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

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Juan Parkin Abstract

Advancing Food Protection Technology

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It has been said that in times of change, learners inherit the earth, while the learned will find themselves beautifully equipped to deal with a world that no longer exists. In the case of microorganisms, their ability to learn or adapt to a changing world has certainly been a key factor in the emergence of new foodborne pathogens that were not of concern even a decade or so ago. The rapid globalization of the food processing and retailing industries, consumer demand for more natural and more convenient products and an overall increase in the susceptibility of the population are believed to be the most important factors that have led to fundamental changes in the nature of foodborne disease itself. Industry, government and academia have responded by advancing the science and technology of food protection in an attempt to combat these new food safety challenges.

Advances in microbial genomics, proteomics, metabolomics and single cell responses are being channelled into bioinformatic networks to bring a new systems biology approach to food protection. Through the integration of statistical analysis, databases, pattern recognition and whole cell simulations, these networks hold the promise for the development of new preservation and intervention strategies, measurement of biovariability within microbial population, better interpretation of microbial resistance and stress data, and improved detection of microorganisms in complex matrices. Increasingly, the systems biology approach will influence food safety management strategies introducing a new dimension to the validation of new food safety control measures, risk assessment procedures, and regulation.

Developments in the areas of predictive modelling and risk assessment now offer the potential to link exposure to a microbial hazard to the likely number of cases of illness in the population and are leading to nothing less than a paradigm shift in the way that food safety risks are managed based on the new concept of Food Safety Objectives (FSOs). Although quantitative aspects of the scheme are still being advanced, the framework will facilitate the transparent communication of food safety responsibilities of different stakeholders across the food chain.

Finally, advances in non-thermal preservation technologies such as ultra-high pressure processing and pulsed electric field treatment as well as renewed interest in food irradiation offer the promise of foods that have improved freshness, in terms of flavor, color, texture and nutritional value closer to non-heated products while at the same time exhibiting enhanced microbiological safety.

Developments in food science offer exciting new possibilities to meet the consumer drivers of health, convenience, pleasure and environment. In delivering these possibilities, it is important that we do not introduce new food safety hazards. Food protection technology will therefore play a crucial role in trying to predict and prevent new concerns as well allowing us to respond quickly and effectively to emerging threats. This will require not only the use of the technologies described but also an intricate networking and collaboration among all stakeholders involved.

Poster Abstracts

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DSC – Developing Scientist Competition

P001 DSC The Combined Effects of Temperature, Sodium Lactate, Sodium Diacetate and Pediocin on the Heat Inactivation of *Listeria monocytogenes* on the Surface of Frankfurters

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The effects and interactions of heating temperature (55 to 60°C), sodium lactate (NaL; 0.0 to 4.8%), sodium diacetate (SDA; 0.0 to 0.4, w/w) and pediocin (0 to 10,000AU) on the heat resistance of *L. monocytogenes* 101M on the surface of frankfurters were examined. Thermal death times were determined by dipping vacuum packaged frankfurters in a circulatory water bath set to required temperatures. The samples were plated on selective modified oxford formulation and nonselective brain heart infusion agar to determine injury and total survivors. Decimal reduction times (D-value) were calculated. The D-values were analyzed by first order response surface regression for temperature, NaL, SDA and pediocin. The observed D-value ranged from 1.6 to 32 min for franks having different combination of NaL, SDA and pediocin treated at different temperatures according to central composite design. Temperature had the most prominent impact on decreasing the D-value of *L. monocytogenes*. However, a combination of temperature with pediocin, NaL and SDA was more effective at decreasing the D-value of *L. monocytogenes* as compared to control. A mathematical model describing the combined effects of temperature, Nal, SDA and pediocin on the thermal inactivation of *L. monocytogenes* was developed. The model can predict D-values for any combination of temperatures, Nal, SDA and pediocin that are within the range of those tested. This model will benefit the industry and consumers by providing possibilities of milder thermal treatment of antimicrobial incorporated ready-to-eat meat products while insuring their safety.

P002 Hot Water Post-process Pasteurization of Cook-in-Bag Turkey Breast Treated with Potassium Lactate and Sodium Diacetate (Opti-Form®) and Acidified Sodium Chlorite for Control of *Listeria monocytogenes*

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The following treatments were evaluated for control of *Listeria monocytogenes* (Lm) in cook-in-bag turkey breast (CIBTB): 1) Opti-Form® (1.54% potassium lactate and 0.11% sodium diacetate) added to the formula in the mixer and 150 ppm acidified sodium chlorite (ASC) applied by surface spraying or 2) Opti-Form® or 3) No Opti-Form® or ASC. Each CIBTB was inoculated (20 ml) with approximately 5 log CFU of a five-strain mixture of Lm and then vacuum-sealed. In two trials, half of the CIBTB were exposed to 203°F water for 3 min in a pasteurization tunnel and then all CIBTB were stored at 4°C for 45 to 60 days and Lm were enumerated by direct plating onto MOX agar. Heating resulted in an initial reduction of about 2 log CFU of Lm per CIBTB. For heated CIBTB, Lm increased by about 2 log CFU per CIBTB in 28 (treatment 1), 28 (treatment 2), and 14 (treatment 3) days. Thereafter, pathogen levels reached about 7 log CFU per CIBTB in 45, 45, and 21 days for treatments 1, 2, and 3, respectively. In contrast, in non-heated CIBTB, Lm levels increased from about 5 log CFU per CIBTB to about 7 log CFU per CIBTB in 28, 21, and 14 days for treatments 1, 2, and 3, respectively. These data establish that hot water post-process pasteurization alone is effective in reducing initial levels of Lm on CIBTB, and Opti-Form® and ASC were somewhat effective at controlling the subsequent outgrowth of Lm during storage.

P003 Partial Control of *Listeria monocytogenes* on the Surface of Full Fat Turkey Frankfurters Held at 4°C Using Zein Coatings Containing Nisin, Sodium Lactate and Sodium Diacetate

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The objectives were to determine whether zein, nisin, lactate and diacetate alone or in combination could control the growth of *L. monocytogenes* on full fat turkey frankfurters at 4°C and to determine whether lactate or diacetate had any synergistic effect on the activity of nisin. Turkey frankfurter pieces, surface inoculated with strain V7 of *L. monocytogenes*, were treated with zein-ethanol-glycerol (ZEG), zein-propylene-glycol (ZPR), ethanol-glycerol (EG), propylene glycol (PR), nisin (N), sodium lactate (L) or sodium diacetate (D) alone or in combination. Over 28 days, frankfurters treated with nisin or diacetate

alone had *L. monocytogenes* counts reduced by 6.6 or 6.3 log CFU/g, respectively. N-D gave a kill of 6 log CFU/g. Since the zein solvents, EG or PR, showed a good kill of about 5.6 or 5.2 log CFU/g, respectively, similar to ZEG or ZPR, zein powder per se had no antimicrobial activity. PR and the ZPR combination treatments gave mean survivor counts that were 0.5 to 1 log CFU/g higher than the EG and ZEG treatments. Over 28 days, ZEG-N-D, ZEG-N-D-L, ZPR-N-D or ZPR-N-D-L yielded no detectable CFUs. Sodium lactate alone was ineffective. No synergies were observed. Collectively, the data indicate that sodium diacetate or nisin when used singly, and N-D, ZEG-N-D, ZEG-N-D-L, ZPR-N-D, ZPR-N-D-L, EG or PR, showed promise as barriers against the growth of recontaminating *L. monocytogenes* cells on this food substrate.

P004 Control of *Listeria monocytogenes* in Wiener and Turkey Slurries by Combinations of Antimicrobials

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Fifty combinations of food-approved antimicrobials were screened for antilisterial activity in slurries prepared with 25% (w/v) uncured turkey (5% fat) or cured wiener batter (30% fat; 30 ppm nitrite). Antimicrobials (alone or up to 3 antimicrobials combined) included diacetate, lactate, nisin, monolaurin, polyphosphates, sorbate, benzoate, propionate, ascorbic acid, several plant extracts, organic acid blends, and lysozyme. Slurries with antimicrobials were adjusted to pH 6.2 and pasteurized by heating to 65°C for 10 min, then cooled to 4°C, and inoculated with a 5-strain mixture of *L. monocytogenes* (LM) to yield 5-log CFU/ml. Duplicate samples of each treatment were enumerated for LM on Modified Oxford Agar at 0-time and after 1, 2, 3, and 4 weeks storage at 4 or 10°C. LM did not grow in wiener or turkey slurries supplemented with 0.25% potassium sorbate, propionic acid, or benzoic acid and stored at 4 or 10°C. Nisin alone (50 or 100 ppm) reduced LM 1 to 1.5-log CFU/g within 5 min of inoculation, but resistant populations increased > 3-log during the 4-week storage. Combining 50 ppm nisin and 0.3% diacetate prevented recovery of LM at 4 or 10°C in both meat types throughout the 4-week period. Certain treatments (0.1% sorbate, propionate, or benzoate, 0.5% organic acid blend, or 0.1% diacetate-25 ppm nisin) prevented listerial growth in wiener slurries with 30 ppm nitrite, but not in uncured turkey slurries. None of the other combinations with < 100 ppm nisin or with < 0.3% diacetate significantly delayed listerial growth compared with the treatments using the single antimicrobial. Additional studies will be completed to verify efficacy of select combinations of antimicrobials in wieners and uncured turkey products.

P005 DSC Effects of Frankfurters Manufactured Using Cellulose Casings Impregnated with Buffered Sodium Citrate on *Listeria monocytogenes* and Shelf Life

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Frankfurter casings impregnated with buffered sodium citrate (BSC) were evaluated. The study consisted of two parts: 1) an inoculated study to evaluate *L. monocytogenes* outgrowth during storage and 2) a non-inoculated study to evaluate treatment effects on microbiological and color shelf life. Frankfurters commercially manufactured using cellulose casings impregnated with BSC were compared to control frankfurters (regular cellulose casings) during 90-day storage. Color and pH changes were monitored during simulated retail storage at 4°C and 10°C. The inoculated study was performed using a low (10^4) and a high (10^6) inoculum level comprised of a five-strain cocktail of *L. monocytogenes*. Changes in instrumental color, pH and microbial spoilage populations during simulated retail distribution and display at 4°C were monitored on non-inoculated packages starting on days 40 and 90 of boxed storage. Samples drawn from chilled box storage on these days were placed into lighted retail storage cases and monitored. Microbial enumeration was done using USDA package rinse method. The inoculated study showed that untreated casings showed lower outgrowth of *L. monocytogenes* compared to the treated casings ($P < 0.05$) at both 4°C and 10°C. In the non-inoculated study, the untreated casings showed significantly lower outgrowth at 10°C ($P < 0.05$), but not at 4°C. No change in pH was observed in the test frankfurters at either of the storage temperatures over time. Cured color deteriorated over time comparably in both the test and control frankfurters at both storage temperatures. Impregnation of BSC in the frankfurter casings, under the conditions of this study, was not beneficial for controlling *L. monocytogenes* outgrowth during storage.

