescence marker coupled with immunomagnetic separation. Magnetic beads coated with anti-*Salmonella* Enteritidis antibodies were employed to selectively capture the target bacteria and biotin-conjugated anti-*Salmonella* antibodies were added to form sandwich immunocomplexes. After magnetic separation, the immunocomplexes were labeled with Qdot via biotin-streptavidin conjugation. This was followed by a fluorescence measurement using a RF-5301 PC fluorometer. The detection limit in Qdot method was 10^3 CFU/ml for the cell concentration of *Salmonella* Enteritidis, while the FITC-based method required over 10^5 CFU/ml. The total detection time was within 2 h. In addition to the nanotechnology developments, the results could play a role in the new rapid detection of various pathogenic bacteria.

P4-03 Methods for the Detection of *Salmonella* spp. in Animal Feed Ingredients and Finished Feed

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Animal feed contamination with human pathogens such as *Salmonella* has been recognized as a contributing factor in colonization of animals and subsequent carcass contamination during slaughter. Attempts to reduce and/or eliminate microbial safety hazards from animal foods should include interventions at both pre- and post-harvest stages of food production. Use of rapid, accurate methods for detection of pathogens is required to minimize colonization of animals with foodborne pathogens such as *Salmonella*. Traditional FDA-BAM method was compared with an immunomagnetic separation (IMS) method for detection of *Salmonella* spp. in animal feed ingredients and finished feed. In addition, a lateral flow enzyme immunoassay (EIA, Neogen Reveal), along with isolation on XLT4 agar from pre-enrichment broth, was used for *Salmonella* spp. screening. A total of 3875 samples of feed ingredients and finished feed were tested over 2 years from various Midwestern feed mills. Samples included whole grains, grain meal, meat/bone meal and feed collected at different stages of processing. *Salmonella* spp. prevalence in animal feed and feed ingredients was 3.7 and 2.3%, using IMS and BAM methods, respectively. *Salmonella* prevalence was greatest in meat and bone meal (32% positive). Use of EIA methods may not be practical for screening animal protein based feed ingredients as they may contain non-viable *Salmonella* spp. cells, resulting in greater false positive test results. Overall prevalence of *Salmonella* spp. in finished animal feed was 3.2%, indicating that either their levels are reduced due to dilution or that animal feed processing steps were sufficient to destroy the pathogen.

P4-04 Dissemination of *Salmonella* sp. in the Swine Production Chain

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Brazil is the fourth largest pork meat producer in the world, with 2.8 thousand ton produced in 2003, and industrial pork meat production is an economically important activity in Southern Brazil. Intensively rearing swine units are divided in 2 areas: piglet production unit (PPU) where 21-day weaners stay up to 40 days, and growing and finishing unit (GFU) where animals stay until sent to the slaughterhouse (approx. 14 wks). The objective of this trial was to determine the possible *Salmonella* sources in industrial pork meat production. Two lots of animals were followed for approx. 155 days from their transfer to the PPU to the slaughterhouse. Twenty nine sampling points (environment, feces, feed, water, equipments, carcasses) were monitored using the Assurance Gold *Salmonella* EIA (BioControl) and the conventional cultural procedure. *Salmonella* was isolated from feces, feed leftovers, floor of the breeding pens, and from drinking water at the production site. In the abattoir, *Salmonella* was isolated from feces collected at the holding pens, mesenteric lymphonodi, pork jowl, carcasses after evisceration, residual carcass washing water, and from the inspection table. The serotypes most frequently found were Mbandaka and Derby (12 and 11 isolates, respectively), followed by Typhimurium (7), Agona (3), Panama (2) and Give, Worthington, Meleagrides and *S. enterica* subsp. *enterica* 4,5,12:i (1 each). One rough *S. enterica* subsp. *enterica* isolate was also found. Up to now, no correlation between the serotypes found in the breeding pen and those from the abattoir could be found.

P4-05 Enhancement of *Salmonella* Enteritidis Isolation from Shell Eggs by Use of Ferroxiamine E

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The effect of iron supplementation on the recovery of *Salmonella* Enteritidis (SE) in white large shell eggs by use of Ferroxiamine E (FE) was determined. It was then evaluated using a direct plating method and enrichment methods. Two bulk lots of blended, pooled eggs were thoroughly mixed manually and artificially contaminated with SE (0.1 CFU/ml). Twenty samples containing 500 ml of liquid eggs were withdrawn from each of the inoculated bulk lots. One set of twenty samples was supplemented with FE to make a final concentration of 200 ng/g of egg and incubated for 24 h at room temperature. The other set of twenty samples was mixed only with sterile distilled water as a control and incubated. For the direct plating method, each sample was cultured by direct plating onto brilliant green (BG), xylose lysine desoxycholate (XLD), and xylose lysine agar Tergitol 4 (XLT4). For the enrichment methods, 25-g portions from each pool were enriched in modified tryptic soy broth (FeSO4, 30 mg/L). After 24 h of incubation, the preenrichment samples were subcultured to tetrathionate and Rappaport-Vassiliadis selective broths, and streaked to the agar plates. SE was confirmed biochemically and serologically. At 25°C, SE concentrations in FE-supplemented egg pools reached 10^5 CFU/ml by 24 h. From a total of 40 pools of eggs analyzed, all supplemented or unsupplemented samples were positive (100%) when enrichment steps were combined. However, with direct plating, 15 out 20 (75%) FE-supplemented samples were positive for SE while 4 out of 20 samples (20%) were positive. These results demonstrated the potential use of Ferroxiamine E to detect low numbers of SE in eggs without enrichment steps in less than two days.

P4-06 Detection of *Salmonella* Enteritidis in Incubated Pools of Shell Eggs Supplemented with Ferrioxamine E by Lateral Flow Test Kit

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Shell eggs and egg products have been associated with many foodborne outbreaks caused by *Salmonella* Enteritidis (SE). A rapid and simple method for detecting SE from poultry samples is critical for the effective implementation of such testing strategies. However, rapid methods have been reported to consistently detect SE in egg pools only at levels of $>10^7$ CFU/ml. Therefore, sample preparation for the rapid methods is critical to detect low numbers of SE cells in eggs. In the present study, an optimum incubation method was determined for the promotion of the multiplication of small initial numbers of SE to permit efficient detection of SE with a rapid lateral flow test kit. The lateral flow device for the detection of SE utilized in this study was manufactured by Neogen, Lansing, MI. A series of iron supplementations using ferrioxamine E and ferric sulfate were conducted to optimize the test procedure for raw eggs in combination with different incubation temperatures (25, 37, and 42°C). Detection of SE was 100% only in FE-supplemented raw egg pools inoculated with 10 SE cells per pool of 10 eggs when combined with an enrichment step at a 1:10 ratio in mTSB for 24 h at 37 or 42°C. The lateral flow test kit could provide a simple, rapid, and inexpensive method for egg producers and processors to test specifically for *Salmonella* group D1 serovars, such as SE, in egg samples.

P4-07 Incidence of *Salmonella* spp. on Processed Poultry

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Several investigators reported the incidence of *Salmonella* spp. in processed poultry and poultry products. However, little research has been conducted on the effect of the chilling process on the incidence of *Salmonella* spp. in chicken. The objective of this study was to determine the effect of the chilling process on the incidence of *Salmonella* spp. contamination. A total of 240 pre- and post-chill whole broiler carcasses were collected from a poultry processing plant during July through December, 2004. Water samples were collected at the entrance and exit of the chiller. Temperature, pH, total and free chlorine were measured in the water samples. Carcasses were pre-enriched overnight by the whole carcass enrichment method. Pre-enriched samples were screened for *Salmonella* spp. by use of the BAX-PCR system. Samples positive for *Salmonella* by the BAX-PCR system were confirmed by cultural methods and serology. Water samples were enriched overnight and analyzed by the above mentioned methods. Ninety two percent of pre-chill and 93% of post-chill carcass samples were positive for *Salmonella* spp. A greater incidence of *Salmonella* was observed in pre-chill than post-chill carcasses only in August. All water samples collected at the exit of the chiller were positive for *Salmonella*. No *Salmonella* was detected in water samples collected at the entrance of the chiller. There was no correlation between presence of *Salmonella* on post-chill carcasses and the levels of free and total chlorine in chiller water. These results indicate that the chilling process did not have a significant effect on the incidence of *Salmonella* spp.

P4-08 Biotypes and Serotypes of *Campylobacter* spp. Isolated from Broilers in Trinidad

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A study was undertaken to determine the biotypes of *Campylobacter jejuni* and *C. coli* and the serotypes of *C. jejuni* present in broilers from selected small retail processors in six counties in Trinidad. A total of 1424 *Campylobacter* isolates consisting of 743 (52.2%) and 681 (47.8%) of *C. jejuni* and *C. coli* respectively were biotyped using the Lior scheme. Fifty-two *C. jejuni* isolates randomly selected from all sources were serotyped using 25 Penner heat-stable antisera (Denka Seiken Co. Ltd., Japan) by the passive hemagglutination method. Amongst *C. jejuni* isolates, 634 (85.3%) and 109 (14.7%) belonged to biotype I and II respectively, compared with *C. coli* isolates, of which 574 (84.3%) were grouped as biotype I and 107 (15.7%) were biotype II. The frequency of both biotypes amongst *C. jejuni* and *C. coli* was statistically significant ($P < 0.05$; +2). Of the 52 isolates of *C. jejuni*, 48 (92.3%) were typable. Twenty-nine (55.8%) isolates reacted with only one antiserum while 19 (36.5%) cross-reacted with at least two antisera. Overall, 19 serotypes were identified with 6 of these occurring in cross-reactions. The predominant serotype Penner HS31 accounted for 19.2% of the isolates tested, while Penner HS2, the predominant serotype recovered from clinical cases worldwide, accounted for 5.8%. The biotypes detected in *Campylobacter* isolates from chickens are similar to those earlier reported from other livestock in Trinidad but this is the first documentation of serotypes of *C. jejuni* from any source in the country.

P4-09 The USDA-FSIS Intensified Verification Testing Program for *Listeria monocytogenes*: Ready-to-Eat Product, Food-contact, and Environmental Sampling Results

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The Intensified Verification Testing (IVT) Program was developed by USDA-FSIS to support regulatory programs for control of *Listeria monocytogenes* in ready-to-eat (RTE) products. Under the IVT program, RTE product, food-contact, and environmental samples are collected from establishments with ongoing *L. monocytogenes* contamination and/or repeated sanitation problems. From the start of the program in December 2002 to November 2004, 70/1238 samples from 31 establishments tested positive: 2.9% (8/278) of product, 3.6 % (14/393) of food-contact, and 8.5% (48/567) of environmental samples. The 8 positive product samples were: sliced (6), chopped and formed (1), and multi-component product (1). The 14 positive food-contact samples were from: conveyer belts (5), tables (3) and other areas (6). The 48 positive environmental samples were from: drains (17), wheels (7), floors (7), and other areas (17). There were positive IVT samples in 21 out of 31 establishments sampled. Nine establishments had positive sample results in more than one sample category: 5 establishments had positive environmental and food-contact results, 1 establishment had positive environmental and product results, and 3 establishments had positive product, food-contact, and environmental results. If a product or food-contact sample tests positive, regulatory action is taken. Results from the IVT program can be used to identify sites of contamination, focus resources on establishments with *L. monocytogenes* harborage, and remove adulterated product from the marketplace, thereby helping to protect public health.

P4-10 DSC Inhibition of *Listeria monocytogenes* in Processed Meat and Poultry by Combinations of Sorbate, Benzoate, and Propionate

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The control of *Listeria monocytogenes* (LM) by use of combinations of food-approved antimicrobials was evaluated in processed poultry and meat products. Preliminary research suggested that >0.1% total potassium sorbate, propionic acid, or benzoic acid, alone or in combinations, prevented the growth of LM in turkey and wiener slurries when stored at 4°C and 10°C for 4 weeks. This study further evaluated their combined anti-listerial effect in uncured turkey and cured beef-pork bologna manufactured with 156 ppm sodium nitrite. Three treatments of both turkey and beef-pork bologna products were formulated using the "Least Cost Formulation" program. Treatments included 1) control with no antimycotics, 2) combination of 0.05% sodium benzoate and 0.05% sodium propionate, 3) combination of 0.05% sodium benzoate and 0.05% potassium sorbate. Ingredients were mixed, stuffed into fibrous, moisture-impermeable casings, cooked to an internal temperature of 73.9°C, chilled, and sliced. The final product was surface-inoculated with LM (4-log CFU/package), vacuum-packaged and stored at 4°C for 91 days. The antimycotic addition to uncured turkey Treatments 2 and 3 initially decreased the rate of LM growth for 1 week, but LM grew >5-logs by 42 days. In contrast, the addition of antimycotic combinations in the cured bologna prevented growth of LM for 91 days at 4°C, compared with >3.5-log increase in listerial populations in the control bologna without antimicrobials. These data suggest that antimycotic agents can prevent LM growth in cured process meats. Additional research is needed to determine levels needed to prevent growth of LM in uncured poultry and for USDA approval for use in formulations.

P4-11 Fate of *Listeria monocytogenes* in Ham Treated with an Acid Dip and Subsequently Used as an Ingredient in Ham Salad

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In a mayonnaise-based ham salad, ham is more likely than mayonnaise to introduce *Listeria monocytogenes* into the product. The objective of this study was to evaluate a surface acid treatment for *L. monocytogenes*-contaminated ham to prevent the possible growth of this pathogen in ham salad during storage at refrigerated and abuse temperatures. Pieces of cooked ham were surface inoculated with an 8-strain mixture of *L. monocytogenes*, and then dipped in sterile water containing 0% (control), 0.3%, 0.6%, 0.8% and 1.25% acetic acid for 30 s. The ham was then mixed with mayonnaise and stored at 4, 8, or 12°C. The initial cell counts of *L. monocytogenes* in ham salad were 2.0 to 3.0 log CFU/g. The counts increased 5.0 log CFU/g in the control after 3 days of storage at 12°C, 5 days at 8°C, and 13 days at 4°C. In salads that supported the growth of *L. monocytogenes*, an increase of 1.0 log CFU/g and 2.0 log CFU/g after 21 days at 8 and 12°C, respectively, were observed in salads containing ham treated with 0.3% acetic acid, and an increase of 1.0 log CFU/g in salad containing ham treated with 0.6% acid after 21 days at 12°C. Regardless of the storage temperature, *L. monocytogenes* was unable to grow in salads containing ham treated with 0.8% or 1.25% acid after 21 days. Results indicate that an acetic acid (=0.8%) dip treatment has potential in treating ham used in salad preparation to reduce the concerns of *L. monocytogenes* growth in ham salad.

P4-12 The Identification of an *Escherichia coli* O157:H7 Meat Processing Indicator for Fresh Meat through the Comparison of the Effects of Selected Antimicrobial Interventions

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Pre rigor lean and adipose beef carcass tissue was artificially contaminated with stationary phase cultures of five generic *Escherichia coli* beef cattle isolates or a composite culture of five *E. coli* O157:H7 strains suspended in a fecal inoculum. Each tissue sample was processed with one of the following antimicrobial interventions: 90°C water; 90°C water followed by 55°C, 2% lactic acid; 90°C water followed by 20°C, 2% lactic acid; 20°C water followed by 20°C, 2% lactic acid; 20°C water followed by 20°C, 20 ppm chlorine; or 20°C water followed by 20°C, 10% trisodium phosphate. The ability of the generic *E. coli* isolates to predict the response of *E. coli* O157:H7 was found to be dependent upon the microbial intervention. For all microbial intervention methods applied, irrespective of tissue type, the mean log reductions of at least two *E. coli* isolates were not significantly different ($P > 0.05$) from the mean log reduction of the *E. coli* O157:H7 composite culture. Due to the frequent employment of multiple microbial intervention methods in industry, it is unlikely that a single isolate can realistically represent the effectiveness of all microbial interventions on *E. coli* O157:H7. Thus, the use of a combination of *E. coli* isolates evaluated here may be required to accurately predict the effectiveness of the total microbial interventions on the reduction of *E. coli* O157:H7 from beef carcass tissue.

P4-13 Validation of Time and Temperature Values as Critical Limits for Ground Beef Processing and Storage – *Escherichia coli* O157:H7

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In order to provide beef processors with valuable data to validate critical limits set for temperature during grinding, a study was conducted to determine *Escherichia coli* O157:H7 growth at various temperatures in raw ground beef. Ground beef samples were inoculated with a cocktail mixture of streptomycin-resistant *E. coli* O157:H7 to facilitate recovery in the presence of background flora. Samples were held at 4.4°C, 7.2°C, 10°C and room temperature (22.2°C to 23.3°C) to mimic typical processing and holding temperatures observed in meat processing environments. *E. coli* O157:H7 counts were measured, on TSA with streptomycin (1000 μg/ml), at 2 h intervals over 12 h for samples held at room temperature. Samples held under refrigeration temperatures were sampled at 4, 8, 12, 24, 48 and 72 h. Less than one log of *E. coli* O157:H7 growth was observed at 24 h for samples held at 10°C. Samples held at 4.4°C and 7.2°C showed less than one log of *E. coli* O157:H7 growth at 72 h. Samples held at room temperature showed no significant increase in *E. coli* O157:H7 counts for the first 4 h. These results illustrate that meat processors can utilize a variety of time and temperature combinations as critical limits to minimize *E. coli* O157:H7 growth during the production and storage of ground beef.

P4-14 Comparison of Indicators of Hygienic Meat Processing

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Various indicator organisms have been used for monitoring the hygienic processing of animal carcasses. Total Viable Count (TVC), *Escherichia coli* and *Enterobacteriaceae* are among the most widely favored. The choice of indicator organism has been generally a national or geographic preference rather than being based on utility. For example, monitoring for regulatory purposes is often conducted using *E. coli* as an indicator. The value of this organism as an indicator decreases when it is rarely isolated, usually at very low concentrations. During a large survey of beef carcasses in Australia (550 samples), comparisons between TVC, *Enterobacteriaceae*, coliform and *E. coli* counts from identical samples were made. There was a high degree of correlation ($r = 0.91$) between coliform and *Enterobacteriaceae* counts, and less than 10% of samples were positive for *Enterobacteriaceae* but negative for coliforms. The correlation between *E. coli* and coliforms and between *E. coli* and *Enterobacteriaceae* was less strong ($r = 0.76$ and 0.69 respectively). *Enterobacteriaceae* or coliforms were present in 17% of those samples negative for *E. coli*. Amongst samples in which both species/groups were enumerated (40 positive for both *E. coli* and coliforms and 39 positive for both *E. coli* and *Enterobacteriaceae*) the correlation improved slightly ($r = 0.79$ and 0.80 respectively). These data indicate that while coliforms or *Enterobacteriaceae* may appear to be more sensitive indicators of hygienic processing, the correlation between *E. coli* and the other enteric indicators is only moderate. Each indicator organism provides information relevant to various industry stakeholders (processors, regulators and consumers).

P4-15 Genetic Diversity of *Pseudomonas* spp. Isolated from Retail Displayed Beef

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The objective of this study was to genetically characterize *Pseudomonas*, isolated from beef, using the Random Amplification of Polymorphic DNA (RAPD) method. Beef loins were purchased from a local supermarket and steaks were cut under aseptic conditions in the laboratory. The steaks were displayed under simulated retail conditions at 4°C. Pseudomonads were recovered from loins on day 0 and from steaks on day 0, 2, 4, 6, 8 and 10 using a hydrophobic grid membrane filtration method with direct plating on CFC media. A total of 309 isolates comprising 29 from loin on day 0 and 46, 47, 48, 45, 46, and 48 from steaks on day 0, 2, 4, 6, 8, and 10, respectively, were selected for RAPD analysis. As *Pseudomonas* grew on steaks, sensory analysis showed a progressive decrease in the retail appearance and a progressive increase in the surface discoloration and odor intensity. Using RAPD analysis, a total of 51 genetic types (>85% similarity) were identified among 309 isolates. One major genetic type contained 45% of the isolates. Sixteen genetic types contained isolates that were shared between >2 sampling times whereas the remaining 34 types were unique to one particular time. These data suggest that although *Pseudomonas* spp. associated with the beef loin were transferred to the steaks prepared from it, a genetically diverse *Pseudomonas* population emerged during the retail display of beef. Understanding the heterogeneous nature of *Pseudomonas* spp. on beef during storage would help in the development of intervention strategies to effectively control the growth of the prevalent strains causing spoilage.

P4-16 Microbiological Quality of Beef Carcasses and Trim in Australia

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Microbiological monitoring contributes to validating the regulatory system. Samples of chilled beef and sheep carcasses and frozen boxed trim were collected from processors accounting for approximately 75% of Australia's beef and 65% of sheep throughput. Beef carcasses were found to have an average total viable count

(TVC, 25°C) of 1.33 log CFU/cm². *E. coli* was isolated from 4.9% carcasses with an average count of -0.42 log/cm² on positive samples. Sheep carcasses were found to have an average total viable count of 2.28 log CFU/cm². *E. coli* was isolated from 32.9% carcasses with an average count of 0.28 log /cm² on positive samples. For frozen beef trim the average TVC was 1.19 log/g and the average count for the 1.1% of samples with detectable *E. coli* was 1.90 log/g. *E. coli* O157:H7 was isolated from 1/1143 carcasses and 0/1082 boxed samples. *Salmonella* was isolated from1/1082 samples of boxed product. No *Campylobacter* sp. were isolated. *S. aureus* was isolated from 20.1% and 2.6% of carcass and boxed beef samples respectively. For frozen sheepmeat trim the average TVC was 1.81 log/g. The average *E. coli* count for the 4.3% of positive samples was 2.02 log/g. *E. coli* O157:H7 was isolated from 0.6% carcasses and 0.2% boxed samples. *Salmonella* was isolated from 0.5% samples of boxed product. *Campylobacter* sp. were isolated from 0.4% of carcasses and 0.2% of boxed samples. *S. aureus* was isolated from 15.9% and 14.1% of carcase and boxed samples. These results are a significant improvement over the survey conducted in 1998, reflecting the investment made by the Australian industry in food safety systems.

P4-17 Efficacy of the Grovac™ System for Decontamination of Retail Beef Trimmings: Process Validation against *Escherichia coli* O157:H7 and *Salmonella* spp.

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The Grovac™ antimicrobial intervention system (Pine Bluff, AR) was evaluated for its effectiveness in reducing *Escherichia coli* O157:H7 and *Salmonella* spp. inoculated onto surfaces of raw beef trimmings. Designed to be used in a batch process, the Grovac system involves treating beef trimmings in a mixture of citric acid and hypotonic salt solution while tumbling under vacuum. Beef trimmings were inoculated with a five-strain cocktail of *E. coli* O157:H7 or *Salmonella* spp., then subjected to no treatment, water with 1 h drain treatment, water with overnight drain treatment, Grovac with 1 h drain treatment, and Grovac with overnight drain treatment. All treatments were applied in the Grovac unit for 2 min. The inoculum level for each pathogen was ca. 6 log CFU/g. *Salmonella* spp. reductions (average of 4 replications) on XLD agar were 0.28 (water only-1 h drain), 0.64 (water only-overnight drain), 0.45 (Grovac™-1 h drain), and 0.92 (Grovac™-overnight drain) log CFU/g. Reductions on XLDD recovery medium were similar. *E. coli* O157:H7 reductions (average of 5 replications) were 0.39 (water only-1 h drain), 0.47 (water only-overnight drain), 0.42 (Grovac™-1 h drain), and 0.69 (Grovac™-overnight drain) log CFU/g.

P4-18 Fate of Acid-adapted and Nonadapted *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella* on Ground or Whole Beef Jerky

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The objective of this study was to determine the fate of acid-adapted and nonadapted *Escherichia coli* O157:H7, *Salmonella* and *Listeria monocytogenes* on ground and whole beef jerky strips during the home-style jerky process. Each organism and meat type was compared separately and analyzed using a split-plot experimental design. To achieve acid-adapted and nonadapted cultures, each pathogen was grown in tryptic soy broth with and without dextrose, respectively. After incubation, the pH of the acid-adapted culture was 4.88 and the nonadapted was 6.97. Inoculated strips were dried in a vertical dehydrator with an air temperature of 60.0°C. For ground beef strips, samples were taken at time 0, 2, 4, 6, and 10 h. After 10 h, population reductions of acid-adapted and nonadapted *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* were 5.86 and 5.30, 4.73 and 3.96, and 4.28 and 4.51 logs, respectively. When population reductions were compared for the same organism, there was no significant difference ($P > 0.05$) between acid-adapted and nonadapted *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* on ground beef strips. Whole beef strips were sampled after inoculation, after marination and at 4, 8, 12, and 14 h. Population reductions after 14 h for acid-adapted and nonadapted *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* were 5.25 and 5.13, 4.85 and 4.82, and 4.81 and 4.87 logs, respectively. When population reductions were compared for the same organism, there was no significant difference ($P > 0.05$) between acid-adapted and nonadapted *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* on whole beef strips.

P4-19 Antibiotic Resistance and Cross-contamination of *Enterococcus* Isolated from Live Cattle, Hides and Carcasses

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To determine patterns of cross-contamination and antibiotic susceptibility of bacterial isolates in a commercial cattle-processing system, a total of 60 cattle were shipped to a commercial abattoir, 20 in each of three separate trial periods. The same animals were followed through the process and bacterial isolates were collected from these animals immediately before shipping, at the abattoir after exsanguination, after hide removal, and in the cooler. Samples were cultured for *Enterococcus* spp. Out of all hide samples at the feedlot 77.8% were positive for *Enterococcus*, compared to 96.1% of the hide samples from the commercial abattoir,

which was a significant increase in hide culture positives ($P < 0.001$). *Enterococcus* spp. were recovered from 58.3% of pre-evisceration carcasses. *Enterococcus* isolates were frequently resistant to the antimicrobials tested. Of 279 confirmed *Enterococcus*, all were resistant to at least one antimicrobial. Interestingly, 179 (64.15%) of these isolates were resistant to at least six agents. The most common resistance was to chloramphenicol (100%), followed by flavomycin (90.32%), lincomycin (87.81%), tylosin (78.49%), erythromycin (76.34%), tetracycline (58.87%), synercid or quinupristin/dalfopristin (47.67%), bacitracin (17.92), streptomycin (8.96%), ciprofloxacin (1.43%), linezolid (0.72%) and salinomycin (0.36%). *Enterococcus* spp. were also analyzed for pulsed-field gel electrophoresis profiles from all sampling points. Similar or indistinguishable PFGE patterns were found from isolates recovered at the feedlot and in the commercial abattoir. These data provide evidence that bacterial isolates originating at the feedlot are passed to the processing environment and onto the final processed carcasses.

P4-20 Evaluation of Ferrioxamine E as a Selective Iron Source for *Enterobacter sakazakii* in Iron-limited Nutrient Media and Egg White

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This study was conducted to find the optimal concentration of iron sources to detect *Enterbacter sakazakii* and the effectiveness among Ferric Ammonium Citrate (FAC), Ferrous Sulfate (FS), and Ferrioxamine E as an iron supplement. FE has been known as a selective iron source for certain groups of bacteria such as *Salmonella* and *Enterobacter*. In recent years, *E. sakazakii* has been associated with necrotizing enterocolitis, bacteremia, and infant meningitis through the ingestion of contaminated powdered infant formula. The iron-free medium was composed of NaCl-glucose with the amount of iron source added to give a final concentration from 200 ng/ml to 5 mg/ml, and was inoculated with 0.1 ml of 10^2 CFU/ml of *E. sakazakii*. After 24 h of incubation at 37°C, the *E. sakazakii* populations recovered from iron-free media supplemented with FAC, FS, and FE were log 6.2, 6.3 and 6.4 CFU/ml respectively, at the concentration of 200 ng/ml. However, only log 1.8 CFU/ml *E. sakazakii* were isolated from iron-free media without supplementation. Also, in egg white known as a perfect iron-free media, when the same procedure was performed, the *E. sakazakii* populations in the egg white media supplemented with FAC were log 8.7 CFU/ml in 5 mg/ml, log 8.6 CFU/ml in 2 mg/ml, and log 5.0 CFU/ml in 200 μg/ml; FS were log 6.0 CFU/ml in 2 mg/ml and log 4.2 CFU/ml in 200 μg/ml; and FE were log 6.5 CFU/ml in 200 μg/ml, log 6.4 CFU/ml in 20 μg/ml, and 3.0 CFU/ml in 2 μg/ml. No *E. sakazakii* was detected from the media without supplementation in egg white. FE could be used as the stimulator to detect *E. sakazakii* in selective media by accelerating the growth of the bacteria that is usually present in low number in infant formula or dairy food.

P4-21 Detection of Virulence Genes of Atypical Non O157 *Escherichia coli* Isolates from Feedlot Cattle Treated with Growth Promoters

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Eighty steers were used to evaluate the effect of 3 growth promoters (Rumensin, Revalor-S and Liquamycin) on the variability of fecal *Escherichia coli* in a 2 × 2 × 2 factorial design. Rectal fecal samples were collected nine times between December 2002 and June 2003. All *E. coli* isolates were identified by API 20E. Detection of virulence genes was performed by PCR and their susceptibility to ampicillin, tetracycline, spectinomycin and ceftriaxone was determined using the agar dilution assay. Of the 156 *E. coli* isolates recovered, 84% were sorbitol negative but none were confirmed as O157. Isolates were grouped into 14 biochemical *E. coli* types with 51% being G type. Among the 133 isolates further screened, 40% were positive for *eaeA* and e-*hlyA* and 2% were positive for *stx1*, *eaeA* and e-*hlyA*. Virulence genes were not detected in 64% of isolates. No significant effect of treatment was observed on biochemical characteristics and the virulence type ($P > 0.05$). A small percentage of ampicillin resistant isolates was observed. A high frequency of tetracycline resistant isolates of *E. coli* were found in all the 8 groups, but were not associated with any specific treatment. The administration of Rumensin, Revalor-S and Liquamycin alone or in combination does not seem to be associated with an increase in the prevalence of a particular *E. coli* biotype. However, the presence of atypical virulent *E. coli* strains in feedlot cattle fecal material may subsequently contaminate carcasses at slaughter and the environment.

P4-22 Survival of *Listeria monocytogenes* on Uncured Sliced Roast Beef and Turkey Products Manufactured with Different Levels of Antimicrobials during Refrigerated Storage

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Different levels of antimicrobials were evaluated in uncured sliced roast beef and uncured sliced turkey. Four treatments of each product type were evaluated, 1 control (Test 1) and 3 test formulations with sodium lactate and sodium diacetate (Tests 2, 3, and 4 containing 3.0% NuLacR, 3.5% NuLacR, and 2.3% NuLacR plus

1% sodium lactate, respectively). Slices were surface inoculated with 4 log CFU/g of a 5-strain mixture of *Listeria monocytogenes*, vacuum-sealed in gas-impermeable bags and stored at 4°C for up to 12 weeks. Populations of *L. monocytogenes* were enumerated on package contents rinsed with Butterfield's phosphate buffer (100 ml/100g) using MOX. Triplicate samples were assayed at 0-time, after 2 weeks, and weekly thereafter. Sampling was discontinued for a formulation if populations of *L. monocytogenes* increased by >2-log CFU/pkg. Turkey and roast beef products had similar moisture, pH and salt (%NaCl) values (70.8 to 75.6%, 6.0 to 6.4 and 1.3 to 1.8%, respectively). All turkey formulations tested in this study supported >2-log growth of *L. monocytogenes* within 2 to 4 weeks of storage. NuLacR was effective in controlling the growth of *L. monocytogenes* on roast beef product at 3.5% and at 2.3% in combination with 1% sodium lactate. Tests 1 and 2 supported >2-log growth of *L. monocytogenes* within 2 to 4 weeks. *L. monocytogenes* showed sporadic growth on Formulation 3 (3.5% NuLacR) starting at week 8; however, the average log increase was <1 log CFU/pkg throughout 11 weeks of storage. Formulation 4 (2.3% NuLacR and 1% sodium lactate) inhibited the growth of *L. monocytogenes* throughout 12 weeks of testing. This study identified antilisterial combinations of sodium lactate and sodium diacetate that could be used in uncured roast beef.

P4-23 Delayed *Clostridium perfringens* Growth from a Spore Inocula by Sodium Lactate in Sous-vide Chicken Products

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Clostridium perfringens growth from a spore inoculum was investigated in vacuum packaged, cook-in-bag marinated chicken breast that included 0, 1.5, 3, or 4.8% sodium lactate (NaL; w/w). The packages were processed to an internal temperature of 71.1°C, ice chilled and stored at 4, 19 and 25°C. The total *C. perfringens* population was determined by plating diluted samples on tryptose-sulfite-cycloserine agar followed by anaerobic incubation for 48 h at 37°C. At 25°C, addition of 1.5% NaL was effective in delaying growth for 29 h. Increasing the NaL level to 4.8%, *C. perfringens* growth from a spore inoculum during storage at 25°C for 480 h was not observed. At 19°C, the growth was >6 log CFU/g by 288 h in control samples. In samples with 3.0 or 4.8% NaL, the growth of *C. perfringens* from spores was dramatically restricted with little or no growth in 648 h at 19°C. *C. perfringens* growth was not observed at 4°C regardless of NaL concentration. The D-values at 55°C ranged from 47.40 (no NaL) to 57.58 min (1.5% NaL). Cyclic and static temperature abuse of refrigerated products for ≤ 20 h did not permit *C. perfringens* growth. However, temperature abuse of products for periods 24 h or longer in the absence of NaL led to growth of *C. perfringens* from a spore inoculum. An extra degree of safety may be assured in such products by supplementation with NaL at 1.5 to 4.8% NaL level.

P4-24 A Risk-Based Approach to the Development of a National Through-chain Seafood Standard

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An assessment of public health risks posed by the consumption of seafood was undertaken to provide a scientific basis for the development of a through-chain seafood standard. The assessment considered the public health risk posed by microbiological hazards across all major seafood commodities and along the entire seafood supply chain, from capture or aquaculture through to consumption. The approach adopted for evaluating risk involved the use of a risk-ranking matrix, and was consistent with the principles for risk analysis adopted by the Codex Alimentarius Commission. The severity of illness was determined using a three-level ranking that ranges from events that are life-threatening to those that are self-limiting and of short duration. The likelihood of illness was estimated on the basis of data on the consumption of seafood, the prevalence and level of hazards in seafood and available epidemiological data. A decision matrix was applied to the severity and likelihood parameters and provided a broad relative risk ranking (high, medium or low) for each commodity:hazard combination. Overall the risk from seafood is well managed, with only a small number of commodities considered to present a high risk to public health and safety. The ranking successfully merged scientific and technical information on food safety hazards in seafood and assisted in the identification of risk management approaches for those higher risk commodities. The seafood standard being drafted includes generic provisions to control hazards in commodities ranked low and medium risk, and specific measures to manage higher risk seafood.

P4-25 DSC Daily Variability of *Listeria* Contamination Patterns in a Cold-smoked Salmon Processing Operation

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Understanding *Listeria* transmission and contamination patterns in Ready-To-Eat (RTE) food processing environments is critical to improved control of *Listeria monocytogenes*. A cold-smoked fish processing operation was used to probe variability in *Listeria* contamination in RTE processing environments throughout one production week (five days) by using extensive testing of finished product and environmental samples collected at the beginning, middle, and end of each work day. A total of 20 finished products and 22 to 36 environmental samples were collected at each time, yielding a total of 300 finished product and 470 environmental samples collected from processing steps after smoking, including skinning, trimming, slicing, and

packing. A total of 28 finished and 56 environmental samples were positive for *Listeria* spp. (including one and four samples positive for *L. monocytogenes*, respectively). DNA sequencing of sigB allowed differentiation of eight *Listeria* subtypes. *Listeria* prevalence varied significantly between days and a high prevalence at day 3 could be linked to contamination by a single *L. welshimeri* subtype. Regression analysis revealed a significant relationship between the environmental and finished product contamination ($P < 0.01$). *Listeria* prevalence did not show consistent differences between the beginning, middle, and end of a production day, but subtype data often revealed unique contamination patterns for samples collected at different times of a given day. While our data further confirm a relationship between environmental and finished product *Listeria* contamination, high variability in contamination patterns between days and within a given day clearly confound our ability to define *Listeria* transmission patterns in RTE processing environments.

P4-26 Influence of Packaging Atmosphere on Growth of *Listeria monocytogenes* on Refrigerated Ready-to-Eat Shrimp

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Cooked, refrigerated ready-to-eat (RTE) shrimp is a value-added product not widely exploited in the U.S. despite increasing popularity elsewhere, primarily due to concerns for psychrotrophic pathogen growth during storage. Thus, the objective of this study was to evaluate the growth potential of *Listeria monocytogenes* on RTE shrimp stored in different atmospheres at different chill temperatures. Cooked, peeled, and deveined shrimp were inoculated with a 5-strain mixture of *L. monocytogenes*, packaged in air, vacuum, or 100% CO_2, and stored at 3, 7, or 12°C for 15 days. Populations of *L. monocytogenes* and psychrotrophic bacteria, and appearance and aroma sensory attributes, were periodically measured. Regardless of temperature, CO_2 packaging was most effective in slowing growth of *L. monocytogenes* and psychrotrophs, followed by vacuum packaging, with air packaging least effective. The most effective packaging and temperature combination to control bacterial growth was CO_2 combined with storage at 3°C. This combination produced *L. monocytogenes* and psychrotroph growth rates of 0.0039 and 0.11 log CFU/g per day, respectively. In contrast, the least effective combination, air packaging and storage at 12°C, exhibited growth rates of 0.64 and 0.65 log CFU/g per day, respectively. CO_2 packaged shrimp remained unspoiled for 15 days at any temperature, while shrimp stored in other atmospheres spoiled before 15 days at 12°C. It is notable that temperatures of 3°C or less will also control growth of psychrotrophic *Clostridium botulinum*. Therefore, for best quality and safe RTE shrimp, CO_2 packaging and storage temperatures at or below 3°C are recommended.

P4-27 A Novel, Enzyme-based Assay for the Rapid and Simple Detection of *Vibrios* in Shellfish and Seawater

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We discovered an enzyme activity present in members of the *Vibrionaceae* family, but not present in non-*Vibrio* pathogens. This enzyme serves as a lysyl aminopeptidase and is associated with phosphoglucose isomerase of all *Vibrio* species tested to date. A colony overlay procedure for peptidases (COPP) was developed for the rapid detection of bacteria containing this enzyme activity. Cellulose acetate membranes containing a synthetic fluorogenic substrate were overlayed onto bacterial colonies on trypticase soy agar plates containing 1% NaCl to detect and quantify *Vibrios* in seawater and shellfish. Within 10 min, *Vibrionaceae* family members produced fluorescent spots on the membranes where the substrate was cleaved by the enzyme. We used this COPP technique in a monthly survey of oysters and seawater from moderate and low salinity areas of the Delaware Bay from May through December, 2004. Six sets of oysters and 6 seawater samples were collected for analysis each month. Results indicated the presence of $>10^4$ *Vibrionaceae* per gram of oyster during the summer months. Biochemical testing of isolates showed the presence of *Vibrionaceae* family members including species of *Vibrio*, Aeromonas, Plesiomonas, Photobacterium, and Shewanella. During June and July, *Vibrio vulnificus* was most frequently isolated and was at levels 10^4/g of oyster and 10^2/ml of seawater from the Delaware Bay. The COPP technique is rapid, simple, and inexpensive. It was useful for monitoring rising levels of *Vibrionaceae* family members in shellfish and seawater as water temperatures increased. Subsequent correlation of pathogenic species and shellfish-associated disease with total *Vibrio* levels could lead to the application of the COPP assay in the establishment of regulatory guidelines for total *Vibrio* levels in shellfish.

P4-28 *Vibrio vulnificus* Septicemia Associated with Clam Consumption, 1988 – 2003

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Vibrio vulnificus causes septicemia and death, typically following raw oyster consumption. Florida requires restaurants to warn consumers about risks associated with raw oysters but not clams; regulations in other states vary. We extracted information from CDC's Cholera and Other *Vibrio* Illness. We defined primary septicemia as a blood culture that yielded *V. vulnificus* in a patient without a wound. We identified 11 patients

with primary *V. vulnificus* septicemia between 1988 and 2003 for whom recent clam but not oyster consumption was reported. Ten were men, and all were either older than 50 years (6 patients) or reported an underlying medical condition (9). Patients resided in Florida (3), New York (3), Pennsylvania (3), and California (2). Nine consumed raw clams, and no other seafood consumption was reported for five of these. Five patients obtained clams from a restaurant or bar, 1 from a market, and 1 from an unlicensed caterer. Harvest site information was available for six cases: Indian River, Florida (4 patients), Virginia (1), and Washington state (1). Six died; five had consumed raw clams and one consumed them cooked. Clams, particularly eaten raw, may have caused serious illness and death. Most patients had underlying medical conditions. Clams associated with these infections were harvested from both Atlantic and Pacific coasts. Raising awareness about the health risks associated with raw clam consumption may reduce *V. vulnificus* infections.