P006 Antimicrobial Treatments to Control the Growth of *Listeria monocytogenes* following Post-processing Contamination of Commercial Bologna and Ham

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Listeria monocytogenes is associated with frequent and highly publicized recalls of potentially contaminated ready-to-eat meat and poultry products. In this study, the antilisterial effects of antimicrobial treatments, applied as dipping solutions, on commercial bologna and ham inoculated post-processing were investigated during storage in vacuum-packages at 10°C. Slices of bologna (58 cm^2) and ham (40 cm^2) inoculated

with a 10-strain composite of *L. monocytogenes*, were dipped (2 min) in acetic acid (AA; 2.5%), lactic acid (LA; 2.5%), potassium benzoate (PB; 5%), or Nisaplin (commercial form of nisin; 0.5%) solutions, either singly or sequentially, and then vacuum-packaged and stored (10°C) for 48 days. Initial populations of *L. monocytogenes* (PALCAM agar) in control bologna and ham samples increased from 3.4 log CFU/cm^2 to 7.4 and 7.8 log CFU/cm^2, respectively, in 8 days. Initial reductions (day-0) of the pathogen, compared to the controls, on bologna and ham samples treated with single applications of AA, LA or PB ranged from 0.4 to 0.7 log CFU/cm^2. Higher initial reductions were obtained for samples treated with Nisaplin (2.4 to 2.9 log CFU/cm^2). However, Nisaplin, applied singly, allowed proliferation of the pathogen during storage to levels comparable to the controls. The remainder of the treatments inhibited growth of *L. monocytogenes* on both products during the 48 day storage period. The combination treatment of Nisaplin followed by LA, reduced *L. monocytogenes* to undetectable levels in both products at the end of storage. Post-processing application of antimicrobial treatments were, thus, very effective in controlling growth of *L. monocytogenes* during storage at 10°C and may be considered by the industry as alternatives under the new regulation for control of the pathogen.

P007 Application of Glucono-delta-lactone (GDL) and Other Antimicrobial Ingredients to Control *Listeria monocytogenes* in Ready-to-Eat Meat and Poultry Products

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The use of lactate and diacetate in cured meat products to control *Listeria* growth is well documented. However, identifying compounds to effectively suppress *Listeria* growth in uncured meat and poultry products continues to be a challenge. This has stimulated work in our laboratory to investigate the effectiveness of other antimicrobial ingredients against *Listeria* in both cured and uncured meat and poultry. Beef frankfurters and uncured sliced turkey breast were formulated with various antimicrobials including 0.25% GDL, 0.2% potassium sorbate (PS), 0.1% sodium benzoate (SB), 0.1% sodium proprionate (SP) and the combination of 2% sodium lactate (SL) and 0.1% sodium diacetate (SD). A higher concentration of SL (3%) and SD (0.25%) was included as an additional treatment for the uncured turkey breast. Products were surface inoculated (10^2 to 10^3 CFU/package) with a 5-strain cocktail of *L. monocytogenes*, vacuum-packaged and stored under refrigerated (4°C) and abuse (10°C) temperatures for the duration of the storage time. *Listeria* growth was prevented on beef frankfurters formulated with 0.25% GDL, 0.2% PS, 0.1% SB, 0.1% SP and 2% SL/0.1% SD for 90 days at 4°C and 56 days at 10°C. To date, no antimicrobials tested limited *Listeria* growth to < 2 logs on uncured turkey breast during refrigerated storage. Research is still needed to minimize the risk of *Listeria* growth in uncured meat and poultry products.

P008 DSC The Reduction of *Listeria monocytogenes* on Ready-to-Eat Roast Beef Treated with Acidified Sodium Chlorite

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On October 2, 2003 the USDA FSIS issued a new directive for the control of *Listeria monocytogenes* (LM) on ready-to-eat (RTE) products. In order to help processors meet this new requirement, we have been investigating Acidified Sodium Chlorite (ASC) as a possible method for the control of LM on 5-gram cubes of RTE roast beef cubes (RBC). An 18-h LM 1/2a culture was decimally diluted and 1 ml inoculated on to RTE-RBC and allowed to air dry. The RTE-RBC were then sprayed with ASC solutions at 0 ppm, 250 ppm, 500 ppm, 750 ppm, and 1000 ppm. The samples were bagged and refrigerated at 4°C. Bacterial counts were examined at 0, 7, 14, 21, and 28 days by spread plating onto Oxford agar plates, then incubating at 37°C for 48 h and determining CFU/g. An initial inoculation of 6.0 log CFU/g of LM was reduced by 5.0 log CFU/g with 1000 ppm ASC, by 4 logs CFU/g with 750 ppm and 500 ppm ASC, and by 3 log CFU/g with 250 ppm ASC for the spicy and regular RBC by day 7. At day 28 the 500, 750, and 1000 ppm ASC treated spicy RBC had greater than 4.00 log CFU/g reductions in LM counts whereas the same concentrations of ASC treated regular RBC only had about 2.5 log CFU/g reductions in LM counts compared to the controls. Our study indicates that ACS could be effective as a processing aid for inhibiting the growth of LM counts on RTE roast beef.

P009 DSC Fate of *Listeria monocytogenes* in Different Vanilla-flavored Products Stored at 4°C

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Listeria monocytogenes is a foodborne pathogen of major concern to the food processing industry. The inhibitory action of the flavoring agent "vanillin" against *L. monocytogenes* was investigated using various vanilla flavored food products stored at 4°C. Commercial products including soy yogurt, rice beverage, 2% milk, skim milk, and cheesecake filling were used as the test foods. These were inoculated with 10^4 CFU/ml of *L. monocytogenes* and stored at 4°C. Soy yogurt and the rice beverage were sampled for up to 30 days. Skim milk, 2% milk and cheesecake filling were sampled for up to 20 days. Samples were serially diluted and plated on modified Oxford agar and brain heart infusion agar. After 30 days, soy yogurt and vanilla soy yogurt showed 3 and 3.6 log reductions, respectively, in viable population. Listerial populations increased by 4.0 log and 3.7 log in the rice beverage and vanilla rice beverage, respectively. There was an increase of 2.2 log and 0.25 log in the viable *Listeria* population in 2% milk and vanilla 2% milk, respectively. An average

increase of 0.21 log in the viable population in skim milk was observed, but the population declined by 0.21 log in vanilla skim milk. The population remained constant in cheesecake filling, but a 1.7 log reduction was observed in vanilla cheesecake filling. The differences in survival patterns of the organism in 2% milk and skim milk may be due to the protective effect of fat on *Listeria*. The results indicate that "vanillin" has a potential inhibitory action against *L. monocytogenes*.

P010 Preparation of Polylactic Acid Nanoparticles and Their Effect on the Growth of *Listeria monocytogenes*

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Nanoparticles are formed by causing a particular compound to become entrapped within or associated with a polymer. A lot of attention has been paid to these particles in the pharmaceutical industry as a delivery method for various drugs, peptides, or proteins. The process of forming them lends stability to the encapsulated compound and allows controlled release of the compound at a specific site. The overall goal of this study is to develop a procedure by which antimicrobials, such as nisin and lysozyme, are encapsulated in polylactic acid (PLA) nanoparticles, thus creating a delivery system that will increase the efficacy of these compounds in complex food systems. The initial objectives were to determine the feasibility of preparing PLA nanoparticles and to determine if empty PLA nanoparticles had any effect on the growth of *L. monocytogenes*. PLA nanoparticles were constructed using a modified emulsion-diffusion technique. Briefly, an organic phase containing PLA is combined with an aqueous phase containing polyvinyl alcohol to form an emulsion. Addition of water to this emulsion causes the PLA to form nanoparticles as it diffuses into the aqueous phase. Prepared particles ranged in diameter from 350 to 700 nm. These empty PLA nanoparticles were evaluated in a microtiter assay against the growth of 4 strains of *L. monocytogenes* for 24 h at 32°C. The nanoparticles did not inhibit the growth of *L. monocytogenes* at concentrations up to 1600 μg/ml. These results indicate the PLA nanoparticles can be produced and that the PLA will not interfere with the antimicrobial assays.

P011 Evaluation of Short-chain Fatty Acids on *Listeria monocytogenes* Cell Invasion and Cell Association to Green Monkey Kidney Cells

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In this study, the role of short chain fatty acids (acetic acid, butyric acid and propionic acid) to influence the ability of *Listeria monocytogenes* Scott A to associate with and invade cultured green monkey kidney cells and cause cell damage were investigated. Exponential phases of cells of *Listeria monocytogenes*, grown at 37°C in brain heart infusion broth supplemented with 25, 50, and 100 mM of short chain fatty acids or a mixture (SCFA) for 12 h, were sedimented by centrifugation (10,000 x g, 10 min, 4°C), washed with phosphate buffered saline (PBS, pH 6.8) and added to cultured green monkey kidney cells for 2 h; virulence response of *L. monocytogenes* was tested by a cell association and invasion assay and cytotoxicity tested with use of an in vitro lactate dehydrogenase (LDH) release assay. The virulence response to SCFA was dependent on the concentration of the medium. Cell association decreased by 4 and 18% for strains treated with 25 mM of butyrate and acetate, respectively. However, when compared to 100 mM of SCFA, there was only a 4 to 7% reduction in cell association. Cell invasion also decreased by an average of 11% when the bacteria were cultured in the presence of all concentrations of acetate and 25 and 100 mM of butyrate. At all concentrations of SCFA, low to intermediate levels (0 to 31%) of LDH release from Vero cells was observed. These results may indicate different concentration of SCFA may directly influence the association and/or invasion of *L. monocytogenes*.

P012 Antilisterial Activities of Vanillin and Vanillic Acid

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Minimum inhibitory concentrations (MIC) of vanillin and vanillic acid against several strains of *Listeria monocytogenes*, *L. grayi*, *L. innocua* and *L. seeligeri* were determined by a broth dilution method in Tryptic Soy Broth adjusted to pH 5, 6, 7 and 8. Vanillin concentrations ≤ 30 mM inhibited all *Listeria* strains tested and the effect was slightly enhanced at lower pH. In contrast, the antilisterial effect of vanillic acid was highly dependant upon pH. Although none of the experimental strains were inhibited in 100 mM vanillic acid at pH 7 and 8, MIC values were 10 mM at pH 5 and 20 mM at pH 6. Tryptic Soy Broth supplemented with vanillin or vanillic acid was inoculated with *L. monocytogenes* to determine the mode of inhibition for both compounds. Growth of *L. monocytogenes* was inhibited in media supplemented with 30 mM vanillin at pH 5, 6 and 7, revealing a bacteriostatic mode of inhibition. Extensive loss of viability in 60 mM vanillic acid at pH 5 was indicative of bactericidal activity. No viable cells could be recovered by enrichment after 24 h at 25°C. The results suggested that both compounds could play a role in the ecology of *Listeria* spp. in foods containing these common flavoring agents.

P013 Enhancing the Bactericidal Effect of Lactoferrin against *E. coli* O157:H7

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Considerable interest has developed regarding the use of lactoferrin (LF) as a natural food preservative, but cations interfere with its antimi-

crobial activity and limit food applications. The objective of this study was to determine whether sodium lactate (SL), sodium hexametaphosphate (SHMP), sodium bicarbonate (SB), or quercetin (a bi-flavonoid) could be used to enhance LF activity against *E. coli* O157:H7 in Lauria broth (LB) containing 0.5 or 2.5% NaCl (as a meat model system) during incubation at 37°C or 10°C. At 32 mg/ml LF was bacteriostatic at 2.5% NaCl and 37°C or 10°C, but had no activity at 0.5% NaCl. At 0.5% NaCl, 3% SL alone caused a 4 log reduction but this effect was eliminated at 2.5% NaCl. Addition of LF to SL at 2.5% NaCl restored the bactericidal effect of SL. Combination of LF with 3 mg/ml SHMP at 2.5% NaCl and 37°C also yielded a 4 log reduction after 4 h exposure, but this was reduced to 2 log at 10°C and required 10 days to achieve. The LF + SHMP combination was ineffective at either temperature with 0.5% NaCl. The combination of LF and SB was more effective at 10°C and 2.5% NaCl, causing a 4 log reduction at 0.02M SB. At 37°C a 3 log reduction required 0.16M SB. Quercetin (250 μg/ml) was also more effective at 10°C with 2.5% NaCl but poor solubility in LB limited its usefulness. Combination of LF with either SL or SB shows potential for reducing viability of *E. coli* O157:H7 and work is under way to evaluate mixtures in meat systems.

P014 DSC Chitosan as an Antimicrobial Coating to Control *Escherichia coli* O157:H7 on the Surface of Lettuce

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Research was designed to determine the antimicrobial effects of chitosan against enterohemorrhagic *Escherichia coli* O157:H7 (EHEC) on the surface of lettuce stored at 4 or 25°C. A high (HMW) or low (LMW) molecular weight chitosan at two different concentrations (1.0 or 0.5%) was dissolved into lactic or acetic acid. Lettuce was cut into 5 g pieces and dipped into a decimally diluted culture of EHEC for 1 min. The lettuce samples were then dipped into the different chitosan solutions for 1 min and stored at 4°C for 0, 2, 4, and 6 days or at 25°C for 0, 1, 2, and 3 days. Lettuce samples were stomached for 2 min, serial diluted, plated onto SMAC and CFU/g determined. Our results showed at 4°C an initial 6.3 log CFU/g of EHEC was reduced to non-detectable levels on the surface of lettuce by day 6 for all chitosan coatings. By day 3 at 25°C the most effective chitosan coatings were both concentrations of the acetic acid HMW or LMW that produced a 3.5 to 4.0 log CFU/g reduction in EHEC counts from control levels. For the lactic acid chitosan coatings at 25°C on day 3 the 1.0% LMW reduced EHEC counts by 3.0 log CFU/g from control levels. All the other lactic acid chitosan treatments reduced EHEC counts on the surface of lettuce by 2.0 log CFU/g at 25°C on day 3. Chitosan could possibly be used as an antimicrobial coating on the surface of lettuce to reduce the risk of *E. coli* O157.

P015 Decontamination Interventions to Reduce *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on Beef Carcass Tissue

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It is well known that enteric pathogens are present in fresh beef processing plants and have the ability to cross-contaminate meat. Samples of beef carcass tissue were inoculated (10^5 CFU/cm^2) with four-strain composites of *Escherichia coli* O157:H7 or *Salmonella* Typhimurium and dipped (30 s in 1.5 L at 23°C unless otherwise indicated) either singly or in combinations of acidic oxidative water (0.005%, AOW - pH 2.67); basic oxidative water (BOW - pH 11.21); lactic acid (2.5%, LA - pH 2.12 at 55°C); water (W, pH 7.01), ammonium hydroxide (0.1%, AH - pH 10.89); or, sodium metasilicate (4%, SM - pH 12.35 at 82°C) as well as in a multiple-hurdle system approach to evaluate the effectiveness of such solutions in reducing contamination. The multiple-hurdle system incorporated: SM (1%, 82°C), followed after 20 min by hot water (W, 82°C), and 10 min later by 5.0% LA (pre-chill) before the carcass tissue was exposed to spray-chilling (for 48 h), and lastly by a post-chill 2.5 or 5.0% LA (55°C) application. Regardless of pathogen, the effectiveness of single treatments was SM > LA > AH=AOW=BOW=W, while sequential treatments combined with LA or SM did not have any additional effect beyond that of the individual treatments. Reduction of pathogens by 5% pre-chill LA was comparatively lower than that of only 5% pre- and 5% post-chill LA but similar to that of only 5% pre- and 2.5% post-chill LA. Single use of 5% pre-chill LA followed by 5% post-chill LA was as efficacious as a multiple-hurdle system with all treatments including 2.5% post-chill LA, and only slightly lower than the same system incorporating 5% post-chill LA.

P016 Ethanol-mediated Variations in Cellular Fatty Acid Composition and Protein Profiles of Genotypically Different Strains of *Escherichia coli* O157:H7

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The influence of ethanol on fatty acid composition and protein profiles of two genotypically different stains of *Escherichia coli* O157:H7 was studied. Growth rates of *E. coli* O157:H7 at 30°C in tryptic soy broth (TSB, pH 7.1) supplemented with 0, 2.5, and 5.0% ethanol decreased with an increase in ethanol concentration. The pH of TSB containing 5.0% ethanol decreased to 5.8 within 12 h, then increased to 7.0 at 54 h. The ethanol content in TSB decreased between 36 and 54 h, indicating utilization or degradation of ethanol. Glucose was depleted in TSB supplemented with 0,

2.5, or 5.0% ethanol within 12 h. With the exceptions of cis oleic acid and nonadecanoic acid, higher amounts of fatty acids were present in stationary phase cells of the two strains grown in TSB supplemented with 5.0% ethanol for 30 h compared to cells grown in TSB without ethanol. The trans oleic acid content was 10 fold higher in the cells grown in TSB with 5.0% ethanol than in TSB without ethanol. In contrast, cis oleic acid was not detected in ethanol stressed cells but was present at concentrations of 0.32 and 0.36 mg/g of cells grown in TSB without ethanol. Protein content was higher in ethanol stressed cells than in non stressed cells. An ethanol mediated protein (MW 28 kDa) was observed in the ethanol stressed cells but not in control cells. The potential for cross protection of ethanol-stressed cells against heat and acidic environments is under investigation.

P017 The Influence of Trisodium Phosphate Adaptation on Changes in Membrane Lipid Composition, Verotoxin Secretion, and Acid Resistance of *Escherichia coli* O157:H7

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The objective of this study was to measure changes in *Escherichia coli* O157:H7 membrane lipid composition, verotoxin secretion, and acid resistance in simulated gastric fluid (pH 1.5) as affected by cell adaptation to various concentrations of trisodium phosphate (TSP). *E. coli* O157:H7 (HEC), *E. coli* O157:H7 *rpoS* mutant (HEC-RM), and nonpathogenic *E. coli* (NPEC) were TSP adapted by continuous incubation in broth containing 0% to 0.6% TSP at 37°C for 24 h. After incubation at each concentration, each population was measured for acid resistance (D-value) in simulated gastric fluid (pH 1.5), membrane lipid composition, and intracellular and extracellular verotoxin concentrations. Membrane lipids were measured by gas chromatography and verotoxin was measured by optical density (450 nm) of ELISA reactions. The ratio of cis-vaccenic acid (18:1ω7c) to palmitic acid (16:0) increased with increasing concentration to 0.4%, but decreased at 0.6%. HEC and HEC-RM adapted at 0.4% had highest verotoxin concentrations, 1.8 and 1.9 mg/ml, respectively. In addition, the ratio of extracellular to intracellular verotoxin concentration decreased at higher TSP concentrations in HEC. In contrast, the ratio for HEC-RM increased at 0.4% TSP. TSP adapted HEC cells exhibited greatest acid resistance, with HEC adapted to 0.4% TSP having the highest D-value of 58 min. For HEC, the increase in membrane fluidity increased acid resistance and extracellular verotoxin concentration for cells adapted to 0.4% TSP. In contrast, the increase in membrane fluidity decreased acid resistance but increased extracellular verotoxin concentration for TSP adapted HEC-RM. Therefore, the deletion of the *rpoS* gene appeared to affect the changes in membrane fluidity, verotoxin concentration, and acid resistance of *E. coli* O157:H7.

P018 Inhibitory Effects of Buffered Sodium Citrate and Buffered Potassium Citrate on *Clostridium perfringens* Spore Germination and Outgrowth during Chilling of Roast Beef

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Inhibition of *Clostridium perfringens* spore germination and outgrowth by buffered sodium citrate (BSC) and BSC plus sodium diacetate (SD), buffered potassium citrate (BPC) and BPC plus SD was evaluated during exponential chilling of roast beef. Beef top rounds were injected to yield final concentrations of 0.8% NaCl, 0.2% potato starch and 0.2% potassium tetra-pyrophosphate. Roast beef was ground and mixed with each antimicrobial at 0.75%, 1.0%, 1.3% concentrations, along with a control with no antimicrobial. Each product was inoculated with a three-strain *C. perfringens* spore-cocktail to a final concentration of ca. 2.9 log spores/g. Inoculated product was packaged into cook-in bags, vacuum sealed, cooked for 15 min at 75°C, and chilled from 54.5 to 7.2°C in 12, 15 and 18 h, following exponential chilling rates. Products were sampled immediately after cooking and after chilling. Chilling of roast beef following 12, 15, and 18 h chill rates resulted in germination and outgrowth of *C. perfringens* spores in control samples by 2.67, 4.32 and 3.73 log CFU/g, respectively. Incorporation of antimicrobials substantially inhibited germination and outgrowth of *C. perfringens* spores compared to controls. Low levels (0.75%) of antimicrobial were less bacteriostatic during 15 and 18 h chill rates. BPC treatments when used at 1.0 and 1.3% were effective in inhibiting germination and outgrowth of *C. perfringens* spores to < 1.0 log CFU/g, and were more effective compared to BSC for all three chill rates evaluated. Use of buffered sodium or potassium citrate salts alone or in combination with sodium diacetate can minimize the risk of *C. perfringens* spores germination and outgrowth during chilling of cooked roast beef.