P4-29 Changes in Reported *Vibrio vulnificus* Infections in the USA following California's Ban on Gulf Coast Oysters

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Vibrio vulnificus infection is a serious health threat, causing hospitalization in 90% of cases, and death in 40%. Cases are due to wound exposure to Gulf of Mexico water or due to eating contaminated food, most frequently raw oysters from the Gulf Coast. In 2003, California banned the sale of raw or untreated oysters harvested from the Gulf of Mexico during April through October. We reviewed cases reported to CDC's Cholera and Other *Vibrio* Infection Surveillance System from 1998 through 2004. Foodborne *V. vulnificus* infection was defined as isolation of *V. vulnificus* from a patient who exhibited no evidence of having sustained a wound that came in contact with coastal water, raw seafood, or marine wildlife. Between 1998 and 2002, 270 (55/year) foodborne *V. vulnificus* infections were reported from 29 states. California reported 37 of these. Food exposure information was available for 231 (86%); 167 (72%) reported eating oysters, of which 94% were raw or untreated. Between 2003 and 2004 (preliminary data), 104 (52/year) foodborne *V. vulnificus* infections were reported from 20 states. California reported no foodborne *V. vulnificus* infections in this time period. Since the ban on the sale of raw Gulf Coast oysters in California, the frequency of foodborne *V. vulnificus* cases in the U.S. did not change importantly, but no cases were reported from California. Foodborne *V. vulnificus* infections continue to occur in the rest of the country.

P4-30 Genetic Variation within the *Vibrio vulnificus* Capsular Polysaccharide Operon

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Vibrio vulnificus is indigenous to estuaries worldwide and produces rare but rapidly fatal septicemia in susceptible persons. Disease is associated with either consumption of raw oysters or exposure of wounds to shellfish and seawater. Virulence factors include capsular polysaccharide (CPS) expression, which correlates with opaque (O) colony morphology and shows phase variation to translucent (T) colony types with reduced CPS and virulence. Characterization of T phase variants suggested multiple mechanisms for phase variation: T1 strains had point mutations in the CPS operon, reduced CPS gene expression, and were able to revert back to O phenotype; T2 strains showed deletions of one or more CPS genes, did not express surface CPS, and were stable in the T phenotype. High frequency switching of O to T phenotype was induced under specific growth conditions, and rates were dependent on strain, media, and temperature, and increased with extended incubation. Deletions in T2 strains were mediated by tandem directly repeated oligonucleotides flanking the deleted genes. Strains without repetitive elements did not show T2 deletion, but, instead, deletion mutations in the CPS operon probably resulted from recombination with the second chromosome. The transition from opaque to translucent phase promotes *V. vulnificus* attachment and biofilm formation (Joseph and Wright, 2004). Therefore, these genetic variations may represent adaptations for increased survival in estuarine environments.

P4-31 The Incidence of Pathogenic Microorganisms in Aquacultured Rainbow Trout (*Oncorhynchus mykiss*)

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Quantitative levels of six known pathogens (*Listeria monocytogenes*, *Clostridium botulinum*, *Salmonella* species, *Vibrio cholerae*, *Yersinia enterocolitica*, and *Y. pseudotuberculosis*) and aerobic plate counts were measured at five aquaculture facilities. Four of the facilities employed a flow-through aquaculture system while the fifth used a system that recirculated approximately 80 percent of its water. Facility 1 had a mud raceway, facilities 2 and 3 had fiberglass raceways, facility 4 had a concrete raceway, and facility 5 had fiberglass recirculating tanks. The farmed rainbow trout (*Oncorhynchus mykiss*) and trout fillets were sampled at two different growing seasons to monitor for potential microbial hazards. *Listeria* spp. was identified in both whole trout and trout fillets from all five facilities sampled from both growing seasons. Presumptive *Clostridium botulinum* spores were also identified from all five facilities for both seasons. The growing season did not affect pathogen levels and there was no evidence that any one aquacultural system was superior to the others. *Salmonella* spp., *Vibrio* cholerae, and *Yersinia* spp. were not isolated from any of the trout samples analyzed.

P4-32 Effects of Acidified Sodium Chlorite, Grapefruit Seed Extract, and Storage Conditions on Recovery of *Listeria monocytogenes* and *Staphylococcus aureus* from Smoked Trout

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Antibacterial activities of acidified sodium chlorite (ASC, Sanova®) or Citricidal™ (grapefruit seed extract, GSE) were evaluated against *Listeria monocytogenes* (LM) and *Staphylococcus aureus* (SA) on smoked trout. Fillets inoculated with five log CFU/g of LM and SA were dipped for one min. in sterile distilled water (SDW), ASC, or GSE with or without subsequent neutralization of the antimicrobials. Treated fillets were stored at 4°C or in ice with (VP) or without (non-VP) vacuum packaging for up to 21 days. LM numbers increased during storage of untreated fish on ice and at 4°C. LM numbers decreased to three (0.5% GSE) or four log CFU/g (SDW or 50 ppm ASC) on VP fish stored at 4°C for two days but then increased to five to six log CFU/g after 14 days. LM numbers dropped to four log CFU/g during storage of fish in ice regardless of treatment. SA numbers were reduced to three (4°C) and two (ice) log CFU/g during storage of untreated fish and treated VP fish. Lack of neutralization of 2% GSE permitted growth of LM from one log CFU/g (day 0) to only two log CFU/g on day 14; numbers increased to five log CFU/g on untreated fish. Numbers of SA decreased to less than one CFU/g in the presence of 2% GSE. The approved concentration (50 ppm) of ASC had minimal effect in reducing LM, while GSE, in combination with VP and iced storage, shows promise in mitigating this public health concern.

P4-33 Quality and Safety of X-ray Treated Fresh Catfish Fillets

DSC

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Consumer skepticism of irradiated foods limits widespread use of this technology. Although lacking consumer appeal, this technology could be the answer to improving food quality and safety of perishable fresh foods. As with any processing technology, if the quality of the treated foodstuff is noticeably different from similar but untreated products, consumers will likely reject the alternative product. The present research was designed to determine if X-ray irradiation treatment results in changes in selected quality and safety parameters (color, texture, microbial load, and *Listeria monocytogenes* incidence) of fresh aquacultured channel catfish fillets. Fresh catfish fillets (*Ictalurus punctatus*) were obtained from a local processor, trimmed to 50 g portions, then sealed individually in Ziploc® bags. Samples were dosed to 0, 0.5, 1.0 and 1.5 kGy using an RS 2000 X-ray irradiator (Rad-Source Technologies) and stored at 4°C for 17 days. Periodically during storage, samples were removed and measured for color, texture, aerobic plate count (APC), psychrotrophic plate count (PPC), total coliform count (TCC), and *L. monocytogenes* incidence. Color and texture did not change over time or with irradiation dose. Microbial counts were significantly different with time, irradiation dose, and the interaction of the two. TCC, APC, and PPC increased with time as expected, with slower rate increases with higher irradiation doses. Overall *L. monocytogenes* incidence was 40%, 27%, 0%, and 7% at 0, 0.5, 1.0, and 1.5 kGy, respectively. This study indicated that X-ray irradiation improved the quality and safety of fresh catfish fillets without negatively affecting consumer purchase attributes.

P4-34 Rapid PCR-RFLP Method for the Identification of Five Billfish Species

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Billfish meats are highly favored in Taiwan. They are commonly used as a sliced meat (sashimi), dried fish floss and fish minced products. Due to an increase in consumption demand and high cost, other cheaper fish meats are occasionally used to adulterate it. So the species identification of these mislabled products is necessary to prevent fraudulent substitution. A PCR-RFLP method has been developed for the detection of five billfish species, Xiphias gladius (Swordfish), Makaira nigricans (Blue Marlin), Makaira indica (Black Marlin), Istiophorus platypterus (Sailfish) and Tetrapturus audax (Striped marlin). The primers L-CYTBF and H-CYTBF were designed on a conserved DNA sequence in the mitochondrial cytochrome b (cytb) gene and molecular weight of amplified fragments was 348 bp. The sequence of amplified DNA fragment in five species were analyzed and then were further subjected to restriction enzyme active sites analysis. The DNA sequence and restriction enzyme analyses could differentiate five billfish species. The results obtained from the Bsa JI and Cac 8I enzyme digestion could be used to distinguish the five billfish meats. The method is sensitive, rapid and valid to detect fraudulent billfish products replaced by less valuable fish.

P4-35 Application of PCR-RFLP Analysis to Species Identification of Raw Material and Canned Products of Tuna

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Tuna is an important pelagic capture in Taiwan; the familiar species in Taiwan are *Thunnus thynnus*, *T. alalunga*, *T. obesus* and *T. albacares*. In order to establish the gene identification of fresh meat of familiar tuna species in Taiwan, in this study we used the directed sequencing and the polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) technology to determine the genetic variation of 358 bp

fragment of the mitochondrial cytochrome b gene of four Thunnus species. Then we developed gene probes to identify the species of frozen, fresh and canned tuna meat. After analyzing the sequence differences in four Thunnus species, we chose three restriction enzymes with specific cutting sites, Bsp1286 I, Hinc II and Rsa I. The polymorphic pattern in the DNA electrophoretic gel could identify four fresh Thunnus species precisely and quickly. In the case of canned product, we designed 7 pairs of primers for PCR amplifying, but there were only two pairs of primer, CbBRs126L/H and CbHi146L/H, which could successfully amplify the PCR products, short fragments of 126 bp and 146 bp, respectively. Then we used three restriction enzymes, Bsp1286 I, Hinc II and Rsa I, to analyze the cutting sites of processed products and could differentiate the diversity between four species by the DNA electrophoretic map.

P4-36 Growth and Histamine Formation of *Enterobacter aerogenes* Inoculated in Sailfish and Milkfish during Storage

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Enterobacter aerogenes was studied for its growth and its promotion of the formation of total volatile base nitrogen (TVBN) and histamine in sailfish (*Istiophorus platypterus*) and milkfish (*Chanos chanos*) stored at various temperatures from -20°C to 37°C. The optimal temperature for bacterial growth in both fish species was 25°C, while the optimal temperature for histamine formation was 37°C. The two fish species inoculated with *E. aerogenes*, when not properly stored at low temperatures such as 15°C, formed histamine at above the FDA hazardous guideline level of 50 mg/100 g. Milkfish was a better substrate than sailfish for histamine formation by bacterial histidine decarboxylation at elevated temperatures (>15°C). Although higher contents of TVBN were detected in the spiked sailfish than milkfish during the same storage time at temperatures above 15°C, the use of the 30 mg TVBN /100 g as a determination index for fish quality and decomposition was not a good criterion for assessing potential histamine hazard for either fish species. Bacterial growth was controlled by cold storage of the fish at 4°C or below, but histamine formation was prevented only by frozen storage. Once the frozen fish samples were thawed and stored at 25°C, histamine started to accumulate rapidly and reached levels greater than the hazardous action level in 36 h.

P4-37 Modeling the Inactivation of *Vibrio parahaemolyticus* in Oysters by High Pressure Processing

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Vibrio parahaemolyticus is a leading cause of diarrhea due to consumption of raw or undercooked seafood. High pressure processing (HPP) can be used to inactivate this organism while retaining desirable product quality attributes. Using pilot-scale HPP equipment, we developed response surface (RS) models to predict inactivation of *V. parahaemolyticus* in oyster homogenates. Different models were developed to reflect changes in response due to altered metabolic status of the microorganism through growth under optimal and sub-optimal conditions. No injury of *V. parahaemolyticus* apparently resulted from HPP following either optimal or sub-optimal growth of cultures, but *V. parahaemolyticus* was more sensitive to HPP when grown sub-optimally than when grown under optimal conditions. Pressure played the most important role in the inactivation of *V. parahaemolyticus*, followed by time and temperature, whereas temperature of HPP is only a nuisance factor for sub-optimally grown cultures. When scaled up HPP equipment was used, the models adequately predicted inactivation of the organism in oyster homogenate, but over-predicted inactivation in whole oysters. When compared with kinetic models of inactivation, the RS models generally predicted similar levels of inactivation.

P4-38 Mapping of Spoilage Indicators and Some Pathogenic Microbes in Three Estonian Dairies

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The aims of this study were to find critical points in processing that may contribute to contamination of dairy products, to compare visual inspection with microbial sampling in hygiene surveys and to determinate the occurrence of the pathogens *Bacillus cereus*, *Listeria monocytogenes* and *Mycobacterium* spp. in process samples. Surfaces were examined with Hygicult-dipslides (TPC, Y&F and E/-gur) and RidaCount (Total, Yeast/Mold and Coliforms) agar sheets. DryCult TPC was used for the determination of microbial counts in liquid samples. The detection of *L. monocytogenes* was performed according to ISO 11290-1 using half Fraser and Fraser broths for enrichment and Oxford and LMBA agars for culturing. The API *Listeria* test was used for identification. Detection of *B. cereus* was performed according to ISO 7932, using Mossel agar for culturing. Before identification with API test, colonies of *Bacillus* spp. were checked for αhemolysis on sheep blood agar. The detection of *Mycobacteria* spp. was performed using Middlebrook 7H10 agar and commercially available *Mycobacterium* tubes 1 and 2 in culturing. The confirmation of *Mycobacteria* was carried out with Ziehl-Neelsen staining. The general results from these three Estonian dairies showed that food contact surfaces were mostly clean from all microbes examined, but non-contact surfaces in food processing were in most cases

contaminated with Enterobacteria, fungi and aerobic bacteria. As a routine hygiene control method, sampling of surfaces for Enterobacteria with contact agar is a good indicator of contamination, which correlates with the aerobic bacterial counts obtained. The presence of pathogens in all three dairies was minor.

P4-39 Efficacy of Multiple Heat Treatments to Inactivate Bacterial Spores in Milk

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Heat-resistant bacterial spores survive high-temperature, short-time (HTST) pasteurization processes, but may be stimulated to germinate so that vegetative cells can be inactivated by a second HTST. The primary objective of this study was to evaluate the efficacy of multiple heat treatments, germinants, and storage and pasteurization temperatures to inactivate *Clostridium botulinum* or *Bacillus cereus* spores in milk. The sporocidal effect of double HTST treatments was evaluated for 1-ml portions of whole milk inoculated with 6-log spores/ml *C. botulinum* or *B. cereus* and heated to 72°C for 15 s, with a 1- or 4-h hold at 4, 30 or 40°C between HTSTs. Spore populations remained unchanged after the second HTST regardless of hold temperature or time. The effect of germinants was similarly tested by adding L-alanine, L-lactate, L-cysteine, sodium bicarbonate, nitrite, nisin, lysozyme, or hydrogen peroxide to inoculated whole milk. Populations of spores in the 0.5% hydrogen peroxide treatments decreased 0.5- to 1-log spores/ml after the first HTST at 71.7°C and an additional 1- to 1.5-log spores/ml after the second HTST. No significant decrease in spores was observed for any other treatment. Further testing with 0, 0.25, and 0.5% hydrogen peroxide compared the effect of pasteurization temperatures (15 s at 72, 81 and 85°C for both spore types and at 75 and 78°C for *B. cereus* only) and hold temperatures before heating (4 or 30°C; 1 h). In order to generate a 2-log decrease in viable *B. cereus* spores, combinations of 0.25% hydrogen peroxide with 78°C double HTST or 0.5% hydrogen peroxide with 75°C double HTST was required. *C. botulinum* spores were more resistant to inactivation, requiring 0.5% hydrogen peroxide and 81°C double HTST to generate a 2-log decrease. This study identified a potential method to inactivate bacterial spores in pasteurized milk and will be replicated using a lab-scale pasteurizer.

P4-40 Collaborative Evaluation of a Fluorometric Method for Measuring Alkaline Phosphatase Activity in Cow, Sheep and Goat Milk

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Pasteurization of raw milk was introduced in order to extend product shelf life and destroy pathogens. The measurement of alkaline phosphatase (ALP) activity has been used as an indicator of proper pasteurization in dairy products for over 65 years. This study was undertaken to evaluate 6 different fluid dairy products at lower phosphatase levels than previously verified using the Fluorophos Test System, a sensitive and precise method for ALP activity detection. Thirteen laboratories participated in this collaborative, international study to evaluate the fluorometric test at 20, 40, 100, 350 and 500 mU/l, and extend the scope of the method to not only include milk from cows but also goat and sheep. Initially, the statutory level of ALP measured fluorometrically was set to equivalent levels of colorimetric test standards (500 mU/l). The European Union recently announced its intention of lowering the legal limit from 500 mU/l to 350 mU/l and, in addition, setting a target value of 100 mU/l, which if exceeded, would trigger an investigation into the pasteurizer plant performance. At 500 mU/l ALP, this trial generated RSDR% values of 6.48, 5.69 and 1.74, and RSDR% values of 14.66, 13.30 and 5.33 for all cow, sheep and goat milk samples, respectively. Data from this study are comparable to data from previous studies and indicate the suitability of the Fluorophos method for measuring ALP activity in milk from cows, sheep and goats not only at the current European statutory level of 500 mU/l but also at much lower levels.

P4-41 Evaluation of Decontamination Protocols, Media Enrichments and Improved Template DNA Preparation Procedure for the Detection and Recovery of *Mycobacterium avium paratuberculosis* from Cow Milk and Feces

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Mycobacterium avium paratuberculosis (MAP) is the etiologic agent of Johne's disease (JD), a chronic enteritis affecting many animal species. JD is prevalent in North America and one of the most significant causes of economic losses to dairy industry. Cows with JD shed this organism into both feces and milk. There is a great interest to improve the procedures for the detection of MAP in these matrices. In this study we compared the effect of decontamination and enrichment in various broth formulations on the recovery, and time-to-detection of the live bacterium. Cow feces and milk seeded with MAP were decontaminated for 4 to 16 h in (a) 2 to 4% NaOH alone or in combination with 0.5 and 5% oxalic acid, (b) NaLc in combination with 2 to 4% NaOH, (c) HPC 0.4 to 1.2%. Treated samples were subjected to 24 h antibiotic treatment, followed by incubation in various mycobactinJ-7H9 formulations. On day 11, samples were seeded to BACTEC and HEYM slants. Contamination was not observed in fecal and milk samples decontaminated with (a) 2 to 4% NaOH-5% oxalic acid, (b) HPC 0.4 to 1.2% for 6 to 12 h. The 7H9 broth formulations containing glycerol, NAD, serum, egg and

lacking all detergents were superior in MAP recovery. An optimized PCR procedure using pooled cream and pellet fractions from 50 ml of seeded milk sample heated to 85°C and cooled fractions treated with 0.75% hexadecylpyridinium chloride (HPC) prior to template DNA extraction by beadbeating and precipitation procedures resulted in MAP detection limit of 15-50 CFU/50 ml, or <1 CFU/ml.

P4-42 Prevalence of *Salmonella enterica* in Bulk Tank Milk from USA Dairies as Determined by PCR

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Samples of bulk tank milk from dairies across the United States, taken as part of the NAHMS Dairy 2002 survey, were analyzed for the presence of *Salmonella enterica* by use of a commercially available real-time PCR kit. Samples from 854 farms in 21 states were collected and enriched in tetrathionate broth to amplify any salmonellae present, and DNA was isolated from the resulting biomass. One-hundred and one samples (11.8%) were shown to contain *Salmonella enterica* by use of the real-time PCR assay whereas conventional culture techniques detected the pathogen in only 22 (2.6%) of the samples. A conventional PCR assay targeting a different gene from *Salmonella enterica* confirmed the presence of the organism in 94 of the real-time PCR-positive samples. Thus, assay of milk samples by real-time PCR indicates that the prevalence of *Salmonella enterica* in US bulk tank milk is substantially higher than previously reported.

P4-43 Prevalence of *Mycobacterium avium* subspecies *paratuberculosis* in Retail Cheese Curds from Wisconsin and Minnesota

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Mycobacterium avium subspecies *paratuberculosis* (MAP) is the etiological agent of Johne's disease, a severe gastroenteritis in ruminants. MAP is also suspected to be associated with Crohn's disease in humans. Recent research has focused on detection of viable MAP in pasteurized milk. Pasteurized milk is a key ingredient in a variety of food products. We investigated whether viable MAP was present in commercially available cheese curds. A total of 101 retail cheese curd samples were collected over a 6-month period from northern and southern regions of Wisconsin and Minnesota. Five grams (in duplicate) of each sample was homogenized in 30 ml of 2% (w/v) sodium citrate and heated at 35°C for 15 min. One milliliter of the resulting solution was placed in Dubos media overnight followed by hexadecylpyridinium chloride and antibiotic decontamination. Samples were then cultured on Herrold's egg yolk agar slants supplemented with mycobactin J, amphotericin B, naladixic acid, and vancomycin. DNA was extracted and PCR confirmation was performed using the IS900 and hspX primer sets. No viable MAP was cultured from commercially available cheese curds. However, 9.2% (6/65) of the samples tested positive for MAP genetic material. These data suggest that the combination of pasteurization and cheese production processes is effective at killing MAP. This study may warrant further investigation of MAP survival in retail cheese curds with a larger sample size, as well as determining MAP survival in other pasteurized and unpasteurized milk products.

P4-44 The Microbiological Quality of Cheese Made from Raw or Thermized Milk from Production and Retail Premises in the UK

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The foodborne pathogens *Salmonella* and *Listeria monocytogenes* are of concern in the dairy industry, especially in the manufacture of soft cheeses. European Commission Recommendation 2004/24/EC required Member States to undertake a program of sampling cheeses made from raw or thermized milk from production and retail establishments in 2004 for the presence of *Salmonella* and *Campylobacter*, and levels of *Staphylococcus aureus*, *Escherichia coli* and *Listeria* spp. including *L. monocytogenes*. Examination of 1,842 cheese samples in the UK and comparison with criteria stipulated in 2004/24/EC revealed that most (98%) were of satisfactory or acceptable microbiological quality. However, 2% of samples were of unsatisfactory quality due to high *E. coli* ($\geq 10^5$ CFU/g), *S. aureus* ($\geq 10^4$ CFU/g) and/or *Listeria* spp. including *L. monocytogenes* ($\geq 10^2$ CFU/g) levels. *Campylobacter jejuni* was detected in one sample, which was of unacceptable quality while *Salmonella* was not. Most (88%) cheese samples collected were made from cows' milk, and almost all (99%) were made using raw milk, with only 1% made using thermized milk. Labelling on 25% of cheeses contained no information as to whether the cheese was made from raw milk. Cheeses collected included unripened soft (fresh) (4%), ripened soft (44%), and semi-hard (52%) cheeses. A greater proportion of unripened soft cheese samples (9%) were of unsatisfactory microbiological quality compared to ripened soft (3%) and semi-hard cheeses (1%). Although most of the cheeses examined were of an acceptable microbiological quality, evidence from this study also indicates that labelling of cheese providing information on whether the cheese was prepared from raw or thermized milk requires improvement.

P4-45 Evaluating the Safety of Raw Milk Cheeses

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Australian food standards do not generally permit the use of raw milk for the production of cheese. Typically, all milk used in cheese making must be pasteurized or thermized. In recent years, Food Standards Australia New Zealand (FSANZ) has evaluated and approved applications and proposals to exempt specific cheeses from the milk heat treatment requirements of the Code. This has lead to permissions for selected raw milk cheeses, viz. gruyere, sbrinz, and emmental cheeses from Switzerland and extra hard raw milk grating cheeses such as parmigiano reggiano and grana padano. Currently, FSANZ is evaluating an application to permit the import of Roquefort cheese. The scientific assessments underpinning these approvals have been based on a equivalence framework, i.e., can these cheeses be produced to an equivalent level of safety as cheese made from pasteurized milk? The assessments have examined the efficacy of milk production and processing operations to adequately control contamination and outgrowth of pathogenic microorganisms. This involves assessing the fate of these organisms during cheese making and maturation. Some assessments have also considered the capacity of regulatory arrangements and enforcement agencies to effectively control raw milk inputs, cheese making operations and microbiological screening of the final product prior to sale. The paper will describe the FSANZ approach and provide a case study contrasting recently completed scientific evaluations of extra hard grating cheese and Roquefort cheese.

P4-46 Effect of Cheese Substitution on the Botulinal Safety of Process Cheese Products

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A current strategy to reduce production costs of process cheese products is to formulate products with <50% cheese, which is the level used for standard process cheese. The objective of this project was to determine the effect of substituting cheese with other dairy solids on the botulinal safety of nonstandard process cheese products. Using a Box and Benken statistical design, over 75 formulations were manufactured using five levels of Cheddar cheese (0, 15, 25, 35, 45%), three moisture levels (55, 60, 65%), three pH levels (5.5, 5.75, 6.0) and three levels of total salts (3, 4, 5% NaCl plus disodium phosphate emulsifier). Cheese solids were substituted with a mixture of dry dairy ingredients (whey, whey protein concentrate, and nonfat dry milk; ratio 1:1.5:3) and fat levels standardized to 18 to 22% with anhydrous milkfat. Formulated products were inoculated with 3-log/g *Clostridium botulinum* spores during manufacture in a lab-scale cheese cooker, hot-filled into glass vials, and incubated at 30°C for up to 6 months; five replicate samples were assayed for botulinal toxin, pH and sensory changes at designated sampling points. Data revealed that products formulated with all dry dairy ingredients (0% cheese) tended to have slightly lower water activity and delayed botulinal toxin production compared with formulations having similar moisture, pH, and total salts, but manufactured with 15 to 45% cheese. No significant difference was observed in time to toxicity between products formulated with 25 to 45% cheese. These results suggest that whey, whey protein concentrate, or nonfat dry milk can be used as substitutes for cheese solids without negatively affecting the botulinal safety of process cheese products provided the water activity is equivalent or lower than similar products manufactured with standard cheese levels.

P4-47 Classifications of Process Cheese Formulations to Prevent Toxin Production of *Clostridium botulinum* by Support Vector Machine

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Formulation control is an effective way to prevent toxin production by *Clostridium botulinum* in process cheese products. The objective of this study was to separate potentially toxic formulations from safe formulations to assist with process cheese formulation adjustments. For this purpose, a support vector machine (SVM) was developed. A SVM results from a computational procedure. Its intention is to separate data points based on the presence or absence of a characteristic (i.e., toxin production) associated with the data. Experiments to determine toxin production of *Clostridium botulinum* in response to various process cheese formulations were previously conducted. The developed SVM generated a hyper-plane boundary to separate safe formulations from potentially toxic formulations. The performance of this SVM was evaluated by comparing predictions with laboratory observations. The results showed that an SVM can separate formulations that are potentially toxic from those that are safe, thus suggesting formulation adjustment region for cheese manufacture. Formulations classified in error are used to make the toxicity prediction strategy more conservative. When the error is that toxin is predicted but no toxin is observed the formulation is treated as potentially toxic for decision making purposes. The accuracy of the decision making before such adjustments was 88%. The SVM also produced an estimate of the probability of toxin production. These results suggest that a support vector machine is a reasonable strategy to help identify useful process cheese formulations. It is a model free approach that classifies formulation alternatives to support safe formulation choices.

P4-48 Detection of Food Allergens by Quantitative and Qualitative ELISA

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Food allergens are increasingly being recognized as a serious health issue. In North America, an estimated 1 to 2% of the adult population, and 5 to 8% of children, are allergic to one or more foods. Reaction to food allergens can range from a slight itching of the mouth to death through anaphylactic shock. In the USA, Congress has enacted the Food Allergen Labeling and Consumer Protection Act of 2004 (108 to 282). This law, which comes into force on 1-1-06, makes the declaration of the major food allergens mandatory within packaged foods, including incidental additives, flavors and colors. To enable the food industry to easily detect these food allergens, TECRA has developed a range of Immunoassays, This presentation details results of preliminary studies to determine the suitability of assays for the quantitative and qualitative analysis of residual egg and peanut in processed foods. Data is presented for the analysis of key food matrices. The Egg Assay detected the presence of both egg yolk and white proteins in food, with a limit of detection below the proposed regulatory action level. The Peanut assay was shown to detect both raw and roasted peanut material in processed foods below the 5 ppm action level. Quantitative results for both assays were read from the standard curve. Visual interpretation of the assay was possible, using a "cut-off standard" below the 5 ppm action level. These assays will provide food processors with the means to meet the new labeling requirements, and to provide greater certainty to allergic individuals.

P4-49 Screening Method to Rapidly Distinguish between Chemical Contamination, Microbial Spoilage, or Simple Deterioration in Fruit Juice and Other Drinks

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There is a need for quick screening methods when cases of suspected contamination or potential food poisoning arise. In fruit juice, carbonated beverages and other drinks, a broad multi-residue extraction followed by GC-MS and other analyses can reveal the chemical differences between a normal control sample and the complaint sample. Some recurring patterns encountered in past cases can help identify the ingested chemicals and determine the cause of the problem. For example, simple product deterioration over time will show chemical changes in the monoterpenes (loss of limonene, increase in a-terpineol), color change (browning, loss of Red # 40 or carotenoids), aspartame hydrolysis. Microbiological spoilage on the other hand will usually show several additional early eluting peaks on GC compared to the normal product. Another chemical change characteristic of microbial growth is the metabolism of flavor aldehydes (e.g., octanal, benzaldehyde, furfural) to their corresponding alcohols, which have blander aromas and longer retention times. Depending on the available precursors, a variety of other metabolites are produced from the normal ingredients, e.g., fusel alcohols, esters, free fatty acids, 2-alkanones, diacetyl. When contamination by extraneous chemicals is the problem, those that are amenable to analysis by GC can be detected at the ppb level without the need for concentrating the extract. The common petroleum contaminants (aliphatic and aromatic hydrocarbons) usually originate from separate sources. Various cleaning compounds are another major cause of consumer complaints. Solvent-like odors can come from several things besides solvents, including terpenes, esters, ketones, fusel alcohols, and sorbic and benzoic acid catabolism.

P4–50 Rescheduled to P1-58

P4–51 Rescheduled to P1-59

P4-52 Distributions and Thermal Behaviors of *Enterobacter sakazakii* in Ready-to-Eat Agricultural Products and Infant Formula in Korea

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Enterobacter sakazakii, designated as a unique microbial species in 1980, is also referred to as 'yellow pigmented *Enterobacter cloacae*' and may cause bacteremia, necrotizing enterocolitis and infant meningitis. The distribution of *E. sakazakii* in unprocessed ready-to-eat (RTE) agricultural products and its behavior in infant formula food (IFF) treated at different temperatures were analyzed in this study. Forty seven percent, 50%, and 57% of brown rice, pumpkin, and potato samples, respectively, had aerobic plate counts (APC) in the range of 4 to 5 log CFU/g. Almost all the other products had APC of approximately 2 log CFU/g. Forty two percent of pea samples and 21% of carrot samples tested had *Enterobacteriaceae* counts approximating 4 log CFU/g, and 44% of brown rice samples were at 5 log CFU/g. The level of *Enterobacteriaceae* contamination was <1 log CFU/g for the other products. *E. sakazakii* was isolated from 3/16 (19%), 4/23 (17%), 1/24 (4%), and 1/27 (4%) of IFF, brown rice, laver, and tomato samples, respectively. D50 and D70 values for *E. sakazakii* isolated from the foods ranged from 2 to 5 min, however, the D50 for *E. sakazakii* isolated from brown rice was 6.7 min. The thermal inactivation of four *E. sakazakii* strains in rehydrated IFF was determined. At 50°C, the

levels of *E. sakazakii* decreased about 1 log CFU/g for 4–6 min and thereafter the levels remained stable. At 60°C, one log inactivation occurred for 4 to 8 min and further inactivation, by about 2 log CFU/g, occurred after an additional 20 min. The results suggest that RTE agricultural products can be contaminated with *E. sakazakii*, and that care should be taken to assure appropriate time/temperature combinations are used to adequately inactivate this organism in IFF.

P4-53 Bugs in Spuds: *Bacillus cereus* in Mashed Potato

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Potato products prepared from dehydrated potato flakes have been implicated as the vehicle in foodborne illness incidents involving *Bacillus cereus* intoxications. This pathogen is often present in potato flakes in low numbers where it survives as spores, and can germinate and multiply in the rehydrated product. To assess the risk to New Zealand consumers, we enumerated *B. cereus* in potato flakes and in ready-to-eat mashed potato products under hot-holding conditions. Of 50 packets of potato flakes tested, eight (16%) contained *B. cereus* greater than 100 CFU/g. No sample exceeded 400 CFU/g. Of the potato portion of 44 hot-held commercial potato products (savory-filled potato-topped pastries, mashed potato with gravy, etc.), only two samples contained *B. cereus* greater than 100 CFU/g, a potato-topped pastry (1000 CFU/g) and a container of potato and gravy (120 CFU/g). The temperature of the hot-held products at purchase was usually between 40 and 60°C. Only six (14%) achieved a safe hot-holding temperature of >60°C. To assess multiplication of *B. cereus* in this food, rehydrated potato flakes with naturally occurring *B. cereus* were held at 37, 42 and 50°C and tested at 2, 4 and 6 h. By 6 h, the number of *B. cereus* in potato stored at 50°C had exceeded 10^4 CFU/g (the point at which food is considered unsafe for consumption) and at 42°C the mean count was close to 10^6 CFU/g.

P4-54 BT Crops and Pesticide Reduction: Economic, Environmental and Health Impacts

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Because biotechnology (Bt) crops such as corn and cotton are genetically modified to produce their own insecticides, one important benefit from planting these crops is reduction in conventional pesticide use. Reduction of pesticide applications has a number of beneficial outcomes: reduced health risk to farm laborers and to wildlife; improved environmental quality; and economic savings in terms of reduced material, labor, and equipment costs. This study explores the benefits that are accrued through use of Bt corn in the United States, from reduced pesticide use. The first part of the study contains a risk assessment of the current types of pesticides that are used to treat lepidopteran insect pests in corn. Human and animal health impacts, and exposure to these pesticides, are discussed. The second part of the study evaluates the reduction of pesticide use through Bt corn planting in the US, and estimates the value of planting these crops in terms of improved health, environmental quality, and cost savings. It is found that at current levels of Bt corn planting in the U.S., the total valuated benefit of pesticide reduction from Bt corn is estimated at $17.4 million ($12.5 million to $22 million): $16.8 million through savings in materials, labor, and equipment; $400 thousand in benefits to wildlife; and $200 thousand in benefits to human health. Thus, the pesticide reduction afforded by Bt corn planting has significant economic benefits to farmers but negligible health and environmental impacts. Benefits of pesticide reduction through Bt cotton planting are also discussed.

P4-55 Inactivation of *Listeria monocytogenes* in Ready-to-Eat Deli Salads

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The ICMSF has proposed a preventative scheme for managing microbiological hazards in foods. The scheme uses the concept of food safety objectives that allows equivalence of different control measures to be assessed. Control measures for *Listeria monocytogenes* contamination of ready-to-eat (RTE) deli salads can encompass several strategies. These include prevention of contamination prior to packaging or growth inhibition in packaged product during storage using a preservative system such as a high acid environment. This study evaluated the survival of *L. monocytogenes* in RTE deli salads in this context. A five-strain mixture of *L. monocytogenes* was inoculated into five different deli salads, consisting of coleslaw, shrimp, pasta, potato and bean salad. Inoculated and treated salads were stored at 5 and 10°C for 14 days. Salads were sampled initially then at 1, 2, 4, 7, 10 and 14 days in triplicate. Samples were analyzed for *L. monocytogenes* by enumeration and enrichment. Studies showed that inoculated salads did not support the growth of the pathogen and that there were differences ($P < 0.05$) between salad types with respect to *L. monocytogenes* survival. A 0.4 to 2.0 D and 0.5 to 2.0 D reduction at 5 and 10°C respectively after 14 days of storage was observed. The data may be used to assist manufacturers of deli salads to produce products that satisfy a theoretical food safety objective of <100 CFU/g at the point of consumption.

P4-56 Inhibition of Microbial Growth on HabaGUARD® Conveyor Belt Surfaces

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Sanitary food contact surfaces are critical to food safety. Incorporation of antimicrobial compounds may help make cleaned surfaces less likely to support microbial growth. These experiments aimed to assess attachment and growth of bacterial cells on commercial belting materials containing HabaGUARD® antimicrobial additive and to assess durability of the effect. Initial laboratory experiments used new samples of belting materials suspended in an annular biofilm reactor through which a dilute inoculum (ca 3×10^3 CFU/mL) of *Escherichia coli* (ATCC 8739) was circulated. Tests of growth medium and belt surfaces showed that belting with antimicrobial supported 4.6 logs less growth in the medium and at least 4.7 logs less growth on its surface relative to control, significant inhibition of bacterial attachment and of growth in the reactor. Field samples provided cleanability and durability measurements. During four months of use, modules from belts containing antimicrobial and control segments were removed directly after cleaning and examined for attached bacteria (aerobic plate count). While overall bacteria levels were low, counts from antimicrobial segments were lower in 6 of 7 sets, averaging 1 log reduction and confirming that the belts were readily cleaned. Finally, field samples from conveyor belts that had been used in frozen food (one- and two-year) and in pork processing (one-year) were compared with new belting. A film of nutrient broth containing ca. 2×10^5 CFU/mL of the test organism, *Listeria monocytogenes* (ATCC 19114) or *E. coli* (ATCC 8739), was spread over a controlled area and incubated. Microorganisms were taken up in peptone physiological saline and enumerated by pour plate. Compared to control, counts were reduced by 1 to >4 logs, depending on microorganism and sample, showing microbial inhibition that persists after extended use.

P4-57 Consumer Attitudes towards Food Irradiation

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The purpose of this study is to determine if an educational intervention increased public understanding and acceptance of a controversial technology such as irradiation. Three hundred consumers participated in an educational program consisting of viewing an eight-min film on food safety and irradiation produced by Purdue University, followed by a question-and-answer period. Consumers were surveyed about their general knowledge and attitudes toward food safety and irradiation before and after viewing the film and participating in the discussion. This program increased awareness of harmful bacteria and the risk of foodborne illnesses. The percentage of participants expressing concern about food safety significantly increased after the program. Prior to the film, over half of the respondents had not heard of irradiation, and those against irradiation reported that they knew little about it. This indicates that the initial negative attitudes may be due to lack of information, and the connotation that the term irradiation creates in people's minds. After the film, most respondents were supportive of irradiation. The majority of respondents would choose irradiated over non-irradiated products, in contrast to only 18% who would initially. Almost 40% of the respondents stated that they would be willing to pay more for irradiated meat. The film provided important information, but people wanted more details as to how FDA assessed safety and the impact of irradiation on nutritional value, food safety and food quality. Overall, informing consumers about the risks of foodborne illness and the benefits of irradiation increases public acceptance.

P5-01 A Comparative Study of VIDAS ECO and the ISO 16654 Method for the Detection of *Escherichia coli* O157 in Food Products

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This study was conducted according to ISO 16140 norm recommendations, to evaluate the efficiency of VIDAS ECO method for the detection of *Escherichia coli* O157 in food products in comparison with the ISO 16654 reference method. A total of 324 food samples, including 150 artificially contaminated and 174 uncontaminated products, were tested by both methods. For the ECO method, samples were pre-enriched for 6 to 7 h at 41°C in m-TSB broth plus acriflavin (dairy products) or novobiocin (other products) and then sub-cultured into 9 ml of MacConkey broth for 17 to 9 h at 37°C, before testing in the VIDAS instrument. Positive samples were further confirmed after isolation on a chromogenic plate medium specific for O157:H7 (O157:H7 ID). The VIDAS ECO method detected 145 positive samples and the ISO method 148 positive samples with an agreement of 95.2%. The difference between the two methods was not statistically significant. The 50% detection limit, established from 5 products spiked at different levels, each with a different strain of *E. coli* O157:H7, was found to be between 0.3 and 2.2 cells per 25 g sample for both methods. This study showed that the VIDAS ECO method is comparable to the ISO 16654 method for the recovery of *E. coli* O157 in food samples. In addition, it allows release of negative products within 24 h compared with 4 days for the standard cultural method. Furthermore, the combination of the VIDAS ECO screening method with the use of O157:H7 ID chromogenic medium leads to a specific identification of *E. coli* O157:H7.