P019 Inhibition of *Clostridium perfringens* Type A Associated with Chicken and Its Alpha Toxin Activity by Hen Egg White Lysozyme

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Clostridium perfringens type A poisoning is among the most common foodborne diseases in North America and Europe. Most cases and outbreaks are associated with foods of animal origin. In this study the inhibitory effect of hen egg white lysozyme on the vegetative form of *C. perfringens* and the alpha toxin it produced in vitro was investigated. A micro-broth dilution assay was used to evaluate the minimal inhibitory concentrations (MIC) of lysozyme against three

C. perfringens type A strains in 96-well microtitre plates. The plates were inoculated with 10^5 CFU/ml of *C. perfringens* from an overnight culture and the bacteria were tested with a gradient of lysozyme concentrations ranging from 0 to 10,000 μg/ml in Luria broth. The plates were incubated anaerobically at 41°C for 24 h and the bacterial growth was measured by a change in optical density. The MIC of lysozyme against *C. perfringens* was found to be between 150 and 200 μg/ml. Scanning electron micrographs of the cells treated with 100 μg/ml of lysozyme revealed intensive cell wall damage. A quantitative sandwich ELISA for alpha toxin produced by *C. perfringens* was developed based on a commercial ELISA kit allowing only qualitative detection. Addition of 200 μg/ml of lysozyme to a standard preparation of the alpha toxin resulted in 33% reduction of the toxin level. In another experiment, 50 μg/ml of lysozyme did not inhibit the growth of *C. perfringens* but inhibited the toxin production.

P020 Cross Contamination Determined by PFGE and the Antibiotic Susceptibility Patterns of Enteric Bacteria Recovered from Feedlot Cattle and Carcasses

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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 *Salmonella*, non-type specific *Escherichia coli*, and enterococci. *Salmonella* was identified in 33.9% (n=20) of fecal samples and on 37.3% (n=22) of hides prior to shipment. At the abattoir, the proportion of hides from which *Salmonella* was isolated increased ($P < 0.01$) to 84.2% (n=48). Generic *E. coli* was recovered from 40.4% of preevisceration carcass samples, while *Salmonella* was recovered from 8.3% and enterococci from 58.3% of these carcasses. No *Salmonella* or generic *E. coli* were recovered from hotbox carcass samples. Enterococci, however, were recovered from 8.6% of the hotbox carcass samples. Isolates were also tested for antimicrobial drug susceptibility. For generic *E. coli* 80.3% (n=270) of isolates were resistant to at least one antimicrobial. For *Salmonella*, 97% (n=101) of the isolates were resistant to at least one antimicrobial; however, only 4.0% were resistant to two or more. The most common resistance for gram-negative bacteria was to Sulfamethoxazole. Antibiotic susceptibility patterns were fairly consistent across sampling points. *Enterococcus* isolates interestingly showed a higher degree of resistance to the antimicrobials tested and 72.9% (n=284) were resistant to four or more antimicrobials. The most common resistance was to Flavomycin (88.0%), Erythromycin (77.1%), Tylosin (75.4%), Lincomycin (73.9%), and Tetracycline (61.3%). Enterococci were also analyzed for pulse field gel electrophoresis profiles from all sampling points. Similar or identical PFGE patterns were found from isolates recovered at the feedlot and in the commercial abattoir. This presents strong evidence that bacterial isolates are being propagated from the feedlot to the processing environment and onto the final processed carcasses.

P021 Activated Lactoferrin Effects against Resident Microflora of Ready-to-Eat Chicken

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Activated lactoferrin (ALF) is a USDA-approved, generally recognized as safe (GRAS) natural antimicrobial with a broad spectrum of antibacterial activity. ALF prevents proliferation and attachment of microbial pathogens at tissue surfaces of foods such as beef or poultry. Ready-to-eat (RTE) products are often consumed without further cooking and therefore pose risks of food safety and spoilage. Microorganisms, particularly species of *Pseudomonas*, *Enterobacter*, *Kluyvera*, *Acinetobacter*, *Shewanella*, *Serratia* and *Edwardsiella*, have been implicated in the spoilage of RTE chicken. This study was designed to evaluate the antimicrobial efficacy of ALF against the growth of 21 resident bacteria isolated from RTE chicken breast halves. Isolated strains of resident microflora were grown in trypticase soy broth at 26°C for 24 h and tested for susceptibility at 4 log CFU/mL to ALF and native lactoferrin (LF) at 5, 2.5, 1.25, 0.625, and 0.3125 (mg/mL) concentrations by optical density. ALF showed a strain-dependent inhibitory activity with varying degrees of antimicrobial efficacy in vitro. ALF (0.3125 mg/mL) inhibited microbial growth of most RTE chicken microflora > 3 days. However, native LF failed to elicit any antimicrobial effects at low concentrations and required > 1.25 mg/mL to cause any noticeable inhibition. ALF at higher concentrations (2.5 and 5 mg/mL) demonstrated total microbial inhibition that extended to > 4 and 5 days, respectively. In conclusion, ALF was highly efficacious in the inhibition of most of the resident microflora on RTE chicken. ALF could be used as a potent antimicrobial intervention in multi-hurdle sanitizing systems for food processing applications, in particular for ready-to-eat foods.

P022 Efficacy of Nisin as a Preservative in Pasteurized Tofu

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Tofu, a vegetarian food prepared by soymilk coagulation, has become popular in Western countries mainly because of its perceived health and nutritional benefits. It is now often sold as a mass-produced, pre-pasteurized packaged product. Heat-resistant spore-forming bacteria such as *Bacillus* and *Clostridium* can survive and grow

even at low temperatures. Spoilage and/or safety problems can be exacerbated by consumers' or retailers' failure to understand the perishable nature of tofu. Analysis of commercial pasteurized tofu found that samples were of varying microbiological quality with potential for the growth of spore-formers. The preservative nisin, added to the soybean milk prior to the coagulation, became concentrated in the curd. After incubation at 8°C for 51 days, nisin levels dropped only slightly. *Bacillus* and *Clostridium* spores inoculated into the soybean milk were detected after pasteurization. In controls not containing nisin, *Bacillus* counts reached 10^6 CFU/g after 7 days at 8°C. In the presence of nisin, bacterial growth was inhibited. A level of 10^6 CFU/g was not detected until after approx. 21 days in the presence of 3.125 M/g nisin, and 41 days with 6.25 M/g nisin. No bacterial growth was observed during 51 days incubation with 12.5 M/g nisin. Inoculated *C. sporogenes* spores failed to grow in tofu incubated at 8°C until the temperature was moved to 37°C. No clostridial growth was observed in samples containing nisin levels as low as 1.25 M/g. These experiments demonstrate the potential of nisin as a preservative in pasteurized soybean products such as tofu.

P023 Shelf-life Extension of Farm-raised Atlantic Salmon Fillets by the Application of SANOVA® (Acidified Sodium Chlorite)

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SANOVA® (acidified sodium chlorite) was evaluated on farm-raised Atlantic salmon fillets to determine the effect on microbial and organoleptic shelf life. Whole gutted Atlantic salmon were immersed in a 1000 ppm SANOVA solution for 60 s then filleted and portioned into 4 to 6 oz fillets. The portioned fillets were then treated in SANOVA for 15 s. Untreated fillets were produced in the same manner. After portioning, fillets were individually packaged and sealed, then stored at 1.5°C. Samples from each group were removed on days 0, 7, 12, 14, 17, 19 and evaluated for total aerobic plate count (TPC). Organoleptic quality was evaluated by a trained panel. Color, odor, texture, and appearance of the raw fillet was rated on a scale of 1 to 5, (5 being the best and 3 being the critical level of acceptance). Microbiological results showed that control fillets reached 6 log at 10 days, whereas the SANOVA treated samples did not reach the same level until 13 days. Organoleptic analysis revealed that the control fillets steadily decreased in quality, reaching a score of 3 at day 17. SANOVA treated fillets' quality remained relatively stable, above 4, until day 17 and did not drop below 3 until day 19. These results show that SANOVA treatment of Atlantic salmon during processing can extend the microbiological shelf life of the resulting fillets. In addition, SANOVA treatment of the whole fish and fillets had no adverse organoleptic impact, and treated samples actually scored higher in overall acceptance during shelf life.

P024 DSC Validation of Controlled-phase Carbon Dioxide against *Listeria monocytogenes*, Generic *Escherichia coli*, *E. coli* O157:H7 and *Salmonella* spp. on Paper Disks

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A novel controlled phase carbon dioxide ($cpCO_2$) antimicrobial decontamination system was tested against a multiple-strain cocktail of *L. monocytogenes*, *Salmonella* spp., generic *E. coli*, and *E. coli* O157:H7 inoculum on sterile 5.5 cm filter paper #1 disks. The disks were dipped into the inoculum and cultures were allowed to attach to disk surfaces for 10 min. Initial bacterial populations on disks were greater than 6.6 logs. Disks were exposed to $cpCO_2$ inside an experimental laboratory model pressure vessel at four different pressure ranges (700 to 800, 1100 to 1300, 1600 to 1800, and 2000 to 2200 psi). Liquid phase carbon dioxide was applied until -10°C and 250 psi were reached inside the vessel; carbon dioxide in gaseous phase was then applied and the isolated vessel was heated with water at 50°C until the targeted pressures were reached and held for 3 min, then pressure was released. After treatment, filter paper disks were analyzed to determine bacterial populations. Log CFU/cm² reductions ranged from 5.4 to 6.0 for *Salmonella* spp., from 2.7 to 5.2 for *L. monocytogenes*, 1.9 to 5.8 for generic *E. coli*, and 4.4 to 6.4 for *E. coli* O157:H7 in four replications. The use of $cpCO_2$ as an antimicrobial intervention system for food products appears promising in the case of low organic loads.