P5-02 A Simple Colorimetric Assay for Rapid Detection of *Escherichia coli* O157:H7

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There are many methods used to detect *Escherichia coli* O157:H7 in food and water. However, most of these tests require long incubation times and sample manipulation prior to the testing process. Reporter bacteriophages represent a novel alternative for the detection of bacteria within food. A rapid colorimetric method for detection of *E. coli* O157:H7 was developed, based on bacteriophage T4 that was genetically modified to carry a beta-galactosidase gene. The assay was developed in a one-tube format. The reporter phage and the beta-galactosidase substrate chlorophenol red -D-galactopyranoside were included within a single test compartment, and separated from one another by a layer of polyethylene glycol distearate. This wax has a melting temperature of 37°C. When a sample to be tested was added to the compartment, the reporter phage infected any viable *E. coli* O157:H7 present within the sample, forcing the bacteria to make large amounts of -galactosidase. Following the infection process (approximately 1 h), the temperature of the tube was increased by placing the tube in warm water, causing the polyethylene glycol distearate to melt, releasing the colorimetric substrate, which reacted with the beta-galactosidase to produce a visible red precipitate. The assay was capable of detecting 10^5 CFU/ml of *E. coli* O157:H7 within 2 to 3 h. When coupled with immunomagnetic separation, a detection limit of 10^3 to 10^4 CFU/ml was possible. The nature of this detection method is such that it effectively detects *E. coli* O157:H7 in a simple and rapid manner, without the need for instrumentation to interpret the test result.

P5-03 The Envisio™ System: Magnetic Detection Technology for *Escherichia coli* O157:H7

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A new assay system has been developed for the rapid detection of *Escherichia coli* O157:H7 in beef samples. The system incorporates the use of antibody-coated superparamagnetic nanoparticles in a lateral flow immunoassay format. The assay devices are analyzed using a recently developed magnetic detection technology which detects the presence of a particle's magnetic field. The assay exhibits a detection threshold of 2000 target cells per milliliter of enrichment, which enables enhanced sensitivity and shorter enrichment times. The data is automatically interpreted and the result printed, eliminating potential subjectivity associated with conventional, visually-interpreted immunoassays. Internal and external evaluations of the new system were performed using low level inoculation of target in ground beef samples ranging in size from 25 to 375 grams. The recovery rate of the assay was compared to the USDA-FSIS reference method in an external study of 65 gram samples inoculated with approximately 1 CFU of *E. coli* O157:H7 to obtain fractional-positive results. A total of fifty samples were analyzed. Five uninoculated and 20 inoculated samples were tested by each method. The magnetic immunoassay system detected 18 positives compared to 12 positives for the reference method. All positive results were culturally confirmed by the reference method. All uninoculated samples were negative by both methods. The data demonstrate that the new assay system is a rapid, simple and accurate method for detection of *E. coli* O157:H7 in raw beef.

P5-04 Comparison of Immunomagnetic Separation and a Commercial Enzyme-linked Immunosorbent Assay for the Detection of *Escherichia coli* O157 on Hides of Feedlot Cattle

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ELISA test kits are used to detect *Escherichia coli* O157 in beef products. Processors also use kits to detect the pathogen on hides or in feces although this is not an approved use. The objective of this study was to compare a commercially available ELISA test kit with immunomagnetic separation (IMS) methods to detect *E. coli* O157 from beef hides. *E. coli* O157 was detected in 1 sample out of 389 samples (0.23%) using IMS. These same samples were subjected to the commercial ELISA in our laboratory and in 139 samples out of 389 samples (35.7%) a positive result for *E. coli* O157 was indicated. The same samples were analyzed in another laboratory and 125 samples out of 345 samples (36.2%) were reported positive using the same commercial ELISA. The IMS data did not correlate to either of the commercial ELISA data. Even though a similar percentage of ELISA assays were positive in each laboratory, the agreement between the laboratories was poor (Kappa of 0.27). A selection of isolates from the pre-enrichment broth that tested positive as *E. coli* O157 were sent to an outside laboratory for identification by DNA sequencing. Among the isolates that tested positive using this test kit were *Enterobacter cowanii*, *Aeromonas media*, *Aeromonas hydrophi*, *Escherichia coli* C3, and *Aeromonas hydrophila*. All of these organisms gave false positive results on the commercial test kit. This data suggests that the commercial ELISA is not useful in detecting *E. coli* O157 from hide swabs when compared to the accepted IMS method.

P5-05 DSC Rapid Detection of Low Numbers of *Escherichia coli* O157:H7 in Apple Cider by Real-time Polymerase Chain Reaction

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Apple cider contaminated with *Escherichia coli* O157:H7 has been implicated in several foodborne illness outbreaks, but due to the presence of reaction inhibitors, detection by PCR is often difficult. The objective of this study was to evaluate techniques to improve detection of *E. coli* O157:H7 in apple cider using a fluorogenic probe-based real-time PCR assay without prior enrichment. Apple cider was inoculated with *E. coli* O157:H7 (6, 4, 2 and 1 log CFU/mL) and plated in duplicate on TSA to verify initial inocula. For particulate removal, apple cider (100 mL) was vacuum filtered through Whatman No.1 filter paper, followed by a 100 mL wash with distilled water. Filtered cider was centrifuged and pellets resuspended in 5 mL of phosphate buffer (0.1 M, pH 7.2) for neutralization. The suspension was filtered a second time through Whatman No. 4 filter paper, washed with 5 mL distilled water, and plated in duplicate on TSA to ensure that cells were not lost during filtration. The filtrate was centrifuged, resuspended in 1 mL phosphate buffer, and *E. coli* O157:H7 cells were concentrated by immunomagnetic separation. PrepMan™ Ultra was added to the magnetic bead/*E. coli* O157:H7 complex for DNA extraction. Extracts were combined as appropriate with primers, probe, and other reagents, and real-time PCR was conducted. Inoculum levels of 5.9, 3.9, 2.2 and 1.5 (35 CFU) log CFU/mL were detected based on *stx*1 and *stx*2 genes at cycle number 24, 30, 35 and 37, respectively, during a 40 cycle program with no prior enrichment. This method can be used for rapid detection (~ 5 h) of *E. coli* O157:H7 in apple cider and potentially other foods.

P5-06 PCR Detection of *Escherichia coli* O157:H7 in Ground Beef: Individual and Pooled Samples

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The ability to pool samples for pathogen testing is very appealing to many companies. Pooling across lots can reduce overall costs of testing while pooling samples within lots can increase confidence in reported results. Combining the Matrix Pathatrix system and the Roche Lightcycler® with hybridization probe technology produces a rapid, accurate, sensitive testing platform that can be used for pooling samples. We tested the sensitivity and accuracy of this platform using *Escherichia coli* O157:H7. Ground beef (375 g) samples (N = 42) were enriched for 5 h at 42°C in buffered peptone water (1L). 18 samples were initially inoculated with *E. coli* O157:H7 ranging from 8 to 800 CFU. After 5 h of incubation broth/meat mixture (250ml) from each individual sample was tested. Broth/meat mixture (50 ml) from each inoculated sample was also pooled with broth/meat mixture (50 ml) from 4 non-inoculated samples and tested. When tested individually all non-inoculated samples tested negative and 100% of the samples that were inoculated, regardless of the inoculate level, tested positive. When any inoculated sample, regardless of inoculum level, was pooled with 4 other negative samples, we detected *E. coli* O157:H7 100% of the time. The combination of these two platforms produces an accurate and sensitive method for testing. We were able to detect initial inoculum levels as low as 8 CFU in both individual and pooled samples within 8 total h. Due to the accuracy and sensitivity pooling across lots may be a way for companies to reduce testing costs.

P5-07 Detection of Very Low Levels of Stressed *Salmonella* in Food in 8 to 10 H with the Alaska AK-phage™ Method

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A novel rapid microbiological method has been developed for the detection of *Salmonella* in food. It consists of four main stages: (i) resuscitation of food samples; (ii) immunomagnetic separation; (iii) bacteriophage lysis of target cells; (iv) detection by AK assay. (i) Several resuscitation conditions were tested; the protocol selected was modified Tryptone Soya Broth supplemented to reduce oxidative stress, incubation at 41.5°C for 6 to 8 h. (ii) Ten ml of the pre-enrichment broth are used to perform the immunoseparation. (iii) Felix 01 bacteriophage and 32 environmental isolates have been screened against 159 *Salmonella* serovars and 54 non-*Salmonella* bacteria, mainly *Enterobacteriaceae*; a cocktail of three bacteriophages was selected, with a sensitivity of 96.9%. (iv) The AK assay uses cell-derived adenylate kinase to convert added adenosine diphosphate to adenosine triphosphate for bioluminescence detection. The detection limit of the Alaska AK-phage™ method in broth is between 10^2 to 10^3 CFU/ml. Over 400 samples of a variety of raw, processed and RTE food were analysed. The TVC background of the foods ranged from < 10 CFU/g to 10^7 CFU/g and the pH from 5.42 ± 0.05 to 7.56 ± 0.05. The food samples were spiked with seven different salmonellae at three levels: 0 CFU/25 g, 0 to 5 CFU/25 g obtaining fractional recovery, and 1 to 20 CFU/25 g. Some salmonellae were heat stressed by incubating the pure cultures at 51.5°C for 10 min; others were cold stressed in the food samples by overnight refrigerated storage. All samples were confirmed by traditional methods, and the reference ISO 6579 method was run in parallel in half of them. As shown in our earlier work on *E. coli* O157, this method is as effective as, or in some cases more effective than, the reference methods for the detection of *Salmonella* in food and can be performed within a working day.

P5-08 Application of Real-time NASBA and Molecular Beacon to the Detection of *Salmonella* sp. in Food

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Salmonella sp. (SLM) is one of the most common bacterial causes of foodborne illness for humans. Since the traditional method of detection of SLM is time-consuming, molecular testing appears as an attractive alternative, thanks to the rapid and specific amplification and detection of the targeted bacterial nucleic acids. We describe the application of the RNA amplification technology called Nucleic Acid Sequence Based Amplification (NASBA) to the detection of SLM. A molecular beacon-based Real-Time NASBA assay was set up to be used with the Nuclisens EasyQ Analyser instrument. Artificially contaminated buffered peptone water enrichments were treated by heat-lysis with a home-brew lysis buffer before NASBA detection. Inclusivity of our NASBA assay was shown on a panel of 24 serotypes (mostly *S. enterica*) while exclusivity was verified with 12 non-*Salmonella* species. Analytical sensitivity was shown to be 10 cell equivalents of purified total RNA. This assay was then tested with lysates of contaminated overnight enrichments; a positive detection was obtained for low contamination levels (1 to 10 CFU/25 g) with a selection of foods including meat and poultry. This preliminary study suggests that we designed a simple and specific protocol for the molecular detection of *Salmonella* sp. in food using the Real-Time NASBA technology.

P5-09 Next-day Detection of *Salmonella* spp. in Select Foods by DNA Hybridization Assay in Conjunction with Abbreviated Sample Enrichment

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Abbreviated enrichment protocols have been developed that permit detection of *Salmonella* spp. in select foods by DNA hybridization assay within 26 h of the start of sample enrichment. The enrichment scheme is comprised of a non-selective pre-enrichment in lactose broth or buffered peptone water for 18 h, followed by transfer to Gram negative broth (GN) and incubation for an additional 6 h. The DNA hybridization assay, utilizing specific DNA probes targeted to *Salmonella* spp. ribosomal RNA, is performed on an aliquot of the final GN culture. Performance of the abbreviated method was assessed in comparison to that of reference culture methods (BAM or USDA-FSIS) for pasteurized egg products, cooked poultry products, chocolate, walnuts, dry pet food, and margarine. A total of 20 trials were conducted using food samples artificially contaminated with low levels of salmonellae, dessication-injured where appropriate. Of 974 samples overall, 530 were confirmed positive by at least one method (DNA hybridization or reference culture). The overall sensitivity of the DNA hybridization method was 98.3%, while that of the reference culture methods was 97.5%. Specificity of the DNA hybridization assay was 99.6%. In separate trials, it was determined that the abbreviated enrichment scheme is not suitable for use with cooked poultry products preserved with nitrite. DNA hybridization assays conducted after 24 h of enrichment yielded low detection rates, while assays performed after an additional 18 h incubation of the GN cultures (42 h total incubation time) showed detection rates equivalent or superior to those of the reference culture procedure. Further work is in progress to validate the abbreviated enrichment protocol for other food types.

P5-10 A Recovery Study of *Salmonella* spp. from the Surfaces of Tomatoes and Packinghouse Materials

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In this study, tomatoes and packing line materials (stainless steel, polyvinyl chloride, conveyor belt, sponge roller and untreated oak wood) were artificially inoculated with a rifampicin resistant, 5-strain *Salmonella* cocktail. Inoculated tomatoes and packing line materials were maintained for 28 days at temperature and relative humidity (RH) combinations which simulated the Florida fall/winter tomato season (20°C/60%RH), the Florida spring tomato season (30°C/80%RH), and standard ripening room conditions (20°C/90%RH). Inocula were recovered from surfaces by a vigorous shake-rub method. After 28 days, *Salmonella* populations remained detectable on tomato surfaces at all three environmental conditions investigated. Inoculated *Salmonella* populations declined to undetectable levels on all packing line materials tested at Florida spring conditions by day 11, with the exception of the unfinished oak, which reached undetectable levels by day 21. In contrast, inoculated *Salmonella* populations declined to undetectable levels on sponge rollers and conveyor belts tested at fall/winter conditions by days 7 and 21, respectively. Stainless steel, polyvinyl chloride (PVC), and unfinished oak surfaces supported the survival of detectable populations of *Salmonella* over the entire 28 day sampling period. Results of this study demonstrate the potential for *Salmonella* to persist on tomato and packing line surfaces at environmental conditions in Florida.

P5-11 A Real-time PCR Assay for the Detection of *Salmonella* spp. in Food and Food-animal Matrices

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Conventional culture methods have traditionally been considered to be the 'gold standards' for the isolation and identification of foodborne pathogens. However, culture methods are labor intensive and time consuming. We have developed a real-time PCR assay for use as a screening method for the detection of *Salmonella* spp. in a variety of food and food-animal matrices. The real-time PCR assay incorporates both primers and hybridization probes based on the sequence of the *Salmonella invA* gene and uses fluorescent resonance energy transfer technology to ensure highly sensitive and specific results. This method correctly classified 51 laboratory isolates of *Salmonella* and 28 non-*Salmonella* strains. The method was also validated using field samples including porcine feces and ceca, pork carcasses, bovine feces, poultry ceca and carcasses, equine feces, animal feeds, and various food products. The samples (3395) were pre-enriched in buffered peptone water followed by selective enrichment in tetrathionate and Rappaport Vassiliadis broths. Aliquots of the selective enrichment broths were combined for DNA extraction and analysis by the real-time PCR assay. When compared to the culture method the sensitivity of the assay ranged from 97.2 to 100.0% and specificity ranged from 95.4 to 100.0%. Kappa values ranged from 0.87 to 1.0 indicating excellent agreement of the real-time PCR assay to the culture method. With the high sensitivity and specificity of the assay and the reduction in time and labor, real-time PCR may be an excellent alternative to culture for detecting the presence of *Salmonella* spp. in a wide variety of matrices.

P5-12 Effect of Growth Temperatures on Rapid Detection of *Listeria*

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Technologies for rapid detection of pathogens have advanced rapidly in recent years. In all methods, however, enrichment is still critical to successful detection of low level pathogens in foods. Enrichment media and temperatures are two of most important factors determining the total time required for enrichment. This work examined the effect of temperatures on the growth of *Listeria* and their detection in foods. Growth curves of 12 *Listeria* strains were obtained at 30, 35 and 42°C, respectively, with 8 different media, including tryptic soy broth (TSB), buffered *Listeria* enrichment broth (BLEB), UVM *Listeria* enrichment broth (UVM), Demi Fraser broth, and Rapidchek *Listeria* broth. Pure culture of *Listeria* was also inoculated in TSB in 250 ml and grown at various temperatures. Total counts and the minimum detection limits were determined by dilution plating and lateral flow strip assay. *Listeria* cells were also spiked in hot dog and raw ground turkey samples for recovery at 30 and 35°C. The results showed that growth at 35°C and above is significantly faster than at 30°C or below, with at least two-h difference in first 10 h in all the media. With an initial 0.2 CFU/ml, after 24 h in TSB, cell levels reached 7.3×10^2, 1.1×10^8, 1.6×10^9, and 1.7×10^9 CFU/ml and the minimum detectable concentration for a lateral flow device was 5×10^4, 1.1×10^5, 1.8×10^6 CFU/ml, and non-detectable for cultures from 25, 30, 35 and 42°C, respectively. This work indicates that growth temperature plays an important role for efficient enrichment and detection of *Listeria*.

P5-13 Validation of the Use of Composite Sampling for the Detection of *Listeria monocytogenes* in a Variety of Food Products

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The current Food and Drug Administrative sampling procedures for *Listeria monocytogenes* specify a 25-gram analytical sample. Frequently cited sampling plans specify 15 to 60 analytical samples per production lot. Multiple test samples have the disadvantage of increasing the testing cost to food manufacturers. This study evaluated the effect of composite sampling on the detection of *L. monocytogenes* when analyzing hummus, surface-ripened cheese, cold-pack cheese, cream cheese, smoked salmon and blue cheese by the FDA cultural method and a PCR method. Composite sizes included 125 g and 375 g. Inoculation levels remained constant for each enrichment and targeted low levels. For each food type, 3 samples for each of 3 strains at 2 inoculum levels and composite size were analyzed. Composite results were compared to the non-composited, 25-g result. Compositing 125-g portions proved acceptable for analysis by the FDA method in all 6 food types and at the 375-g size with 3 food types (hummus, surface-ripened cheese, cream cheese). Compositing 125-g portions proved acceptable in 2 of the 6 food types by the PCR method (cream cheese, smoked salmon) and at the 375-g size with 1 food type (cream cheese). Detection of *L. monocytogenes* in surface ripened cheese and blue cheese may require different enrichments. Further research is recommended.

P5-14 Impact of Stomaching, Pulsifying and Dilution Ratio on Growth of *Listeria monocytogenes* in University of Vermont Medium

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The current USDA *Listeria* enrichment protocol requires that foods be enriched 1:10 in University of Vermont Medium (UVM), giving a detectable limit of 0.4 CFU/g. However, 125-g samples (e.g., five 25-g

composited samples) enriched in 1125 ml of UVM (detectable limit of 0.08 CFU/g) are now being used in collecting incidence data for *Listeria* risk assessments. In an attempt to circumvent problems associated with large enrichment volumes, 125-g samples of delicatessen turkey breast were surface-inoculated with a 6-strain *L. monocytogenes* cocktail to contain ~1 x 10^3 CFU/g. After 30 min, samples were diluted 1:1 in UVM and processed in a Stomacher or Pulsifier for 2 min. Thereafter, sufficient volumes of UVM were added to obtain dilution ratios of 1:2, 1:3, 1:5, and 1:10. All enrichments were assessed for growth of *L. monocytogenes* at 0, 2, 4, 6, 9, 11.5, 13.5, and 24 h of incubation at 37°C by plating on Modified Oxford Agar with generation times calculated using the 6 to 13.5 h time points. Generation times for the 1:2, 1:3, 1:5, and 1:10 dilutions processed using the Stomacher were 49.8, 49.7, 48.3, and 49.2 min, respectively; whereas the Pulsfier yielded generation times of 42.6, 47.5, 52.5 and 45.2 min for the 1:2, 1:3, 1:5 and 1:10 dilutions. Generation times for stomached samples varied by < 1.5 min, indicating that all four dilutions yielded equivalent growth.

P5-15 Rescheduled to P1–56

P5-16 Comparison of Four Half-Fraser Enrichment Broths for the Detection of *Listeria monocytogenes* in Foodstuffs

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The goal of this study was to compare the performances of 4 half-Fraser enrichment broths for the detection of *Listeria monocytogenes* in food products with the VIDAS LMO2 method. Most of the products included in this study were raw products showing a high risk of contamination by *L. monocytogenes*. 145 food products were studied, which included 54 meat products (43 raw meat products, cooked pork meat and uncooked poultry), 81 dairy products (among which 69 were raw milk-based) and 10 seafood products (among which 8 were smoked salmon). Twenty one of these products were artificially-contaminated and 124 were naturally-contaminated or uncontaminated. Out of the 70 samples found positive with the immunoassay, 58 were found positive after enrichment in half-Fraser A, 35 in half-Fraser B, 32 in half-Fraser C and 36 in half-Fraser D. A significant difference was observed in performance with the half-Fraser broth (A) compared to the three other broths (B, C & D). This may be explained by factors such as the choice and quality of peptones and the purity and stability of the selective agents which are known to have a significant influence on fertility and/or selectivity. The presence of high levels of competitive flora in the critical products tested requires the use of a half-Fraser broth with sufficient selectivity to promote the growth of *L. monocytogenes* while inhibiting competitive flora. In conclusion, the choice of half-Fraser should be subject to a comparative study which includes both the food matrices analysed in the laboratory and the detection method used.

P5-17 Evaluation of a Novel Enrichment Broth and Chromogenic Medium for the Detection of *Listeria monocytogenes*

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Rapid and consistent detection of *Listeria monocytogenes* remains an important priority in control of food safety. Conventional culture methods can take 5 days for a confirmed negative result, typically involving use of two enrichment media, two isolation media and two plating intervals. Chromogenic isolation media have been introduced that significantly improve the overall test method but little has been done to improve the enrichment stage. In this study, a new enrichment medium (Oxoid Novel Enrichment Broth – *Listeria*; ONE Broth) combined with a chromogenic plating medium (Oxoid Chromogenic *Listeria* Agar; OCLA) were compared with the ISO method for the detection of *L. monocytogenes* in naturally contaminated and spiked foods. Enrichment in ONE Broth followed by plating onto OCLA detected more positive samples (159/182) after a single overnight incubation than the Half-Fraser and Full-Fraser combination (156/182). In 153 of the samples positive by ONE Broth, there were consistently in excess of 100 *L. monocytogenes* colonies per OCLA plate compared with only 139 samples by Half-Fraser. Direct comparisons of the growth of two strains of *L. monocytogenes* spiked into a range of foods showed that ONE Broth supported at least a ten-fold higher increase in target cell numbers than Half-Fraser. ONE Broth in combination with OCLA detected more positive samples than the ISO method and consistently generated higher numbers of *L. monocytogenes* than Half-Fraser. The One Broth and OCLA method is recommended as a rapid culture-based alternative to conventional two-part enrichment combinations for the detection of *L. monocytogenes*.

P5-18 Evaluation of New BBL™ CHROMagar™ *Listeria* for Isolation of *Listeria monocytogenes* in Foods

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The new BBL™ CHROMagar™ *Listeria* was evaluated for its ability to detect *Listeria monocytogenes* in various food matrices. The performance of the medium was compared to other non-chromogenic selective media commonly used for *Listeria* isolation (Oxford, Modified Oxford, and PALCAM). The sensitivity of BBL™

CHROMagar™ *Listeria* for detection of *L. monocytogenes* in spiked and naturally contaminated samples from 50 different food matrices was 99% and 100% respectively, whereas the specificity was 100% in both spiked and naturally contaminated samples. BBL™ CHROMagar™ *Listeria* produced similar colony characteristics for both *L. monocytogenes* and *L. ivanovii* forming a white precipitation zone (halo) around blue colonies. Since *L. monocytogenes* is a known human pathogen while *L. ivanovii* is pathogenic to animals (with some human cases reported), it is advantageous to be able to detect both species and use simple biochemical tests (L-Rhamnose and D-Xylose utilization) to differentiate the two species. The sensitivity of the non-chromogenic media was 98-99% with some spiked samples producing negative results for *L. monocytogenes*. These media were not differential for *L. monocytogenes* or *L. ivanovii*. The use of BBL™ CHROMagar™ *Listeria* offers the ease of detecting *L. monocytogenes* and *L. ivanovii* as early as 24 h of incubation, with relatively high sensitivity and specificity of detection.

P5-19 Evaluation of a New Chromogenic Media, the Ottaviani and Agosti Agar, for the Detection of *Listeria monocytogenes* in Food Products and Environmental Samples

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The performance of a new rapid method for *Listeria monocytogenes* detection using the bioMérieux prepoured chromogenic medium, Ottaviani and Agosti Agar (OAA), in comparison with the ISO method, has been validated. OAA was also compared to ALOA agar, for specific *L. monocytogenes* isolation as part of the ISO 11290-1 reference method. 263 food and 72 environmental samples were tested by both the rapid OAA method and ISO 11290-1. For the OAA rapid method, samples were pre-enriched 24h at 30°C in half-Fraser broth. Then 100 µl were streaked onto OAA and incubated for 24 h at 37°C. Presumptive positive colonies (blue turquoise with an opaque halo) were further confirmed either by traditional tests or directly with the Accuprobe™ *Listeria monocytogenes* assay. The ISO method detected 150 positive products with OAA medium and 149 positives with ALOA medium. 138 of these were positive after 10 µl isolation of the half-Fraser pre-enrichment broth on OAA medium, and 133 after isolation on ALOA. After Fraser broth enrichment, 148 products were positive on OAA medium and 147 on ALOA. Rapid OAA detected 148 positive samples, 98% in agreement with ISO. In conclusion, the rapid OAA method was comparable to the ISO 11290-1/A1:2004 for the recovery of *Listeria monocytogenes* in food and environmental samples. Rapid OAA protocol allows release of negative products within 48 h compared with 5 days for the standard cultural method. Furthermore, the use of the Accuprobe *Listeria monocytogenes* assay allows the same day confirmation of presumptive *Listeria monocytogenes* colonies. The OAA medium also compared favourably to the ALOA medium in terms of sensitivity and specificity when used as part of the ISO 11290-1/A1:2004 isolation method.

P5-20 DSC Comparison of 3M™ Petrifilm™ Environmental *Listeria* Plate vs. Standard Methods in Detecting *Listeria* from Environmental Surfaces

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New Federal regulations are stressing the importance of detecting *Listeria* on environmental and food contact surfaces to eliminate contamination of ready-to-eat food products. Standard methods can take up to a week for results whereas the 3M™ Petrifilm™ Environmental Listeria Plate, a no-enrichment culture medium with a chromogenic indicator, provides results within 27 to 31 h (1). This study was conducted to evaluate the performance of Petrifilm™ Environmental Listeria Plates when recovering *Listeria* from dairy plant environmental samples. Samples were taken from 68 environmental and food contact surfaces using Bacti-sponge kits (Hardy Diagnostics) and placed in coolers. Upon arrival at the lab, 15 ml of buffered peptone water was added to each sampling bag, stomached and incubated at room temperature (20°C) for 1 hour. After 1 h, 100 µl of the BPW was plated on Oxford for direct enumeration and 3 ml on the Petrifilm Plate and incubated at 35°C for 24 h and 28 h respectively. For the standard method, 1/2 of the BPW and the sponge were added to 90 ml UVM broth and the other 1/2 to 90 ml Listeria Repair Broth (LRB). From this point, standard FDA methods were followed. Listeria positive samples from the standard methods were ribotyped. Sensitivity was > 90% and the accuracy was > 95% for the two methods. Results indicated that 3M™ Petrifilm™ Environmental Listeria Plates are a time-saving alternative to standard methods.

P5-21 Concentration of *Listeria monocytogenes* from Artificially Contaminated Mayonnaise-based Deli Salads Using Cultural and PCR Detection Methods

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Rapid detection of pathogens in food has been difficult due to the necessity of large, representative samples, which complicate DNA extraction and PCR amplification without prior enrichment. In this study, we have successfully incorporated a 2-step centrifugation as a means to concentrate *L. monocytogenes* from

commercial deli salads, while eliminating much of the food matrix. DNA extraction and PCR amplification were conducted targeting the iap (371 bp) gene and a unique region of 16S rDNA (287 bp). Growth/survival of *L. monocytogenes* in the salads was also determined. For 2-step centrifugation, mean cell recovery in order was potato (67%) > seafood (49%) > chicken (36%) > macaroni (34%) salad. Following 2-step centrifugation, PCR detection limits were 10^4 CFU/g for macaroni and chicken, and 10^3 CFU/g for potato and seafood salad using the iap target and the 16S target. Without sample preparation, PCR detection limits from deli salads were 10 to 100 fold less sensitive than concentrated samples. Higher cell recoveries by cultural methods (potato = 67%, seafood = 49%) were correlated with improved PCR detection limits (potato, seafood 10^3 CFU/g). Seafood salad supported growth of *L. monocytogenes* at 25°C and cell populations remained stable at 5°C for 2 weeks. *L. monocytgenes* numbers remained stable at 25°C (48 h) and declined one log at 5°C (2 weeks) in the other deli salads. This study illustrates the importance of reducing food components (protein, starch, fats) in complex foods such as deli salads prior to DNA extraction and PCR amplification.

P5-22 A New Method for Next Day Detection of *Listeria* in Environmental Samples

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The objective of this study was to evaluate a new "next day" immunoassay for detection of *Listeria* species in environmental samples, the VIDAS® *Listeria* Species Xpress (LSX). Samples are culturally enriched for a minimum of 24 h in a newly formulated *Listeria* enrichment broth (LX broth), before testing in the VIDAS® instrument. Positive results are then confirmed by streaking enrichment broths onto selective agar. The study was designed to compare the performance of the new assay with the USDA Environmental Sponge method. Part 1 of the study included 59 swabs from food manufacturing areas (dairy, poultry and fresh produce); of these naturally contaminated samples, the LSX detected 11 confirmed positives for *Listeria*, compared to 10 confirmed positives with the USDA method. In part 2, an inoculated surface study, 25 replicate areas (5 uninoculated and 20 "low" level inoculum) were tested for each of 4 surfaces (stainless steel, plastic, rubber and ceramic tile). For the 100 samples tested, 52 were confirmed positive by the LSX and 46 were confirmed positive by the USDA method. All positive results in the LSX assay were confirmed by subculture from LX broth. There were no false positive results for the LSX assay. The VIDAS *Listeria* Express provides a very rapid, sensitive and convenient method for the detection of *Listeria* in environmental samples, allowing a presumptive result within 26 h of sample set up.

P5-23 A New Method for the Simultaneous Detection of *Listeria monocytogenes* and *Listeria* Species in Food Products

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The goal of this study was to verify the performance of VIDAS *Listeria* DUO, a new automated immunoassay which allows the simultaneous detection of *Listeria monocytogenes* (LMO) and *Listeria* species (LIS) in food products. Food samples were culturally enriched in a 2-step enrichment including a specific *Listeria* enrichment broth (LX broth), prior to VIDAS detection. The VIDAS reagent includes monoclonal antibodies directed specifically against a soluble LMO antigen and monoclonal and polyclonal antibodies directed specifically against *Listeria flagellar* antigens. The reagent performed satisfactorily when tested with pure cultures as the 30 LMO strains tested positive, the 38 *Listeria* non monocytogenes strains tested negative in the LMO response and positive in the LIS response and the 20 non-*Listeria* strains tested negative in both responses. 161 food products, including 69 meat products and 92 dairy products (mainly raw milk-based) were studied by the new method in comparison with the cultural reference method ISO 11290-1/A1:2004. Overall, for the 161 samples tested, the *Listeria* DUO method detected 21 confirmed positives for *L. monocytogenes* and 38 confirmed positives for *Listeria* spp., compared to 18 and 32 positives respectively, with the reference method. There were no false positives for the *Listeria* DUO assay. The new method compared favourably with traditional method in sensitivity and specificity. Furthermore, it allows the detection of both *L. monocytogenes* and *Listeria* spp. in a single test and it requires only 2 days for negative or presumptive positive results compared to 4 days for the traditional method.

P5-24 A Comparative Study of VIDAS LSX Method for the Next Day Detection of *Listeria* sp. in Food Products and the ISO 11290-1 Reference Method

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We describe the results of an inter-laboratory study of the VIDAS LSX immunoassay method for the detection of *Listeria* sp. in food products, in comparison with the ISO 11290-1 reference method. For the LSX method, samples were pre-enriched for 22 to 24 h at 30°C in LX broth. 3 ml of this pre-enrichment were then inoculated into 6 ml of LX broth for 6 to 8 h at 30°C, before testing in the VIDAS instrument. Presumptive positive samples were further confirmed after isolation onto a selective agar medium (PALCAM agar or a

chromogenic agar which differentiates *Listeria monocytogenes* from other *Listeria* species). 790 food products (197 meat products, 242 dairy products, 191 vegetable products and 160 miscellaneous products) were tested by the two methods in three different laboratories. A total of 330 products were found positive (149 naturally contaminated and 181 artificially contaminated products). The LSX method detected 308 positive samples (sensitivity 93.3%) and the ISO method 290 positive samples (sensitivity 87.9%) with an agreement of 92.2%. The difference observed between the two methods was not statistically significant. In this study the VIDAS LSX method compared favourably to the ISO 11290-1 method for the recovery of *Listeria* sp. in food products. In addition it allows release of negative products within 30 h compared with 5 days for the standard cultural method. Moreover, the combination of the VIDAS LSX screening method with the use of a chromogenic plate medium, for confirmation of positive samples, can lead to the rapid identification of *Listeria monocytogenes*.

P5-25 Evaluation of Epidemiological Relevance of Multi-Virulence-Locus Sequence Typing for *Listeria monocytogenes*

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Multi-Virulence-Locus Sequence Typing (MVLST) was proven to have higher discriminatory power and lower cost than PFGE for molecular subtyping of *Listeria monocytogenes* in a previous study. However, the ability of MVLST to investigate *L. monocytogenes* outbreaks (epidemicological relevance) has not been evaluated. To evaluate the epidemiological relevance of MVLST for *L. monocytogenes*, randomly selected 23 *L. monocytogenes* non-outbreak isolates and 18 isolates from 4 major *L. monocytogenes* outbreaks (1985-2002) were subjected to MVLST. Isolates from each outbreak were clustered and non-outbreak isolates were distinguished from outbreak isolates. This study supports the potential use of MVLST as a routine approach for epidemiological investigation of *L. monocytogenes* outbreaks.

P5-26 Integrating Tangential Flow Filtration with Rapid Tests for Detection of *Escherichia coli* O157:H7, *Salmonella* and *Listeria monocytogenes* in Large Volumes of Spent Sprout Irrigation Water

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Microbiological testing of spent sprout irrigation water has been recommended as part of an overall strategy to enhance the safety of sprouts. However, the extra cost required for the testing has hindered the full implementation of this recommendation by the industry. Sample pooling has been proposed to lower the cost of testing. However, there is concern that pooling may reduce the detection efficiency due to dilution of target pathogens. One way to alleviate this problem is to institute a sample pre-concentration step that would allow the entire pooled sample to be analyzed by a single test. We have developed a tangential flow filtration (TFF) system capable of concentrating 10 L of sprout water by 100 fold within 2 h. This study examines the feasibility of integrating TFF sample pre-concentration with existing rapid tests for detection of low levels of *Escherichia coli* O157:H7, *Salmonella* and *Listeria monocytogenes* in large volumes of sprout water. Two aspects of system performance were evaluated. The first was to determine whether the increase in background microflora in the concentrated samples affects the enrichment and subsequent detection of target pathogens by commercial tests, and the second was to determine the lowest level of pathogens that the system can recover. All the test kits evaluated were able to detect the presence of 1 CFU of the target pathogens in 10 ml of concentrated samples (corresponding to 1 L of sprout water sample prior to filtration). Recovery studies showed that as few as 10 CFU of *Salmonella* can be recovered from 10 L of sprout water. These results suggested that incorporation of TFF could improve the detection limit of existing rapid tests to 1 CFU/L and thus allow the testing of 10 L of sprout water with a single test.

P5-27 Evaluation of an Automated Immunomagnetic Separation System for Selective Isolation of *Escherichia coli* O157, *Salmonella* and *Listeria monocytogenes* from Inoculated Spent Sprout Irrigation Water

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The isolation of target pathogenic organisms from sprouts or spent sprout irrigation water is often difficult due to the high level of background microflora and other interfering substances present in the samples. Immunomagnetic separation (IMS) technology has been increasingly used for the selective isolation of pathogens from complex food matrices and culture enrichments. An automated IMS system (Pathatrix system from Matrix Microscience) has become commercially available and has been performance certified by the AOAC International for detection/isolation of *Escherichia coli* O157:H7, *Salmonella* spp. and *Listeria* spp. at levels of 1 cell/25 g. In this work, the efficacy the Pathatrix system to selectively isolate *E. coli* O157:H7, *Salmonella* and *L. monocytogenes* from spent irrigation water collected during sprouting of alfalfa seeds was evaluated. To test the feasibility of combining sample pre-concentration with IMS for pathogen detection in greater volumes of sample, sprout water that has been pre-concentrated 100 fold was also included in the evaluation.

Twenty-five ml of un-concentrated and concentrated sprout water was inoculated with 0, 1, 10, and 10^0 CFU of the pathogens and subjected to the enrichment and capture protocols recommended by the manufacturer. The efficacy of the Pathatrix system was determined by plating the IMS beads after capture on selective media and looking for typical colonies. Using the recommended enrichment protocol, the system was able to isolate *Salmonella* and *L. monocytogenes* from sprout water inoculated at levels of 10 CFU or higher per 25 ml but failed to isolate *E. coli* O157:H7 due to interference from background flora. Modifications of the enrichment protocol could improve the detection limit for *Salmonella* and *L. monocytogenes* to as few as 1 CFU per 25 ml for both unconcentrated as well as concentrated samples. In summary, with modified enrichment protocols, the combination of sample pre-concentration with IMS could allow the selective isolation of *Salmonella* and *L. monocytogenes* from sprout water at levels as low as 1 CFU per 2.5 L.

P5-28 Evaluation of a Novel Sample Pooling Strategy for High Volume Screening and Detection of *Escherichia coli* O157, *Salmonella* spp. and *Listeria* spp. in Food

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We describe the development of a sample pooling strategy for the screening and detection of *Escherichia coli* O157, *Salmonella* and *Listeria* that enables rapid screening of samples for presumptive positives. The principle of the technique involves the simultaneous screening of 5 samples, with the ability to rapidly re-test individual samples within the pool within 1 to 2 h to identify individually positive samples. A novel high sample volume (analyses 250 ml samples) Immunomagnetic Separation (IMS) system called Pathatrix was used to capture and concentrate the pathogens from the pooled samples (5 × 50 ml individual sample enrichments) and this concentrate was then analysed by, agar plating, PCR and in the case of *Salmonella* a colorimetric assay called Colortrix. Only 1 of the 5 samples in any pooled sample was positively inoculated at low levels (1 to 10 CFU/25 g) to provide the toughest challenge to the system. For the *Salmonella* study a total of 103 samples pooled samples (represents 515 individual samples) were analyzed using the Pathatrix+ agar plating, PCR and Colortrix methods. For the *Listeria* study a total of 140 samples pooled samples (represents 700 individual samples) were analyzed using the Pathatrix + agar plating and PCR methods. For the *E. coli* O157 study a total of 24 pooled 375 g samples (represents 120 individual samples) and 7 un-pooled samples were analyzed using the Pathatrix+ agar plating and PCR. Data for all 3 pathogens analyzed indicated that Pathatrix pooling could provide the food industry with a rapid and cost-effective tool for the screening and detection of pathogens.

P5-29 Multiplex PCR for Simultaneous Detection of *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in Meat Samples

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The multiplex PCR system has been considered for the simultaneous detection of pathogens since the expense of reagents and the preparation time are less in multiplex PCR than in systems where several tubes are used. In this study, we describe the development of a multiplex PCR method capable of identifying *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* O157:H7 directly from enrichment culture of meat samples. In addition, we have evaluated the optimization of pre-enrichment medium, DNA extraction method and the multiplex PCR setting. Detection sensitivity of this method showed that DNA from 10^3 CFU/mL of each pathogenic bacteria could be detected. When this protocol was used for the detection of each of the above pathogenic bacterium in spiked pork samples, 10^0 cells/25 g of inoculated sample could be detected within 30 h. Also in the samples of naturally contaminated meat, *Salmonella* spp., *L. monocytogenes*, and *E. coli* O157:H7 were detected by the same time period. Excellent agreement of the results of multiplex PCR with that of conventional culture method suggests that the multiplex PCR is a reliable and useful method for rapid detection of *Salmonella* spp., *L. monocytogenes*, and *E. coli* O157:H7 contamination in meat products. In conclusion, the multiplex PCR assay described in this study for the simultaneous detection of *Salmonella* spp., *L. monocytogenes*is, and *E. coli* O157:H7 is capable of detecting as few as 1 CFU/25 g of any of these organisms in raw meat after enrichment cultivation for 24 h.

P5-30 PCR-based Fluorescent Method for Rapid Detection of *Campylobacter jejuni*, *Salmonella* Typhimurium, *Escherichia coli* O157:H7, and *Listeria monocytogenes* in Poultry Samples

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A DNA binding fluorescence method based on polymerase chain reaction (PCR) products was evaluated for rapid detection of *Campylobacter jejuni*, *Salmonella* Typhimurium, *Escherichia coli* O157:H7, and *Listeria monocytogenes* in poultry samples. Samples of chicken carcasses and ground turkey were separately inoculated with *C. jejuni*, *S.* Typhimurium, *E. coli* O157:H7, or *L. monocytogenes* to obtain final concentrations of 10^0 to 10^5 CFU/ml. One ml of each sample was used to get the DNA template and 5 micro-liter of the sample template was added into 25 micro-liter of SYBR Green PCR Master Mix and two specific primers C1, C4

(*C. jejuni*), S29, S30 (*S.* Typhimurium), UidAa, UidAb (*E. coli* O157:H7), or FP, RP (*L. monocytogenes*). The negative control was the same except 5 micro-liter of each wash solution was added instead of 5 micro-liter sample template. The reaction was carried out in a thermocycler. Finally, the fluorescence signal of each PCR product was measured using a fluorometer. The PCR products were also confirmed by ethidium bromide agarose gel. The results showed that when bacterial cells increased from 0 to 2 CFU/ml, the fluorescence signal increased significantly. The PCR-based fluorescence method could detect the target bacteria in min after PCR amplification compared to h by gel electrophoresis and also could be done at an earlier time during PCR amplification. The detection limit of this method for the poultry samples was 5 CFU/ml for *C. jejuni* and 2 CFU/ml for all *S.* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* without any enrichment.

P5-31 Nanobeads-based Biosensor for Rapid Detection of Pathogenic Bacteria in Poultry and Meat Samples

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Magnetic nanobeads were coated with specific antibodies and used in both separation and detection of target pathogens in poultry and meat samples by use of an impedance immunosensor. Antibodies were immobilized on the surface of the magnetic nanobeads by use of streptavidin and biodin conjugation technique. *Salmonella* Typhimurium or *Escherichia coli* O157:H7 were artificially inoculated into poultry and meat samples. After the sample was mixed with the magnetic immuno-nanobeads, target pathogens were separated by applying a magnetic field and washing away the rest. Then the magnetic nanobeads were sent to the biosensor, consisting of a gold microelectrode in a flow cell and an impedance detector. The microelectrode was immobilized with specific antibodies by the protein A method to capture the target pathogen bond with the magnetic nanobeads. The impedance value of the target pathogen with nanobeads was measured at 10 kHz and was correlated to the cell number of target pathogens in the food sample. The results showed that the magnetic immuno-nanobeads-based impedance immunosensor could detect *E. coli* O157:H7 and *S.* Typhimurium in poultry and meat samples with a detection limit of 2×10^2 cells in 1 h. The detection range was from 10^2 to 10^6 cells/ml with a linear calibration line. The biosensor showed its advantages in highly efficient separation, sensitive detection, and enhanced automatic operation for detection of foodborne pathogens.

P5-32 Detection of an Array of Foodborne Pathogens by Use of Universal Primers and Specific Oligoprobes

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Food safety and security are of continuing concern throughout the world. Unfortunately, most pathogen detection methods are designed to test for a single organism per assay. The purpose of this research is to develop methods aimed at detecting multiple pathogenic organisms in a single assay. The general approach employs non-specific bacterial concentration from the sample matrix followed by specific detection and endpoint confirmation with nucleic acid amplification coupled with oligonucleotide array hybridization. In this phase of the study, we used universal primers to amplify an approximate 1.5 kb product of the 16s rRNA bacterial genome. PCR amplification conditions were optimized for *Escherichia coli* O157:H7, *E. coli* DH5-, *Salmonella enterica* serovar Enteritidis, *Listeria monocytogenes*, *Vibrio vulnificus* and *Shigella flexneri*. Detection limits by gel electrophoresis were 10^3–10^4 CFU/ml for various strains of *E. coli*; 10^2 CFU/mL for *S. enterica* serovar Enteritidis and *L. monocytogenes*; and 10^3 CFU/ml for *V. vulnificus* and *S. flexneri*. Specific digoxigenin-labeled oligoprobes and dot blot hybridization were used to confirm *Salmonella* and *L. monocytogenes* amplicons, with improvement in detection limits to 10^1 CFU/ml. The specificity of these probes was confirmed by testing against amplification products obtained using the universal primers as applied to a variety of foodborne bacteria. Studies continue to identify internal oligoprobes to confirm 16S rDNA amplicons from other foodborne pathogens, and to develop an oligonucleotide array approach for multiple pathogen detection/confirmation. Methods based on the use of universal amplification primers can have advantages, including the ability to detect multiple bacterial targets in a single assay.

P5-33 Rescheduled to P2-02

P5-34 Adaptive Response to Environmental Stresses by *Campylobacter jejuni*

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Campylobacter jejuni is one of the leading causes of acute bacterial gastroenteritis in humans. Poultry has been implicated as a possible source of *C. jejuni* in human infection. During poultry processing, *C. jejuni* may encounter different kinds of environmental stresses and *C. jejuni* cells surviving may become stress hardened. To determine the survival and adaptive responses of *C. jejuni* to environmental stresses, laboratory strains of *C. jejuni* were exposed to sub-lethal stresses of different pH values, different atmospheres, or starvation in 0.1M PBS to induce any adaptive stress response. After the stressed cultures were allowed to recover, both the non-

stressed and stressed cultures were exposed to the same stresses or different stresses. The CFU/ml of each culture was determined and survivor plots constructed. Results indicate that some *C. jejuni* isolates form an adaptive tolerance response to high acid and aerobic environments. Acid and aerobically stressed cells were able to survive homologous stress twice as long (100 min) as non-stressed cells. After being exposed to acid and aerobic stress, some *C. jejuni* did not have protection from further starvation stress. These results indicate stressed isolates may survive further stress in the form of passage through the human gastrointestinal tract better than non-stressed isolates. Comparing the ability of pre-processing and post-processing isolates to survive environmental stress will be the focus of continuing research.

P5-35 Highly Virulent *Campylobacter jejuni* in Retail Raw Chicken Carcass Rinses

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Campylobacter jejuni were easily obtained by direct plating of rinses from retail raw chicken carcasses and such randomly collected *C. jejuni* isolates were tested for virulence against Caco-2 cells. Confluent Caco-2 cell monolayers in 24-well tissue culture plates were exposed to 10^8 CFU/well of *Campylobacter jejuni* for 2 h to allow infection of Caco-2 cells, followed by treatment with 120 μg/ml gentamicin for 2 h to kill extracellular *Campylobacter* cells. After gentamicin removal and wash step, Caco-2 cells were then direct plated on Bolton agar to recover intracelluar *C. jejuni* from Caco-2 infection. The majority of the *C. jejuni* isolates tested successfully infected target Caco-2 cells within 2 h after challenge. As many as 10^3 to 10^4 CFU/well of intracellular *C. jejuni* were recovered from Caco-2 cells which were challenged with 10^7 to 10^8 CFU/well of spiral cells of *C. jejuni* isolates. Under the same test protocol, only two of the 22 isolates of *C. jejuni* tested from retail raw chicken carcass rinses were found to be weakly virulent, yielding less than 10^2 CFU/well of intracellular *C. jejuni* from Caco-2 infection. These results indicate predominance of highly virulent *C. jejuni* in rinses from retail raw chicken carcasses using the Caco-2 invasive assay.

P5-36 Cross-contamination and Enhanced Attachment to Chicken Skin by *Campylobacter jejuni* Biofilm Isolates

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Campylobacter jejuni is a primary cause of diarrheal associated foodborne illness. Our research focused on investigating any enhanced attachment to processing materials and chicken skin by *Campylobacter jejuni* biofilm isolates versus planktonic isolates and survival under processing conditions. Over 150 *C. jejuni* isolates from chicken carcasses, turkey carcasses and humans were screened for biofilm formation, using a 96-well plate assay. Three biofilm and three planktoinc isolates were chosen. Using a 24-well plate, isolates were cultured with 1.5 cm^2 stainless steel, plastic, or rubber coupons or chicken skin in a low temperature, low nutrient, or chlorine concentration (20 ppm) environment. After 48 h, two coupons were removed and stained for direct microscopic observation. The other coupons were rinsed and vortexed with glass beads to remove biofilms. Chicken skin was also rinsed and vortexed but without glass beads. Plates were made of the rinses and counted to determine the number of attached cells. Results indicated an enhancement in attachment (two logs) to the chicken skin and stainless steel by biofilm formers over planktonic isolates. Biofilm formers attached to plastic better than to rubber. In addition, biofilm formers were able to survive processing conditions of low nutrients and chlorine exposure better than planktonic isolates. *C. jejuni* biofilm formation and survival under poultry processing conditions may be possible and may be a source of cross contamination.

P5-37 Identification of *Campylobacter* Isolates from Farms by PSTL Ribotyping

DSC

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The objective of this study was to use automated ribotyping to determine the feasibility of using the Qualicon Riboprinter to identify *Campylobacter* in farm animal isolates. A total of 122 *Campylobacter* isolates were randomly selected from 332 isolates confirmed as *C. jejuni*, *C. coli*, or *C. lari* (2002 to 2004). Isolates were obtained from 5 production facilities (swine, chicken, turkey, dairy and beef farm) in 5 geographical locations (Tennessee, Alabama, North Carolina, California, and Washington State). *Campylobacter* isolates were analyzed by use of the DuPont Qualicon's RiboPrinter® Microbial Characterization System with the PstI restriction enzyme. Three ATCC reference strains (*C. jejuni* 29428, *C. coli* 43133 and *C. lari* 35221) were also analyzed. The ribotype pattern for each isolate was automatically compared to all *Campylobacter* RiboPrint patterns stored in the RiboPrinter® database, which included 135 isolates of *C. jejuni*, 24 of *C. coli*, 11 of *C. lari*, 6 of *C. jejuni* subsp. *jejuni*, and 1 of *C. jejuni* subsp. *doylei*. Only 9 of the 122 (7.4%) animal isolates and *C. coli* ATCC 43133 had DuPont identification threshold similarity of > 0.85 and were automatically identified by ribotyping. One-hundred and thirteen of the isolates (93%) evaluated in our study had DuPont identification threshold similarity of < 0.85 and had to be identified manually by DuPont Qualicon's Manual DUP identification criteria. The DuPont Qualicon database is a work-in-progress and identification of *C. jejuni*, *C. coli*, and *C. lari* isolates from farm samples will require addition of farm isolates to the database before accurate identification of thermophilic *Campylobacter* species from farms can be expedited.

P5-38 A Multiplex PCR Assay for Speciating *Campylobacter coli* and *Campylobacter jejuni* Strains by Partial Sequencing of Virulence Genes

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Campylobacter coli and *C. jejuni* are common causes of gastroenteritis in humans. The objective of this study was to utilize a multiplex PCR assay for concurrent detection of *Campylobacter* spp. and *C. coli* or *C. jejuni*, using probes derived from virulence genes *cad*F and *ceu*E and an undefined gene. A total of 97 *Campylobacter* strains, isolated from turkey litter (n = 74), chicken livers (n = 15) and clinical (n = 8) samples, were speciated using the PCR-based assay. PCR amplification of the isolates identified a 400-bp *cad*F gene, conserved in *Campylobacter* species, an 894-bp *ceu*E gene, specific for *C. coli*, and a 160-bp oxidoreductase gene, specific for *C. jejuni*. The ~35 KDa *cad*F adhesion proteins allow *Campylobacter* to bind to the intestinal epithelial cells and the 37 KDa *ceu*E lipoproteins are involved in siderophore transport. Sequencing and BLAST search of the 160-bp undefined gene yielded a 67% protein identical match with a gene encoding an oxidoreductase subunit in *C. jejuni*. The specificity of the assay was validated on 36 non-*Campylobacter* strains (11 Gram-positive and 25 Gram-negative bacteria). The PCR assay identified 59% of turkey and 47% of chicken isolates as *C. jejuni*, and 41% of turkey and 53% of chicken isolates as *C. coli*. All human isolates were identified as *C. jejuni*. The specificity of this assay to detect *C. coli* or *C. jejuni*, by concurrent detection of the 400-bp (*cad*2B) and 894 (*ceu*E) or 160-bp band was 97%. Rapid identification of *Campylobacter* spp. can be vital in the treatment of campylobacteriosis.

P5-39 Effect of Freeze-stress on Enrichment, Isolation, and Virulence of Plasmid-bearing Virulent *Yersinia enterocolitica* in Pork Chops

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Relatively little is known concerning the ability of plasmid-bearing virulent *Yersinia enterocolitica* (YEP^+) to adapt to freeze stress conditions in raw pork products. Furthermore, the ability to isolate/detect low levels of YEP^+ in pork that is frozen prior to testing may be hindered. Therefore, the influence of freeze-stress at -20°C on the enrichment, isolation, detection, presence of virulence plasmid, and expression of virulence of YEP^+ inoculated on pork chops (PC) was assessed. The PC artificially contaminated with 10, 1, and 0.5 CFU/cm² of YEP^+ (strain GER O:3) were placed in sterile petri dishes at -20°C for 24 h. The PC were swabbed when frozen, and after thawing at room temperature (RT) and 40°C. Swabs were enriched in modified trypticase soy broth containing yeast extract and bile salts at 120°C for 24 h, and then again incubated for 24 h after the addition of 4 μg/ml of Irgasan. The YEP^+ were isolated by Congo red (CR) binding on CR brain heart infusion agarose (CR-BHO), and the presence of virulence plasmid was assessed by the appearance of red pinpoint colonies showing low calcium response (Lcr) and CR binding. The YEP^+ were isolated under all conditions on PC inoculated with 10 CFU/cm² and at a level of 1 CFU/cm² when thawed at RT and at 4°C but not from frozen PC. The YEP^+ was not isolated from PC inoculated with 0.5 CFU/cm² and then frozen, whereas YEP^+ were recovered at this level from PC not subjected to freezing. Results of a multiplex PCR targeting the chromosomal *ail* gene and a plasmid-associated *vir*F gene further confirmed that YEP^+ isolates from CR-BHO possessed the virulence plasmid. Moreover, multiple *in vitro* virulence assays including dye binding, Lcr, autoagglutination, and hydrophobicity indicate that these freeze-stressed isolates are potentially capable of causing foodborne illness.

P5-40 Loss of Virulence by *Yersinia enterocolitica* under Various Environmental Conditions

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Most *Yersinia enterocolitica* strains isolated from natural environments are avirulent, and it has long been recognized that growth at high temperatures can lead to loss of virulence. In this study, we measured loss of virulence during long-term storage of cells under various environmental conditions. Virulent *Y. enterocolitica* cells were inoculated into water, peptone water, various concentrations of salt and sucrose in peptone water, and milk and incubated at 4°C, 25°C and 35°C. At various time intervals, samples were withdrawn and plated onto Congo Red (CR) agar, and the proportion of CR binding cells in the population was assessed. A multiplex PCR for the plasmid-associated virulence determinant yadA and 16S rDNA was used as a confirmation for virulence of colonies. The proportion of virulent cells in the population remained stable during storage in water at 4°C and 25°C for up to 6 months. In peptone water, growth at 4°C did not alter the proportion of virulent cells, but the population lost virulence over time at 25°C and at 35°C. The presence of up to 10% salt or 20% sucrose did not affect the rate of loss of virulence at 25°C. The ratio of virulent cells did not change appreciably during growth in milk at 4°C or at 25°C; however, growth in milk at 35°C caused a rapid loss, with virulent cells comprising less than 10% of the population within 3 weeks. These data will be useful in quantitative assessments of the risk of *Y. enterocolitica* in foods and natural environments.

P5-41 Comparison of Survival of *Yersinia enterocolitica* and *Yersinia pestis* under Various Environmental Conditions

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Deliberate contamination of foods with potential agents of bioterrorism is of concern, but there is a lack of data that describe the behavior of these agents in food. Although much is known about the survival of *Yersinia enterocolitica* in food, little information is available about the behavior of its close relative and potential agent of bioterrorism, *Yersinia pestis*. In this study, we compared long-term survival of *Y. enterocolitica* and an avirulent strain of *Y. pestis* under different temperatures (4°C, 25°C and 35°C), during nutrient limitation (water vs. peptone water) and in varying salt (0 to 15%) and sucrose (0 to 60%) concentrations. Washed, stationary phase cells of each species were inoculated into liquid media at approximately 10^4 CFU/ml, incubated, and at various time intervals, samples were withdrawn and plated onto tryptic soy agar to assess population levels. In general, *Y. pestis* was shown to be more sensitive to environmental stresses than *Y. enterocolitica* by demonstrating faster declines in population levels. In water, *Y. enterocolitica* maintained stable population levels for up to 6 months at 4°C and 25°C but declined within a month at 35°C, while *Y. pestis* showed increasing inactivation rates at 4°C, 25°C and 35°C. *Y. pestis* was more rapidly inactivated by increasing salt or sucrose concentrations than *Y. enterocolitica*. These data will be helpful for assessing the use of *Y. enterocolitica* as a surrogate for *Y. pestis* and for risk assessments in potential contamination events.

P5-42 DSC Production and Characterization of Monoclonal Antibodies against *Clostridium perfringens* Alpha Toxin

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Clostridium perfringens phospholipase c (PLC), commonly known as alpha toxin, is the lethal, dermonecrotic toxin produced by all *Clostridium perfringens* strains and is considered a major virulence factor in clostridial myonecrosis. In this study, hybrodomas secreting monoclonal antibodies specific for *Clostridium perfringens* type A alpha toxin were produced by fusion of SP2/O myeloma cells with spleen cells from BALB/c mice immunized with alpha toxoid. Fifteen ELISA-positive hybridomas were selected and cloned twice by limiting dilution. These hybridomas were then grown as ascitic tumors in mice, and monoclonal antibodies were purified from the ascites fluids with ammonium sulfate. The antibody activity was evaluated by antigen-binding activity in ELISA, by immunoblot assay. These antibodies have sensitivity and specificity, and show promise for application to detection of alpha toxin produced from the foodborne pathogen *Clostridium perfringens*.

P5-43 Influence of Four Retail Foodservice Cooling Methods on the Behavior of *Clostridium perfringens* ATCC 10388 in Turkey Roasts following Heating to an Internal Temperature of 74°C

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The influence of four foodservice cooling methods (CM) on growth of *Clostridium perfringens* ATCC 10388 in cooked, ready-to-eat (RTE) turkey roasts was evaluated. Raw whole turkey roasts were inoculated with *C. perfringens* spores, vacuum packaged, and heated to an internal temperature of 74°C. The cooked roasts were cooled as follows: whole roast cut into 4 quarters and held at 4°C (CM1); whole roast held in a blast chiller (CM2); whole roast loosely wrapped and held at 4°C (CM3); and whole roasts (3 per bag) held at 4°C (CM4). Cooled roasts (5°C) were analyzed for *C. perfringens* by use of Shahidi-Ferguson perfringens (SFP) agar and anaerobic incubation (37°C, 24 h). None of the cooling methods met the amended 2001 FDA Food Code guidelines for safe cooling of potentially hazardous foods. Times taken for roasts to cool from 57°C to 21°C with CM1, CM2, CM3, and CM4 were 2.27, 3.11, 6.22, and 8.71 h, respectively. Times taken for roasts (21°C) to reach 5°C ranged from 6.33 h (CM1) to 19.45 h (CM4). No growth of *C. perfringens* occurred in roasts cooled with CM1 or CM2; however, populations of the pathogen increased by 1.5 log in the single whole roast (CM3) and by 4.0 log in the three bagged roasts (CM4). These findings indicate that certain foodservice cooling methods involving refrigeration (4°C) of whole cooked turkey roasts may result in proliferation of *C. perfringens* that compromises the microbial safety of this RTE meat product.

P5-44 Protein A, A Source of False Positive Results for Staphylococcal Enterotoxins (SET) Detection in Food Products

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Immunoassays are the most commonly used methods for enterotoxin detection in food products. For milk products, concentration of the food extracts is recommended to increase the sensitivity of detection. This is achieved either by dialysis of the food extract against a polyethylene glycol (PEG) solution or precipitation

with trichloroacetic acid also after purification on immunoaffinity gel chomatography. In this study, we compared the results of two immunoassay kits for SET detection (VIDAS SET2 and Transia Plate SET) and the three concentration systems previously described, on two ewes' milk cheeses which previously tested positive in an Industrial Laboratory.The two products were found positive by the Transia Plate reagent without concentration and after concentration by PEG and immunoaffinity purification but not after TCA precipitation. These products tested negative by the VIDAS SET2 reagent whatever the concentration method used. The positive results obtained with the Transia Plate method were totally suppressed by the addition of rabbit serum into the concentrated food extract. Complementary analysis (gel filtration, immunoblot and protein A assay) demonstrated that protein A was at the origin of the positive signal obtained with the Transia Plate kit. In conclusion, it appeared that research of SET in food products should take into consideration the presence of protein A synthesized by the staphylococcal organisms. The VIDAS SET2 reagent does not give false positive results due to the presence of Protein A. The TCA concentration method suppresses interference contrary to the PEG or the immunoaffinity concentration methods.

P5-45 A Comparative Study of Methods for the Detection of *Staphylococcus aureus* in Food Samples

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Staphylococcus aureus are pathogenic bacteria of significance to the food industry. They are associated with many food intoxication outbreaks following ingestion of the enterotoxins produced by these organisms. The presence of *S. aureus* in processed foods or on food processing equipment is usually an indication of poor sanitation practices. Simple, rapid detection methods would aid in screening for these microorganisms. In this study, a rapid method, the TECRA *Staphylococcus aureus* Visual Immunoassay (STAVIA), was compared with two commonly used AOAC Reference Methods, the spread plate and MPN procedures. The study focused on three foods, skimmed milk powder, infant formula and whole egg powder. Each food was tested for natural contamination with *S. aureus*, and also following inoculation with the organism at low level (1 to 5 cells/g) and high level (10 to 50 cells/g). For each food, 36 replicate samples were tested in a blind study, which paired the STAVIA and the two reference methods. Results showed that the unspiked foods were not contaminated with *S. aureus*. For foods spiked at the high level, the TECRA method detected *S. aureus* in all 36 samples, while the spread plate and MPN procedures each detected 34/36 samples. For foods spiked at the low level, the TECRA and MPN methods were equivalent, but the spread plate method detected significantly fewer contaminated samples. This study shows that the TECRA STAVIA is at least as accurate as two commonly used standard methods for the detection of *S. aureus*, and provides a faster result.

P5-46 Detection of *Shigella* spp. by Selective Plating and PCR Methods after Aerobic Enrichment in *Shigella* Broth

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The current FDA method for detecting *Shigella* spp. requires anaerobic incubation followed by presumptive and confirmatory tests that are time and space consuming. Shigellae are usually present in low numbers in foods and difficult to detect by conventional methods, particularly in foods with a high background microflora. This study was conducted to evaluate aerobic enrichment for the isolation and detection of low numbers of *Shigella* spp. from artificially contaminated *Shigella* broth, potato salad and cilantro rinse by selective plating and by PCR. To assess the suitability of aerobic enrichment, samples were cultured in *Shigella* Broth (SB), SB with 0.5% novobiocin and SB with 3% novobiocin at 40°C. Evaluations were performed with *S. sonnei*, *S. flexneri*, *S. boydii* and *S. dysenteriae* at four inoculum levels (0 to 1, 1 to 10, 10 to 100 and 100 to 1,000 colony forming units). After enrichment for 20 h, samples were streaked onto MacConkey, Hektoen Enteric and Xylose Lysine Desoxycholate agar and the presumptive shigellae were confirmed by biochemical tests. Enriched samples were also used to detect *Shigella* spp. by nested PCR. In inoculated SB, both PCR and selective plating detected shigellae at the lowest inoculum. For potato salad enrichments, the PCR procedure was found to be approximately 10 times more sensitive than the selective plating procedure for detecting *Shigella* spp., while for cilantro, a 100-1,000 fold increase of sensitivity was seen for the PCR procedure. Competing microorganisms in the cilantro rinse enrichments made the isolation of shigellae difficult on the selective agar plates. Different novobiocin levels did not improve the isolation.

P5-47 Development of Protocol for the Rapid Detection of Foodborne Virus in Oyster

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Norwalk-like viruses (NLVs) are becoming major causes of foodborne illness in Korea. This study evaluated and modified major steps of virus particle separation and concentration from contaminated oyster. As a surrogate model for NLVs, we used Feline Calicivirus that belongs to the same Caliciviridae Family and is culturable in CRFK cell line. Instead of an ultracentrifugation method, an efficient method based on solvent extraction and PEG precipitation procedure was developed. Direct homogenization of 25 g sample of whole oyster in 175 mL PBS provided simplicity in the actual field of food industry. The acid adsorption step was

eliminated without dropping he RT-PCR detection level. The general overnight PEG pecipitation step was reduced to 3 h by putting the tube on ice and adjusting PEG concentration. The improved method developed in this study could be applied efficiently to detect NLVs from the oysters in commercial markets.

P5-48 RT-PCR Assay for Detecting Noroviruses

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Foodborne viral infections are a common cause of diarraheal disease in the United States and around the world. Norovirus (formally Norwalk-like virus) may account for 60% of these illnesses. Detection of the virus has been difficult because of its genetic variation. Reverse Transcriptase (RT) PCR can be used for the detection of Noroviruses. Several different primer sets have been developed. Five primer sets from published work were selected and evaluated for their effectiveness at detecting Noroviruses from both Genogroup I (GI) and Genogroup II (GII). Control viral RNA was extracted from stool samples from patients known to be shedding the virus. One control was from GI, the other from GII. All primers were testing using a one step RT-PCR procedure. RT-PCR products were run on a 3% agarose gel, stained with ethidium bromide, and visualized using UV light. Detection of noroviruses varied among primer sets. The MON primer set and the NV51/NV3 primer pair both gave a positive result for the GI control and the GII control. The SR primer set gave a positive result for the GI control, but a negative result for the GII control. Both the NVp110/NVp36 and the JV12/JV13 primer pairs gave negative results for the GI and the GII control. One major obstacle in detecting noroviruses is the extreme variability from virus to virus. This may be the reason that some of the primer sets did not work. However, both the MON primers and the NV51/NV3 primer set gave promising results and should be tested further, using various NLV strains to better determine their sensitivity. Further studies will be conducted to evaluate the assay in detecting the viruses in fresh produce samples.

P5-49 Reschuled to P1-57

P5-50 Serological Typing of Pathogenic *Vibrio parahaemolyticus* Isolated from Oregon and Washington Coastal Water

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Vibrio parahaemolyticus is the main causative agent of shellfish related outbreaks and a major cause of diarrhea associated with seafood consumption in the United States. Four recent outbreaks of *V. parahaemolyticus* infections associated with raw oysters consumption occurred between 1997 and 1998 in the Gulf Coast, Pacific Northwest, and Atlantic Northeast regions of the US. However, distribution of pathogenic *V. parahaemolyticus* in US coastal waters has not been extensively studied. This study investigated serological characteristics of pathogenic *V. parahaemolyticus* isolated from Oregon and Washington coastal water. A total of 43 pathogenic *V. parahaemolyticus* containing the thermostable direct hemolysin (tdh) gene and/or the tdh-related hemolysin (trh) gene isolates from oysters, seawater and sediment in Oregon and Washington were serotyped with antiserum (Seiken Corp., Tokyo, Japan) according to the manufacturer's procedures. Seven O (somatic) antigen types (O1, O3, O4, O5, O8, O10 and O11) were identified among the isolates with O5 strains being the most prevalent (22 isolates) followed by O1 strains (9 isolates). Both tdh and trh were found in strains of serogroups O1, O3 and O5. However, only tdh was found in serogroups O4, O8 and O10 while only trh was detected in serogroup O11. Serogroups O1, O3 and O8 were all isolated from Washington, which indicated that distribution of *V. parahaemolyticus* might be tied to geographical locations.

P5-51 Thermal Resistance Characteristics of *Bacillus anthracis* Spores are Similar to Those of Other *Bacillus* Species

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The objectives of this study were to determine thermal resistance characteristics of *Bacillus anthracis* spores in several heating media and to compare these characteristics to those of other *Bacillus* species which might be used as surrogates. The viability of spores suspended in buffer (pH 7.0 or 4.5), milk, or orange juice was determined after heating at 70, 80, or 90°C. Decimal reduction times (D values) were determined from the linear portions of survival curves in duplicate experiments and z values calculated in the usual fashion. The D values for *B. anthracis* strains Sterne, Vollum, and Pasteur ranged from less than one min at 90°C to about two hundred min at 70°C and were lower in acidic media than neutral ones. The *B. anthracis* D values fell within the range of D values obtained from eight strains of *B. cereus*, *B. thuringiensis*, *B. mycoides*, and *B. subtilis* under all conditions tested. The z values for all strains were approximately 10°C in milk and pH 7.0 buffer, but were as high as 19°C under acidic conditions for some strains. These results suggest that *B. anthracis* spores do not have unusual thermal resistance characteristics and that spores from other *Bacillus* species may be appropriate thermal resistance surrogates.

P5-52 Development of Fluorescent Reference Bacterial Strains by Chromosomal Integration of a Modified Green Fluorescent Protein Gene

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Microbiology laboratories maintain reference cultures for quality control of their testing methods. A problem encountered by microbiology laboratories is cross contamination. In order to identify cases of cross contamination with reference cultures, laboratories use bacterial strains that are rarely detected in their tests. However, differentiating these rare reference cultures from contaminating bacteria takes time, and lengthy delays can have serious implications. To significantly reduce the time required to distinguish reference culture strains from contaminating bacterial strains, fluorescent forms of commonly used reference cultures have been developed. A modified version of the green fluorescent protein (GFP) under the control of the strong bacteriophage lambda PL promoter has been isolated by mutagenesis and selection. A gene cassette comprising the promoter, the modified GFP gene, the chloramphenicol gene, and the MuA transposon DNA arms was constructed and integrated via a MuA tranposase-DNA complex into the genomes of two *Escherichia coli* and two *Salmonella* reference cultures. Colonies originating from cultures carrying the integrated GFP cassette can be easily differentiated from their unmodified equivalent by observable fluorescence upon illumination with short to medium wavelength UV light. The developed fluorescent bacterial strains have been shown to be 100% viable when incorporated into a lyophilised BioBall format and their fluorescence properties remain stable in standard non-selective nutrient media free from antibiotics. BioBall is a proprietary delivery matrix for precise numbers of viable bacterial cells into or onto microbiological media and test samples.

P5-53 Precise Reference Standards for Easy Measurement of Uncertainty

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Quality Control (QC) standards are routinely used in microbiology to demonstrate the efficacy of testing methods and culture media. The variability and inaccuracy of the current QC standards increases the potential for false results and makes comparison of method performances difficult. Accreditation to the ISO 17025 standard requires every test result to have a measurement of uncertainty (MoU). This entails giving a value of uncertainty, indicating the range the test result could fall within given all the variables affecting that testing process. Considering the complexity of some testing methods and the diversity of matrices, it is possible that the MoU will be a significant proportion of the result. A new generation of QC standards contains a precise number of bacterial CFU that does not vary from batch to batch. The developed BioBall technology enumerates bacterial cells by flow cytometry and sorts the cells into a single droplet, which is then frozen and freeze dried. The novel freeze drying cycle maintains a 100% viability and stability of the thirty microorganisms. The resulting freeze dried sphere can be re-hydrated onto a culture plate, or into a sample to produce 30 CFU (+/- 3 standard deviations). Using this precise reference standard can considerably reduce a MoU result, as the variation in the seed dose is reduced to 3 standard deviations, compared to up to 10× the variation obtained with dilution prepared spikes. The use of the standard to highlight differences between selective agar recoveries of quality control microorganisms will also be reported.

P5-54 Primer Design for Rapid PCR Detection of *Listeria monocytogenes*

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There is a zero tolerance for *Listeria monocytogenes*, the most frequent cause of ready-to-eat food recalls. This bacterium can cause listeriosis in humans particularly in certain well-defined high-risk groups, including pregnant women, neonates, and immunocompromised adults. Therefore, the development of rapid and accurate methods for the detection of this foodborne pathogen is necessary for the food industry in order to reduce the number of recalls and supply safe food to consumers. The objective of this research is to develop a rapid method for *L. monocytogenes* detection using PCR. A pair of primers from *L. monocytogenes* EGD-e encoding internalin-A was designed specifically to this bacterium from Entrez database in the National Center for Biotechnology Information. This sequence was further analyzed in NCBI Blast, and a 450 bps region was picked up as our target for the PCR. This region satisfied our criteria because it exists in all pathogenic *L. monocytogenes* strains but not in other strains or species. The specificity of the primers has been tested with 61 strains or species of *Listeria*, *E. coli*, *E. coli* O157:H7, *Salmonella*, and *Staphylococcus*, and only *L. monocytogenes* was shown positive. The sensitivity has been performed using a pure culture of *L. monocytogenes* and the detection limit is 10 cells. From these results, it is shown that these primers have high specificity and sensitivity for *L. monocytogenes* detection. Therefore, using the PCR with enrichment and concentration processes has great potential for detecting *L. monocytogenes* in foods within hours.

P5-55 Detection of Bovine DNA by the Warnex™ Real-Time PCR Assay

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A ban on ruminant-derived proteins in ruminant feeds has been introduced as a preventive measure to avoid the spread of bovine spongiform encephalopathy (BSE), as well as to prevent any potential risk of BSE transmission from bovine to human. Current commercial methods to test for the presence of bovine material in animal feeds do not appear to be effective, quick or simple. The aim of the study was to develop a real-time molecular beacon-based polymerase chain reaction (RT-PCR) assay to identify bovine DNA. The DNA was prepared from raw tissue by use of Warnex proprietary methodology. A molecular beacon-based PCR assay was used to amplify a conserve region of DNA from a gene specific for bovine species. The sensitivity of the PCR assay was tested using serial dilutions of bovine DNA, and results showed a detection limit of 0.1 pg of DNA template. The specificity of the assay was tested by comparison with DNA samples from other species. Background cross-amplification was not detected in the presence of purified DNA from other species, such as sheep, pig, horse and goat. The Warnex RT-PCR assay is highly specific and sensitive for bovine, and is suited to be used as a routine control assay to evaluate the presence or absence of bovine-derived meat and bone meals in ruminant feeds.

P5-56 Optimization of Ferrioxamine E Concentration as Effective Supplementation for Selective Isolation of *Salmonella* Enteritidis in Egg White

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Studies show that utilization of ferrioxamine E (FE) as a sole source of iron distinguishes Salmonellae from a number of related species, including *Escherichia coli*. Ferrioxamine E is not able to feed *E. coli* or the *Proteus-Providencia-Morganella*-group. This confers a selective advantage on *Salmonella* Enteritidis in egg white supplemented with FE. The optimum concentration of FE for selective growth of SE in egg white was determined. Four supplementation concentrations were evaluated (500 μg/ml, 200 μg/ml, 50 μg/ml, and 25 μg/ml) in egg white artificially inoculated with proportionally mixed cultures of a Rifampicin-resistant strain of *Salmonella* Enteritidis (0.1 ml of 10^2 CFU/ml) and *E. coli* K12 (0.1 ml of 10^1 through 10^8 CFU/ml). After 24 h incubation at 37°C, *Salmonella* and *E. coli* populations were enumerated. At higher concentrations of FE (>50 μg/ml), both *Salmonella* and *E. coli* were able to utilize the iron supplement (log 1 to 8.5 and log 1.8 to 8 CFU/ml, respectively); however, lower FE concentrations (<50 μg/ml) exclusively promoted *Salmonella* growth. *Salmonella* was unrecoverable without supplementation. This study indicates that optimum levels of FE supplementation in egg can improve selective detection for *Salmonella* Enteritidis against other competitive organisms.

Technical Abstracts

T1-01 An Examination of Media Reporting Trends of Produce-related Food Safety

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Produce has been increasingly linked to cases of foodborne illness in North America. The public discussion of produce safety has also increased, impacting belief formation with respect to safe food consumption. Risk managers addressing produce food safety must monitor and understand media trends to adjust programs and communication strategy relevance. Risk management activities for the produce industry should include the use of risk communication to consumers to potentially minimize the impact of any unpredictable economically damaging crises. News articles culled from representative public agenda-setting sources were examined for reporting risk message trends, using quantitative and qualitative content analysis methods. This database of articles was then compared to actual documented outbreaks to explore information omissions from media reports. Factors leading to media coverage were discovered, including number of cases resulting from an outbreak; geographical footprint of an outbreak; implication of imported produce; information available to media; and the stigma of vehicles and pathogens. Produce industry stakeholders can apply the results of this research and create a template to be used in extension activities to support produce-specific on-farm food safety issues.

T1-02 Microbial Safety Evaluation of Organic and Conventional Fresh Produce at the Pre-harvest Stage

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Microbiological analyses of fresh fruits and vegetables produced by organic and conventional farmers in Minnesota and Wisconsin were conducted to determine coliform and *Escherichia coli* counts and prevalence of *Salmonella* and *E. coli* O157:H7, at the pre-harvest stage. Among the participating farmers, 24 claimed to use organic practices but weren't certified, 8 were certified organic and 14 were conventional growers. A total of 1,182 produce samples (539 from non-certified growers, 295 from certified organic and 348 from conventional growers) were collected during the 2004 harvest season. Major produce types included lettuce, other leafy greens, cabbage, broccoli, pepper, tomato, zucchini, summer squash, cucumber, berries and other minor types like bok choi, pea, cauliflower and cantaloupe. A farmers' survey on relevant farm-level production, handling and management practices were collected at the beginning of the season. The average coliform counts in non-certified, certified organic and conventional produce were 2.2, 2.3 and 1.5 log MPN/g, respectively. *E. coli* counts were 2.4, 2.3 and 1.9 log MPN/g, respectively, in non-certified, certified organic and conventional produce. *E. coli* prevalence was significantly higher in non-certified and certified organic samples compared to their conventional counterpart. *E. coli* prevalence were also significantly greater among leafy greens compared to all other major produce types. None of the produce samples tested positive for *Salmonella* and *E. coli* O157:H7.

T1-03 Results of Microbial Baseline Study of Ontario Grown Fresh Fruits and Vegetables

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A baseline study of selected indicator and pathogenic bacterial prevalence in Ontario-produced fresh fruit and vegetables was carried out between August and October of 2004. This was the first study of its kind in Ontario. Comparison of this study with others that are similar in nature revealed that Ontario had a lower prevalence rate of pathogens, which may be due to Ontario's climate. Samples were collected from locations where consumers most often obtain their produce, including distribution centers (82% of samples obtained), farmers markets (15%) and organic site operations (2%). This sampling plan also enabled the largest percentage of Ontario farms to be sampled. The 1183 samples consisted of 151 cantaloupe, 173 green onions, 263 leaf lettuce, 155 head lettuce, 112 organic leaf lettuce, herbs (61 cilantro and 127 parsley) and 141 fresh market tomatoes. These commodities were chosen because they have a higher attributable risk compared to other foods of plant origin commodities, and there is a limited amount of information available about the prevalence of foodborne pathogens in these commodities in Ontario. Samples were analyzed for *Salmonella* spp., *Shigella* spp. and generic *Escherichia coli*. Any samples positive for *E. coli* were further analyzed for verotoxigenicity. No samples were positive for verotoxigenicity or for *Shigella* spp. Two (2) samples were positive for *Salmonella* spp., resulting in a prevalence rate of 0.17% for the whole study. The prevalence rate for generic *E. coli* was 5.3% of all commodities sampled, and the prevalence rate for generic *E. coli* for all commodities ranked as parsley>organic leaf lettuce>leaf lettuce>green onions>cilantro>cantaloupe>head lettuce and fresh market tomatoes.

T1-04 ***Salmonella enterica*** **and Enterohemorrhagic** ***Escherichia coli*** **O157:H7 Survival on Produce is Altered by the Presence of Epiphytic Bacteria**

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Both *Salmonella enterica* and *Escherichia coli* O157:H7 contaminate fresh produce. In the field, these pathogens can survive in low numbers in soil and on plants. Occasionally conditions may occur in the field or during processing that lead to an outbreak. Survival of the pathogens in the field is controlled to a certain extent by complex interactions with indigenous soil-borne and seed-borne epiphytes. Insight into the nature of these interactions may improve produce safety. Two epiphytes were isolated from pathogen infected plants that displayed opposite interactions with these pathogens. *Ralstonia paucula* enhanced the survival of *E. coli* O157:H7 Odwalla two-fold on lettuce and had no effect on *S. enterica* Newport. In contrast, *Enterobacter asburiae* reduced the survival of both pathogens on lettuce. *E. coli* O157:H7 Odwalla showed a 20 to 30-fold reduction in the foliage when *E. asburiae* and *E. coli* O157:H7 Odwalla were previously co-inoculated on lettuce seed. Competition against *S. enterica* was four-fold less effective. Additionally, *E. asburiae* was also competitive against several other strains of *E. coli* O157:H7 and *S. enterica*. Epi-fluorescent images of co-inoculated plants *in vitro* indicated that the pathogens are restricted to small, isolated colonies. Also *in vitro*, pathogen competition with *E. asburiae* was only seen while the bacteria grew on plant exudate. Hence, *E. asburiae* likely competes with these pathogens for nutrients found in plant exudate. Good agricultural practices that encourage the growth of competing bacteria, like *E. asburiae*, may reduce the incidence of produce contamination.