P025 Antibacterial Activity of Thymol, Eugenol, Vanillin, Carvacrol, Citral, Potassium Sorbate and Sodium Benzoate against *Listeria innocua* and *Escherichia coli*

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Several compounds present in plants are antimicrobial agents. Our objective was to evaluate and compare antimicrobial effectiveness of thymol (Th), eugenol (Eu), vanillin (Vi), carvacrol (Cr), citral (Ci), potassium sorbate (KS) and sodium benzoate (NaB) to inhibit the growth of *Listeria innocua* and *Escherichia coli* at a_w 0.99 or 0.97 combined with a pH 5.5 or 4.5. Tripticase soy agar (TSA) was adjusted to the desired a_w by adding NaCl, sterilized, and adjusted to the desired pH. Amounts to yield concentrations ranging from 50 to 3000 ppm of each antimicrobial were added. TSA plates for every antimicrobial combination were inoculated using a spiral plater with 50 mL of 10^6 cell/uL fresh suspensions, and incubated for 2 to 3 days at 35°C. Increasing antimicrobial concentration decreased *L. innocua* and *E. coli* counts. In general, *E. coli* was more sensitive than *L. innocua* to the evaluated antimicrobials. Concentrations higher than 2400 ppm NaB were needed to inhibit *L. innocua* while

250 to 500 ppm inhibited *E. coli*. Decreasing a_w and pH increased efficacy on NaB or KS. However, Th, Eu, Vi, Cr and Ci inhibitory concentrations were not pH dependent. Inhibitory concentrations of Cr, Ci, Th, and Eu varied from 100 to 600 ppm and from 250 to 3000 ppm for Vi. The natural antimicrobial agents tested were more effective in controlling *L. innocua* and *E. coli* than synthetic antimicrobials.

P026 Bacterial Inhibition with Ternary Mixtures of Phenolic Compounds and Potassium Sorbate

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Phenolic compounds, naturally present in plants, can be used as antimicrobial agents. Individual and combined effects of thymol (Th), carvacrol (Cr), eugenol (Eu), and potassium sorbate (KS) on the growth of *Escherichia coli*, *Listeria innocua*, *Salmonella* Typhimurium, and *Staphylococcus aureus* were evaluated. Trypticase soy agar was prepared with NaCl and hydrochloric acid to give an a_w of 0.99 and pH 5.5 and the necessary amount of Th, Eu, and/or Cr (0, 15, 30, 45, up to 240 ppm) was incorporated into the medium. Over plates of each binary combination, 50 mL of KS solution (5%) 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 bacterium, incubated at 35°C, and observed after 2 to 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. *S. aureus* and *L. innocua* exhibited higher resistance than *E. coli* and *S.* Typhimurium to the antimicrobials evaluated. MICs varied from 600 (KS) to 150 ppm (Th). Combinations of 75 ppm Th, 50 ppm Eu with less than 20 ppm KS inhibited the growth of the four bacteria. Calculated FIC indexes as well as FIC isobolograms showed synergistic effects. Ternary mixtures of phenolic compounds and KS drastically reduced individual inhibitory concentrations, expanding the possibilities to design antimicrobials that include naturally occurring agents.

P027 Lethality of Chlorine, Chlorine Dioxide, and a Commercial Fruit and Vegetable Sanitizer to Vegetative Cells and Spores of *Bacillus* Species

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Chlorine, ClO_2, and a commercial raw fruit and vegetable sanitizer were evaluated for their effectiveness in killing vegetative cells and spores of *Bacillus cereus* and spores of *Bacillus thuringiensis*, with the ultimate goal of using one or both species as a surrogate(s) for *Bacillus anthracis* in studies focused on determining the efficacy of sanitizers in killing the pathogen on food contact surfaces and foods. Treatment with alkaline (pH 11.0) ClO_2 (200 μg/ml) produced by electrochemical technologies reduced populations of five-strain mixtures of cells and spores of *B. cereus* by >5.4 and >6.4 log CFU/ml, respectively, within 5 min. This compares to respective reductions of 4.5 and 1.8 log CFU/ml resulting from treatment with 200 μg/ml chlorine. Treatment with a 1.5% acidified (pH 3.0) solution of Fit7 powder product (FPP) was less effective, causing 2.5 and 0.3 log CFU/ml reductions in the number of *B. cereus* cells and spores, respectively. Treatment with alkaline ClO_2 (85 μg/ml), acidified (pH 3.4) ClO_2 (85 μg/ml), and a mixture of ClO_2 (85 μg/ml) and FPP (0.5%) (pH 3.5) caused reductions in vegetative cell/spore populations of >5.3/5.6, >5.3/5.7, and >5.3/6.0 log CFU/ml, respectively. Treatment of *B. cereus* and *B. thuringiensis* spores in a spent culture medium (3.4 mg of organic and inorganic solids/ml) with an equal volume of alkaline (pH 12.1) ClO_2 (400 μg/ml) for 30 min reduced populations by 4.6 and 5.2 log CFU/ml, respectively, indicating high lethality in the presence of materials other than spores.

P028 Activated Lactoferrin Antimicrobial Efficacy against *Campylobacter jejuni*

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Campylobacter is a major foodborne pathogen that primarily inhabits the gastrointestinal tract of poultry with an array of mechanisms to aid its survival within the avian gut, including iron acquisition and invasion-attachment. Activated lactoferrin (ALF) is an all-natural antimicrobial intervention that inhibits pathogens by iron deprivation stasis and prevents microbial attachment to tissue surfaces. In this study, we have elucidated the in vitro antimicrobial efficacy of ALF against *C. jejuni*. *C. jejuni* (NCTC 11168) grown in NTRL-Campy broth at 42°C under microaerophilic conditions was tested for ALF susceptibility at 5 log CFU/ml bacterial density. ALF effects on *C. jejuni* growth was measured as changes in optical density of the media. Effects of iron supplementation on ALF activity was assessed by adding $FeSO_4$ (0.9 mM) to growth media. In the absence of iron, ALF (0.625 mg/mL) inhibited the growth of *C. jejuni* for 3 days. ALF at concentrations >1.25 mg/mL inhibited *C. jejuni* for >10 days. Supplementation of $FeSO_4$ to the media completely abolished the inhibitory effects of ALF. However, $FeSO_4$ addition to the media after 48 h of *C. jejuni* growth did not affect the ALF activity. These data suggested that the initial stages of ALF-mediated growth suppression are iron-dependent; however, after ALF exposure for an extended period of time, the susceptibility of *C. jejuni* to ALF was iron-independent and irreversible. In conclusion, ALF is a potent antimicrobial intervention against *C. jejuni*. ALF interaction with *C. jejuni* for an extended period of time results in an irreversible and iron-independent inhibition.

P029 Antimicrobial Activities of *Zanthoxylum schinifolium* Extract against *Vibrio parahaemolyticus*

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Recently, the use of nontoxic natural antimicrobial agents has become important. Thus, this study was designed to investigate the possible utilization of *Zanthoxylum schinifolium* as a source of decontamination agents. The antimicrobial effect of *Z. schinifolium* extract against *Vibrio parahaemolyticus*, which is a foodborne disease organism, was investigated. Ethanol extract of *Z. schinifolium* was compared with water extract of *Z. schinifolium* to test antimicrobial activities against *V. parahaemolyticus* by disk method. Ethanol extract was more effective than water extract. It had remarkable antimicrobial activities against *V. parahaemolyticus*. It was very stable over a wide range of temperature and pH (25~100°C, pH 4~10). Estragole (4-allyl anisole) was identified as an antimicrobial component of *Z. schinifolium* extract by GC-MS. The inhibitory effect of estragole on growth of *V. parahaemolyticus* was investigated. The minimum inhibitory concentration of estragole was 10 ppm. These results indicated that *Z. schinifolium* extract could provide bacterial contamination protection and inhibit growth of *V. parahaemolyticus*.

P030 DSC The Effect of Trans-cinnamaldehyde and Carvacrol on Cell Membrane Lipids of *Salmonella* Typhimurium

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Trans-cinnamaldehyde (CIN) and carvacrol (CRV) are major components of cinnamon and oregano essential oils and are inhibitory to many bacteria. The goal of this study was to evaluate changes in membrane composition by observing changes in fatty acid composition associated with exposure and adaptation to CIN or CRV. *Salmonella* Typhimurium DT104 cells were grown for 24 h at 35°C with or without 250 ppm CIN or 1020 ppm CRV in 9ml BHI. Log 5.7 CFU/ml was inoculated into 200 ml broth containing 250 ppm CIN or 1020 ppm CRV. At 2 h and 24 h, samples were centrifuged, washed, lyophilized, and extracted for lipids. Fatty acid methyl esters prepared from phospholipids were quantified by GC-MS. The cell membranes of cells adapted to CIN and exposed to CIN for 2 h were less fluid than non-adapted cells exposed to CIN since they were composed of a smaller mol % C16:1w7c fatty acid (1% versus 12%) and C18:1w7c (29% versus 37%) and higher mol % C18:0 (12% versus 3%). Cell membrane of non-adapted cells exposed to CIN consisted of less C16:1w7 (12%) and more C16:0 (42%) than the non-adapted control (25% and 30%), which also indicated decreased fluidity. Non-adapted cells exposed to CRV for 2 h contained greater mol % of C12:0, C14:0, and C18:0 and lower mol % of C16:1w7c and C18:1w7c than the control. These data show that fatty acid composition changed to decrease fluidity in response to CIN and CRV, probably as a defense mechanism. At 24 h, fatty acid compositions of CIN adapted and non-adapted cells were similar, indicating that disruption in membrane fluidity occurred within the first 24 h after exposure.

P031 Susceptibility of Antibiotic Resistant Strains of *Salmonella* Typhimurium to Lactic Acid at 37°C

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Antibiotic use in human and animal medicine may result in more resistant organisms in the food supply; cross-resistance to acid could result in ineffective processing interventions. Previous studies reported increased acid resistance in antibiotic resistant organisms. To determine the susceptibility of antibiotic resistant strains of *Salmonella* Typhimurium to lactic acid (LA) at 37°C, nalidixic acid resistant (ST140RNal) and tetracycline resistant (ST140RTet) strains were developed from ATCC 14028 (ST140). Strains were incubated in 3 treatments, trypticase soy broth (TSB), TSB with 1% LA, and TSB with 2.5% LA, at 37°C and sampled at: 0, 2, 8, 12 and 24 h. Samples were plated on trypticase soy agar (TSA), TSA with 50 ug/mL nalidixic acid and TSA with 50 ug/mL tetracycline. Three replications were performed. For TSB treatments plated on TSA, all strains exhibited typical bacterial growth curves. For 1% LA treatments plated on TSA, counts were significantly reduced after 2 h for all strains; however, ST140RTet was more ($P < 0.05$) resistant to LA (1.06 versus 0.35 and 0 log CFU/ml for ST140RTet, ST140 and ST140RNal, respectively). For 2.5% LA treatments plated on TSA, a reduction occurred at time 0 in all strains, with ST140 having increased ($P < 0.05$) survival versus ST140RTet (0.68 versus 0 log CFU/ml, respectively). No growth was observed for 1% LA after 8 h and for 2.5% LA after 2 h. Although the strains have high resistance to particular antibiotics, this resistance does not appear to assist in biologically significant cross protection to lactic acid at 37°C.

P032 *Zygosaccharomyces bailii* Inhibition with Selected Antimicrobials, Water Activities, and pHs

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Several compounds present in extracts and essential oils of plants are effective natural antimicrobial agents. Water activity and pH affect

antimicrobial properties of some additives. Our objective was to evaluate and compare antimicrobial effectiveness of thymol (Th), eugenol (Eu), vanillin (Vi), carvacrol (Cr), citral (Ci), and potassium sorbate (KS) to inhibit the growth of *Zygosaccharomyces bailii* at a_w 0.99 or 0.95 combined with a pH of 4.5 or 3.5. Potato dextrose agar was adjusted to the desired a_w by adding sucrose, and 50, 100, 150, up to 3000 ppm of each antimicrobial were added. Plates for every combination were inoculated, using a spiral plater with 50 mL of 10^6 cell/mL fresh suspension, and incubated for 3 to 5 days at 25°C. Inhibitory concentrations were determined as those that inhibit yeast growth. Decreasing a_w and pH reduced the antimicrobial concentration needed to inhibit yeast growth. In general, *Z. bailii* was more sensitive to the antimicrobials at reduced pH and/or a_w. At pH 4.5 and a_w 0.99, 100 ppm of Th and Cr inhibit growth, while 300 ppm of KS were needed. At a_w 0.95 and pH 3.5, 50 ppm Cr or KS, 100 ppm Eu or Th inhibit yeast growth in comparison with 650 ppm Vi or 1100 ppm Ci. Comparing their effectiveness with common antimicrobials (KS), inhibitory concentrations of Th, Eu and Cr are comparable and they are active over a wide range of a_w and pH values.

P033 *Aspergillus flavus* Inhibition with Ternary Mixtures of Thymol, Eugenol and Potassium Sorbate

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Individual and combined effects of eugenol (Eu), thymol (Th), and potassium sorbate (KS) were evaluated as antifungal mixtures, using *Aspergillus flavus* as a target strain. Inhibitor combinations include 2/3 of the minimal inhibitory concentration (MIC) of one agent and 1/6 of MIC of the other two, 1/3 of MIC of each agent, combining 1/6 MIC of each agent or 1/3 MIC of one antimicrobial with 1/12 MIC of the other two. Potato dextrose agar was prepared with sucrose and hydrochloric acid at an a_w (0.99) and pH (3.5), and the specified amount of Th, Eu and KS was added directly to the media. Plates for every combination were surface inoculated with mold spore suspension, incubated at 25°C, and periodically observed for growth. Inhibition was defined as no observable mold growth after 30 days. MICs were 600 (Eu) and 400 ppm (Th or KS). In ternary mixtures of KS-Th-Eu, mold growth was observed only when 1/3 MIC of KS was combined with 1/12 MIC of Th and 1/12 MIC of Eu. Growth was observed when Th or Eu represented the lowest MIC fraction tested (1/12). Combinations that result in synergism (Fractional Inhibitory Concentration = 0.5) include at least one phenolic in a proportion higher than 1/12 MIC. Synergic antifungal combinations were found to be 1/12 MIC < phenolic concentrations < 1/6 MIC. The use of these agents dramatically increased the antifungal effect, significantly reducing the MIC in comparison to the individual agents.

P034 USDA FSIS In-depth Verification Reviews, 2000–2003

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The US Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS) periodically conducted In-depth Verification (IDV) reviews in federally inspected meat and poultry establishments from 2000 to 2003. These reviews were based on Directive 5500.1 "Conducting Targeted In-depth Verification Reviews," published 10-11-01. This report analyzes IDV reviews and summarizes their impact on establishments with *Salmonella* contamination problems. IDVs were conducted by multi-disciplinary teams. The IDV report consisted of team members' observations of nonconformance with regulatory, technical, and scientific standards. Regulatory action may have been taken based upon the IDV report. IDV reviews were held throughout the country for reasons including: failure of Pathogen Reduction Hazard Analysis Critical Control Point (PR/HACCP) *Salmonella* testing (n=60), *Listeria monocytogenes* detected in ready-to-eat food (n=9), *Escherichia coli* O157:H7 detected in raw ground beef (n=4), and other reasons (n=4). Because the majority of IDVs were for *Salmonella* second-set failures (78%), further analysis was performed on the outcomes of these reviews. IDVs for *Salmonella* were held in only 3% of all establishments. Of these, 16 were in large plants, 34 in small plants, and 10 in very small plants (7, 4, and 1 % of plants of each size, respectively). The greatest number of IDVs were held in ground beef (n=19), market hog (n=17), and broiler (n=11) plants (2, 5, and 5% of plants producing each commodity, respectively). PR/HACCP *Salmonella* data show that after an IDV, establishments were more likely to pass subsequent *Salmonella* testing. Therefore, IDVs likely have had a positive impact on food safety.

P035 FSIS *Salmonella* Pathogen Reduction/ Hazard Analysis Critical Control Point Data 1998 to 2003: A Summary

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All federally inspected meat and poultry establishments are subject to random *Salmonella* testing, based on the Pathogen Reduction/Hazard Analysis Critical Control Point (PR/HACCP) rule. Testing is performed in "sample sets" based on performance standards that vary according to commodity. This report shows how the Food Safety and Inspection Service (FSIS) uses PR/HACCP testing data to identify establishments with ongoing *Salmonella* problems and targets investigatory resources at these establishments. PR/HACCP data show that 90.1% of *Salmonella* random "A" sets were passed. Examination of the data on a per-establishment basis shows that 84.6% of establishments have never failed an "A" set, while 15.4% have failed at least one set. FSIS targets investiga-

tory resources at establishments that have failed *Salmonella* sets; therefore, this report focuses on these. Establishments that failed an "A" set (15.4% of all establishments, ranging from 4.0% in steer/heifers to 34.1% in broilers) were required to implement improvements in pathogen reduction programs and were then targeted for a "B" set. The percentage of all establishments that failed "B" sets averaged 4.4%, ranging from 0.8% in steer/heifers to 9.2% in broilers. An In-Depth Verification (IDV) review was conducted at the establishment upon failure of a "B" set. Following the IDV, only 0.5% of establishments failed subsequent *Salmonella* sets ("C" and "D" sets). These data show that PR/HACCP testing, together with pathogen reduction efforts by establishments and focused inspection by FSIS, increases the likelihood that establishments will pass *Salmonella* testing, and contributes to decreasing *Salmonella* levels in meat and poultry.

P036 Effect of Single or Combined Antimicrobial Washes and Their Sequence of Application on Microbial Reduction and Survival during Storage of Beef

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This study evaluated the effect of hot water and lactic acid washes on the behavior of *Listeria monocytogenes* and the spoilage microflora during storage of fresh beef. A five-strain mixture of *L. monocytogenes* was inoculated onto fresh beef, which was then decontaminated by immersion (30 s) in hot water (HW; 75°C), 2% lactic acid (LA; 55°C), hot water followed by lactic acid (HW/LA), or lactic acid followed by hot water (LA/HW) and stored aerobically at 4, 10 and 25°C. Initial populations of *L. monocytogenes* were reduced by application of treatments by 0.82 (HW), 1.43 (LA), 2.73 (HW/LA) and 2.68 log CFU/cm^2 (LA/HW). During storage of HW-treated samples, the pathogen grew with higher rates than in untreated samples at all storage temperatures tested. Storage at 4°C resulted in no growth of the pathogen in all acid-treated samples. During storage at 10°C, treatment with HW/LA did not allow growth, while in samples treated with LA, growth of the pathogen was slower ($P < 0.05$) than in untreated samples. In contrast to low storage temperatures, all treatments tested resulted in a much weaker inhibition of *L. monocytogenes* growth during storage at 25°C. In untreated and HW-treated samples the spoilage microflora was dominated by pseudomonads. Acid treatments resulted in a significant inhibition of pseudomonads and shifted the predominant microflora in the direction of yeasts and lactic acid bacteria. Overall, the results indicated that acid decontamination treatments could limit growth of *L. monocytogenes* and rapid spoilage by pseudomonads on fresh beef. However, in order to optimize the efficacy of such treatments, they must be applied in an appropriate sequence and followed by effective temperature control.

P037 Validation of Multiple Antimicrobial Treatments to Reduce *Escherichia coli* O157:H7 and *Salmonella* spp. in Beef Trim and Ground Beef

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Both *Escherichia coli* O157:H7 and *Salmonella* have been associated with ground beef recalls and outbreaks. There are few interventions for processors to utilize to reduce microbial contamination during the grinding process. The objectives of this study were to validate the effectiveness of acidified sodium chlorite (ASC, 1200 ppm) and acetic and lactic acids (2% and 4%) in reducing pathogens in beef trim and ground beef in a simulated processing environment. The reduction of *Salmonella* spp. and *E. coli* O157:H7 was determined initially, in trim just after treatment, and in ground beef after grinding, after 24 h of refrigerated storage, after 5 days of refrigerated storage, and 30 days after frozen storage. Trim was inoculated with a cocktail mixture of one of the two pathogens at a high (H), 1 x 10^4 CFU/g, and low (L), 1 x 10^1 CFU/g, inoculation level. All antimicrobial interventions reduced the (L) inoculation levels of both pathogens to non-detectable amounts at all sampling intervals. All interventions had similar reductions in pathogen loads in the (H) samples, with the higher concentrations of acid not having additional benefits over the low concentrations. On the beef trim, there was a significant 2 to 2.5 log reduction of both pathogens just after treatment. The reductions were sustained at all sampling intervals of the ground beef, with no additional reductions or increases in pathogen loads. Use of either ASC or organic acids could be of benefit to processors in reducing the pathogen loads in the product.

P038 Reduction of *Escherichia coli* O157:H7 on Vacuum-packaged Ground Rabbit Meat

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The objective of this study is to assess the efficacy of lactic acid in reducing pathogenic bacteria on ground rabbit at 0, 3, 7, 14 and 21 days of vacuum storage. Thirty-six ground rabbit meat (6 cm dia x 0.3 cm height) samples were dipped into an inoculum of *E. coli* O157:H7 (EDL 933). Meat samples were sanitized by dipping in lactic acid (0.5 and 1.0%) for 15 or 30 s at 55°C, vacuum packaged and stored at 4°C for 21 days. At 3, 7, 14 and 21 days of storage, samples were homogenized and plated onto Sorbitol MacConkey Agar supplemented with cefixime and tellurite for *E. coli* O157:H7. For confirmatory purposes, 2 to 3 presumptive colonies were selected and confirmed by PCR and serological testing. Lactic acid at concentrations of 0.5 and 1% resulted in a significant reduction ($P < 0.05$) in counts of *E. coli* O157:H7 attached to the ground meat throughout

the storage period. At 3 and 7 days of storage, significant reductions were observed between the control and both treatments (0.5 and 1%) for *E. coli* O157:H7 dipped for 15 and 30 s, respectively. At 14 days of storage, there was no significant ($P > 0.05$) reduction in the numbers of *E. coli* O157:H7 on the ground meat. However, at day 21, 0.5 and 1% lactic acid inhibited the growth of *E. coli* O157:H7, resulting in a 1.04 and 1.34 log reduction, respectively. Results suggest that lactic acid may be effective in reducing pathogenic bacteria in ground rabbit meat and extending shelf life.