T1-05 **Evaluation of Steam Blanching for** ***Salmonella*** **Enteritidis Reduction on Almond Surfaces**

DSC

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This study was conducted to investigate the effectiveness of steam blanching (204 F) for reducing *Salmonella* Enteritidis on almond surfaces. Batches of 25 g "Nonpareil" almonds were inoculated with 10^{7-8} CFU/ml of either *S.* Enteritidis cocktail (*S.* Enteritidis 43353, ME-13, ME-14) or *S.* Enteritidis PT-30 and allowed to dry overnight. Inoculated almonds were then subjected to steam treatment through a pilot-sized blanching machine for time intervals of 5, 15, 25, 35, 45, 55, and 65 s. Survival of *S.* Enteritidis was evaluated with tryptic soy agar (TSA) and xylose lysine desoxycholate (XLD) for injured and healthy cells, respectively. Results indicate no significant differences ($P > 0.05$) between the two *S.* Enteritidis inoculums. Reduction of *S.* Enteritidis increased as a function of treatment time, with 25 s being sufficient to achieve a 5-log reduction. Though steam blanching resulted in more reduction as treatment time increased, it also resulted in degradation of almond qualities. Discoloration and visible formation of wrinkles were observed after steam blanching of 35 s, agreeing with other almond investigations stating that steam treatments of longer than 30 to 40 s would negatively affect almond qualities. We thus conclude that steam blanching of 25 s is effective to achieve a 5-log reduction of *S.* Enteritidis without compromising almond quality.

T1-06 **Migration of** ***Salmonella*** **Enteritidis PT 30 through Almond Hulls and Shells**

DSC

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Recent outbreaks of salmonellosis from consumption of raw almonds have generated interest in potential modes of contamination of this nutmeat. The ability of *Salmonella* Enteritidis PT 30 to migrate through almond hulls and shells was evaluated in two ways. Shell halves from five varieties of almonds were glued inside Petri dish lids so that the outer shell only was submerged in a suspension of *S.* Enteritidis PT 30 (10^6 CFU/ml in Butterfield's buffer) placed in the bottom half of the Petri dish. Following incubation for 24 h at 24±2°C, the inside of the shell was swabbed, streaked directly onto XLD agar and incubated for 24 h at 35±2°C. The swab was transferred into 25 ml of lactose broth and enriched using standard methods. *S.* Enteritidis was isolated from the inside of almond shells by both direct swabbing (14/30) and enrichment (30/30). Additionally, intact whole almonds (hulls, shells, and kernels) were soaked 24 to 72 h at 24±2°C in buffer containing 10^3 to 10^9 CFU/ml of green fluorescent protein (GFP)-labeled *S.* Enteritidis PT 30. After incubation, populations of *S.* Enteritidis PT30 were 10^8 to 10^9 CFU/ml regardless of initial inoculum level. Almonds were drained and the kernel removed using aseptic techniques. GFP-*Salmonella* was detected on almond kernels, outer and inner shells, and on and within the hull by confocal laser scanning microscopy. These combined data provide direct evidence that wet conditions allow for *Salmonella* multiplication and migration through the hull and the shell, thus providing a means by which almond kernels may become contaminated.

T1-07 **Inactivation of Bacteria, Yeasts and Molds on Palletized Highbush Blueberries Using Chlorine Dioxide Sachets**

DSC

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Chlorine dioxide (ClO_2) gas generated by a dry chemical sachet was tested against three foodborne pathogens, five yeasts and five molds known for spoilage. Initially, five fresh blueberry samples (100 g) were separately inoculated with *Listeria monocytogenes*, *Salmonella*, *Escherichia coli* O157:H7 (3 strains each),

and yeasts and molds (5 strains each) to contain ~10^6 CFU/g and treated with ClO_2 (4 mg/l) for 12 h in a sealed 20-l container (99.9% RH) at ~22°C (3 replicates). After gassing, blueberries (25 g) were diluted 1:5 in neutralizing buffer, pulsified for 1 min and plated using standard FDA procedures to quantify survivors. This treatment yielded reductions of 3.94, 3.62, 4.25, 3.10, and 3.17 log CFU/g for *L. monocytogenes*, *Salmonella*, *E. coli*, yeasts and molds, respectively. Thereafter, 30 lugs of blueberries (~9.1 kg/lug) were stacked on 4 × 4 ft pallets (5 lugs/level × 6 levels) (6 replicates), tarped, and exposed to ClO_2 (18 mg/l) for 12 h. After gassing, significant ($P < 0.05$) reductions of 2.12, 1.61, 0.72, 1.76, and 1.55 log CFU/g were seen for mesophilic aerobic bacteria (MAB), yeasts, molds, coliforms, and *E. coli*, respectively, compared to ungassed controls. No significant differences ($P > 0.05$) in microbial inactivation were seen between lug levels and, with one exception (MAB), between the bottom and top surface of individual lugs. Based on these findings, ClO_2 sachets may provide a simple, economical and effective means of enhancing the microbial shelflife and safety of fresh blueberries.

T1-08 Variability of Virus Attachment Patterns to Butterhead Lettuce

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Enteric viruses are a major cause of foodborne illness in the United States. The objective of this study was to determine whether the isoelectric point (pI) of viruses such as Feline Calicivirus (FCV), Echovirus 11, and bacteriophages phiX174 and MS2 had any effect on their attachment to Butterhead lettuce. The adsorption of virus particles to the lettuce was variable. Bacteriophage MS2 was the only virus showing maximal attachment at its critical pH and was the only virus fitting the DLVO model of virus attachment. Echovirus 11 had the highest affinity to lettuce surface. Echovirus 11 exhibited reversible attachment above its pI, whereas below the pI, a strong adsorption was observed. At low salt concentrations, Echovirus adsorption was strongly influenced by its pI, indicating that at intermediate salt concentrations both electrostatic and hydrophobic forces are responsible for Echovirus adsorption. Adsorption of FCV was at its maximum above the viral pI. Bacteriophage phiX174 exhibited the most complex adsorption pattern with attachment occurring only at the pH extremes (pH 3.0 and pH 8.0). These results suggest the current model for virus adsorption to sediment does not adequately explain the attachment of virus to lettuce. Importantly, the results suggest that current sample processing methods to recover viruses from lettuce can differentially select for certain viral types and that a pathogenic human virus has a high affinity to lettuce.

T1-09 Evaluation of Hepatitis A Virus Inactivation by High Pressure Processing in Strawberry Puree and Sliced Green Onions

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To investigate the potential of high pressure processing (HPP) as an intervention strategy for virus-contaminated fruits and vegetables, strawberry puree and sliced green onions were contaminated with > 10^6 PFU of hepatitis A virus (HAV) and treated with pressures ranging from 225 to 375 MegaPascals (MPa) in 25-MPa increments at ambient temperature. Virus extraction and plaque assay determined that HAV was inactivated in strawberry puree and sliced green onions after 5-min exposures to pressures of 375 MPa, with log PFU reductions of 4.32 and 4.75, respectively. For treatments of < 325 MPa, the virus was more sensitive to pressure in strawberry puree than in sliced onions, with log reductions of 1.2, 2.06, and 3.13 observed for strawberries, and 0.28, 0.72, and 1.42 observed for onions, after 5-min treatments at 250, 275 and 300 MPa, respectively. Results show HPP will inactivate HAV in these foods at these pressures and times.

T1-10 Survey of Biosecurity Practices in Produce Operations in the Southeast

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Fresh produce is potentially vulnerable to tampering because it is usually eaten raw or in a minimally processed state. It is grown, harvested and packed literally "in the open" and there is typically no kill step to destroy microbial pathogens prior to consumption. In a collaborative effort with researchers from Georgia, South Carolina and Florida, this study was undertaken to assess the current status of security at fresh produce facilities in these states. Security audit forms were prepared and used to survey growers, packers, and fresh-cut processing operations. A total of 25 farms, 25 packinghouses and 7 fresh-cut produce processing operations were surveyed. Practically all of the fresh-cut processors have a written security plan, conduct security training for their employees and have restricted access to their facilities. However, only about half of the farm and/or packinghouse operations provide employee security training, and only one farm and one packinghouse surveyed have written security plans. About half (52%) of the packinghouses surveyed have perimeter fencing and only half have locks on the cooler doors. Documentation of any sort of security practice is lacking among both growers and packers. Survey data collected to date indicates that while fresh-cut processing facilities are dealing with current security challenges, farm and packing operations in the tri-state region are lagging behind. More training programs and assistance to increase awareness and to facilitate incorporation of feasible, preventative measures are needed by segments of the industry.

T1-11 Impact of Regulations on Juice-associated Outbreaks

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FDA's 1998 juice labeling regulation required either implementation of process control measures to achieve a 5-log pathogen reduction or a warning label indicating the health risks of drinking untreated juice. These were interim measures until the 2001 juice HACCP regulation became effective between January 2002 and January 2004. This regulation required nearly all juice processors to implement HACCP systems, including a 5-log pathogen reduction. Retail establishments processing and selling directly to consumers are exempt from the 2001 rule but subject to the 1998 rule if they operate in more than one state. We reviewed outbreaks for which the implicated vehicle was fruit juice reported to CDC's Foodborne Outbreak Reporting System. From 1994 to 2004, 26 juice-associated outbreaks (JAOs) were reported to CDC; 38% implicated apple juice or cider, 27% orange juice, 23% lemonade, and 12% other juice. These outbreaks caused 1,370 illnesses, with a median size of 11 cases (range 2 to 398). Among the 17 outbreaks of known etiology, 5 were caused by *Salmonella*, 4 by *E. coli* O157:H7, 3 by Norovirus, 2 by *Cryptosporidium*, 2 by chemical agents, and 1 by *E. coli* O111 and *Cryptosporidium*. Among the 17 JAOs reported from 1998 through 2001, pasteurization information was available for 7, all of which were unpasteurized. All 4 JAOs reported between 2002 and 2004 were associated with apple juice or cider. Fewer JAOs have been reported since the implementation of the juice HACCP rule. Apple juice or cider operations exempt from processing requirements continue to cause illness.

T1-12 Lethality of Vanillic Acid toward *Escherichia coli* O157:H7 in Unpasteurized Apple Juice

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Unpasteurized apple juices made from Granny Smith, Gala, Empire, McIntosh, Red Delicious and Golden Delicious apples were supplemented with 0, 5 or 10 mM vanillic acid and were inoculated with a five strain mixture of *Escherichia coli* O157:H7. Cell populations gradually declined and no viable cells were recovered from control juices made with McIntosh, Golden Delicious, Empire and Granny Smith apples after 7 days storage at 15°C. In contrast, populations were stable in high pH (Gala and Red Delicious) juices stored for 7 days at 15°C and in juices from all cultivars stored at 4°C. Vanillic acid exerted a concentration, pH and time dependant lethal effect toward *E. coli* O157:H7. Supplementation with 5 mM vanillic acid accelerated death of *E. coli* O157:H7 and population reductions ranged from 2 log CFU/ml in low pH juice (Granny Smith pH = 3.18) to <1 log CFU/ml in high pH juices (Gala, pH = 3.90) after 7 days at 4°C; and 5 log CFU/ml in Granny Smith and 2 log CFU/ml in Gala juice after 7 days at 15°C. No sensory difference or preference was detected by triangle testing in two of the six juices supplementated with 5 mM. Supplementation with 10 mM vanillic acid led to a 5 log CFU/ml reduction in *E. coli* O157:H7 populations after 7 days at both temperatures but sensory analysis revealed significant differences from and preference for un-supplemented juices. Adjustment of pH would therefore be required for the effective control of *E. coli* O157:H7 in unpasteurized juice by vanillic acid.

T2-01 DSC Evaluating Microbial Safety of a Slow Partial-cooking Process for Bacon: Use of a Model Based on Small-scale Meat Inoculation Studies

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Pathogen growth and toxigenesis potential during slow partial-cooking of meats should be evaluated in a Hazard Analysis. To aid this evaluation, the safety of slow partial-cooking, *Salmonella* spp., *Escherichia coli* O157:H7, and *Staphylococcus aureus* lag-times were modeled using small-scale (25 g) ground pork isothermal inoculation studies. Lag times ranged from > 9 h at 21°C to 3 h at 35°C and > 6 h at 46°C. To test model predictions, cured pork bellies were inoculated with these pathogens, subjected to commercial slow partial-cooking processes and analyzed for pathogen numbers. Processes lasted 6 h, with the product interior within the 21 to 46°C range of the model for 139 to 325 min (high-humidity processes) and 173 to 245 min in (low-humidity processes). For the pork belly interior, the model predicted growth of *Salmonella* spp., *E. coli* O157:H7, and *S. aureus* for 3 of 6, 1 of 6, and 4 of 6 trials, respectively. The model was fail-safe, as pathogen growth did not occur in 5 trials for which it was predicted. In one high-humidity trial *S. aureus* grew as predicted (0.6 to 0.7 log CFU). Similarly, no meaningful pathogen growth (-1.0 to +0.3 log CFU change) occurred in trials for which growth was not predicted. For the pork belly surface, the model correctly predicted growth/no growth in every trial, except for one trial with slight (0.4 to 0.9 log CFU) increases for *Salmonella* spp. and *S. aureus*. Our study shows pathogen growth is unlikely during several 6 h bacon partial-cooking processes and the usefulness of an inoculation-study model in process safety evaluation.

T2-02 DSC Survival of *Listeria monocytogenes*, *Listeria innocua*, and Lactic Acid Bacteria Species in Chill Brines

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Listeria monocytogenes is the major pathogen in ready-to-eat meat products. Contamination can occur via salt brines used to cool thermally processed meats. Both *L. monocytogenes* and lactic acid bacteria can grow under these brine conditions, and may compete with each other for available nutrients. This study investigates effects of a three strain cocktail of lactic acid bacteria (LAB) *Enterococcus faecalis*, *Carnobacterium gallinarum*, and *Lactobacillus plantarum* on survival of *Listeria monocytogenes* and *L. innocua* in brines stored under low temperatures for up to 10 days. Three brine concentrations (0%, 7.9%, and 13.2% NaCl) were inoculated with 7.0 log CFU/ml of one of five cocktails (*L. monocytogenes, L. innocua,* lactic acid bacteria (LAB), *L. monocytogenes* + LAB, or *L. innocua* + LAB) and stored for 10 days at 4 or 12°C. Three replications of each brine/cocktail/temperature combination were performed. No significant reductions of *L. monocytogenes* were seen in 7.9 or 13.2% brines when LAB were present; however, reductions of *L. monocytogenes* were seen in the 0% brine solution when LAB were present (1.43 log CFU/ml at 4°C and 3.02 log CFU/ml at 12°C). *L. innocua* was significantly less resilient to environmental stresses of the brines than *L. monocytogenes*, both with and without LAB present ($P < 0.05$). Lactic acid bacteria are not effective at reducing *L. monocytogenes* in brines at low temperatures. Under these conditions *L. innocua* should not be used as a model for *L. monocytogenes*. Since *L. monocytogenes* survives in brine, technologies for its elimination in brine solutions need to be investigated.

T2-03 Alternative Cutting Methods to Minimize Transfer of Specified Risk Materials during Steak Preparation from Bone-in Short Loins

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Steaks produced from bone-in loin subprimals where the vertebral column bone is first cut out may inherently leave residual specified risk materials (SRM) on steak surfaces when undercut. The objective of this study was to evaluate current and alternative cutting methods that could be used to minimize the transfer of SRM tissue during preparation of steaks from bone-in short loins. Loins were cut as follows: (i) method I - vertebral column bone removed prior to cutting the loin into steaks from medial (vertebral column) to distal (meat) end; (ii) method II - loin cut into steaks from medial to distal end prior to removal of vertebral column bone; and, (iii) method III - loin cut into steaks from distal to medial end prior to removal of vertebral column bone. Results indicated that surface areas along the vertebral column cut line had detectable (0.100 and 0.215% / 100 cm^2) and, thus, higher SRM contamination compared to resulting steak surfaces or the cutting blade. Overall, there were no detectable (<0.100% / 100 cm^2) differences in SRM contamination of steaks produced by the three cutting methods. Furthermore, immunohistochemical evaluation of areas on steak surfaces excised and ground indicated that none of the cutting methods were responsible for transferring contamination from the medial to distal end of the steak following cutting. These results imply that steaks may be cut from bone-in subprimals prior to removal of vertebral column bone without affecting transfer of SRM tissue at concentrations <0.100% / 100 cm^2 to resulting steaks.

T2-04 Decontamination of Beef Cuts, Intended for Blade/Needle or Moisture-enhancement Tenderization by Surface Trimming vs. Rinsing with Solutions of Hot (82°C) Water, Warm (55°C) Lactic Acid or Activated Lactoferrin Plus Warm (55°C) Lactic Acid

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The objective of this project was to investigate interventions that may minimize the risk of transferring *E. coli* O157:H7 from the exterior, to the interior, of whole-muscle cuts during blade/needle or moisture-enhancement tenderization. Two-hundred outside round pieces were inoculated with an average of 4.17 log CFU/100 cm^2 of *E. coli* O157:H7 with a 24-h culture. Inoculated pieces were vacuum packaged and stored for 10 to 18 days at 2–4°C. Enumeration using CT-Smac was performed on 100 cm^2 samples cut from each outside-round piece before and after interventions were applied and after further processing. Each piece was treated with one of five interventions: Postive control, (1) external trim using GMPs, (2) hot water (82°C) (3) warm 2.5% lactic acid (55°C), (4) warm 5.0% lactic acid (55°C), and (5) activated lactoferrin plus warm 5.0% lactic acid (55°C). Inoculated outside-round pieces were used as positive control. The mean reduction of *E. coli* O157:H7 after interventions were applied were 1.10, 1.00, 0.99, 1.07 and 0.93, respectively. Mean *E. coli* O157:H7 counts from the internal surface of outside-round pieces post-blade tenderization were non-detectable. Mean *E. coli* O157:H7 counts from the internal surface of outside-round pieces post-moisture-enhancement were 2.13, 1.32, 1.23, 1.20, 1.15, and 1.39 The mean percent of *E. coli* O157:H7 transferred from the external surface, post-intervention, to the internal surface, post-blade-tenderization, were 5.85, 3.63, 3.71, 4.52 and 4.68 for external trim, hot water,

5.0% LA and ALF + 5.0% LA. The mean percent transferred from the external inoculated surface before intervention to the internal surface after blade-tenderization were .82, .37, .41, .40, .51 and .38. The mean percent of *E. coli* O157:H7 transferred from the external surface post-intervention to the internal surface, post-moisture-enhancement were 3.88, 4.97, 5.26, 5.55, 6.08, and 4.96. The mean percent of *E. coli* O157:H7 transferred from the external surface of the inoculated outside-round pieces to the internal surface post-moisture-enhancement were 3.88, .65, .59, .82, .53, and .92.

T2-05 Microbial Loads on Subprimals and the Impact on Injection

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Beef subprimals tenderized by marinade injection may allow pathogenic bacteria on meat surfaces to be internalized and consequently increase their likelihood of survival during cooking. The objective of this study was to determine microbial loads and pathogen incidence on incoming raw beef and in processing marinades as well as the potential to distribute such contamination. Incoming beef subprimal samples (n = 982), pre-injection marinade samples (n = 40) (50 ml), and marinade samples during beginning (n = 175), middle (n = 131), and end (n = 137) of the injection process were microbiologically analyzed for levels of microbial populations (total plate [TPC], total coliform [TCC], and *Escherichia coli* counts [ECC]) and presence of *E. coli* O157:H7 and *Salmonella* spp. Samples of incoming beef subprimals yielded levels of 4.0 to 6.2, 0.8 to 2.7, and < 0.8 log CFU/g for TPC, TCC, and ECC, respectively. Incidence rates of *E. coli* O157:H7 and *Salmonella* on incoming raw beef subprimals were 0.3 and 2.2%, respectively. Pre-injection marinade samples yielded levels of 0.8 to 1.7 log CFU/ml for TPC while TCC and ECC were < 0.8 log CFU/ml and was not contaminated with *E. coli* O157:H7 or *Salmonella*. Microbial populations in marinades increased from 1.9 to 5.3 (TPC) and 0.8 to 2.3 (TCC) log CFU/ml during beginning to end of meat injection and became contaminated with *Salmonella* (1.8% incidence rate). These results confirm the presence of pathogens on subprimals and the potential risk of such meat contaminating recirculated marinades as well as highlighting the need to introduce interventions to control the microbiological quality of such marinades.

T2-06 Prevalence and Numbers of *Campylobacter jejuni* and *C. coli* in Uncooked Retail Meats in New Zealand

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A national quantitative survey of *Campylobacter* in uncooked retail meats (beef, unweaned veal, chicken, lamb/mutton and pork) covering four seasons was undertaken from August 2004 to June 2005 to establish baseline proportionality data. Qualitative analysis was performed on each sample by PCR and was followed by quantitative analyses of positive samples using MPN/PCR methodologies. A total of 1011 meat samples were tested. The prevalences of *C. jejuni* and *C. coli* in the meat species (minced or diced) were chicken 89.1% (205/230), pork 9.1% (21/230), lamb/mutton 6.9% (16/231) and beef 3.5% (8/230). Unweaned veal, comprising, in the absence of minced or diced products, frozen or chilled cutlets was 9.5% (21/230). *Campylobacter jejuni* was identified in the majority of positive samples (246/259); seven contained mixed cultures of *C. jejuni/C. coli* and six contained *C. coli* only. *Campylobacter jejuni* counts in chicken meat showed that of 205 positive samples, 84 had counts of < 0.3MPN/g, 104 had counts ranging from 0.3 to 10.9 MPN/g, 12 of 23.7 MPN/g, 1 of 28.8 MPN/g, 3 of 45.9 MPN/g and 1 of 110 MPN/g. Enumeration of *C. jejuni* from the other positive meat species (49 samples) showed that, except for one unweaned veal sample with a count of > 10.9 MPN/g, counts were equal to, or below, 0.3 MPN/g. The results provide benchmark proportionality profiles of *Campylobacter* in the five meat species that will facilitate (a) exposure assessment in the light of other information such as consumption data and (b) subsequent quantitative risk assessment.

T2-07 Enhancement of Food Safety Surveillance in the Republic of Ireland

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The primary responsibility for food safety in Ireland rests with food producers and food processors. Official agencies monitor the effectiveness of controls as well as ensuring that effective systems are in place for rapidly identifying food safety alerts. In many countries data on food safety tests undertaken by industry in private laboratories is generally not collated and made available to the regulatory agencies. While official agencies in all developed countries undertake independent testing of food, it is estimated to account for only 3 to 5% of total testing. In the Republic of Ireland the food industry, under the regulation of Department of Agriculture and Food (DAF), is required to undertake its microbiological analysis of animal foodstuffs in private laboratories approved by DAF. These private laboratories are required to submit monthly reports on tests to the Central Veterinary Research Laboratory; the National Reference Laboratory for Zoonoses. The FoodMicro database, developed in 2001 to collate this information, now contains a significant bank of data on potential sources of

zoonoses pathogens from foods on the island of Ireland. Surveillance is focused on *Salmonella*, *Campylobacter*, *Listeria* and *Escherichia coli* O157. Since 2001 over 500,000 tests have been recorded in this database, making it the largest database on microbiological testing in Ireland. This has allowed the development of a laboratory based food safety surveillance system and facilitated more effective food safety monitoring by combining the test data collected in both official and private laboratories.

T2-08 Indigenous Protein Markers for Evaluation of Prion Inactivation in Processed Meat Products

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Bovine Spongiform Encephalopathy (BSE) is thought to enter the human food chain through beef products contaminated with infectious prion proteins. Studies have demonstrated the potential applications of emerging processing technology in reduction of prion infectivity in meat products. However, lack of a rapid verification measurement to facilitate evaluation of such technology has hampered its further development. Our objective was to identify heat- and protease-resistant protein markers that can be used as an indication of inactivation effectiveness to evaluate/optimize the processing procedures. Indigenous protein markers from bovine muscle were identified based on their resistance to heat and proteolysis. SDS-PAGE of muscle extract showed that the majority of the proteins were insolubilized by heating. The few proteins remaining soluble and stable at higher temperatures were identified as the potential candidates. These proteins were presumptively determined as myosin heavy chain, tropomyosin, and troponin subunits, based on their relative mobility. Among these candidates, tropomyosin exhibited the most promising features. Under a high temperature (133 C/30 psi), tropomyosin remained integral in the solution with minor degradation to another stable fragment (25 kD). As seen in the time-resolved-proteolysis by Proteinase K at 1/1000 (w/w), tropomyosin was completely hydrolyzed in 60 min to form three partially resistant fragments ranging from 15 to 18 kD. One of these fragments (17 kD) retained its resistance up to 240 min. Our results indicated that tropomyosin could be a useful marker for monitoring the inactivation of BSE agent in meat products, if an immunoassay to quantify the denaturation of the protein marker is developed.

T2-09 Distribution and Prevalence of *Salmonella* Serotypes in Maryland Retail Poultry Meat

DSC

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According to the World Health Organization, *Salmonella* is a leading cause of foodborne illness worldwide. Since the implementation of the HACCP system for pathogen reduction in poultry processing plants, the Food Safety Inspection Service (FSIS) of the US Department of Agriculture reports substantial reductions in the prevalence of *Salmonella* contamination of raw poultry at processing plants. However, limited data is available regarding the prevalence and distribution of *Salmonella* contamination in poultry meat at retail grocery stores. Random samples of retail grocery stores within the state of Maryland were selected for this cross-sectional study. The objectives of this study were to estimate the *Salmonella* prevalence in chicken meat products from retail grocery stores throughout Maryland and to identify risk factors for *Salmonella* contamination in retail poultry meat products. Out of 180 poultry meat samples that were collected, the overall prevalence of *Salmonella* contamination was 22.7% (C.I 15.16-30.86). Integrator brand ground poultry (19%) was at increased risk for *Salmonella* contamination when compared to integrator brand non-ground poultry products (7%) but this difference was not significant ($P = 0.053$). Among non-ground products, store brand poultry was 18 times more likely to be contaminated with *Salmonella* than integrator brand poultry products (CI 5.1 to 61.2). 57% (24/42) of positive samples were *S.* Heidelberg, 24% were *S.* Kentucky, and 19% were *S.* Typhimurium (Copenhagen). 88% of positive ground chicken samples were *S.* Typhimurium (Copenhagen) and 70% of positive non-ground chicken samples were *S.* Heidelberg and *S.* Kentucky.

T2-10 Water Spray and Extended Dry Time to Lower Bacterial Numbers on Soiled Flooring from Broiler Transport Cages

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Broiler transport cages soiled with *Campylobacter*-positive feces have been demonstrated to result in cross contamination of processed broiler carcasses. Washing and sanitizing cage surfaces does not always effectively eliminate bacteria. The objective of this study was to examine drying as a means to lower bacterial numbers on transport cage flooring. Small squares (5 × 5 cm) of fiberglass flooring from transport cages were intentionally contaminated with 1 g of *Campylobacter*-positive broiler gut contents. Soiled floor squares were sprayed with water and allowed to dry for 0.25, 24 or 48 h. Unsprayed squares were examined at each time period as controls. All squares were sampled by cotton tipped applicators, which were cultured for *Campylobacter*, coliforms and *E. coli*. Sampling unsprayed squares at 0.25 h yielded 7.3 log CFU *Campylobacter*, 6.2 log CFU coliform and 5.9 log CFU *E. coli*. Water spray alone resulted in a significantly lower number of organisms recovered: 4.1 log CFU *Campylobacter*, 3.6 log CFU coliform and 3.2 log CFU *E. coli*. When water spray was

followed by a 24 h drying period, no *Campylobacter*, coliforms or *E. coli* were detected on the floor surface. However, allowing unsprayed soiled flooring to simply dry for 24 or 48 h also resulted in no recovery of *Campylobacter* and very low numbers of coliforms and *E. coli*. A 24 or 48 h drying period for fecal matter on broiler transport cage flooring may be a viable method to lower bacterial numbers on these surfaces.

T2-11 DSC Evaluation of Water Quality and Prevalence of Bacterial Pathogens and Their Antimicrobial Resistance in Food Fish and Their Pond Water in Trinidad

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In Trinidad, Tilapia (*Oreonchromis* spp.) is one of the most important freshwater food fish and the number of farms has been increasing annually. This study determined the water quality, bacterial pathogens and their antimicrobial resistance in the local food fish industry. 75 apparently healthy fish and 15 pond water samples from 3 fish farms were processed. Chemical analysis of water revealed values outside the recommended standards for pH, unionized ammonia and nitrites in 7 (46.7%), 9 (60.0%) and 8 (53.3%) ponds, respectively. Of 202 bacterial isolates from fish slurry and 88 from water, 13 and 16 genera respectively were identified. The predominant bacteria from fish slurry were *Pseudomonas* spp. (60.0%), *Aeromonas* spp. (44.0%), *Plesiomonas* (41.3%) and *Chromobacterium* (36.0%) ($P < 0.05$) compared with isolates from pond water, where *Bacillus* spp. (80.0%), *Staphylococcus* spp., *Alcaligenes* spp. and *Aeromonas* spp. (60.0%) were most prevalent ($P < 0.05$). Using 8 antimicrobial agents, 168 (97.1%) of 173 bacterial isolates from fish slurry exhibited resistance to one or more antimicrobial agents, compared with 47 (90.4%) of 52 from water. Regardless of farm, 51(98.1%) of 52 isolates of *Aeromonas* spp., 33 (97.1%) of 34 *Chromobacterium* spp., 34 (92.1%) of 35 *Plesiomonas* spp. and 33 (100%) of 33 *Pseudomonas* spp. exhibited resistance ($P > 0.05$). Resistance was highest to ampicillin (78.8%) and erythromycin (51.9%) but lowest to norfloxacin (3.8%) and sulfamethoxazole/trimethoprim (7.7%). In conclusion, the rather high prevalence of bacterial pathogens in fish coupled with their high prevalence of resistance to antimicrobial agents might pose a health risk to consumers.

T2-12 Antimicrobial Resistance in *Salmonella* and *Escherichia coli* Isolated from Commercial Shell Eggs

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Antimicrobial resistance in foodborne and commensal bacteria has become a problem. *Salmonella* and *Escherichia coli* from shell egg samples were analyzed for resistance to 16 antimicrobials. Shell eggs (n = 990) from multiple points in 3 commercial plants were individually sampled using a saline rinse. Pooled samples were pre-enriched in buffered peptone water and selectively enriched in TT and Rappaport-Vassiliadis broths. Presumptive *Salmonella* colonies from selective agar plates (BGS and XLT-4) were identified and serotyped. *Enterobacteriaceae* were enumerated from individual shell rinses by use of Violet Red Bile Glucose agar plates. Randomly selected isolates were identified as *E. coli*. *Salmonella* and *E. coli* antimicrobial susceptibility testing was conducted with a semi-automated system. A greater degree of resistance was observed with *Salmonella* isolates (n = 41) than with *E. coli* isolates (n = 194). Of the *Salmonella* isolates, *S.* Typhimurium was the most prevalent (69.0%) serotype and had the greatest multiple-resistance. *Salmonella* Kentucky, the least prevalent (5.0%) serotype recovered, was also the most susceptible. While 34.1% of the *Salmonella* were susceptible to all compounds, 60.1% were resistant to more than 5. Antimicrobials for which the greatest numbers of *Salmonella* isolates exhibited resistance were tetracycline (63.4%), nalidixic acid (63.4%), and streptomycin (61.0%). A majority of *E. coli* isolates (73.2%) were susceptible to all compounds studied. Other *E. coli* isolates exhibited resistance to tetracycline (29.9%), streptomycin (6.2%), and gentamicin (3.1%). Only 1% of *E. coli* isolates were resistant to 4 compounds. These data indicate that shell eggs can harbor resistant foodborne and commensal bacteria.

T3-01 Multistate Foodborne Outbreaks in the United States, 1973 – 2003

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The US food supply includes many widely distributed food items. Contamination of these items can lead to widespread outbreaks of foodborne illness. To better characterize the epidemiology of multistate outbreaks in the United States, we reviewed outbreaks reported to CDC. We reviewed data from the Foodborne Outbreak Reporting System from 1973 to 2003. We defined a multistate outbreak as a foodborne outbreak caused by a common food that was consumed in two or more states. From 1973 through 2003, 123 (0.6%) of 20890 foodborne outbreaks were multistate outbreaks. These 123 outbreaks resulted in 15,011 illnesses, 1108 hospitalizations, and 72 deaths. The number and size of multistate outbreaks reported increased during this 30 year period, from 28 outbreaks with a median size of 27 persons during 1973 – 1983, to 79 outbreaks with a median size of 47 persons during 1993 – 2003. For 119 multistate outbreaks the etiology was known:

Salmonella (41% of outbreaks), enterohemorrhagic *E. coli* (15%), Hepatitis A (7%), Norovirus (6%), *Listeria monocytogenes* (5%), *Shigella* (4%) and other pathogens (22%). In 110 outbreaks, a specific food vehicle was implicated: fruits and vegetables (35% of outbreaks), beef (17%), shellfish (13%), dairy (10%), fish (4%), non-dairy beverages (4%), poultry (3%), and other foods (14%). The number of multistate outbreaks has increased in the past 30 years. Fruits and vegetables account for over a third of these outbreaks. As more multistate outbreaks are identified, multistate coordination and action is critical to investigate, control, and prevent them in the future.

T3-02 A Review of Foodborne and Waterborne Disease Outbreaks 1998 – 2004

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In response to the information need associating aetiology and food vehicle, data were examined from 1,964 outbreaks reported from 1998 to 2004. Information collected from peer-reviewed journals, newspapers, listservs, press releases, and government websites was entered into a Microsoft Access™ database. The majority of the outbreaks were reported from the USA (59%), the EU (14%), Canada (10%) and Australia and New Zealand (7%). There were 970 deaths and 100,496 confirmed cases of illness reported. Aetiology was identified in 85% of outbreaks: bacterial disease 62%; viruses 18%; and parasites 4%. The most frequently reported aetiologies were *Salmonella* in 31% of outbreaks, norovirus in 14%, *Escherichia coli* in 11% and *Clostridium perfringens* in 4%. Waterborne outbreaks resulted in the most deaths: *Vibrio cholerae* (529) and hepatitis E virus (139). The majority of deaths from mainly foodborne pathogens resulted from *E. coli* (66), *Listeria monocytogenes* (27), *Shigella* (26), *Salmonella* (25) and *Staphylococcus aureus* (16). Restaurants and commercial caterers were associated with 37% of the outbreaks, the home 20%, school 6%, and hospitals/homes for aged 3%. Home/community based outbreaks were associated with 84% of the reported deaths, school/daycare 5%, processing plants 2%, church/community centers 2%, hospitals or homes for the aged 2% and restaurants or catering 1%. Food vehicles were identified in 60% of the outbreaks and included multi-ingredient foods 17%; meats 17%; water 9%; produce 9%; and seafood 6%. The most frequently reported causative factors were water system deficiencies, eating of raw food, ingestion of recreational water, inadequate cooking and infected food handlers.

T3-03 Outbreak Alert! Trends in Foodborne Illness Outbreaks, 1990 – 2003

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The Center for Science in the Public Interest (CSPI) maintains a unique database of foodborne illness outbreaks in the United States, categorized by food vehicles. Linking outbreaks to specific foods alerts consumers to food safety hazards and provides policy-makers with better information for food safety resource allocation. CSPI's database was compiled from sources including the Centers for Disease Control and Prevention (CDC), state health departments, and medical and scientific journals. It is updated regularly, and contains only outbreaks with known or suspected etiology and food vehicles. The database is extremely useful to identify trends, for example, whether food types implicated in restaurant outbreaks differ from those due to home-prepared foods. Between 1990 and 2003, 4,491 foodborne illness outbreaks with 138,696 cases were listed in the CSPI database. Forty percent (n = 1,777) of these outbreaks were identified as occurring in restaurants, while twenty-three percent (n = 1,028) occurred in private households. The food most commonly linked to outbreaks in both settings was seafood, although it constituted a greater proportion of outbreaks within private homes than within restaurants (31% vs. 23%, $P < 0.05$). Produce was linked to the most cases in restaurants (30%), but not within private households (13%), where both seafood (14%) and beef (12%) were also among the foods most commonly linked to cases. These findings suggest that differences exist in the foods associated with restaurant outbreaks and home-prepared food outbreaks. Contributing factors may include consumption trends, the number of people involved in food preparation, and differing risks of cross-contamination.

T3-04 Difference in the Causes of Foodborne Illness among Young Children and Older Adults in the United States, 1998 – 2003

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Background: Young children and older adults are the most susceptible to foodborne illness. Variation in the most important foodborne agents and food vehicles with age has not been described. We reviewed foodborne outbreak data reported through the National Electronic Foodborne Outbreak Reporting System (EFORS) from 1998 through 2003. Outbreaks were included if at least 50% of ill persons were under the age of 5 years or over the age of 49 years. Between 1998 and 2003, 109 outbreaks were reported in which at least half of the cases were under 5 years of age (2710 illnesses in children under 5). Of those 109 outbreaks, 57 had known etiologies: 86% were bacterial (16 *Salmonella*, 8 E coli and 5 *Yersinia enterocolitica* outbreaks), 10% viral, and 3% marine biotoxin. Thirty-three of the 109 (30%) had known vehicles; the most frequent were poultry (42%) and pork (18%) products. There were 1005 outbreaks reported in which at least half of the cases were over 49 years

of age (21,043 illnesses in adults over 49). Of those 1005 outbreaks, 603 (60%) had known etiologies: 49% were bacterial, 38% viral, and 11% marine biotoxin. 301 of the 1005 had known vehicles; the most frequent were seafood (35%), poultry (18%), fruits/vegetables/grains (16%), and beef (15%). Most foodborne outbreaks affecting young children were caused by bacterial pathogens; poultry was the most common vehicle. Both bacteria and viruses were common etiologies in older persons; poultry was a less common vehicle amongst older adults than young children.

T3-05 *Salmonella* Serotype Enteritidis Outbreaks in the United States, 1993–2003

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Salmonella Enteritidis (SE) emerged in the US in the 1980s. SE is the second most common human *Salmonella* serotype and is often associated with egg contamination. National SE outbreak surveillance data was reviewed from 1993 through 2003 to determine outbreak characteristics and trends. A total of 517 confirmed outbreaks of SE were reported. These outbreaks resulted in 17,112 illnesses, 1,370 (8%) hospitalizations, and 22 (< 1%) deaths. A suspected food vehicle was identified for 408 (79%) outbreaks; 230 (53%) of these were confirmed by culture or statistical association. Eggs were ingredients in 172 (74%) of the 230 outbreaks with confirmed food vehicle. In addition, eggs were ingredients in 113 (63%) of 178 outbreaks in which the suspected food item was not confirmed. Commercial foods were implicated in 276 (53%) outbreaks. Egg traceback investigations were conducted for 73 (42%) of 172 outbreaks in which the suspected or confirmed vehicle contained eggs. The most common phage types were 8 (32%), 13a (24%), and 4 (19%). The two regions with the highest number of SE outbreaks reported were the Mid-Atlantic and the Pacific states. There was a decline in reported outbreaks from 66 in 1993 to 34 in 2003. The number of cases in outbreaks decreased from 2,215 in 1993 to 578 in 2003. National SE outbreak surveillance revealed a decline in outbreaks in the last decade, possibly because of improved prevention, including improved food handling due to egg traceback investigations and intervention programs which prevented SE contamination to shell eggs.

T3-06 Public Health Investigation of *Salmonella* in Raw Shell Eggs Used in Catering

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The Health Protection Agency in October 2002 initiated an outbreak-related public health investigation (PHI) in response to a dramatic change in the epidemiology of *Salmonella enterica* serotype Enteritidis in England and Wales thought to be associated with raw shell eggs. Raw shell eggs from premises linked to outbreaks, or their sources of supply, were examined for the presence of *Salmonella* spp. It is important to note that the focus of this investigation undoubtedly influenced the rates of contamination reported. Eggs sampled were those in use in food premises or suppliers at the time of sampling. Between October 2002 and November 2004, 12,615 shell eggs were sampled, and *Salmonella* spp. was recovered from 4.1% of eggs. *Salmonella* was isolated from 5.5% of Spanish eggs used in catering premises linked to outbreaks — a level significantly higher than that (1.1%) found in non-Lion Quality UK eggs sampled. The small sample of UK Lion Quality eggs tested (reflecting their lack of use in premises visited) did not contain *Salmonella*. Eighteen different strains of *S.* Enteritidis were isolated from Spanish eggs and often, egg and human *Salmonella* isolates were indistinguishable by molecular typing techniques. A number of *S.* Enteritidis strains recovered from these eggs has never been hitherto reported. The use of Spanish eggs by the catering sector has been identified as a consistent causal factor in many of the outbreaks of *S.* Enteritidis non-PT4 infection in England & Wales during 2002-2004. The significance of these findings for public health will be discussed.

T3-07 Analysis of Food Commodities Recalled for Microbial Reasons 2003 – 2004

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Outbreak investigations attempt to associate specific food or water vehicles with the causative pathogen. Information from recalls confirms the source of foodborne disease and identifies new commodity-pathogen combinations. Analysis of country-of-origin data for recalled products identifies areas where contamination occurs. In response to the information need associating aetiology and food vehicle, data were examined from 940 recalls reported for 2003 and 2004. Information collected from peer-reviewed journals, newspapers, listservs, press releases, and government websites was entered into a Microsoft Access™ database. Most recalls were reported from the EU (57%), the USA (27%) and Canada (4%). The majority of recalled meat, dairy, poultry and seafood products originated in Europe. North America was the origin of the majority of recalled produce and nuts. Bacterial contamination caused 97% of all recalls, reflective of testing bias. The most frequently reported pathogens were *Salmonella* (43%), *Listeria* (30%), *Vibrio parahaemolyticus* (6%) and *Escherichia coli* (6%). Food groups most frequently associated with recalls were meats (38%); seafood (27%); dairy (11%); produce (6%) and nuts (5%). *Salmonella* was associated with the largest number of recalls in all meat categories: poultry 58%, beef 43% and pork 76%. Seafood contaminants included *Listeria* (33%), vibrios

(24%) and *Salmonella* (17%). Dairy products were most often recalled because of *Listeria* (66%). Half of all produce recalls were due to *Salmonella* and 29% due to *Listeria*. Analysis of recalls focuses allocation of inspection and testing resources on commodities of greatest risk. Identification of novel commodity-pathogen combinations can prevent foodborne illness.

T3-08 *Salmonella* Serotypes in Selected Classes of Food Animal Carcasses and Raw Ground Products, 1990 – 2000

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From 1990 through 2000, the United States Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS) conducted fifteen separate microbiological baseline studies/surveys for selected classes of food animal carcasses and raw ground products to estimate the prevalence of *Salmonella* spp. contamination. Here, we report the frequencies of *Salmonella* serotypes recovered from these baseline studies/surveys and describe the relative rankings for individual serotypes within and among certain product classes: young chicken carcasses, young turkey carcasses, goose carcasses, ground chicken, ground turkey, cattle carcasses (cows, bulls, steers, and heifers), ground beef, swine carcasses, and fresh pork sausage. Sampling and microbiological methods are described in official FSIS reports and laboratory guidelines. In total, serotype information was obtained for 1,955 *Salmonella* isolates. The observed distribution of 70 discrete serotypes reveals the diversity and variety of serotypes both within and among specific classes of red meat and poultry products. For example, in carcasses and raw ground product originating from chicken, *S.* Kentucky, *S.* Heidelberg, and *S.* Hadar represented 50.3% of serotyped isolates; in swine carcasses and fresh pork sausage, *S.* Derby, *S.* Typhimurium (Copenhagen), and *S.* Anatum represented 47.6% of serotyped isolates. This report is the first to present data regarding the specific serotypes isolated during the course of FSIS baseline microbiological testing for *Salmonella*, thus presenting important regulatory and public health information.

T3-09 Studies to Evaluate Chemicals/Conditions for Lowering Microbial Counts on Cattle Hides

DSC

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Contamination of beef carcasses with *Escherichia coli* O157:H7 has been attributed primarily to transfer of such contamination from the hide during its removal. This study evaluated the efficacy of antimicrobials for reduction of contamination on cattle hide samples. The samples (25 cm^2) were inoculated with a 5-strain composite of rifampicin resistant *E. coli* O157:H7, and then sprayed (7 s) with acetic acid (AA, 10%; 23 or 55°C), lactic acid (LA, 10%; 23 or 55°C), sodium hydroxide (SH, 3%; 23°C), sodium metasilicate (SM, 4 and 5%; 23°C), or distilled water (DW; 23°C). Each chemical solution was also applied before or after spraying with distilled water (7 s). Two min following final spraying, samples were placed into D/E neutralizing broth, pummeled (2 min) and plated on tryptic soy agar (TSA) + rifampicin (100 μg/ml) plates. Initial populations (6.3 ± 0.5 log CFU/cm^2) were reduced by 1.8 to 2.5 log CFU/cm^2 when subjected to AA (55°C), LA (55°C), SH or SM (4 or 5%) sprays followed by water rinse. These, the most effective treatments, were then evaluated for reductions of aerobic plate (APC), total coliform (TCC) and *E. coli* (ECC) counts by spraying specified areas of whole uninoculated cattle hides. Initial populations were 3.9 to 4.3 and 3.7 to 4.1 log CFU/100 cm^2 for TCC and ECC, respectively. Average ECC reductions for all treatments were 2.2 to 2.9 log CFU/100 cm^2. Of treatments evaluated, the organic acids are the least corrosive and the most worker-safe treatments. Thus, they may be worthy of further consideration for application as potential interventions for lowering microbial contamination on hides of incoming slaughter cattle.

T3-10 An Innovative Method for the Recovery of *Escherichia coli*, *Clostridia*, and *Yersinia enterocolitica* from Air Samples

DSC

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Recovering and identifying bacteria from the air can be difficult due to drying stress, nutritional deficiencies, or injuries incurred during air sampling. Previous studies using conventional agars for recovery of bacteria from air samples identified only 2/511 (0.004%) bacterial isolates as *Escherichia coli*, none as *Clostridia*, and only 1/511 (0.002%) isolates as *Yersinia*. As these bacteria may be under-reported, a new method for the recovery of *E. coli*, *Clostridia*, and *Yersinia enterocolitica* from animal confinement air samples was devised. Air samples were collected on Tryptic Soy Agar plates using an impaction air sampler. Depending on the location, 60 to 180 L of air were sampled. The plates were overlaid with 25 ml of either Fluorocult® Brilliant Green 2% Bile (BRILA) broth for *E. coli*, Differential Reinforced Clostridial broth (DRCM) for *Clostridia*, or an in-house-developed broth for *Y. enterocolitica* enrichment. The DRCM plates were sealed with Parafilm® and all plates were incubated for 24 to 48 h at 35 to 37°C. Following incubation, cultures were identified using appropriate microbiological methods and test kits. Using the new approach, 75% of the BRILA plates recovered

E. coli, 80% of the DRCM plates recovered *Clostridia*, and 100% of the plates containing the newly developed broth recovered *Y. enterocolitica*. This research is significant as we successfully identified a novel, effective, and simple method for the recovery of *E. coli*, *Clostridia*, and *Y. enterocolitica* from air samples. Moreover, a highly selective *Y. enterocolitica* enrichment medium was developed.

T3-11 DSC **Sensitive Detection of *Listeria monocytogenes* Using Fully Engineered Recombinant scFv Antibody Fragments**

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Listeria monocytogenes is a major concern to the food industry and has been responsible for many recent well-publicized food poisoning outbreaks. The inclusion of *L. monocytogenes* in the list of organisms subject to HACCP has driven the search for detection methods suitable for on-line monitoring. The aim of this work was the development of a biosensor-based immunoassay for the sensitive detection of *L. monocytogenes*. We have generated murine recombinant scFv antibody fragments against the *L. monocytogenes* invasion-associated protein, internalin B (InlB). The gene encoding InlB was cloned into *E. coli*, biotinylated *in vivo* and used for the selection of anti-InlB antibody fragments by phage display on streptavidin-coated magnetic beads. Random mutagenesis techniques such as DNA shuffling and error prone PCR were employed to affinity maturate selected scFv antibodies. Antibody binding kinetics were analysed, using the Biacore 3000 optical biosensor, to evaluate affinity improved antibody mutants. Epitope mapping studies were performed to define the antibody-binding domain of immobilized metal affinity chromatography (IMAC)-purified recombinant InlB fragments of varying lengths. Antibodies expressed in soluble form were found to interact with the N-terminal region of InlB (region consisting of the cap and leucine rich repeat (LRR) domains) by Western blot analysis. Selected antibodies were characterised by ELISA and used in the development of new biosensor-based immunoassay for the reliable detection of *L. monocytogenes* from enriched samples.

T3-12 DSC ***Listeria monocytogenes* Subtypes Commonly Found in Foods Show Reduced Invasion in Human Intestinal Cells Due to Distinct Non-sense Mutations in inlA**

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Internalin A (InlA), encoded by inlA, is a cell wall anchored surface protein that is sufficient for *Listeria monocytogenes* to invade human intestinal epithelial cells. Previous inlA sequencing data revealed three novel nonsense mutations leading to premature inlA stop codons. To probe the distribution of these mutations, additional *L. monocytogenes* isolates from human clinical cases (n = 46), foods (n = 116), and healthy animals (n = 58) were screened for the presence of premature inlA stop codons. Premature inlA stop codons were observed in six *L. monocytogenes* strains (EcoRI ribotypes). While all isolates belonging to ribotypes DUP-1046B, DUP-1062A, DUP-16645A, and DUP-1031A contained premature inlA stop codons, ribotypes DUP-1052A and DUP-1025A contained isolates with and without premature inlA stop codons. Western immunoblotting showed that these non-sense mutations in inlA resulted in production of an InlA that was truncated in size and secreted rather than cell wall anchored. Searches of the PathogenTracker database, which contains subtype and source information for more than 5,000 *L. monocytogenes* isolates, showed that the six ribotypes known to contain isolates with premature inlA stop codons were common among food isolates and rarely associated with human listeriosis. A Caco2 cell invasion assay *L. monocytogenes* isolates with a truncated and secreted InlA have significantly ($P < 0.0001$) reduced invasiveness in human intestinal epithelial cells as compared to isolates with a full-length InlA. Our data showed that specific *L. monocytogenes* strains, which are common among US food isolates but rarely associated with human listeriosis, carry inlA mutations leading to an attenuated invasion phenotype.

T4-01 DSC **Determination of Minimum Inhibitory Concentrations of Sodium Lactate and Sodium Diacetate Combinations against Individual *Listeria monocytogenes* Strains**

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Processors include combinations of sodium/potassium lactate and diacetate in the formulation of ready-to-eat meat and poultry products for control of *Listeria monocytogenes*. Lactate and diacetate are approved by the Food Safety and Inspection Service for use in these products at levels up to 4.8% and 0.25%, respectively. The objective of the present study was to determine MIC of lactate (0 to 4.8%) and diacetate (0 to 0.25%) combinations in broth, using 96-well microtiter plates; four strains, classified from previous studies as fast- (N7150 and 558) and slow-growing (Scott A and NA1), were studied. Growth responses at 4°C (60 days), 7°C (15 days) and 30°C (24 h) were determined turbidimetrically (630 nm) with a microplate reader. At 7°C, MIC of lactate and diacetate ranged from 0.5 to 4.8%, and 0.1 to 0.2%, respectively, depending on strain. The MIC (7°C) of combinations of lactate/diacetate (%) were 3/0.1 and 2/0.15, 2.5/0.05 and 2/0.1, 0.5/.05, and 2/0.05 for strains N7150, 558, Scott A and NA1, respectively, and the results suggested that lower lactate/diacetate

concentrations were effective for slower growing strains. Overall, growth at 7°C was inhibited by 4.8% lactate or 0.15% diacetate or by combinations of 2.5% lactate and 0.05% diacetate or 2% lactate and 0.1% diacetate, while at 30°C the effective combination consisted of 1.5% lactate and 0.2% diacetate. These results should be useful in efforts to validate optimal concentrations of lactate/diacetate for control of *L. monocytogenes* in broth.

T4-02 DSC Combined Inhibition of *Listeria monocytogenes* Growth Using Lactic Acid, Monolaurin, and Nisin

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Listeria monocytogenes is a foodborne pathogen associated with refrigerated ready-to-eat foods. One approach to control this pathogen is through use of antimicrobial ingredients in the formulation or as decontamination aids. To overcome the sensory limitations of some antimicrobials, such as organic acids, use of combined agents at lower concentrations may be useful. Previous work has shown that lactic acid, monolaurin, and nisin have individual antimicrobial activity against *L. monocytogenes*. The objective of this study was to evaluate *L. monocytogenes* growth inhibition by combinations of lactic acid, monolaurin, and nisin. Using a microtiter plate growth assay, two levels of each antimicrobial, zero and sublethal concentrations (0.24% lactic acid at pH 5.43, 5 μg/ml monolaurin, and 25 IU/ml nisin) were used individually or combined to assess *L. monocytogenes* ATCC 7644 growth at 35°C in trypticase soy yeast extract broth for 40 h. All combinations of factors and their levels resulted in N = 2n experimental design. The four-parameter Gompertz equation was applied to growth curves to calculate detection time and growth rate. Treatments that included lactic acid and nisin, monolaurin and nisin, and the three combined antimicrobials prevented growth. The combination of lactic acid and monolaurin resulted in a slower combined growth rate (0.023 A600/h) and a longer detection time (26 h) than either antimicrobial alone (0.041 A600/h and 10.6 h for lactic acid and 0.210 A600/h and 8.47 h for monolaurin). This study demonstrates that a combination of lactic acid, monolaurin, and nisin may prove useful to control growth of *L. monocytogenes*.

T4-03 DSC The Antibacterial Effects of Decanol with and without Nisin against *Listeria monocytogenes* Strains

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Historically alcohol has been used to control pathogenic bacteria in the meat industry. In the present study the minimum inhibitory concentration (MIC) for decanol, a natural by-product of enteric bacteria, was determined for six strains of *Listeria monocytogenes* (LM) (pH 7 and 30ƒC). The antibacterial effect of decanol in Muller Hinton Broth was determined spectrophotmetrically (660 nm) following 12, 24, 36, 48, and 60 h of incubation. Greater than or equal to 40-ppm decanol concentrations significantly inhibited the growth of all LM strains compared to control samples ($P < 0.05$). Decanol concentrations less than 30-ppm increased the population of LM strains at 24 h. To evaluate the ability of decanol to affect the antilisterial activity of nisin, cultures were grown in the presence of both compounds. Nisin concentrations of 400 and 800 IU in combination with 20-ppm decanol showed significant population reductions of LM following 24 h of incubation compared to a control ($P < 0.05$). Following 24 hrs incubation cell populations increased and reached 10^9 CFU/ml at 60 hrs. Lower concentrations of nisin (100 and 200 IU) did not have any significant effects on reducing LM population ($P < 0.05$). Treatments containing decanol concentrations of 10, 20 and 30-ppm and 50 IU nisin were examined and results showed no significant inhibitory effect on total LM population ($P < 0.05$). These results indicate that decanol is effective in reducing LM populations in broth cultures and may be an effective option for controlling LM in meat products.

T4-04 DSC Multi-drug Resistance in Nalidixic Acid Resistant Mutants of *Salmonella* Typhimurium

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The emergence of multi-drug resistant foodborne pathogens is a concern affecting human and animal medicine as well as the food processing industry. Acquisition of antibiotic resistance can occur through chromosomal mutations or horizontal transfer. To examine the development of multi-drug resistance through chromosomal mutations, 21 nalidixic acid resistant mutants were developed using *Salmonella* Typhimurium ATCC 14028 as the wild type strain. Mutants were developed separately in a stepwise selection with a 50 μg/ml endpoint resistance. Minimum inhibitory concentrations were determined for 15 antibiotics, using a 96 well microdilution plate; resistance was determined by use of the Clinical and Laboratory Standards Institute interpretive standards. The wild-type strain was susceptible to all of the antibiotics tested. Multi-drug resistance was observed in the nalidixic acid resistant mutants; 11 mutants were resistant to two or more antibiotics. One mutant was resistant to 6 antibiotics (nalidixic acid, sulfisoxazole, tetracycline, chloramphenicol, cefoxitin and streptomycin). Mutants with resistance to four and five antibiotics were also observed. Six mutants were resistant to two antibiotics, and 2 mutants were resistant to 3 antibiotics. The most common resistance observed was sulfisoxazole (6 mutants),

followed by chloramphenicol (5 mutants), cefoxitin and streptomycin (4 mutants, respectively) and tetracycline (3 mutants). Studies have shown the number of foodborne pathogens with resistance to nalidixic acid appears to be increasing, and both animal and human isolates of nalidixic acid resistant foodborne pathogens have been observed. The results of this study indicate that chromosomal mutations leading to nalidixic acid resistance can also produce multi-drug resistant *Salmonella* Typhimurium.

T4-05 The Effect of Dried Plum Mixtures on Microbial Shelf Life of Ground Beef

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Spices and essential oils containing phenolic compounds can control or prevent the growth of foodborne pathogens and spoilage organisms in media and food systems. Commercial dried plums and dried plum mixtures (*Prunus domestica* cv. French) contain phenolics and have been shown to reduce foodborne pathogens in liquid medium, ground beef, and pork sausage. The objective of this study was to determine the effect of dried plum mixtures on the microbial shelf life of ground beef when added to meat with low or medium initial aerobic plate count (APC). The effect of dried plum mixtures on microbial shelf life of ground beef was determined by adding plum juice concentrate, dried plum puree, or prune powder to ground beef at 3% and 6% for low initial APC and 6% for medium APC and comparing to ground beef without dried plum mixtures. The meat was stored at 7°C for 18 days, with APC determined every other day for the extent of storage. The dried plum mixtures were able to increase the microbial shelf life of ground beef by maintaining the APC of the ground beef with dried plum mixtures after initial growth with the low APC samples and throughout storage with the higher initial APC samples while the APC of the ground beef without dried plum mixtures continued to increase to greater than 9 log CFU. Because of the dried plum mixtures' ability to control microbial growth, the addition of dried plums to ground beef would be very beneficial.

T4-06 A Comparative Study as Related to the Effects of Glucose Monohydrate, Hot Water and Sodium Pyrophosphate on Some Quality Criteria of Deboned and Matured Brisket

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Organic acids, hot water and chlorine have been commonly used in carcass decontamination for years. Organic acids had adverse effects on color and were corrosive, while high temperature water was discoloring. On the contrary, glucose fermentation by lactic acid bacteria in meat during rigor might be effective in microbial inhibition without adverse effect. Maturation of beef brisket primal cuts occurred for 2 days at 11°C and then at 5°C for 4 days in 85% relative humidity. Briskets were dipped in D(+) Glucose Monohydrate (GM) (16.51 g/100 ml, 15%) on the 7th day of this maturation. Other primals were treated by hot water immersion (HW); Sodium Pyrophosphate (SPP) and HW; GM+SPP+HW; or GM+HW. 1.63 and 1.41 log reductions in coliforms, 1.90 and 1.80 log reductions in *Pseudomonas* spp., and 0.83 and 0.72 log reductions in MAB (Mesophile Aerobic Bacteria) were observed in GM+HW and SPP+GM+HW respectively at day 8 of shelf life under 4°C, indicating a significant protective effect of the concerned applications with their lowest acidity losses, while SPP's increasing effect of heat penetration was not detected during pasteurization, from the standpoint of *Pseudomonas* and coliform inhibitions ($P < 0.05$ and $P < 0.01$).

T4-07 Prevalence and Enumeration of *Escherichia coli* O157 in Beef Steers Receiving Various Strains of Direct-fed Microbials

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The need for effective strategies to control foodborne pathogens in the pre-harvest area is increasing. Previous research has indicated steers supplemented with *Lactobacillus acidophilus* (strain NP 51) have reduced prevalence of *E. coli* O157 and improved animal performance. We conducted a study to evaluate fecal *E. coli* O157 prevalence and concentration in feedlot steers fed four differing *Lactobacillus*-supplemented diets. Fecal samples were collected immediately prior to shipment to the abattoir. *E. coli* O157 was detected with selective enrichment and IMS methodologies. A novel MPN method was used to determine concentration of *E. coli* O157 on positive samples. Prevalence varied among the four treatment diets and the control ($P < 0.01$). The greatest prevalence was in the controls (26.6%), which was greater ($P < 0.05$) than those supplemented with NP51 or NP28. The greatest *E. coli* O157 concentration was also observed in the controls (3.2 log CFU/10g); this concentration was greater than in positive animals receiving NP51 or NP24 ($P < 0.05$). Specific strains and combinations of *Lactobacillus* reduced prevalence of *E. coli* O157 by more than 50% in harvest-ready cattle. In addition, treatment animals had on average, a 99% reduction in *E. coli* O157 concentrations in their feces.

T4-08 Comparison of Petrifilm and ChromAgar ECC for Isolation of *Escherichia coli* from Chicken

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Generic *Escherichia coli* is frequently recovered from processed broiler chickens, and FSIS is requiring meat and poultry slaughter plants to test carcasses for generic *E. coli* as an indicator of the adequacy of the plant's process control for fecal contamination. The majority of *E. coli* enumeration in the poultry and meat industries currently uses Petrifilm as the plating medium of choice. The objective of the current study was to determine if different subpopulations of *E. coli* were selected for by Petrifilm in comparison to ChromAgar ECC, a chromogenic medium for isolation of *E. coli*, and to compare the relative effectiveness of these two media to enumerate generic *E. coli* from processed broiler chicken rinsates. Rinse samples of broiler chickens taken from the processing line immediately after the picker or after the chill tank from eight processing plants were transported to the laboratory and sampled within 24 h. Serial dilutions of the rinses were plated on Petrifilm and ChromAgar, and plates were incubated for 24 and 48 h before counts were determined. Selected colonies were stored to compare antimicrobial resistance profiles and PFGE patterns. No significant difference in *E. coli* counts (log 2.58 vs log 2.49) were found between Petrifilm and ChromAgar. However, analysis of antibiotic resistance profiles and PFGE patterns indicates that the two plating media select for different subpopulations of *E. coli*. In general, ChromAgar selected for *E. coli* which had more antibiotic resistance. Further studies are required to determine the origins and significance of the different *E. coli* populations.

T4-09 The Microbial Efficacy of Commercial Application of CPC Antimicrobial to Prechill Broiler Carcasses

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A 90 day in-plant trial was conducted in an USDA-inspected broiler processing facility to determine the antimicrobial efficacy of Cecure (cetylpyridinium chloride:CPC) as a prechill whole carcass treatment. The trial was designed to evaluate microbial effects of treating inspection-held "visually contaminated" carcasses. Treatment of these carcasses was necessary to determine whether CPC could be utilized as an integral part of a plant's on-line reprocessing procedure for carcasses accidentally contaminated with digestive tract contents. Over the 90 day trial, carcasses were collected on 20 sampling days. On each day carcasses (n = 10) were collected at four sites, including visually clean carcasses before CPC treatment, visually contaminated carcasses before CPC treatment, visually contaminated carcasses after CPC treatment, and visually contaminated carcasses after traditional off-line reprocessing (manual wash-out with chlorinated water). All carcasses were microbiologically evaluated for APC, *E. coli*, total coliform, incidence and level of *Campylobacter*, and incidence of *Salmonella*. Treatment of visually contaminated carcasses with CPC significantly reduced APC by 2.5 logs, *Escherichia coli* by 1.6 logs, total coliforms by 1.2 logs, and *Campylobacter* by 1.2 logs. CPC treatment resulted in greater than 90% and 70% reduction in incidence of *Campylobacter* and *Salmonella*, respectively, on the prechill carcasses. The microbial condition of CPC-treated visually contaminated carcasses was significantly improved in comparison to non-treated "clean" carcasses and traditional off-line reprocessed carcasses. The data suggest that use of CPC as a prechill carcass treatment provides a viable alternative to labor intensive off-line reprocessing as well as providing carcasses with significantly reduced microbial levels.

T4-10 Random Amplified Polymorphic DNA Analysis and Antibiotic Resistance Patterns of *Salmonella* spp. from Whole Broiler Carcass Rinses

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The objective of this study is to compare antibiotype and random amplified polymorphic DNA (RAPD) profiles of *Salmonella* isolates from whole broiler carcass rinses and determine the correlation between antibiotic susceptibility and RAPD profiles. In a previous study, 38 *Salmonella* isolates obtained from whole carcass broiler rinses (51% broiler samples positive for *Salmonella*) were tested for antibiotic resistance patterns against 10 antimicrobial agents. About 68% of the isolates were found to be resistant while 24 % were sensitive to antimicrobial agents. The isolates were further analyzed by random amplified polymorphic DNA (RAPD) analysis by use of five decamer universal primer pairs and compared with antibiotics susceptibility profiles. Five isolates from each susceptibility group or antibiotype (resistant, intermediate resistant and sensitive) were subjected to RAPD and a dendrogram was generated. The data showed that 66% of the strains resistance to antimicrobial agents had similar profiles in comparison to intermediate resistant (52%) and sensitive strains (38%). Overall, sensitive strains exhibited fewer polymorphic bands (11) compared to intermediate (18) or resistant (19) strains. This study indicates that the strains with increased resistance to antibiotics may have acquired antibiotic resistance genes during the evolutionary process, therefore showing higher genomic polymorphism or mutational divergence than the sensitive *Salmonella* strains.

T4-11 Antimicrobial Resistance Profiles of *Salmonella*, *Campylobacter*, and *Escherichia coli* from Chickens Reared without Exposure to Antibiotics

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The antibiotic resistance profiles of *Salmonella*, *Campylobacter*, and *Escherichia coli* from retail broiler chickens reared without exposure to subtherapeutic or therapeutic antibiotics were determined. For *Salmonella* and *E. coli*, resistance profiles to the antibiotics used in the National Antimicrobial Resistance Monitoring System (NARMS) were compared to those previously reported for NARMS poultry isolates. Overall resistance of *Salmonella* isolates from chickens reared free of antimicrobials was less than observed in poultry isolates from NARMS. Only ampicillin resistance was found more frequently among the current study isolates and this was attributed to one sampling period from one company. Overall, *E. coli* isolates also demonstrated less resistance than NARMS isolates, with the exception of kanamycin and trimethoprim/sulfamathoxazole, which were resistant at approximately the same levels as NARMS isolates. *Campylobacter* were tested for resistance to eight antimicrobials. *C. jejuni* from chickens reared free of antimicrobials were susceptible to azithromycin, clindamycin, and eythromycin. Resistance was only observed for ciprofloxacin (n = 33; 15.2%), naladixic acid (n = 33; 15.2%), and tetracycline (n = 33; 48.5%). Overall, isolates of *C. coli* were more resistant than isolates of *C. jejuni*. While less antimicrobial resistance was observed in isolates from chickens reared without exposure to antibiotics, resistance was still observed for all major classes of antimicrobials. This suggests that rearing of chickens without antimicrobials decreases, but does not eliminate, the prevalence of resistance.

T4-12 Decontamination of Whole Cantaloupes Using a Pilot-scale Chlorine Dioxide Gas Treatment System

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Multi-state cases of salmonellosis from cantaloupes have raised concern. Our objectives were to evaluate a pilot-scale ClO_2 gas treatment system to reduce *Salmonella* on cantaloupes and its effects on microbial quality and skin color after the treatment and during refrigerated storage. Each cantaloupe was spot-inoculated with 7 to 8 log CFU of a mixture of five *Salmonella* strains on an area of 4 × 4 cm^2 of cantaloupe surface, stored for 24 h at 22°C, treated with 5 to 12.5 mg/l ClO_2 gas for 10 min at 22°C and 80 to 95% relative humidity with an automated pilot-scale treatment system, and then stored for 1 to 4 weeks at 4°C. Populations of *Salmonella*, aerobic plate count (APC), and yeast and molds (YM) were determined by surface-plating on xylose lysine desoxycholate agar, plate count agar, and dichloran rose bengal chloramphenicol agar, respectively. Surface color was measured using a Hunter colorimeter. The levels of *Salmonella* were reduced by 3.31 log CFU immediately after 5 mg/l treatment and further reduced by 4.98 log CFU in total after one-week storage. The 12.5 mg/l treatment led to a reduction of 4.70 ± 1.07 log *Salmonella*, 3.0 ± 1.18 log on APC, and ≥ 3.80 ± 0.4 log on YM. Further storage for 4 weeks at 4°C resulted in a total reduction of 6.0 ± 0.12 log *Salmonella*, 4.17 ± 2.0 log on APC, and 3.77 ± 0.62 log on YM. During the storage, *Salmonella* level decreased and APC and YM populations increased on untreated samples. No significant color changes were observed after ClO_2 gas treatments. ClO_2 gas technology is promising for reducing *Salmonella* and maintaining quality on cantaloupes.

T5-01 Foodborne Illness Litigation and How to Avoid It

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Pathogens in food cause an estimated 76 million cases of human illness in the United States each year. CDC estimates that 325,000 individuals are hospitalized and 5,000 people die as a result of consuming contaminated food. Only a small percentage of these injured persons pursue legal action as a result of their illness but when they do, it can mean catastrophe to a food service establishment and its suppliers. Based on the rule of strict liability, food service administrators can be held liable if the food they serve is unsafe and causes injury. The speaker will discuss issues such as liability and how it is determined, the discovery process, and why ignorance is a bad defense. Real-life examples from past foodborne illness litigation will be used to illustrate how food service establishments can avoid catastrophe and survive in the event a foodborne outbreak occurs.

T5-02 Framework for Identification and Collection of Data Useful for Risk Assessments of Microbial Foodborne or Waterborne Hazards

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The ILSI Risk Science Institute (RSI) has established an Advisory Committee on Data Collection for Microbial Food Safety Risk Assessment (MRA) in developing countries. The committee includes representatives from FAO, WHO, governments, non-profits and academics from Asia, Europe, Africa and the Americas as well as ILSI branches. There is a need for capacity building in developing countries, both in how to do MRA, and on

the types of data needed to ensure that the risk assessments are meaningful. At present, data for international risk assessments come mainly from North America, Western Europe, Australia, New Zealand and Japan, as few data are available from other regions of the world. Risk assessments will be more relevant and useful if a broader data set is used, particularly when collecting data for exposure assessment or epidemiological food attribution. The Committee is developing a framework for data collection for MRA that could be used by national governments worldwide. The framework includes specific information on how governments can use existing data, and what types of new data may need to be generated in order for an assessment of risk to be made. The document lists both the minimum and the optimum data sets needed for MRA, focusing on data to determine burden of foodborne and waterborne disease, contamination of food/ water and food consumption patterns. Case studies on *Vibrio* in shellfish in Thailand and Malaysia and on *Campylobacter* in chickens in Uganda are used to illustrate the framework.

T5-03 The OIE Standard for BSE: A Case of International Risk Management

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The World Organization for Animal Health (OIE) standard for bovine spongiform encephalopathy (BSE) is reviewed, focusing on the criteria for classifying the risk status of a country's cattle population. The decisional criteria used in crafting the international BSE standard are compared with those prescribed for national standards under the World Trade Organization (WTO) Sanitary and Phytosanitary Agreement (SPS Agreement). The SPS Agreement may be interpreted to require purely risk-based national standards designed to achieve a country's appropriate level of protection (ALOP). The OIE standard for BSE is not based on a numeric risk estimate or safety standard. Instead, it qualitatively classifies the risk status of a cattle population based on a combination of numeric and narrative criteria and identifies restrictions on trade in live animals and animals products that are considered commensurate with the BSE risk classification. The OIE standard-setting involves balancing the risks and benefits of trade and consideration of other factors, such as enforcement aspects of regulatory design and maintaining the confidence of consumers and trading partners. Thus, risk management decisions such as the OIE standard for BSE inherently require judgments that extend beyond the scope of science. Consequently, some tension is to be expected between the decisional criteria prescribed under the SPS Agreement and those applied by the international risk management agency.

T5-04 RTE Food Product Exposure from Cross-contamination Vectors

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Currently, very little information is available for use in exposure assessments tackling the complex area of post heat-treatment, microbial cross-contamination transfer to ready-to-eat (RTE) foods during production. Consequently, CCFRA, together with a Working Party of UK and multinational RTE food manufacturers, have established a workplan to evaluate product exposure from studies both in the laboratory and in selected food manufacturing environments. The objective of this on-going 3 year program is to produce a Guideline Document that contains information, experimental data, case studies, expert opinion and selectively developed models that can be used to help assess the exposure of RTE foods to microbial cross-contamination. This will include a ranking of selected vectors, in order of significance, with regard to microbial transfer to selected RTE foods. In addition, cross-contamination data will also be available in a format that will be useful in other risk assessment models. Sources of microbial contamination and vectors of transfer have been determined for three manufacturing process lines: cooked and sliced ham, cooked pasta (as an ingredient on a lasagne line) and leaf salad. Factory studies are gathering data on the levels of TVC and selected microorganisms associated with selected vectors (the air, liquid aerosols, stainless steel contact surfaces, foodhandlers hands) and the frequency/duration of the contact of these vectors with the products during production periods. Laboratory studies are being undertaken to examine and model microbial transfer rates from the selected vectors to the selected product surfaces. Transfer variables of e.g. contact time, contact pressure, degree of surface moisture, product surface characteristics and microorganism type are being investigated.

T5-05 Withdrawn

T5-06 Impact of Contact Time and Product Weight on Transfer of *Listeria monocytogenes* from Different Conveyor Belt Surfaces to Ham and Bologna

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Given *Listeria monocytogenes* concerns surrounding delicatessen meats, transfer rates from interlocking conveyor belts composed of high-density polyethylene (HDPE), acetyl resin (A) and polypropylene (PP) to sliced ham and bologna were determined using different contact times and product weights. Sterile HDPE, A and PE conveyor belt coupons (5 × 8 cm) were inoculated with a 6-strain *L. monocytogenes* cocktail (~10^5 CFU/coupon). After 30 min, one ham slice (20 g) was placed on the inoculated surface followed by a 12 oz (340.2 g) deadweight and held for 30 to 90 s. The ham was then appropriately diluted and plated on Modified Oxford Agar overlaid with Trypticase Soy Agar + 0.6% yeast extract to quantify *Listeria*. Contact times of 30, 60

and 90 s yielded statistically similar transfer rates of 86.5%, 88.2% and 87.0% for all three materials, respectively. Using deadweights of 4 (113.4 g), 8 (226.8 g) and 12 oz (340.2 g) and a contact time of 1 min, fewer listeriae ($P < 0.05$) were transferred using a 4 (71%) compared to an 8 (79%) or 12 oz (82%) deadweight. When *L. monocytogenes* transfer from HDPE conveyor belt coupons to bologna was assessed using contact times of 15 s to 5 min (1 pound deadweight) and 60 s (1, 3, and 5 lb. deadweights), no significant differences ($P > 0.05$) were seen between results with different contact times. However, transfer was greater ($P < 0.05$) at 3 and 5 compared to 1 lb. Thus, *Listeria* transfer rates were primarily dictated by product weight rather than contact time or surface material.

T5-07 Validation of the Safety of Bacon Processing from Biological Hazards of *Clostridium perfringens* and *Staphylococcus aureus*

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It is unclear how rapidly heat-treated, not fully cooked meat products, such as bacon, should be cooled to prevent outgrowth of sporeforming bacterial pathogens while also limiting growth of vegetative cells. Pork belly substrates with or without 1.25% liquid smoke (LS) were inoculated with *Clostridium perfringens* spores and vegetative cells and *Staphylococcus aureus*. Substrates underwent thermal profiles of industrial smoking to 48.9°C and cooling (3 h) of bacon, or a 15 h-cooling phase, until reaching 7.2°C. Laboratory-scale smoking and 15 h cooling using whole belly pieces was also performed. Under industrial smoking and cooling thermal conditions, growth of *C. perfringens* in substrates was <1-log regardless of smoke. Increase of *S. aureus* was 2.38 log CFU/g, but only 0.68 log CFU/g with LS. When cooling of non-LS substrates spanned 15 h, both pathogens grew by about 4-log and staphylococcal enterotoxins were detected in 5 of 6 samples. LS inhibited *C. perfringens*, but *S. aureus* grew by 3.97-log, though without enterotoxin production. In whole belly pieces, initial *C. perfringens* populations of 2.23 log CFU/g were reduced during smoking to 0.99 log CFU/g, and were 0.65 log CFU/g after 15 h of cooling. Smoking reduced populations of *S. aureus* from 2.00 log CFU/g to a final level of 0.74 log CFU/g after cooling in 15 h. LS plays a critical role in preventing pathogen growth during smoking and cooling of bacon. If smoked bacon is cooled from 48.9 to 7.2°C within 15 h, a food safety hazard from *C. perfringens* or *S. aureus* is not likely to occur.

T5-08 Variation among Batches of Freshly Ground Chicken Breast Meat Complicates the Modeling of *Salmonella* Growth Kinetics

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The objective of the current study was to develop and verify a tertiary model for predicting the high-density (3.7 log CFU/g) growth of *Salmonella* on freshly ground chicken breast meat as a function of time and temperature. A multiple antibiotic resistant strain of *Salmonella* Typhimurium was inoculated onto the surface of one-g portions of freshly ground chicken breast meat. Changes in pathogen density over time of incubation at 10 to 40°C were assessed by viable cell counts on a selective medium with four antibiotics. Kinetic data were fit to the modified Gompertz primary model to determine lag time (LT), maximum specific growth rate (SGR) and the total log cycles of growth (C). Secondary models for LT, SGR and C as a function of temperature were developed and combined with the primary model in a computer spreadsheet to create the tertiary model. Ability of the tertiary model to predict the CFU data (n = 928) used in model development (verification) was evaluated using the recently published acceptable prediction zone method. The proportion of prediction errors (pD) that fell in an acceptable prediction zone from a log cycle difference (D) of -0.5 (fail-safe) to 0.25 (fail-dangerous) was 0.552, which was below the acceptable value of 0.700 for pD. The failed verification of the tertiary model was not explained by performance of the primary model, which had an acceptable pD of 0.934, or by improperly fitting secondary models. Rather, it resulted from variation of *Salmonella* growth kinetics among batches of ground chicken.

T5-09 Correlation of Visual Perception of Cleanliness and Reported Cleaning Practices with Levels of Microbial Contamination in Home Refrigerators

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Consumers are the final line of defense against foodborne illness. Consumers' food handling and storage practices greatly impact the degree of microbial contamination in the home refrigerator and the risk of foodborne illness for family members. Trained observers used a cleanliness scale to assign scores for the apparent condition of four areas of over 100 in-home refrigerators, and swabs were collected from the surface of those areas. Additionally, each consumer completed a home refrigeration practices survey. Aerobic plate counts (APC), psychotrophic plate counts (PPC) and a microbial ATP bioluminescense procedure (mATP) were carried out on swabs to assess microbial contamination. The highest correlation (r = 0.895) was found between

mATP and the average of APC and PPC. Microbial ATP was not highly correlated with consumer's self-reported refrigerator cleaning practices. Cleanliness scores for several refrigerator compartments were correlated with mATP found on the bottom shelf. Microbial ATP in the vegetable bin was correlated with the cleanliness score for that compartment. Fewer vegetable bins contained nondetectable mATP (14%) while over 22% had higher levels of microbial contamination. Microbial ATP appears to be a suitable rapid method for assessing the degree of microbial contamination of home refrigerators. Consumers should regularly engage in adequate cleaning of their refrigerators regardless of visible soiling.

T5-10 An Evaluation of the Medical Screening Methods Used for Employees and Visitors to Food Manufacturing Plants in the USA, Canada, UK and Mainland Europe

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Humans can be a source of foodborne pathogens and food handlers have been identified as the original source of the pathogen in a number of outbreaks. Control measures include testing and identification both sufferers and symptomless carriers. Food safety systems vary in the demands for medical screening both for employees and visitors to food processing sites.The varying expectations of international food safety audit programs have been studied. The practices and requirements for medical screening to assess the levels of control over employee and visitors health were analyzed using the BRC (Global Food) Technical Standard as a benchmark. The methods for reporting health status were also analyzed. Key areas were training; screening techniques for both visitors and employees; reporting structure for illnesses while employed; documented policies on the above. The effects of culture on medical screening and reporting behaviors and practices are also discussed. Strategies on how these may be overcome are reported. In total the practices of 20 companies were studied across the two continents. Fifty percent had documented training materials outlining the controls employed during medical screening. Sixty percent failed to screen visitors with a questionnaire on recent health and exposure to foodborne illness or food poisoning possibilities. Thirty percent had no ongoing assessment of employee health and no method of reporting foodborne illness and exposure through contacts to such risks. For those companies that did utilize checks, many only operated a restricted range of enquiries and requirements likely to miss important foodborne pathogens. Thirty-five percent operated a visitor screening questionnaire, with 25% specifying food poisoning or foodborne diseases within the screening process. Visits to some countries have been reported as a risk factor in some outbreaks of food poisoning and is of particular importance given the global nature of current food trade, however, only 25% specifically mentioned foreign travel in their screening protocol. The findings of the study will be discussed within the context of food safety and international trade.