P039 A Novel Continuous Intervention System for the Reduction of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in Ground Beef

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The Rinse & Chill™ Technology (R&C) involves the vascular transfer of a chilled solution of sugars and salts. R&C is a process that has been demonstrated to significantly reduce the presence of microorganisms, particularly coliforms and generic *Escherichia coli* on carcasses. R&C also provides a continuous intervention in the reduction of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in ground beef. Ground beef was prepared from the *Triceps brachii* (TB) muscle collected from 20 (10 controls; 10 rinsed) cattle slaughtered over two days. Control, rinsed and 50/50 blend of control and rinsed ground beef were inoculated with *E. coli* O157:H7 and *S.* Typhimurium, placed in both vacuum-packaging and tray packaging environments, and then stored at 4°C until sampled. The average daily log ratio (ADLR) of reduction of *E. coli* O157:H7 in vacuum-packaged ground beef over 42 days storage at 4°C was 2.27 for rinsed and 1.27 for the 50/50 blend versus the control; in tray-package the ADLR was 7.42 for rinsed and 2.66 for the 50/50 blend versus the control. The ADLR of reduction of *S.* Typhimurium in vacuum-packaged ground beef over 42 days storage at 4°C was 5.50 for rinsed and 2.31 for the 50/50 blend versus the control; in tray-packaged ground beef the ADLR was 4.18 for rinsed and 2.82 for the 50/50 blend versus the control.

P040 Reduction of *Salmonella* Enteritidis in Powdered Egg, Dehydrated Egg Yolk and Egg White Using Gamma Irradiation

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Eggs are considered a major risk factor for gastroenteritis caused by *Salmonella* spp. worldwide. Several processes can be used to control the pathogen in this food. However, these methods can interfere with some physical and sensorial characteristics of eggs and of products such as egg yolk and egg white. The combination of dehydration and irradiation was evaluated in this study in order to improve the microbiological safety of powdered egg, dehydrated egg yolk and egg white. Samples were spiked with ca. 7 log of a combination of different strains of *S.* Enteritidis and were irradiated at room temperature at doses ranging from 0.5 to 4.0 kGy with 0.5 kGy increases. Non-spiked irradiated powdered egg, dehydrated egg yolk and egg white were also submitted to sensory evaluation to determine the feasibility of the process. D10 values for *S.* Enteritidis varied from 0.67 to 0.76 kGy in powdered egg, from 0.71 to 0.76 kGy in dehydrated egg yolk and from 0.66 to 0.77 kGy when in dehydrated egg white. Sensory parameters such as odor, taste and color were not affected when dehydrated egg yolk and egg white were exposed to doses up to 3.0 kGy. Doses up to 4.0 kGy could be applied to powdered egg without impairing color, odor and taste. These results show the potential use of irradiation as a process for improving the microbiological safety of powdered egg as well as dehydrated egg yolk and egg white.

P041 Low-dose Ionizing Irradiation to Control *Listeria* spp. and *Escherichia coli* O157:H7 on Meat Trimmings Used for Dry Fermented Sausage Production

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Irradiation may be used for meat decontamination and assist in minimizing bacterial pathogens during sausage fermentation. To evaluate this, trimmings (40% pork, 30% beef, 30% lard) were inoculated with ca. 6 log CFU/g of a four-strain mixture of *Listeria* innocua plus a non-pathogenic *L. monocytogenes* strain and *Escherichia coli* O157:H7 ATCC 43888 and were irradiated at 0 (control), 2 and 4 kGy. Trimmings were then mixed with curing salts and ingredients, starter cultures were added, and the batter was formulated into 45-mm diameter sausage sticks. Sticks were fermented at 23 to 18°C and 96 to 80% RH for 7 days followed by ripening at 15°C and 80% RH for up to 28 days. Survival of *Listeria* spp. and *E. coli* O157:H7 was determined before and after irradiation and at 0, 4, 7, 14, 21 and 28 days of ripening. Irradiation at 2 and 4 kGy reduced initial contamination with *Listeria* and *E. coli* O157:H7 by 1.3 and 2.1, and 2.4 and 5.5 log CFU/g, respectively. In fact, *E. coli* O157:H7 was minimized by 4 kGy at formulation as compared to 7 and 21 d of ripening in samples treated at 2 and 0 kGy, respectively. Despite the fact that irradiation assisted in faster declines of listeriae during fermentation, these bacteria showed a strong tailing, and their survival in 28-day sausages irradiated at 2 or 4 kGy (2.0 ± 0.6 CFU/g) was similar to that in non-irradiated samples (2.1 ± 0.3 CFU/g). Thus, irradiation showed promise for controlling *E. coli* O157:H7 in fermented sausages.

P042 Evaluation of a Peroxyacetic Acid-based Antimicrobial Treatment for the Reduction of *Escherichia coli* O157:H7 on Beef Trimmings

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Ground beef processors face the challenge of finding effective interventions to control pathogens in their processes. The objective of this study was to determine the efficacy of a commercially available peroxyacetic acid-containing carcass wash for reducing *Escherichia coli* O157:H7 on beef trimmings to be used for ground beef production. A combination of lean and fat beef trim was obtained locally. Trimmings were inoculated with a cocktail of streptomycin-resistant *E. coli* O157:H7 to facilitate recovery in the presence of background flora. A 200 ppm solution of the antimicrobial treatment was prepared and brought to 37°C prior to use. Samples were sprayed and held for 0 s, 30 s, 60 s, 5 min or 30 min before the treatment was neutralized in sterile BPW. In addition, an inoculated sample sprayed with sterile water as well as inoculated and uninoculated controls receiving no treatment were evaluated. Sampling intervals were designed to mimic the period that would most likely elapse between spraying of trim and grinding, in a commercial environment. *E. coli* O157:H7 counts were determined on TSA with streptomycin. The experiment was replicated three times. *E. coli* O157:H7 counts remained constant with a difference of less than 0.1 log CFU/ml between all treatments and inoculated controls. There were no significant reductions for any treatment or holding time compared to the controls. Based on these results, we conclude that the use of this peroxyacetic acid-based antimicrobial treatment at 200 ppm is ineffective for the reduction of *E. coli* O157:H7 on beef trim.

P043 Use of Ultraviolet Light for the Inactivation of *Listeria monocytogenes* and Lactic Acid Bacteria in a Model Meat Brine Chiller System

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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 meat brine chiller system. Two concentrations of brines (7.9% w/w and 13.2% w/w) were inoculated with a ~6.0 log CFU/ml cocktail of *L. monocytogenes* or lactic acid bacteria and passed through the ultraviolet (UV; peak output at 254 nm) treatment system for 60 min. At 0, 1, 5, 15, 30 and 60 min UV exposure, duplicate samples were withdrawn, pour-plated in Trypticase Soy Agar + 0.6% yeast extract (TSAYE) and Modified Oxford Agar followed by incubation at 32°C for 48 h [*L. monocytogenes*] or All-Purpose Tween Agar + 0.0032% bromcresol purple (APT+BCP) [lactic acid bacteria] with incubation at 30°C for 48 h. UV treatment of chiller brines resulted in at least a 4.5 log reduction in bacterial populations for both types of microorganisms in all treated brines. Bacterial populations were significantly reduced after five min exposure to UV light in the model brine chiller as compared to the control, which received no UV light exposure ($P < 0.05$). The maximum rate of inactivation for both microorganisms in brines occurred between min 1 and 15 of UV exposure. Overall, results indicate that inline UV treatment shows promise for inactivation of *L. monocytogenes* and lactic acid bacteria in chill brines.

P044 Poultry Disinfection Using Gaseous Ozone

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The main objective of this project was to evaluate the efficacy of a gaseous ozone process on disinfecting chicken breasts during the tumbling phase of processing. This included identification of the process parameters necessary to achieve an adequate reduction in foodborne pathogens (i.e., *Salmonella* Enteritidis, *Listeria monocytogenes*) and acceptable quality attributes from sensory analysis. The surface of skinless boneless irradiated chicken breasts was inoculated with a food borne pathogen using the spread method. The inoculated chicken breasts were then placed in a tumbler along with 13% (w/w) sterile deionized water. Then ozone was added and the tumbling process started at a speed of about 18 revolutions per min. The results of our experiments showed that there is a challenge between chicken quality and microbial reduction. A concentration of 0.25 mg O_3/g chicken and a treatment time of 10 min resulted in a reduction of *S.* Enteritidis between one to two logs. The sensory results for this same set of parameters indicated that a lower concentration would be required in order to achieve a more favorable consumer acceptance. Results showed that a concentration of 0.1 mg O_3/g chicken and a processing time of 5 min will be accepted by most consumers. This treatment still resulted in the comparable range of microbial reduction.

P045 DSC Multistate Study to Determine the Presence of *Salmonella* in Poultry and Dairy Cattle Fecal Swabs and Environmental Samples

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Human cases of salmonellosis have long been linked to the consumption of poultry and unpasteurized dairy products. Whereas fecal oral contamination among animals has been well

established, less is known about contamination through the environment at the farm level. The objective of this study was to compare isolation of *Salmonella* in chickens, turkey and dairy cattle and the farm environment based on temporal, spatial, and environmental factors. Samples from 8 locations were collected over 16 months. Environmental samples (n=216) and fecal swab samples (n=648) were analyzed to determine the presence of *Salmonella* using modified BAM protocols. *Salmonella* was recovered in 13% of the dairy samples, and 15% of poultry samples. *Salmonella* isolation was higher in dairy environment (15% bedding, soil and feed) than fecal swabs (5%). Among states, the prevalence of *Salmonella* from dairy samples was: Alabama (20%), Washington (15%), Tennessee (10%), and California (5%). No *Salmonella* were isolated from poultry fecal swabs, whereas the environmental isolation rate was 31% from feed, 23% from litter, and 15% from soil. Among states, Washington had the highest isolation rate (20%) followed by Tennessee (19%) and North Carolina (6 %). Turkey samples were collected from North Carolina with an isolation rate of 38%. It is significant that there was low correlation between *Salmonella* in fecal swabs and environmental samples in dairy, chicken or turkey since sampling error may affect risk management decisions. To determine the prevalence of *Salmonella* in dairy cattle, combining soil-bedding or feed-bedding sampling gave the highest prevalence (25%) of *Salmonella*. For poultry, the combinations feed-litter or feed-soil sampling gave highest recovery for *Salmonella* (38%).