T5-11 In Situ Sensor Technology

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In situ sensor technology is increasingly being utilized as a panacea to protect public safety and infrastructure. We have combined molecular biological techniques with Microsystems technology to allow in-field genetic analysis, thereby removing the separation between sample collection and analysis. These sensors are open platform and tunable. Development of the sensors has used a combination of micro-fluidic management systems, Micro-Electro-Mechanical-Systems (MEMS), non-standard Printed Circuit Board (PCB) technology and off-the-shelf components to produce systems that allow in-field application of molecular biological techniques. Using Nucleic Acid Based Sequence Amplification (NASBA) as a base methodology we have developed a fully automated in-field analyzer and semi-autonomous systems that require varying degrees of user input. We have utilized a Personal Data Assistant (PDA) as a user interface for a handheld real-time NASBA detection system that allows a user to manually prepare and analyze samples in the field. Amplifications are displayed to the user on the PDA as a real-time plot of fluorescence with time. The device can be tuned to virtually any nucleic acid sequence and is comparable to current instrumentation. Similarly, use of PCB/MEMS technology sample preparation and amplification modules are integrated into a "match-box" size sensor. The eventual target for this research is to enable integration of automated and semi-autonomous sensors with the aim of producing multifunctional (chemical/biological) in situ adaptive sensor arrays. Combined with microbial growth models and RFID-technology, these instruments will help provide a secure and safe food supply and provide cost effective in-house quality assurance monitoring capabilities.

T5-12 Assessment of Temperature Fluctuation in Multiple Locations of In-home Refrigerators in Four USA States

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Refrigerators help maintain the microbiological safety of the food supply by inhibiting the growth of bacteria that reproduce rapidly when above 40°F. Yet, little current information is known about actual temperatures at which cold foods are stored within the home and the variation of those temperatures. Thus the

objective of this project was to evaluate the temperatures within refrigerators within 200 homes in four states in the US. Temperature loggers were used to record temperatures each min in 2–3 places for 3–7 days. In some homes a thermocouple was also placed in a commercially sealed hot dog and a cup of yogurt, both of which were provided by the researchers. Mean temperatures ranged from 35°F for the top shelf to 38°F on the bottom shelf. Four percent had average temperatures above 45°F and 25 percent were above 40°F. The mean temperature on the door was 41.3°F; however, 61 percent had average temperatures above 40°F, and 15 percent had average temperatures above 45°F. The majority of the refrigerator doors (71%) were above 40°F for more than six h per day. The temperature of the hot dogs (mean 36.3°F) and the yogurt (mean 37.1°F) fluctuated less than the temperature of the surrounding air. Therefore, a large percentage of refrigerators were not maintaining the recommended temperature continuously, and remained in the danger zone for more than two h a day.

T6-01 Research on Consumer Attitudes and Behaviors as a Foundation for Educational Programs in FightBAC!

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The basic FightBAC! messages form the framework for baseline measures of food safety behaviors of consumers and foodservice workers for Healthy People 2010, the public health blueprint for the USA. Because they are research-based, consistent, collaborative messages, they are the backbone of public food safety campaigns in the USA. These messages originate in the scientific literature and proprietary research on consumer attitudes and behaviors. The Partnership for Food Safety Education (PFSE) reviews this research to determine what consumer attitudes and behaviors are barriers to optimal consumer food safety practices. Using expertise of government, professional, consumer, and trade association partners, PFSE develops a range of alternative messages and consumer tests them for clarity and motivation. Once developed, the messages are available to partners and others for dissemination through government outreach, private industry initiatives, school education programs, and media placements. The partnership component of FightBAC! is critical to developing, disseminating, and evaluating food safety messages that will make a public health and personal health difference in the USA. During this presentation, we will explore the development and application of effective partnership strategies as a force for establishing research needs, developing priority messaging, disseminating messages through grassroots membership of partner organizations and public members of the BAC fighters, and evaluating the success of food safety campaigns both large and small. We will also share the next steps of FightBAC! and describe how you and your organization can participate in those steps and share the success.

T6-02 Influencing Factors for the Adoption of Food Safety Controls in the Mexican Meat Sector

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This study explores the factors that motivated the adoption of quality and food safety controls in Mexican enterprises, Type Federal Inspection (TIF) by the implementation of HACCP System. A questionnaire was sent to 160 meat processing plants in the 25 Mexican States. Principal Component Analysis identified four key factors that have motivated the adoption of HACCP, that is, internal efficiency and profitability of plant, being a good practice, to improve quality of meat products and to reduce product wastage in plant. The results indicate that there are differences in the HACCP adoption process between individual firms. Domestic market will be protected by gradual adoption of HACCP by Mexican meat processing firms.

T6-03 Evaluation of Consumer Food Hygiene Initiatives in the UK

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It is estimated that 3.5 million UK consumers are annually affected by episodes of foodborne disease. The majority of cases are thought to be sporadic, with the domestic kitchen a significant origin. Thus, consumer education concerning risks and correct domestic food-handling behaviors is essential. The purpose of this research is to attain an understanding of the extent and diversity of consumer food hygiene education in the UK. Self-complete questionnaires were distributed to > 2500 UK organisations including Environmental Health (EH), Health Promotion (HP) and Public Health departments within Local Authorities (LAs) (response rate > 75%) and the National Health Service (response rate 34%). In addition, product-specific and industry organisations, supermarkets and local consumer groups were also contacted. Questionnaires were designed to identify specific details of recent, current and proposed initiatives. Cumulatively, findings suggest that a wide range of organisations in the UK do undertake consumer food hygiene education. The largest coordinated initiatives are those undertaken by the Food Standards Agency and Food and Drink Federation. Furthermore, 94% of EH/HP departments in LAs currently provide food-handling advice for consumers. Intervention materi-

als collected from LAs and other organisations were reviewed in terms of content, format and intended audience. The most common food hygiene issue currently addressed in UK initiatives was cross contamination (82% of LAs). Other common food safety issues identified will be discussed. Intervention materials provided in UK initiatives included mainly leaflets, posters, bookmarks, reminder-aids and magnets as well as advice on the Internet. Evaluation of initiative effectiveness was reported in only 22% LAs, most commonly using self-report questionnaires. Cumulative results from this study will be discussed in the context of generic recommendations for strategy development of consumer education applicable for use in different countries.

T6-04 Comparison of Computer-based and Face-to-face Personal Hygiene Training Methods in Food Processing Facilities

DSC

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Because of convenience, self-pacing ability, and ease of use, computer-based training may be an option for educating workers in the food processing industry. The objectives of this project were to determine if personal hygiene training through a computer-based method is as effective as a face-to-face format in knowledge gain and improved food safety attitude. Employees from four food processing facilities (n = 99) were randomly assigned to a control group, a face-to-face training group, or a computer-based training group. Evaluation instruments consisted of a pretest and a posttest to measure knowledge gain, a step-scale to measure attitude after training and retrospectively before training, and a brief set of interview questions to gauge participants' reactions to the computer-based training. The instruments were reviewed by a panel of experts and pilot tested prior to actual data collection in food processing establishments. Results for both treatments indicated increases in knowledge compared to the control, although the difference was not significant ($P > 0.05$). However, there was a significant increase in attitude scores for both groups, although the difference between groups was not significant. Interviews after training demonstrated that the computer-based group reacted positively to the training, thus indicating this method may be an acceptable alternative to face-to-face training. Issues discovered that may limit application of computer-based training in processing environments include low literacy, language barriers, and time constraints within companies. These and other variables should be taken into account in future research projects.

T6-05 Consumer Attitudes, Self-reported and Observed Behaviors Relating to Cloth-wiper Usage in the Domestic Kitchen

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Cross contamination of bacterial pathogens in domestic kitchens is an important food safety issue. Research has shown that domestic cloth-wipers may harbor large numbers of microorganisms, including pathogens, and may also be significant intermediary vectors for transferring microbial contaminants within the kitchen. Relatively little is currently known about domestic usage of cloth-wipers and perceptions of associated microbial risks. Therefore, consumer attitudes and domestic cloth-wiper behaviors, including decontamination events, have been investigated. Self-reported behaviors were assessed using structured telephone interviews (n = 100) and attitudes toward microbial risks were evaluated by use of a self-complete postal questionnaire (response rate 91%). Cloth-wiper behaviors were observed during meal preparations (n = 30) undertaken in a model domestic kitchen. Survey and observational data suggest that consumers frequently use different types of cloth-wipers in the domestic kitchen for a multitude of different purposes. Discrepancies between attitudes, self-reported and observed behaviors have been identified and will be discussed. The majority (> 90%) of consumers believed that cloth-wipers are frequently contaminated with potentially large numbers of bacteria, yet cloth-wipers were used on average 20 times per meal preparation. High-risk cross contamination practices associated with cloth-wiper usage have been identified, for example, direct contact between raw chicken and cloth-wipers was observed in 30% of meal preparations and 7% of respondents reported wiping their hands on cloth-wipers immediately after handling raw chicken. Decontamination events, methods and timing were highly variable and were frequently ineffective. Cumulatively, findings from this research indicate the need for an improvement in cloth-wiper behaviors in the domestic kitchen. The implications of these results will be discussed in the context of knowledge provision for consumer risk communication.

T6-06 Teaching Food Safety Principles to Children Using Smart Kids FightBAC!® Computer Games

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The University of Georgia, Mississippi State University and North Carolina State University, in cooperation with The Partnership for Food Safety Education, FSIS-USDA and CSREES-USDA, have developed food safety computer games based on the Smart Kids FightBAC!® animated video. Six games offer fun-filled, interactive environments for learning food safety principles while learning math, science, language arts and computer skills for K-3rd grade curriculum standards. Three difficulty levels of play in one- and two- player formats with

support materials including a "BAC-tionary," "BAC-lopedia," etc. make this a rich tool for a variety of skill levels, including gifted learners. Focus groups with teachers and students ensured appropriate content, design and usability. Final pilot testing occurred in three states. Children in test groups watched the animated video, completed workbook activities from the Smart Kids FightBAC!® curriculum and played the computer games. Control groups followed the same format without exposure to the computer games. Results of pre-, post- and post-post tests indicate that the program is most effective in increasing knowledge of food safety at the K-2nd grade levels. The control group (n = 614) scored significantly higher than the CD-ROM group (n = 620) on the pre-test ($P < 0.05$). Immediately following implementation, there was no significant difference between the post-test means for the two groups ($P > 0.05$). However, by the end of the 8-week study, the CD-ROM group had a significantly greater increase in knowledge ($P < 0.05$) over the course of the study, indicating that these computer games may stimulate children's interest and ability to learn food safety information.

T6-07 Food Safety Practices of Vendors at Farmers' Markets in Florida

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Fresh produce consumption is on the rise in the US. Although fresh produce is considered a low risk product, an increase in produce-related foodborne illness outbreaks has prompted increased interest among professionals and consumers. According to a recent USDA ERS report, one market channel for fresh produce is through direct markets, such as farmers' markets. The goal of this study is to assess current food safety practices among vendors at farmers' markets in Florida in order to understand the current situation and to identify any educational gaps. A statewide farmer's market survey with 16 questions was conducted during the peak season. A total of 47 vendors returned the completed surveys. Overall, more than 50 per cent of products sold at Florida farmers' markets are produce-related items. Other outlets for their products include U-pick, restaurants, and produce stands. The majority of vendors use either compost or manure to amend the soil used in growing those products. Even though more than 50 per cent think food safety is important for their operation, only 32 per cent of respondents have completed food safety training. The majority of the respondents provide the farm labor themselves, or family members are the primary labor source. Very few vendors use permanent or temporary workers. When asked about the FDA's Guide to Minimize Microbial Food Safety Hazards for Fresh Produce (GAPs), the majority (44%) of the vendors were not aware of this publication. The results of this survey will provide insights for educators to create a food safety educational program for this segment of the produce industry.

T6-08 Thermometer Education Program

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USDA-FSIS has developed educational materials to promote the use of thermometers with consumers to determine the proper doneness of foods. A thermometer educational display was developed with a 3 question survey for use at health fairs and other educational events to promote thermometer usage by consumers. The educational display contained pictures of different thermometers, pictures and directions for proper insertion of thermometers in food items and the FightBAC! message. The survey was developed by a group of extension educators. One question focused on types of food thermometers that the participants have in their homes. The second question focused on what food items the consumer uses a thermometer to determine doneness and the third question was a knowledge question. The educational display was used at two major events during the summer of 2004. To encourage participation, a drawing for a grill was held. Participants received a Thermy magnet as a reward for completing the survey. From these events, 862 participants completed the survey. Approximately half (50.3%) of the participants had candy thermometers, while 33.2% had refrigerator thermometers and only 18.1% had a dial thermometer. The food item with which participants most often used a thermometer was turkey (24.2%) while 36.2% did not use a thermometer to determine doneness. Forty (40%) of the participants correctly answered the question on the proper temperature to cook hamburgers. The results of the survey indicate that more education of consumers is needed on the use of thermometers to determine doneness.

T6-09 Educating Consumers to Use Food Thermometers with Thin/Small Meat Items

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Most consumers are aware of the need to adequately cook meats to kill pathogens, but few use a thermometer to check doneness of small meat items. Particularly for ground beef patties, color is an unreliable indicator of endpoint temperature, yet only 6% of consumers use a thermometer with hamburgers. In this research, four consumer focus groups were convened to probe for barriers and motivators regarding food thermometer use when cooking small/thin meat items, such as hamburgers. Participants were asked to read two prototype, informational brochures and to cook a small/thin meat item, using both dial and digital instant-read food thermometers to assess endpoint temperature. The participants provided a variety of views on this use of a

thermometer, a new behavior for most, and made suggestions about the brochures. Information provided aided the preparation a full-color brochure and recipe cards, which were sent, along with a thermometer, to a random sampling of 100 Idaho consumers. Via telephone survey, before receiving the materials, 37 of the 100 respondents thought it was very important to use a thermometer to determine doneness in hamburger. Six weeks after receiving the materials, 54 of the 79 consumers (68%) we could contact for the follow-up survey said it was very important; the difference was statistically significant. Thermometer use increased from 3% before the materials were received to 19% afterwards. Although increasing consumer thermometer use will be a challenge, many individuals responded positively to the educational message regarding improved safety and palatability when cooking thin/small meat items.

T6-10 School-related Foodborne Illness Outbreaks Low – The Food and Nutrition Service Collaborates with Other Agencies and Works to Assure Safe Food in Schools

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Recent national media outlets have questioned the safety of food served in school lunches. The Government Accountability Office's (GAO) Report to Congress in May 2003, however, cited data from the Centers for Disease Control and Prevention (CDC) and concluded that meals served in schools are generally safe. The report also stressed that the opportunity exists to enhance data collection and food safety practices. The Food and Nutrition Service (FNS) is responsible for administering the National School Lunch Program (NSLP), School Breakfast Program and other nutrition assistance programs. Roughly 28 million lunches and 8 million breakfasts are served daily at participating schools. FNS has responsibility for and is committed to ensuring the safety of foods served in schools. The Food Safety Unit of FNS was created to address all aspects of food safety related to USDA nutrition assistance programs and has formed strong relationships with other federal agencies and private organizations involved in food safety education and the prevention of foodborne disease. These partnerships have enhanced the safety of foods in the NSLP. In particular, our complaint process involves data sharing with the USDA Food Safety and Inspection Service and Agricultural Marketing Service, which has strengthened our ability to protect school lunches. The partnerships have also improved communication and public health monitoring through coordination with CDC and strengthened food safety education efforts through cooperative projects with the Food and Drug Administration, such as Hazard Analysis and Critical Control Points (HACCP) and food biosecurity for school food service operations.

T6-11 Knowledge and Routines in Equipment Hygiene in Finnish Food Factories

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Food processing equipment has been shown to contaminate food products. In Europe, the most important legislation giving criteria for hygienic design is Council Directive 89/392/EEC revised in 1998 (98/37/EC) and the EN 1672-2 standard. They contain safety requirements and basic principles of hygienic design. National standards and/or directives applicable to the hygienic design of food machinery are also available, but few international standards exist and they are mainly directed at the dairy industry. Guidelines and methods for the design of new hygienic equipment published by EHEDG, 3-A Sanitary Standards Inc. and NSF International are also available. A questionnaire on equipment hygiene in the Finnish food industry was sent to 184 companies. The expert in equipment hygiene in each company was asked to answer the questionnaire and the respondents were able to remain anonymous. The questions were categorized into seven topics: hygienically problematic equipment, acquisition of the equipment, cleanability of the equipment, cooperation between parties affecting equipment hygiene, lubricants used in the equipment, information acquisition on equipment hygiene and background information on the respondent plant. Packaging machines, conveyers, dispensers, slicing and cooling machines were recognized as the most problematic equipment. Hygiene problems were mainly caused by poor hygienic design. It was also found that responsibilities in hygiene matters should be clearly defined. Furthermore, the equipment in the process lines must be hygienically integrated. This study also showed that the information flow between equipment manufacturers, food processors, cleaning and maintenance personnel, and cleaning chemical manufacturers must be improved.

T6-12 Unification of Retail and Process HACCP

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Today, HACCP is mandated in USDA and fish plants, but not in FDA processing or retail food. However, in retail food operations, it is optional, unless operators want to do a new process. Then, they must prepare a variance and submit a HACCP plan for approval to their local regulatory authority. Actually, the biological, chemical, and physical hazards and process control (performance) criteria for kill and growth are identical, whether food is prepared in a home kitchen, a restaurant, or a process plant. Many regulatory rules are based on historical precedent. The only analytical, science-based way to know that a process in a food operation will be safe is to prepare a HACCP plan. It also leads to uniformity. This presentation will show how the principles

of the NACMCF HACCP plan can be merged with the process control science of the ICMSF to form a unified set of documents, language, and science. HACCP documentation for any process begins with the HACCP recipe and includes the HACCP flow process chart; the HACCP plan; and the food safety system policies, procedures, and standards manual. Adopting a unified set of HACCP documents with an ICMSF science and terminology base will mean that, once food preparers have learned the HACCP documentation and control rules, they will be able to work in any food process, large or small, and be able to produce a product meeting a desired Food Safety Objective with an Appropriate Level of Protection.

T7-01 DSC **The Direct Detection of *Salmonella* and *Escherichia coli* O157:H7 from Raw Alfalfa Sprouts and Spent Irrigation Water by Use of Polymerase Chain Reaction**

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Raw seed sprouts have been recognized as an important cause of foodborne disease. Consequently, the U.S. Food and Drug Administration recommended that spent irrigation water from each lot of raw seed sprouts be tested for *Salmonella* spp. and *Escherichia coli* O157:H7 as a means of assuring the safety of the product. In an effort to streamline such testing, the purpose of this study was to develop a method to pre-concentrate pathogens from sprouts and spent irrigation water to facilitate the direct (without prior cultural enrichment) detection of pathogens by use of the polymerase chain reaction (PCR). Alfalfa sprouts and spent irrigation water were seeded with *S. enterica* serovar Typhimurium and *E. coli* O157:H7 in the range of 10^1 to 10^6 CFU/g or ml. Samples were then centrifuged to sediment the total bacterial population. The precipitate was processed for DNA isolation, PCR amplification, and amplicon confirmation by Southern hybridization. Recoveries after centrifugation ranged from 96 to 99% for both pathogens in both matrices. Using primers targeting the inv A gene for *S.* Typhimurium and the *stx* genes of *E. coli* O157:H7, it was possible to detect both pathogens in alfalfa sprouts at seeding levels as low as 10^1 CFU/g. PCR detection limits for both pathogens from spent irrigation water were 10^{-1} CFU/ml, the equivalent of 10^2 CFU/l. This study demonstrates progress toward a rapid and sensitive method for the detection of pathogens associated with this commodity, and one that is relevant to current industrial practices and needs.

T7-02 **Location of Bung Bagging during Beef Slaughter Influences the Potential for Spreading Pathogen Contamination on Carcasses**

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Pre-evisceration carcass washing prior to bung bagging during beef slaughter may allow pooling of wash water in the rectal area and consequent spread of potential pathogens. The objective of this study was to compare protocols for bung bagging after pre-evisceration washing to an alternative method prior to pre-evisceration washing on the potential to spread enterohemorrhagic *Escherichia coli* (EHEC), *E. coli* O157:H7, and *Salmonella* spp. on carcass surfaces. The study evaluated incidence rates of pathogens in pre-evisceration wash water (10 ml) samples (n = 240) and on surface (100 cm^2) sponge samples (n = 240) in the immediate bung region when bagging occurred before (post-bag) and after (pre-bag) pre-evisceration washing. Pre-bag surface sampling yielded incidence rates of 29.4, 2.5, and 4.0%, while wash water sampling yielded 14.2, 0.8, and 2.4% for EHEC, *E. coli* O157:H7, and *Salmonella*, respectively. Post-bag surface sampling yielded incidence rates of 16.7, 0.8, and 0%, while wash water sampling yielded 8.9, 0, and 4.0% for EHEC, *E. coli* O157:H7, and *Salmonella*, respectively. Results indicate that bung bagging, as proposed in this study, prior to rather than after pre-evisceration washing was more effective ($P < 0.05$) overall in controlling potential pathogen spread on carcasses. Although regulatory authorities do not permit bung bagging and dropping inside the body cavity before pre-evisceration washing, the proposed protocol suggests bagging the bung to form a plug that when receded to its natural location covers the rectal opening. These data will assist the beef industry in decisions regarding Hazard Analysis Critical Control Point (HACCP) reassessments.

T7-03 DSC **Impact of Bacterial Stress and Biofilm Forming Ability on Transfer of Surface-dried *Listeria monocytogenes* Cells during Slicing of Delicatessen Meats**

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Listeria contamination of delicatessen slicer blades can lead to cross-contamination of luncheon meats. In the present study, six previously identified strong and weak biofilm-forming strains of *L. monocytogenes* were grown at 37°C/18 to 24 h on trypticase soy agar containing 0.6% yeast extract, harvested in 0.1% peptone and then combined into two 3-strain cocktails. The cocktails were resuspended in turkey slurry with or without prior cold-shock at 4°C/2 h and then used to inoculate flame-sterilized stainless steel delicatessen slicer blades at a concentration of 10^6 CFU/blade. After incubation at 22°C / ~78% relative humidity for 6 and 24 h, the inoculated blades were attached to a gravity-fed delicatessen slicer and used to generate 30 slices from retail chubs of roast turkey breast or Genoa salami. Slices (~25 g) were diluted 1:5 in phosphate buffered saline or

University of Vermont Medium and then pour-plated (5 ml) into 150-mm dia. Petri plates using 20 ml of Tryptose Phosphate Agar containing esculin and ferric ammonium citrate with results reported as the average of 30 slices. Overall, strong biofilm-formers transferred more readily (3.8 log CFU/slice) than weak biofilm-formers (2.7 log CFU/slice) with transfer greater to turkey (3.8 log CFU/slice) than to salami (2.7 log CFU/slice). Previous cold-shock significantly increased *Listeria* transfer (3.9 log CFU/slice) compared to healthy cells (2.7 log CFU/slice). Length of blade incubation (6 vs. 24 h) did not significantly affect transfer. Greater transfer for the strong biofilm cocktail suggests that these strains are better adapted to survive stressful conditions when dried on stainless steel.

T7-04 Transfer of *Listeria monocytogenes* during Slicing of Turkey Breast, Bologna, and Salami Using Kitchen Knives

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Knife blades are now recognized as an important vector for *Listeria* contamination in delicatessens. To assess *Listeria* transfer rates, grade 304 and 316 electropolished stainless steel knife blades were inoculated with a 6-strain *Listeria monocytogenes* cocktail to contain ~10^8, 10^5, 10^3 CFU/blade. Chubs of bologna, salami, and roast turkey (3 replicates) were sliced (30 slices) at a cutting speed of 50.8 cm/min using knife blades attached to an Instron compression analyzer. Slices (25 g) were diluted 1:5 in PBS, stomached for 2 min, pour-plated (5 ml) in 20 ml of Modified Oxford Agar and/or enriched in University of Vermont Medium. Transfer from knives inoculated at 10^8 CFU/blade was logarithmic ($R^2 = 0.92$) with populations decreasing 2 logs after 12 slices. At inoculation levels of 10^5 and 10^3 CFU/blade, transfer was more sporadic for all products with direct counts out to 13 to 20 and 5 slices, respectively. Normalizing data on a log scale for the first 10 slices demonstrated significantly greater *Listeria* transfer and a greater tailing effect for grade 304 as opposed to 316 stainless ($P < 0.05$). Surface profilometry measurements on 1-year old blades also showed that grade 304 blades were significantly rougher ($P < 0.001$) than those prepared from 316 grade stainless. While cutting speed was not significantly affected by stainless grade ($P > 0.05$), significant differences were seen between salami and turkey ($P < 0.05$). Given the extended tailing seen for *Listeria* on grade 304 stainless, use of grade 316 stainless for knives and food contact surfaces in retail delicatessens would be preferred.

T7-05 Evaluation of Hot Water and Sanitizer Dip Treatments of Contaminated Meat-cutting Knives

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Hot (82.2°C) water [HW] is used for sanitation of meat cutting implements in most slaughter facilities, but validation of actual practices against meat-borne bacterial pathogens and spoilage flora is lacking. Observed implement immersions in HW in two large pork-processing plants were found to typically be 1 s. Knives were inoculated with raw pork residues and *Escherichia coli* O157:H7, *Salmonella* Typhimurium DT104, *Clostridium perfringens*, and *Lactobacillus* spp. and were sampled before and after 1 or 15 s dips of blades in HW, warm (48.9°C) water [WW], or warm sanitizers (neutral or acid quaternary ammonium compounds at 400 ppm [QAC], or peroxyacetic acid at 700 ppm H_2O_2 and 165 ppm peroxyacetic [PAA]). Simultaneous scrubbing and 15 s dipping in HW or acid QAC was also evaluated. Reductions on knives dipped for 1 s were usually < 1 log and were not significantly different ($P > 0.05$) between treatments. Reductions of *E. coli* O157:H7 after 15 s in HW, neutral QAC, acid QAC, or PAA were 3.02, 2.38, 3.04, and 1.52 log, respectively. Reductions of other bacteria due to HW were not significantly different from sanitizers and were significantly greater than WW for all bacteria except *C. perfringens*. Combined scrubbing and 15 s dipping in HW resulted in a 2.91 and 2.25-log reduction of *E. coli* O157:H7 and *S.* Typhimurium DT104, respectively, while reduction caused by acid QAC was significantly less at about 1.7 log each. Brief dip treatments of contaminated knives have limited efficacy, but longer immersions cause greater reductions that were not enhanced by scrubbing. QAC is a suitable alternative to HW in this application.

T7-06 Evaluation of the VERIcleen Hygiene Indicator Test, Compared with Traditional Microbiological Methods, to Assess the Efficacy of Hand Washing

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Hand hygiene is extremely important in the food industry and the potential for inadequately washed hands to cause cross contamination, resulting in food poisoning, is of major concern. To monitor compliance, the results of hand decontamination should be available in time to implement corrective action. This project compares the VERIcleen hygiene indicator test with microbiological testing, as a method of assessing hand decontamination efficiency. The VERIcleen test and dipslides (a microbiological method) were performed on 100 participants before and after handwashing. Performance of handwashing was compared with the UK Food Standards Agency recommended procedure by observation. Data were analysed using the Mann-Whitney and Kruskal-Wallis tests. Of the 100 participants, 95% failed the VERIcleen test before hand washing. Fifty-four

percent did not wash their hands in accordance with the full FSA procedure. Of these 61% passed the VERIcleen test and 39% were microbiologically satisfactory. Of the 46% who did fully wash their hands, 74% passed the VERIcleen test and the remaining 26% marginally failed. The number testing positive with the VERIcleen before handwashing was significantly higher than those after handwashing. A similar trend was seen for dipslides where microbial growth was less in 95% of cases after hand washing. There was no significant difference in the ability of dipslides or the VERIcleen test to measure the effectiveness of hand washing. The VERIcleen system is a cheap, rapid (2 min) alternative to traditional microbiological methods for assessing the efficacy of hand washing and may be valuable in improving compliance.

T7-07 Restructuring Employee Health Requirements in the FDA Food Code to Reduce the Risk of Transmitting Viral and Bacterial Pathogens from Infected Food Employees in Food

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When the Food Code was first developed in 1993, there was little appreciation of the importance of Norovirus (NoV) as a foodborne pathogen. However, NoV, known previously as "Norwalk-like virus", is now recognized as the single most common cause of gastroenteritis in all age groups, and one of the most important foodborne pathogens in the western world. A number of studies have now demonstrated that consuming food contaminated by infected food workers is a leading risk of NoV foodborne illness. The ease of Norovirus transmission as an aerosol, or through the hands of infected food workers to ready-to-eat food items, combined with the low infectious dose emphasizes that more must be done to control the spread of this pathogen in a food environment. New data on the transmissibility and extreme infectivity of NoV has allowed us to develop improved guidance in the Food Code to further reduce the risk of transmitting NoV at the retail level. These changes in the food code represent a trilateral approach to food safety by exclusion of workers exhibiting specific symptoms, washing hands and avoiding bare hand contact. This presentation will provide an overview of the revised structure of Employee Health and review both the data that led to the changes in the 2005 Food Code as well as the changes themselves.

T7-08 Rapid Determination of Bacterial Load for Assessment of Water Quality

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A safe water source is essential for food and beverage processing, effective animal production, and safe recreational activities, as well as maintaining human health. Except for emergencies relating to disasters, such as the Tsunami in the Indian Ocean, most assume safe water is a given. With the aging of the water distribution systems, contamination of bottled mineral water and even contaminated water on aircraft, there are renewed concerns as to water quality. Currently, the current methods for assessing water quality include heterotrophic plate counts (HPC), acridine orange direct count (AODC), direct viable count (DVC), fecal coliform tests and others. These methods take from 1 to 7 days to obtain results. There remains a need for a rapid on site evaluation of the bacterial quality of water. A rapid luminescence based system has been developed that can effectively measure generic total bacteria (TBC) and, with utilization of specific antibodies, identify generic *Escherichia coli*. The TBC result can be available within 10 min with a detection limit of less than 1000 cells. The specific *E. coli* assay system can detect less than 20 CFU/100 ml of water in less than 1 h. Specificity for the *E. coli* system depends on the antibody utilized. This study compares this luminescence field system with standard methods. Hundreds of data points comparing these systems will be presented. The equipment is portable and the reagents field stable. The system is suitable for laboratories and food processing plants as well as farms.

Symposium Abstracts

S01 Laboratory Response to Food Bioterrorism: How Prepared Are We?

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In December 2004, Health and Human Services Secretary Tommy Thompson issued a stark warning about the vulnerability of the food supply in the United States to deliberate acts of contamination with chemical or biological agents. Because of the high degree of centralization of food production and processing, wide distribution of food products, and increased importation of foods from all parts of the globe, very large numbers of people in a country can be affected by a food contamination event. Depending on the magnitude of a food bioterrorism event, clinical, public health, food regulatory agency, and food industry laboratories may be faced with the analysis of tens of thousands of food and environmental samples for the suspected etiologic agent(s). Prioritization of specimens for analysis, use of the best available screening and confirmation methods, standardization and harmonization of analytical methodology across state and federal agencies and commercial organizations, and rapid and secure exchange of results among those who need to know are some of the critical factors for successful, rapid identification of the source of contamination and for implementation of appropriate control measures. This symposium will explore the factors that will be required to mount a cohesive and well-coordinated laboratory analysis strategy to support the investigation of a food bioterrorism event.

S02 Microbiological Predictive Models: Development, Use and Misuse

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The purpose of the symposium is to present the latest developments in microbiological predictive models, practical and regulatory uses and limits of predictive models, and an international perspective. The audience is industry, regulators and academics who use, develop or validate microbiological predictive models. Three active model developers in the field will present their latest developments. The ARS Pathogen Modeling Program has been continuously improving since its early versions as a spreadsheet. Combining it with the European ComBase has greatly expanded its utility and made ComBase available to many more workers. Modeling microbial responses to continuously changing conditions has been a challenge. Two developers will present their work on a cooling model and a thermal inactivation model. For context, two regulatory microbiologists will add industry and government perspectives on the utility of current predictive models and their limits for practical and regulatory use. Finally, one of the pioneers of microbial predictive modeling will sum up with his insights on optimizing the use of predictive models as well as offering the experience of the Australian meat industry. Thus, this symposium will also give model developers additional insight on improving the utility and application of models that they are developing. The audience will also have a greater knowledge of what current and future models can offer them.

S03 Food Allergens: Concerns for the Packaged Food and Food Service Industries

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Recent studies indicate that food allergy continues to rise in the United States and other industrialized countries. In the United States, an estimated 11 million Americans have food allergy, with 6.5 million reporting

an allergy to fish or shellfish and 3 million believed to be allergic to peanuts and tree nuts. These four foods account for the majority of severe or fatal allergic reactions, accounting for 30,000 emergency room visits and up to 200 deaths each year. There is no known cure; strict avoidance of the allergen is the only way for consumers to prevent an allergic reaction. Their need for accurate ingredient information is impacting the packaged food industry, restaurants, the retail food industry, government, and others. This workshop will provide the latest scientific and consumer research information regarding label reading and causes of reactions; regulatory approach to food allergens; and allergen control strategies from leaders in the packaged food and retail food services arena. A discussion on the latest studies to establish threshold levels and allergen detection test kits will provide a view into the future of managing food allergies from the industry and regulatory perspective.

S04 Global Water Quality Concerns

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The quality of ingredient water is a global issue in the food industry and one that is attracting heightened awareness in food safety discussions. The impact of the downtime from a water emergency has far reaching implications in food manufacturing, catering, and retail industries. The differences in the issues faced during a water emergency by the United States and Europe will be discussed in this symposium. Water recycling in the food industry will be addressed from an international perspective. Water's role in foodborne outbreaks is a global concern. The US Center for Disease Control (CDC) will discuss the increased awareness of foodborne disease outbreaks actually being tied to water quality issues. There will be a discussion of the complex water quality challenges to food safety in the global market of cruise ships, airplanes and train travel. From a Canadian perspective, we will hear about ensuring safe ingredient water by protecting water sources used for food processing and manufacturing as the first step in a multi-barrier approach. Lastly, the impact of the aging distribution systems of public water systems on ingredient water quality will be addressed.

S05 Recent Regulatory Changes and Issues Affecting Your Dairy Operation

RON SCHMIDT, University of Florida, Food Science & Human Nutrition Dept., P.O. Box 110370, Gainesville, FL 32611-0370, USA; MARLENA BORDSON, National Conference on Interstate Milk Shipments, Illinois Dept. of Public Health, 525 W. Jefferson St., Springfield, IL 62761-0001, USA; PHILLIP WOLFF, USDA, 9604 Ellicott Lane, Manassas, VA 20110-5703, USA; ALLEN SAYLER, International Dairy Foods Association, 1250 H St. NW, Washington, D.C. 20005, USA; LOUIS CARSON, DHHS-FDA-CFSAN-OCD-OFSDO, HFS-032 5100 Paint Branch Pkwy., College Park, MD 20740, USA

Multiple agencies and many regulations govern today's United States dairy industry. Whether you are fairly new to the dairy industry, an international attendee or a seasoned US dairy veteran – this symposium is for you! Attendees unfamiliar with the industry will learn which US agencies have responsibility for certain dairy products and how regulations are drafted and implemented. International impacts such as Codex and IDF will be discussed. Presenters from USDA and FDA will bring you up-to-date on recent regulatory decisions such as the Bioterrorism Preparedness Act, which affects domestic plants and importers alike. Hear about the most recent Pasteurized Milk Ordinance decisions made at the National Conference on Interstate Milk Shippers held in May of this year. Attendees will leave having a much better understanding of the complex regulatory world of the US dairy industry.

S06 Update on Foodborne Disease Outbreaks

SUNDEEP GUPTA, CDC-CID, MS A-38, Foodborne & Diarrheal Diseases Branch, 1600 Clifton Road, Atlanta, GA 30333, USA; THOMAS HILL, FDA, 5100 Paint Branch Pkwy., HFS-600, College Park, MD 20740, USA; LARRY BEUCHAT, University of Georgia, Center for Food Safety, 1109 Experiment St., Griffin, GA 30223-1797, USA; MICHAEL LYNCH, CDC-CID, MS A-38, Foodborne & Diarrheal Diseases Branch, 1600 Clifton Road, Atlanta, GA 30333, USA; DAVID WHITE, FDA, 8401 Muirkirk Road, Laurel, MD 20708, USA; KRISTIN HOLT, CDC, Food Safety Office, MS G-24, 1600 Clifton Road, Atlanta, GA 30333, USA; JEFF FARRAR, California Dept. of Health Services, Food and Drug Branch, 1500 Capitol Ave., P.O. Box 997413, MS 7602, Sacramento, CA 95899-7413, USA

Foodborne outbreak investigations must be viewed as an opportunity to determine: (1) the specific food or foods serving as the vehicle for contamination, (2) how the food became contaminated, (3) how to stop the exposure, and (4) how to prevent the recurrence of a similar incident. Thorough, complete, and standardized epidemiological and environmental investigations are required, yet they are not generally viewed or conducted as a multidisciplinary effort among responsible agencies. Closely coordinated, standardized investigations are

critical to determine factors that may have contributed to the introduction, growth, and/or survival of pathogens in foods implicated in foodborne outbreaks. In this symposium, presenters will review recent foodborne outbreak investigations, including those linked to *Salmonella* Branderup in tomatoes and *E. coli* O157:H7 in lettuce. The epidemiological phase and environmental phase of both investigations will be presented. Additionally, we will provide an overview of an emerging foodborne pathogen (*Salmonella* 4,5,(12):i:.

S07 Safety Concerns of Food Chemical Contaminants

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Today's consumer, more than ever, is paying attention to trace levels of contaminants in foods and is concerned about the chemical safety of the food supply. Improved detection methods are capable of finding lower and lower levels of chemicals in a variety of matrices. Chemicals that have been in the spotlight recently for potential health and safety concerns have included acrylamide, furan, perchlorate, dioxins, PCBs, flame retardants, and mercury. Nearly every manufacturer is impacted by one or more of these chemicals. These toxicants can enter the food supply at several points in the supply chain, including harvesting, production, storage, and transportation. This symposium will touch on many areas of toxicological concerns, from dietary supplements to packaging to seafood. It will provide a basic understanding of food toxicology, risk extrapolation, legal concerns, and research to address emerging issues in food toxicology. This symposium will provide insight into chemical food safety vulnerabilities.

S08 Data for Decision Making

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Data, data, data, we say it, we want it, but what does it really mean? Data is like ice cream and potato chips. Everyone wants to have some: consumers, regulators, scientists, producers, processors, suppliers and customers (I scream, you scream, we all scream for data!). The more data the better, as you can never get enough (You can't eat just one!). Data makes us feel good. Data gives us something tangible to hold on to. Data gives us something to measure each other by – or so we think. Merriam-Webster's online dictionary defines data as "factual information (as measurements or statistics) used as a basis for reasoning, discussion, or calculation." "Ask Jeeves" defines data as a "representation of facts, concepts, or instructions in a formalized manner suitable for communication, interpretation, or processing by humans or by automatic means." Just as valuable as the numbers (raw data) themselves is how the data were collected (what methods were used), how they were analyzed, and how they will be interpreted. This symposium will cover several aspects of data use, from developing and implementing sampling plans to collect data, to the use and interpretation of data for process validation and in meeting customer specifications. Presenters will also focus on the historical use and value of data and how to insure (or question) confidence in your testing program and thereby in your data. The information covered in this symposium will be of value to anyone in industry, academia, and government who develops and/or reviews sampling plans and collects, manages, and/or interprets data.

S09 Materials for Multi-Use Food Contact Surfaces: Characteristics, Fabrication, and Evaluation

STEVE SIMS, FDA, 5100 Paint Branch Pkwy., College Park, MD 20740-3835, USA; JOHN TVERBERG, Metals and Materials Consulting Engineers, 135 Fruitlyn Drive, Dallastown, PA 17313, USA; JEFFREY A. JANSEN, Stork Technimet, 2345 S. 170th St., New Berlin, WI 53151, USA; SARA RISCH, Michigan State University, 135 Packaging, East Lansing, MI 48824, USA

The symposium will focus on the sanitary design characteristics and fabrication of materials used in multi-use food contact surfaces of dairy and food equipment, recommended use applications, and evaluation testing. The principles of sanitary and hygienic construction and fabrication will be emphasized, along with current issues regarding the advantages and disadvantages of the various materials. An overview will be presented of the current knowledge of the characteristics of metal materials (e.g., stainless steel, aluminum, and other metals) and non-metal materials (e.g., plastics, rubber and rubber-like materials). An in-depth evaluation will be given regarding compositional formulation; surface treatment and finish; fabrication; and methodology for determining cleanability and absence of toxic residues. The symposium will conclude with a panel discussion involving the speakers, regulatory officials, and industry stakeholders.

S10 Foodborne Diseases: Discovery of Causes and Reduction Strategies

JACK GUZEWICH, FDA-CFSAN, HFS-600, 5100 Paint Branch Pkwy., College Park, MD 20740, USA; DAVID GOLDMAN, USDA-FSIS-OPHS, Room 341-E, Whitten Bldg., 1400 Independence Ave., SW, Washington, D.C. 20250, USA; JENNY SCOTT, Food Products Association, 1350 I St. NW, Suite 300, Washington, D.C. 20005, USA; CAROL SELMAN, CDC, National Center for Environmental Health, 4770 Burford Hwy, NE (F28), Atlanta, GA 30341-3724, USA; CHRISTOPHER GRIFFITH, University of Wales Institute, Cardiff, Room D104, Llandaff Campus, Western Ave., Cardiff, South Wales, CF5 2YB, UK; TRENT WAKENIGHT, Michigan State University, National Food Safety & Toxicology Center, 165 Food Safety Bldg., East Lansing, MI 48823, USA; ELISA ELLIOT, FDA-CFSAN-OC-ERC, Staff HFS-600, 5100 Paint Branch Pkwy., Room 3B069, College Park, MD 20740-3835, USA

Each year, foodborne disease affects billions of people worldwide. However, causes of illnesses and outbreaks are often not discovered. This is due to the inherent difficulties involved in linking etiological agents to a food vehicle and then determining the original source of contamination. Even if the cause is discovered, the information is not always used to prevent future illnesses from occurring. This symposium will focus on the work of several groups who are helping to protect public health at the national and state level by identifying contamination pathways of food and by developing strategies to reduce the incidence of foodborne disease. Speakers will address how regulatory agencies use information from foodborne disease investigations to set priorities, identify new interventions, and measure policy effectiveness; how the food industry has focused resources on validation and verification to ensure hazard control; how data from the CDC's EHSNet program can be used to prevent foodborne disease; how new tools such as social cognition models, notational analysis, risk assessment, cost-benefit analysis, and social marketing can be used to improve food safety; and how efforts to increase food safety education at the state level can contribute to reductions in illness. Finally, Healthy People 2010 goals and their impacts on reducing incidence of foodborne disease will be discussed.

S11 Safety of Raw Milk Cheeses – A Global Perspective

CATHERINE DONNELLY, University of Vermont, 200 Carrigan Hall, Dept. Nutrition and Food Science, Burlington, VT 05405, USA; JOHN BRUHN, University of California-Davis, Dept. Food Science & Technology, 101B Cruess Hall, Davis, CA 95616, USA; JEFFREY FARBER, Health Canada, Tunney's Pasture Banting Research Centre, Postal Locator 2203G3, Ottawa, ON, K1A 0L2, Canada; PETER SUTHERLAND, NSW Food Authority, P.O. Box 6682, Silverwater, NSW, 1811 Australia; MELCHIOR SCHÄLLIBAUM, Agroscope Liebefeld-Posieux (ALP), Eidg. Forschungsanstalt für Milchwirtschaft FAM, Schwarzenburgstrasse 161, 3003 Bern-Liebefeld, Switzerland; SYLVIE LORTAL, INRA-AGROCAMPUS, 65, rue de Saint Brieuc, Rennes Cedex, 35042 France; GIUSEPPE LICITRA, CORFILAC, S.P. 25 Ragusa mare Km 5, 97100 Ragusa, Sicily, Italy; CATHY STRANGE, Whole Foods Market, 2700 Wilson Blvd., Arlington, VA 22201, USA

The safety of cheeses made from milks that have not received the legally defined pasteurization treatment (a minimum of 71.7 C for 15 seconds or more, United States) has been increasingly questioned by health authorities and researchers. Further, questions have arisen as to whether aging of these raw-milk cheeses is sufficient to inactivate target pathogens of public health significance. This symposium continues a discussion started at the 2004 IAFP raw milk cheese symposium on safety of these raw milk cheeses, primarily from a United States perspective. In this instance, the symposium will focus on global perspectives of the safety of milk raw cheeses. Discussants from Canada, Italy, France and Australia will review their science and safety perspectives regarding the manufacture, aging and distribution of raw-milk cheeses. Alternatives to pasteurization, such as stringent microbiological criteria and sanitary standards implemented by the EU, will be reviewed. Several speakers will also address issues facing their national cheese industry relating to Codex Alimentarius and perspectives on the impact of global regulations on the international cheese trade will be discussed.

S12 Yeasts and Molds: When Fungi Go Bad, Who Do You Call?

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The USDA's Economic Research Service has reported that up to 27% of the edible food supply in the US is lost every year at the retail, foodservice, and consumer levels. A significant portion of this loss is due to spoilage of the edible food supply by yeasts and molds. Consumer demand for more natural, fresher food products with

fewer preservatives, combined with industry productivity initiatives and the global sourcing of ingredients, have exacerbated the problem in recent years. In addition to the economic and aesthetic impact of yeast and mold spoilage, public health can be impacted through increased allergic responses or through sequelae related to consumption of mycotoxin-contaminated foods. Presentations in this symposium will include an overview of the problem, detailed case studies on the spoilage of processed foods and beverages by yeasts and molds, an examination of mycotoxins, their control, both in pre-harvest commodities and in processed products, and approaches for the rapid detection of yeast and molds in food products.

S13 They Said What? – The Risky World of Risk Communication

TOBY TEN EYCK, Michigan State University, 433B Berkey Hall, East Lansing, MI 48824, USA; GORDON MERIWETHER, The Uriah Group, 7700 Leesburg Pike, Suite 270, Falls Church, VA 22043, USA; EDWARD GROTH, Groth Consulting Services, 75 Clifford Ave., Pelham, NY 10803-1702, USA; SHELLEY GOLDBERG, International Food Information Council Foundation, 1100 Connecticut Ave., NW, Suite 430, Washington, D.C. 20036, USA; TODD PRITCHARD, University of Vermont, Dept. of Nutrition and Food Science, 204 Carrigan Hall, 536 Main St., Burlington, VT 05405-0004, USA; WILLIAM HUESTON, University of Minnesota, Center for Animal Health and Food Safety, 136 Andrew Boss Lab, 1354 Eckles Ave., St. Paul, MN 55108, USA

Communicating on food safety issues can be a perplexing and complicated process. Those within the food safety arena have risk analysis information that must be presented to the public, scientists, regulators, politicians, consumers and the media. How each of these groups hears information, processes that information and then re-communicates to others influences the perception of risk. Whether the hazard is biological, physical or chemical, whether the outcome is acute or chronic, whether the risk is significant or insignificant, and numerous other factors also impacts on the perception of risk by many audiences. The symposium will introduce the social/cultural dynamics of risk and then, through the use of case studies, evaluate the historic success of risk analysis within specific areas of food safety. Using the broad topic areas of agricultural production, agricultural processing, food processing and bio-security, the speakers will identify and analyze the difficulties of staying on message while under fire from the many stakeholders. Utilizing the components of risk assessment, risk management and risk communication, each of the case studies will provide a historic picture of the changing perception of risk and society's reaction to that change. In addition, strategies for reducing message modification will be presented.

S14 Pre-Harvest Issues Associated with the Transmission of Viruses and Parasitic Protozoa – The Problems and the Solutions

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Human enteric viruses and parasitic protozoa are recognized as significant causes of foodborne disease, responsible for as much as 68% and 3% of all foodborne illness in the United States, respectively. Although they have been recognized for years, the human enteric viruses and parasitic protozoa can be considered "emerging" agents of foodborne disease, largely because scientists have only recently been able to detect and study these pathogens. In fact, with improvements in epidemiology, molecular biology and molecular epidemiology, tools have been developed to investigate the origin of viral and parasitic outbreaks and contamination. Various viruses and parasites have been isolated or implicated in outbreaks involving fresh produce, shellfish, and meats. Many questions remain as to the sources of these pathogens in the environment, the routes of contamination, the pathogenic genotypes and the best strategies to minimize the risks of contamination at the pre-harvest level of food production. This symposium will address specific topics that are currently of interest in the field of food virology and parasitology, focusing on the pre-harvest issues associated with transmission of these pathogens and mechanisms to prevent or eliminate contamination so as to result in a safe food source. The latest foodborne outbreaks from new and emerging pathogens will be discussed, including outbreaks due to avian influenza virus and *Cyclospora*. This symposium will provide some insights into the problems of maintaining a variety of foods, such as shellfish, meat and produce, virus- and parasite-free, for human consumption.

S15 Managing the Risk of *Listeria monocytogenes* at Retail and Restaurants

ANN DRAUGHON, University of Tennessee, 2605 River Road, 105 Food Safety and Processing Bldg., Knoxville, TN 37996, USA; MARTIN WIEDMANN, Cornell University, Dept. of Food Science, 412 Stocking Hall, Ithaca, NY 14853, USA; JON WOODY, FDA, 5100 Paint Branch Pkwy., College Park, MD 20740, USA; STEVEN GROVER, Burger King Corporation, 5505 Blue Lagoon Drive, Miami, FL 33126, USA; JILL HOLLINGSWORTH, Food Marketing Institute, 655 15th St. NW, Washington, D.C. 20005, USA; KATHERINE SWANSON, Ecolab, 545 Huber Drive, Mendota Heights, MN 55120-1902, USA

Manufacturers of ready-to-eat foods have made significant improvements in the design and manufacturing processes of sensitive products to prevent contamination and/or growth of *Listeria monocytogenes*. Control measures include reformulation with antimicrobial agents to inhibit *L. monocytogenes* growth, installing post-lethality treatments to eliminate potential contamination, plant design focused on the isolation of individual producing lines, and increased environmental and finished product testing for *Listeria monocytogenes* or *Listeria* spp. Recent studies demonstrated that *L. monocytogenes* can persist in some retail environments. However, knowledge of the potential transmission of *L. monocytogenes* to foods, and of effective control measures in retail and restaurants establishments, is limited. This symposium will cover the latest information on the incidence of *L. monocytogenes* in retail and restaurant environments and the opportunities for additional control measures to prevent product contamination. Presenters will focus on defining the problem of *L. monocytogenes* in retail and restaurants, identifying current control measures in place, providing insight on additional control measures potentially needed, and providing some of the questions and issues that have arisen about the methodologies to monitor this pathogen in retail and restaurant settings to prevent harboring of *L. monocytogenes* in their environment. The information covered in this symposium will provide valuable tools to food safety professionals, personnel at regulatory agencies, and retail and restaurant managers and operators.

S16 Risk and Control of *Salmonella* in Raw Nuts

BILL HOSKINS, Blue Diamond Growers, 1802 C St., Sacramento, CA 95812, USA; JACK GUZEWICH, FDA-CFSAN, 5100 Paint Branch Pkwy., Room 3B-074, College Park, MD 20740-3835, USA; LINDA HARRIS, University of California, One Shields Ave., Davis, CA 95616-8598, USA; KAREN BATTISTA, Kraft Foods, 200 DeForest Ave., East Hanover, NJ 07936, USA; GUANGWEI HUANG, Almond Board of California, 1150 Ninth St., Suite 1500, Modesto, CA 95354, USA; MERLE JACOBS, Almond Board of California, 1150 Ninth St., Suite 1500, Modesto, CA 95354, USA

Recent outbreaks of salmonellosis associated with the consumption of raw almonds have caused concerns about the risk and control of *Salmonella* in raw nut production and consumption. This symposium will discuss a range of topics associated with the risk and control of *Salmonella* in raw nuts. Speakers from government, industry and academia will provide information on the occurrence of *Salmonella* in nut production and processing environments, wet vs. dry cleaning, and sanitation practices in postharvest environments. The speakers will provide an overview of the 2004 *Salmonella* outbreak in almonds and its impact on the industry. The symposium will also address the latest in thermal, non-traditional thermal, and non-thermal processes for the control of *Salmonella* in raw nuts.

S17 Oceans and Human Health: Trends and Practical Tools for Seafood Safety

JULI TRTANJ, National Oceanic and Atmospheric Administration, Office of Global Programs, 1100 Wayne Ave., Suite 1225, Silver Springs, MD 20910, USA; MARLENE JANES, Louisiana State University, Dept. of Food Science, 111 Food Science Bldg., LSU Ag Center, Baton Rouge, LA 70808, USA; LINDA ANDREWS, Mississippi State University, Coastal Research and Extension Center, Biloxi, MS 39532, USA; DAVID GREEN, North Carolina State University, Center for Marine Sciences and Technology, 303 College Circle, Morehead City, NC 28557, USA; PAW DALGAARD, Danish Institute for Fisheries Research, Dept. of Seafood Research, Ministry of Food Agriculture and Fisheries, DTU, Bldg. 221, 2800 Kgs. Lyngby, 2800, Denmark

Little is known about the direct impact that the ocean has on human health. In 2004, NOAA established the Oceans and Human Health Initiative (OHHI) to address this concern. This symposium will give an overview on the research goals of the OHHI. We do know that environmental conditions of the ocean have a strong influence on public health. Pathogenic organisms can be spread by circulation of waters through estuaries and coastal areas, such as harmful algal blooms, and through freshwater runoff from sewers, rivers, and streams. Pathogens can invade new areas of the ocean and become concentrated in filter-feeding shellfish, resulting in a burden on the seafood industry to control them. During this symposium, new processing technologies for detection and control of pathogens in seafood will be discussed. Emphasis will be placed on rapid methods to determine problem areas during sanitation of seafood processing facilities, new processing trends to control pathogenic bacteria in seafood, and bioluminescence for predicting microbial spoilage. Furthermore, with the globalization of fish trade and consumer demand for safe fish, HACCP-based systems and scientifically based risk assessment methods have been adapted. However, now, the emphasis is turning to an integrated, multidisciplinary approach to food safety and quality that includes the entire fish food chain. Presentations related to science-based strate-

gies for histamine control in fish and the need for a holistic approach to ensure safety and quality will be given. This symposium will give insights into target research areas needed to better understand how the ocean affects human health and new approaches to ensuring the safety and quality of seafood.

S18 Risk Ranking for Foodborne Pathogens

GREG PAOLI, Decisionalysis Risk Consultants, Inc., 1831 Yale Ave., Ottawa, ON, K1H 6S3, Canada; ROB LAKE, ESR Food Safety Programme, P.O. Box 29-181, Christchurch, New Zealand; MICHAEL BATZ, Resources for the Future, 1616 P St. NW, Washington, D.C. 20036, USA; ROSETTA NEWSOME, Institute of Food Technologists, 525 West Van Buren St., Suite 1000, Chicago, IL 60607-3814, USA; JOHN PAINTER, CDC-NCID-DBMD, Foodborne Outbreak Response and Surveillance Unit, Foodborne and Diarrheal Diseases Branch, Mailstop A-38, Atlanta, GA 30333, USA; DEON MAHONEY, Food Standards Australia New Zealand, P.O. Box 7186, Canberra, BC, ACT 2610, Australia

In order to reduce the burden of foodborne disease with the limited resources available, it is important that risk management activities be directed to areas of greatest risk and greatest opportunities for risk reduction. This exercise requires ranking of risks associated with food safety issues. The symposium will (1) introduce the problem broadly, including international issues such as the need for priority setting in developing countries, problems in ranking across both microbial and chemical hazards, and the importance of the 'measure' that is chosen, (2) identify data requirements for risk ranking and means of assembling necessary data, (3) examine approaches to attributing the total burden of foodborne disease to particular foods, (4) present different international approaches to risk ranking, (5) demonstrate how risk ranking exercises can be used to assess opportunities for risk reduction, and (6) give specific examples of how risk ranking has been applied to regulatory decision-making. Important risk ranking exercises have been undertaken or are underway in different parts of the world and this symposium tries to capture some of the main international developments.

S19 Enrichment Media and Sample Preparation: What's New?

CATHERINE DONNELLY, University of Vermont, 200 Carrigan Hall, Dept. Nutrition and Food Science, Burlington, VT 05405, USA; JINGKUN LI, Strategic Diagnostics, Inc., 128 Sandy Drive, Newark, DE 19713, USA; LEE-ANN JAYKUS, North Carolina State University, Dept. of Food Science, Campus Box 7624/339D Schaub Hall, Raleigh, NC 27695, USA; BARRY PYLE, Montana State University, Dept. of Microbiology, Bozeman, MT 59717, USA; JAY ELLINGSON, Marshfield Clinic Laboratories, 1000 North Oak Ave., Marshfield, WI 54449, USA; SRINAND SREEVATSAN, Ohio State University, Dept. of Verterinary Preventive Medicine, Food Animal Health Research Program, OARDC, 1680 Madison Ave., Wooster, OH 44691, USA

In the race to develop rapid and novel microbiological identification methods, most of the developmental efforts have focused on the detection phase. These methods work well if sufficient target organisms are presented to the detection system, but, as is often the case when levels of contamination are low, the ability to find the organism in the food matrix become more complicated. Indeed, the usefulness of rapid detection technologies could be expanded if (i) the levels of the target organism(s) and/or their molecular components could be increased more rapidly and/or (ii) the target organism(s) could be separated, concentrated, and purified from the sample matrix before detection. This symposium will present and review those methods which are available to simultaneously address upstream sample processing and rapid detection, including the use of improved enrichment media, immunomagnetic separation in combination with PCR, surface sampling linked with scan cytometry, and other novel approaches for combined pathogen concentration and detection. Taken together, the collaborative efforts of microbiologists, molecular biologists, and chemists are resulting in significantly reduced detection time while still enabling scientists to detect pathogens at the low levels anticipated in naturally contaminated products.

S20 A Behavioral Approach to Performance-based Food Safety Management – Theory, Practice and Outcome for Successful Retail Food Safety Programs

STEPHANIE OLMSTED, Safeway Inc., 32727 193rd Ave. SE, Kent, WA 98042, USA; PAUL MARRA, Wegman's Food Markets, 1500 Brooks Ave., Rochester, NY 14603-0844, USA; TOM CHESTNUT, Darden Restaurants, P.O. Box 593350, Orlando, FL 32859-3330, USA; FRANK YIANNAS, Walt Disney World, P.O. Box 10,000, Lake Buena Vista, FL 32830, USA; LARRY COHEN, Kraft Foods, NA, 801 Waukegan, Glenview, IL 60025, USA; ALAN TART, FDA, Southern Region, 60 8th St. NE, Atlanta, GA 30328, USA; HARRY FIELD, Law Office of Harry S. Field, 77 W. Washington, Suite 605, Chicago, IL 60602, USA; ROBERT GRAVANI, Cornell University, Dept. of Food Science, 11 Stocking Hall, Ithaca, NY 14853, USA

Retail Food Safety concerns continue to elevate each year as they have ownership of the food and are the last point of transfer before consumption by the customer. This makes them the first to be suspected in alleged food borne outbreaks. While retail food safety systems in the past may have not received the same emphasis placed on food safety in manufacturing facilities, success in today's retail food systems makes food safety practices a high priority needing everyone's support. Behavioral change is necessary to achieve food safety success.

This symposium will review how to change employee and management food safety behavior and provide positive success stories from selected IAFP Black Pearl recipients. Kraft Foods will provide an overview on their behavioral-based food safety training program on how Best Practices can carry from manufacturer to retail. FDA will cover how intervention strategies using an operational approach towards performance management can lead to successful retail food safety programs. While there is a positive side to having effective food safety programs, there is a negative side when changes are not applied. The legal, economic and social consequences of not changing these behaviors will be addressed from a legal aspect. Dr. Robert Gravani will tie all this together on how retail establishments can apply this theory and practice to "do it right the first time" and achieve successful retail food safety.

S21 Produce Packinghouse Sanitation: Designing and Implementing Effective Food Safety Programs

JACK GUZEWICH, FDA-CFSAN, 5100 Paint Branch Pkwy., College Park, MD 20740-3835, USA; JUAN LEON, Emory University, Dept. of Global Health – RSPH, 1518 Clifton Road, 7th Floor, Atlanta, GA 30322, USA; JENNIFER TONG, United Fresh Fruit and Vegetable Association, 1901 Pennsylvania Ave. NW, Suite 1100, Washington, D.C. 20006, USA; RON SCHMIDT, University of Florida, Food Science and Human Nutrition, P.O. Box 110370, Gainesville, FL 32611-0370, USA; LES LIPSCHUTZ, Food Safety, Inc., 2301 Calle de Real NW, Albuquerque, NM 87104, USA; JUAN MUNIZ, PrimusLabs.com, 2810 Industrial Pkwy., Santa Maria, CA 9345, USA

Assuring and enhancing produce food safety is a top priority for the fresh produce industry. Fresh fruits and vegetables are increasingly packed directly into boxes in production fields to reduce postharvest handling and minimize the potential for mechanical damage to the products. However, significant quantities of fresh fruits and vegetables are still commercially harvested, placed in bulk containers, and transported to packinghouse facilities where sorting and packing occurs. Each product handling step, if done inappropriately, provides an opportunity for produce contamination to occur. Commingling of fresh produce and extensive direct product contact at packinghouses by employees, machinery and aqueous solutions also serve as potential means of cross contamination. Produce packinghouse operations are extremely diverse in their product handing sophistication and food safety procedures. Raw agricultural commodity postharvest handling practices in packinghouse operations are currently not as clearly defined and commonly agreed upon as are GMPs and HACCP in the food processing industry. Food safety programs, which are well defined and function well within the control environs of a food processing plant, are not always appropriate in production agriculture situations such as a packinghouses. There is also little quantitative data regarding the risk associated with many of the postharvest handling practices commonly used in fresh produce packinghouses, and it is not commonly agreed upon as to exactly what the most effective risk management strategies may be. This symposium will offer the current perspectives of industry, academia and regulators on designing and implementing effective produce packinghouse food safety programs.

S22 International Food Safety Opportunities and Challenges in the Developing World

MARIA DE LOURDES COSTARRICA, FAO, Food Quality Liaison Group, Food Quality and Standards Service, Food and Nutrition Division, Via delle Terme di Caracalla 00100, Office C-280, Rome, Italy; MALCOLM MCDONALD, Kraft Foods, P.O. Box 2000, Cobourg, ON, K9A 4L4, Canada; MARIA TERESA DESTRO, University São Pãulo, Av Prof. Lineu Prestes 580 B114, São Pãulo, SP, 05.508-900, Brazil; LOUIS LALEYE, United Arab Emirates University, Dept. of Food Sciences, College of Food Systems, P.O. Box 17555, Al-Ain, UAE; MARIA DEUG-DEEB, 525 Wilde Greene Drive, Roswell, GA 3007, USA; ROBERT TAUXE, CDC, Mailstop A-38, Atlanta, GA 30333, USA

According to the World Health Organization, the leading causes of death in developing countries originate from foodborne or waterborne diarrheal illnesses. Approximately 1.8 million people, mostly children, become ill or die annually. The developing world faces many challenges attempting to ensure the safety of their food supply. Food safety issues developing countries encounter are significantly different from those of developed countries, for many reasons. An introduction to the food safety differences between the developing and the developed world will be given. Suggestions will be offered regarding the future of food safety in these areas, including the need for international surveillance networks, comparative research, and collaborative investigations to research outbreaks when they occur. Political, cultural, and economic barriers affecting these areas will be also be addressed, and will include the objectives of the Kraft-UN Unistar program. This program provides technical assistance to small food companies in developing countries aiding in development of local food businesses. Food safety and pathogen control perspectives in South America will be introduced. A training program developed in part by the United Arab Emirates that educates employees in food safety and sanitation, including technical writing and research and presentation skills, will be presented. Finally, information regarding compliance standards for exportation of foods to developed countries will be discussed. The symposium will conclude with a round table discussion to encourage discussion regarding the different areas which were discussed.

S23 Recent Advances in Intervention Strategies for Pathogen Control

VIJAY JUNEJA, USDA-ARS-ERRC, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA; KATHERINE SWANSON, Ecolab, 840 Sibley Memorial Hwy., Mendota Heights, MN 55118, USA; JOHN SOFOS, Colorado State University, Dept. of Animal Sciences, 1171 Campus Delivery, Fort Collins, CO 80523-1171, USA; LARRY BEUCHAT, University of Georgia, CFS, 1109 Experiment St., Griffin, GA 30223-1797, USA; JOSEPH MEYER, Kraft Foods - Oscar Mayer, 910 Mayer Ave., Madison, WI 53704, USA; ROBERT BUCHANAN, FDA-CFSAN-DHHS, HFS-06, 5100 Paint Branch Pkwy. #2B64, College Park, MD 20740-3835, USA

Despite stricter regulatory standards, commercial standards, and better methods for controlling microorganisms, the increasing numbers of illnesses associated with foodborne pathogens such as *Listeria monocytogenes* and *Escherichia coli* O157:H7 has renewed concerns about food safety. Accordingly, the need for better control of foodborne pathogens has been paramount in recent years. Recent research has explored or employed interventions to kill, remove or reduce foodborne pathogens on foods, including carcasses, meat and poultry products, fruits and produce. This symposium will discuss recent developments in intervention strategies for control of foodborne pathogens. This includes the interventions applied to the carcass surface and post-slaughter interventions; new technologies for decontamination of produce and novel means of applying sanitizing agents; multiple food formulation factors, and the use of predictive models to ensure microbiological safety of foods. The discussion will include national and international issues related to differences in conceptions of food, perceptions of risk and demand for food safety, and justifiable differences in regulatory systems. Also, this symposium will discuss verification and validation of plant sanitation intervention approaches, and management of foodborne pathogens for HACCP application and documentation.

S24 Microarray Technology: An Emerging Tool in the Food Microbiologists' Toolbox

MARTIN WIEDMANN, Cornell University, Dept. of Food Science, 412 Stocking Hall, Ithaca, NY 14853-7201, USA; ANDREW BENSON, University of Nebraska, Dept. of Food Science and Technology, 330 Food Industry Complex, Lincoln, NE 68583-0919, USA; KATHRYN BOOR, Cornell University, Dept. of Food Science, 413 Stocking Hall, Ithaca, NY 14853-7201, USA; CLAUDE MABILAT, bioMérieux, Parc Club du Moulin à Vent, 33, rue Docteur Lévy, 69693, Vénissieux, Cedex, France

Microarray technologies provide the microbiologist with an advanced tool to characterize microorganisms at the whole genome level, including the ability to simultaneously determine the presence or absence or expression of thousands of genes. Microarray-based expression monitoring provides the opportunity to gain a complete understanding of the response of food associated bacteria to different environmental conditions, which can facilitate the development of novel bacterial control and intervention strategies. Characterizing gene presence or absence by microarrays, on the other hand, provides a novel tool for sensitive bacterial subtyping, which not only allows subtype classification but also provides information on the presence or absence of relevant genes (e.g., virulence genes, antibiotic resistance genes, etc.). Finally, microarrays provide an opportunity for highly multiplexed detection of target genes, which can facilitate detection of pathogens and spoilage organisms as well as allow the detection of undesirable food components (e.g., not labeled animal species, etc.). Microarrays also provide unique challenges with regard to experimental design and statistical data analysis, which need to be addressed to maximize the benefits food microbiologists can derive from the use of this technology. This symposium will provide food microbiologists with an introduction to the microarray technology and different applications of this technology, including commercial and practical applications in the food industry.

S25 Pathogen Survival in Dried Fermented Meat and Partially Cooked Products

CARL CUSTER, USDA-FSIS-OPHS-MD-MIB, 902 D St. SW, Washington, D.C. 20024, USA; MARK HARRISON, University of Georgia, Dept. Food Science Technology, 340 F. S. Bldg., Athens, GA 30602-7610, USA; RICHARD HOLLEY, University of Manitoba, Dept. of Food Science, Winnipeg, MB, R3T 2N2, Canada; CATHERINE CUTTER, Penn State University, Dept. of Food Science, 111 Borland Laboratory, University Park, PA 16802, USA

E. coli O157:H7 can survive the American Meat Institute (1992) guidelines for the manufacture of fermented and dried meat products. The more recent recommendations from the Blue Ribbon Task Force (Nickelson et al., 1996) will increase manufacture cost and may compromise product quality if lethal thermal treatments are required. This symposium will identify the food safety problems associated with these products and the difficulty in dealing with issues associated with eliminating pathogens. It will explain why these products, which represent about 10% of processed meat production, are currently a risk to human health. The importance of microbial adaptation to environmental stress will be discussed and the symposium will present information on how "secondary" natural preservatives such as *Lactobacillus reuteri* and allyl isothiocyanate can enable continued safe production of these products without lethal thermal treatments, and overcome adaptive responses. The symposium will be concluded by a presentation that involves personal experience with small producers of dried meat products and the problems they face.

S26 Food Safety Objectives – Now That We Have Decided to Have Them, How Do We Think They Will be Used in Food Safety Management?

ROBERT BUCHANAN, FDA-CFSAN-DHHS, HFS-06, 5100 Paint Branch Pkwy., #2B64, College Park, MD 20740-3835, USA; PATRICIA DESMACHELIER, Food Science Australia, CSIRO, P.O. Box 52, North Ryde, NSW, 1670, Australia; LEON GORRIS, Unilever, SEAC - Risk Analysis Group, Colworth House, Sharnbrook, Bedford, MK44 1LQ, UK; S. ANDREW STARBIRD, Santa Clara University, Operations & Management Information Systems, 215 Kenna Hall, Santa Clara, CA 95053, USA; TANYA ROBERTS, USDA-ERS, 1800 M St. NW, Washington, D.C. 20036, USA

A prominent global development in recent years within food safety management is the move towards a more risk-based approach at the governmental level. Lead by Codex Alimentarius, under the auspices of WHO and FAO, governments around the world are adopting risk analysis as a framework for making decisions on food safety. Within this framework, Microbiological Risk Assessment (MRA) is used to establish a view on the prevailing risk in a population, for instance the risk associated with a food product/hazard combination. New concepts have been introduced in the process: Appropriate Level of Protection (ALOP), Food Safety Objective (FSO), Performance Objective (PO) and Performance Criterion (PC). Governments can use these to communicate their public health protection policy to particular food chains, e.g., through setting an ALOP (or comparable public health goals) and deriving an FSO from this. A number of questions remain to be answered, however. Which process is best followed in deriving the FSO from the ALOP and what science is called upon to underpin this process? Having an FSO, how will the related parameters be derived within an industrial context and how can industry prove to be in compliance with the FSO? Again, what science do we have to back up all this? The symposium intends to give a critical but positive outlook on issues related to setting and complying with FSOs.

S27 Current Practices and Innovations in Cold Chain Management for Food Products

KATHRYN BOOR, Cornell University, Dept. of Food Science, 413 Stocking Hall, Ithaca, NY 14853, USA; MARK SALIMBENE and CHUCK STOFFERS, Safeway Inc., 2800 Ygnacio Valley Road, Walnut Creek, CA 64598-3592, USA; STEPHEN NIGHTINGALE, Burntside Partners, Inc., 9607 Doctor Perry Road, #110, Ijamsville, MD 21754, USA; CATHERINE GOLDSMITH, SIRA Technologies, 345L E. Foothill Blvd., Suite 460, Pasadena, CA 91107, USA; THOMAS MCMEEKIN, University of Tasmania, School of Ag Science, Private Bag 54, Hobart, Tasmania, 7001, Australia

Many foods in the diets of people around the world are dependent upon management of temperature to assure safety and quality. As the length and complexity of the in-country and international food chains grow, the importance of temperature management becomes the predominant factor in delivering a safe and consumable product. Temperature management for foods is primarily cold chain management. Cold chain management has progressed from storing food outside during the cold winters to the harvesting of ice that artificially extended the winter season to the invention and extended applicability of mechanical refrigeration, which allowed an artificial food winter to exist year around in any part of the globe that had power to drive the refrigeration units. Because microbes are the primary food contaminant affecting safety and quality, the symposium will focus on the specifics of how cold chain management affects human pathogens and food spoilage organisms. In order to address "Current Practices and Innovations in Cold Chain Management," existing methods, challenges and technologies will be reviewed by international and domestic food distributors. Finally, new technologies will be shared that improve the tracking and temperature control of foods, ending with a panel question-and-answer session open to attendees.

Abstract Book Addendum

as of August 8, 2005

MONDAY MORNING – AUGUST 15, 2005

S01 **Laboratory Response to Food Bioterrorism: How Prepared are We?**
Sponsored by ILSI N.A.

8:30 Title Change – Food Bioterrorism Response Plan and CDC National Laboratory Capacities – Arthur P. Liang, CDC, Atlanta, GA, USA

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S02 **Microbiological Predictive Models: Development, Use and Misuse**

11:30 Presenter Affiliation Correction – Tom McMeekin, University of Tasmania, Australian Food Safety Centre of Excellence, Hobart, Tasmania, Australia

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P1-12 Withdrawn

MONDAY AFTERNOON – AUGUST 15, 2005

Late Breaking Session
Avian Influenza and Managing Risk
Harborside DE
Organizer/Convenor: Mike Robach
Sponsored by Cargill Inc.

1:30 **History and Classification of the H5N1 Virus** – David Swayne, US Department of Agriculture, Southeast Poultry Research Laboratory, Athens, GA, USA

2:00 **Risk Assessment, Risk Communication and Consequences** – William Hueston, University of Minnesota, St. Paul, MN, USA

2:30 **Risk Management Strategies in Southeast Asia** – Mike Robach, Cargill, Minneapolis, MN, USA

3:00 **Break**

3:30 **Risk Management Strategies in the United States** – Bruce Stewart-Brown, Perdue Farms, Salisbury, MD, USA

4:00 **Decontamination Technologies** – Katherine M. J. Swanson, Ecolab, Mendota Heights, MN, USA

4:30 **Avian Influenza – a Global Perspective** – Alex Thiermann, International Office of Epizootics, Paris, France

This late-breaking symposium will provide food safety professionals with an overview of avian influenza and its potential effect on public health worldwide. Presentations will offer a history of avian influenza, including the recent H5N1 epizootic, natural reservoirs of the virus, surveillance and monitoring efforts (including diagnostics), and approaches to understand and control the spread of the virus. Presentations will focus on public health implications and the impact on food safety and security. Risk management strategies, including the effect of food processing will also be discussed. Speakers will address experiences in Thailand and other parts of Southeast Asia, as well as the United States industry experience.

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S06 **Update on Foodborne Disease Outbreaks**
Sponsored by IAFP Foundation Fund

3:50 Presenter Affiliation Correction – FSIS Perspective on This Emerging Pathogen – Kristin Holt, USDA-FSIS, Atlanta, GA

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S07 **Safety Concerns of Food Chemical Contaminants**

2:00 Presenter and Title Change – The Role of a Standard in Ensuring Safety Dietary Supplements – Clif McLellan, NSF International, Ann Arbor, MI, USA

3:30 Presenter Affiliation Correction – How Do Toxicologists Extrapolate Risk? – Stephen S. Olin, Risk Science Institute, ILSI Research Foundation, Washington, D.C., USA

4:00 Title change – Environmental Contaminants in Seafood – Charles R. Santerre, Purdue University, West Lafayette, IN, USA

4:30 Presenter Affiliation Correction – Legal Concerns Related to Chemical Food Safety Issues and the Impact on the Food Industry – Mark F. Nelson, Grocery Manufacturers Association, Washington, D.C., USA

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T02 **Foods of Animal Origin Technical Session** – Convenors for the session are Mark Berrang and Doris D'Souza

T2-02 Survival of *Listeria monocytogenes, Listeria innocua,* and Lactic Acid Bacteria Species in Chill Brines – Bridget Archibal Meadows should be listed as an author

T2-03 Alternative Cutting Methods to Minimize Transfer of Specified Risk Materials during Steak Preparation from Bone-in Short Loin – will be presented by Rob Yemm, Institute for Environmental Health, Seattle, WA, USA

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P2-45 Evaluation of an Ozonated Water System and a Steam Pasteurization System for Controlling *Listeria mono-cytogenes* and *Salmonella* spp. on Raw Whole Shelled Almonds – will be presented by R. K. Phebus, Kansas State University, Manhattan, KS, USA

P2-46 Evaluation of a Commercial Steam Pasteurization System for Controlling *Listeria monocytogenes* and *Salmonella* spp. on Raw Whole Shelled Pistachios – will be presented by R. K. Phebus, Kansas State University, Manhattan, KS, USA

P2-52 Withdrawn

P2-54 In Vitro Inhibition of Adsorption of Foodborne Viruses by Human Bifidobacterial Isolates – will be presented by Julie Jean, Universite Laval, Quebec, QC, Canada

TUESDAY MORNING – AUGUST 16, 2005

S12 Yeasts and Molds: When Fungi Go Bad, Who Do You Call?
Sponsored by ILSI N.A.

9:00 Presenter Affiliation Correction – Case Studies on the Spoilage of Processed Foods and Beverages by Yeasts and Molds – Paul A. Hall, Kraft Foods Global, Inc., Glenview, IL, USA

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S13 They Said What? – The Risky World of Risk Communication
Sponsored by IAFP Foundation Fund

8:30 Presenter Change – Global Differences in Risk Communication – GMOs and Irradiation – William K. Hallman, Cook College, New Brunswick, NJ, USA

10:30 Presenter Change – Lost in Translation: Communicating Trans Fat to the Consumer – Tony Flood, International Food Information Council Foundation, Washington, D.C., USA

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P3-15 Survival and Growth of *Enterobacter sakazakii* on Fresh-cut Fruits and Vegetables – will be presented by Larry R. Beuchat, University of Georgia, Griffin, GA, USA

P3-43 Attachment and Biofilm Forming Abilities of Persistent and Non-persistent *Listeria monocytogenes* Isolates – will be presented by Joseph F. Frank, University of Georgia, Athens, GA, USA

TUESDAY AFTERNOON – AUGUST 16, 2005

S19 **Enrichment Media and Sample Preparation: What's New?**
Sponsored by IAFP Foundation Fund

3:30 Presenter Change – A Novel Surface Sampling Method Combine with Rapid Detection – Bruce Bradley, Microbial-Vac Systems, Inc., Jerome, ID, USA

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T04 Antimicrobials Technical Session
Convenors will be Karen Killinger Mann and Amy Simmone

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P4-28 *Vibrio vulnificus* Septicemia Associated with Clam Consumption, 1988–2003 – will be presented by Patricia Yu, CDC, Atlanta, GA, USA

P4-48 Detection of Food Allergens by Quantitative and Qualitative ELISA – will be presented by Phil Myers, Tecra International Pty Ltd., Frenchs Forest, NSW, Australia

P4-49 Withdrawn

P4-54 Withdrawn

WEDNESDAY MORNING – AUGUST 17, 2005

S20 A Behavioral Approach to Performance-based Food Safety Management – Theory, Practice and Outcome for Successful Retail Food Safety Programs

9:00 Presenter Change – Positive Success Stories on Implementing Behavioral Changes in Food Safety Culture – Panel of IAFP Black Pearl Recipients – Peter Hibbard, Darden Restaurant, Orlando, FL, USA will be replacing Tom Chestnut.

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S21 Produce Packinghouse Sanitation: Designing and Implementing Effective Food Safety Programs

9:30 Presenter Change – GAPs, GMPs, and Guidance: An Industry Update on Assuring Produce Food Safety – James Gorny, United Fresh Fruit & Vegetable Association, Davis, CA, USA

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T5-01 Foodborne Illness Litigation and How to Avoid It – will be presented by Patti Waller, Marler Clark LLP, PS, Seattle, WA, USA

T6-10 School-related Foodborne Illness Outbreaks Low – The Food Nutrition Service Collaborates with Other Agencies and Works to Assure Safe Food in Schools – will be presented by Margaret Venuto, USDA-FNS, Alexandria, VA, USA

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P05 Method Development for Pathogen Testing Poster Session

Convenors will be Laura Bauermeister and Vanessa Teter

P5-05 Rapid Detection of Low Numbers of *Escherichia coli* O157:H7 in Apple Cider by Real-time Polymerase Chain Reaction – will be presented by David A. Golden, University of Tennessee, Knoxville, TN, USA

P5-37 Identification of *Campylobacter* Isolates from Farms by PSTL Ribotyping – will be presented by F. Ann Draughon, University of Tennessee, Knoxville, TN, USA

WEDNESDAY AFTERNOON – AUGUST 17, 2005

S24 Microarray Technology: An Emerging Tool in the Food Microbiologists' Toolbox

Sponsored by ILSI N.A.

2:00 Title Correction – Use of DNA Microarrays for Subtyping Microorganisms – Andrew K. Benson, University of Nebraska, Lincoln, NE, USA

3:00 Presenter Affiliation Correction – Microarray as a Diagnostic Tool – Claude Mabilat, bioMérieux SA, Marcy l'Etoile, France

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S27 Current Practices and Innovations in Cold Chain Management for Food Products

Organizers: John Rushing and Allen Sayler

Convenors: Lori Ledenback and Allen Sayler

2:30 Presenter Affiliation Correction – New Technologies for Monitoring and Managing Product Temperatures – Tom McMeekin, University of Tasmania, Australian Food Safety Centre of Excellence, Hobart, Tasmania, Australia

WEDNESDAY AFTERNOON – AUGUST 17, 2005

Round Table Discussion on Identifying Food Safety Research Priorities for Developing Countries

Chasseur Room

1:00 p.m – 4:00 p.m.

The Consultative Group on International Agriculture Research (CGIAR) has been charged with expanding their research efforts to include food safety. The mission of the CGIAR system is to mobilize national and international agricultural science to reduce poverty, foster human well being, promote agricultural growth, and protect the environment. The goal of this round table discussion is to aid the CGIAR Science Council in identifying food safety research priorities as they mobilize resources to expand their research agenda.

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