P046 Microbial Analysis of Chicken Carcasses in Ontario Poultry Processing Plants

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Bacterial contamination on chickens processed in Ontario's provincial abattoirs was evaluated. Volume throughput from the abattoirs was utilized to stratify large and small groups and assign sampling frequency. 1480 samples from randomly selected carcasses, shaken for 1 min in 400 mL BPW, were analyzed at University of Guelph (UG) for aerobic colony count (ACC), total coliform count (TCC), *Escherichia coli* count (ECC), *Listeria monocytogenes* (Lm), *Salmonella* spp. (S), *Campylobacter* spp. (C) and verotoxigenic *E. coli* (VTEC). Quantification of S and C and VTEC serotyping were conducted at UG; S and C serotyping was provided by Health Canada. HPB methods were used for ECC, S, Lm, ACC, C and VTEC and USDA Most Probable Number (MPN) procedures for S and C enumeration. Contamination rates were 99.86%, 98.99%, 29.95%, 31.55%, 63.85% and 0% for TCC, ECC, Lm, S, C and VTEC, respectively. Median log CFU/ml for ACC, TCC and ECC were 3.95, 2.71 and 2.40, respectively; median values for S and C were 0.64 and 0.63 log MPN/mL, respectively. 96.62% of samples contained 10^5 CFU/mL ACC. No association was detected ($P > .05$) between strata, season, location and TCC prevalence. ECC was significantly lower in small plants ($P < .05$) and NE region ($P < .01$). ECC values were not associated with season ($P > .05$). Significantly higher C ($P < .05$) and S ($P < .01$) were associated with hot/dry seasons. Small plants had less Lm and S than large ($P < .001$), but higher C ($P < .001$). Significant positive correlation was detected between ECC (log CFU/ml) and C (log MPN/ml) values ($r = .37$; $P < .05$).

P047 The Microbiological Examination of Modified Atomosphere Packed and Vacuum-packed Cooked Ready-to-Eat Meats at End of Shelf Life

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Modified Atmosphere Packed (MAP) and vacuum-packed meats have become increasingly popular due to consumer demands for longer shelf-life convenience foods that are safe and organoleptically pleasing. During September to October 2003 a microbiological study of MAP and vacuum packed meats at end of shelf life was undertaken in the UK to determine their microbiological quality. Examination of 3000 samples from retail premises revealed that 1% (29) were of unacceptable microbiological quality due to the presence of *Listeria monocytogenes* at ≥100 CFU/g (28 samples; range of 10^2 to 10^6 CFU/g) and *Campylobacter jejuni* (one sample) indicating a risk to health. A further 5% of samples at the end of shelf life contained *L. monocytogenes* at levels below 10^0 CFU/g. Ten different *L. monocytogenes* typing characters (serotyping/AFLP/phage typing) were obtained from meat samples of unacceptable quality, with the 1/2/IX/NT type recovered from over a third (35%) of these samples. In each case the manufacturer and the UK Food Standards Agency were immediately informed and remedial action taken. Unsatisfactory results were largely due to high levels of Aerobic Colony Counts (predominantly lactic acid bacteria), which can lead to spoilage. *Salmonella* spp. were not detected in any samples examined. The results from this study suggest that the shelf life assigned to some MAP and vacuum packed meats may not be appropriate. Temperature control (=8°C) in domestic kitchens and small retail premises during the storage of these products may differ from that recommended by the manufacturer (=5°C), which in turn adversely affects the microbiological quality of the product.

P048 Undercooked Chicken Livers as a Vehicle for Campylobacteriosis

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Chicken liver pâté and undercooked chicken livers have been associated with outbreaks of Campylobacteriosis in New Zealand. Investigations indicate that chefs are sometimes trained to cook livers only until they are "pink in the middle", and there is no indication that this mild heat treatment is an adequate cooking regime. Unlike whole pieces of meat where microbial contamination is restricted to the external surfaces, chicken livers are contaminated with *Campylobacter* on both the internal parenchyma and outer surfaces. To assess the level of contamination, thirty fresh chicken livers were tested for both the presence/absence and numbers of *Campylobacter* on external surfaces and internally, following a brief surface sterilization by immersion in boiling water. All chicken liver samples were externally contaminated with *Campylobacter*, with 30% of counts >1000, 23% between 100 and 1000 and 47% <100 MPN/liver. *Campylobacter* was detected in the internal tissue of 90% of livers with 6% >1000, 10% between 100 and 1000 and 83% <100 MPN/g. Chefs from a local chef training college tested a range of cooking methods for their effectiveness at destroying *Campylobacter* (livers sautéed rare, livers sautéed >74°C, liver pâté baked in bain marie). All methods were effective at destroying *Campylobacter*. The internal color of cooked livers was recorded photographically. This showed that color is not a reliable indicator of effective cooking as liver tissue can remain pink after it has reached a safe temperature.

P049 *Campylobacter* spp. in New Zealand Raw Sheep Liver and Human Campylobacteriosis Cases

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Campylobacteriosis is the most frequently notified disease in New Zealand with a current rate of 369.0/100,000/year. Much research has focused on poultry as a transmission route; however, farmed animals may also be a source of these organisms. While red meat is seldom contaminated by *Campylobacter*, studies have suggested that *Campylobacter* can be isolated from offal from a variety of food animals. In this study sheep liver samples were tested for the presence and numbers of *Campylobacter jejuni* and *C. coli* in both the spring and autumn. Simultaneously isolates were obtained from human clinical cases from the same geographical area as where food samples were purchased. A subset of *C. jejuni* isolates was subtyped by both Penner serotyping and pulsed field gel electrophoresis using the restriction enzyme *Sma*I, to estimate the proportion of liver isolate types that were also isolated from human cases of Campylobacteriosis. Of the 272 liver samples tested 180 (66.2%) contained *Campylobacter*. Most of the positive samples contained <3 MPN/g of the organism, and only 12 (6.7%) were contaminated at a level exceeding 100 MPN/g. More than half (61.1%) of the *C. jejuni* isolates from liver were of subtypes that were also isolated from human cases. While the *C. jejuni* present in sheep liver were mostly of types also isolated from human cases, the significance of this food as a vehicle of human Campylobacteriosis cannot be determined until other factors such as dose response information, consumption data, frequency of undercooking and cross contamination are more fully examined.

P050 Comparison of Psychrotrophic Bacterial Flora of Fresh and Marinated Chicken Breast Fillets during Refrigerated Storage

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Differences in populations of psychrotrophic bacteria on non-marinated and marinated chicken breast fillets stored at 4°C were examined. Fresh breast fillets were purchased from a local supermarket. One group of fillets received no marination treatment, while the other fillets were marinated in a solution of 10% NaCl and 4% sodium tripolyphosphate (STPP) in a pilot-scale vacuum tumbler. Fillets were marinated for 30 min under 15 Hg vacuum and tumbled at 15 rpm. Individual fillets were sealed in Ziploc® bags and stored at 4°C. On days 0, 7, and 14, fillets were rinsed in 100 ml of sterile distilled water, rinsates were decanted, and psychrotrophic bacteria in rinsates were enumerated by plating dilutions on Plate Count Agar and incubating at 4°C for 10 days. Bacterial isolates were identified using the MIDI Sherlock Microbial Identification System. Data indicated that significantly ($P \leq 0.05$) more psychrotrophs were recovered from marinated fillets (3.10 log CFU/ml) than from non-marinated fillets (1.59 log CFU/ml) on day 0; however, significantly more psychrotrophs were recovered from non-marinated fillets than marinated fillets on day 7 (6.58 and 5.75 log CFU/ml, respectively) and on day 14 (8.62 and 8.11 log CFU/ml, respectively). Additionally, significant ($P < 0.05$) increases in the psychrotrophic population occurred between days 0 and 7 and between days 7 and 14 on both types of fillets. *Pseudomonas* spp. were the predominant psychrotrophs recovered from non-marinated and marinated breast. Findings indicated that although NaCl/STPP marination significantly reduces growth of psychrotrophs on breast fillets, marination was unable to completely prevent psychrotrophic spoilage.

P051 Application of a Universal PCR Method as a Complementary Tool to the Microscopic Technique for the Detection of Bones and Other Animal Tissues in Animal Feeds

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In this work, a PCR method aimed at detecting a 359 bp DNA target of the cytochrome b gene from mitochondrial DNA was used in parallel with a variation of the official microscopic technique established by the 98/88/EC Directive, to investigate homemade animal meals containing both cereal (wheat or corn) and either hard (bone and plume) or soft animal materials (meat, liver, fat and blood) at different concentrations. In addition, the animal meals were subjected to different temperatures (115°C, 121°C, 133°C) and times (10 min, 20 min, 30 min) of processing. Microscopy successfully detected animal bones with a sensitivity of 1 g kg^{-1}, while the sensitivity of the PCR method was in the 5 to 10 g kg^{-1} range. Microscopy also allowed the detection of animal bones processed at intermediate (115°C) and high temperatures (133°C) as well as plume, while this technique failed to detect other animal materials, such as liver, meat, blood or fat. By contrast, the duplex-PCR method successfully detected cereals (wheat and corn) and either meat, bone, liver, fat or plume after processing at 115°C/20 min. Intense heating of animal meals at 133°C under overpressure conditions resulted in a more intense DNA fragmentation and lower DNA extractability. Nevertheless, bone and liver and homemade animal meals containing wheat and corn were successfully detected, even after severe heat processing at 133°C/20 min.

P052 DSC Survival of *Escherichia coli* O157:H7 in Salami and Roast Beef after Exposure to an Alkaline Cleaner

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Survival and growth of wild-type (EDL 933) and *rpoS*-deficient (FRIK 816-3) strains of *Escherichia coli* O157:H7 after exposure to an alkaline cleaner for 2 min and inoculated into roast beef (pH 6.3) and hard salami (pH 4.9) at low (ca. 2.7 log CFU/g) and high (ca. 5.5 log CFU/g) were determined. At 4°C, untreated cells of both strains showed greater reductions in salami than in roast beef during a 21-day storage period. Populations of untreated cells recovered from roast beef and salami on tryptic soy agar (TSA) were significantly ($P < 0.05$) higher than on Sorbitol MacConkey agar (SMAC). At 12°C, treated and untreated cells grew in roast beef. Growth of treated cells of the FRIK 816-3 strain at 12°C was significantly ($P < 0.05$) slower than that of the EDL 933 strain in roast beef. Populations of both strains decreased at different rates (20°C > 12°C > 4°C) in salami. *E. coli* O157:H7 strain EDL 933 grew at 20°C in a slurry of salami (pH 5.97) on which *Penicillium chrysogenum* had grown; populations within 2 to 3 days were ca. 3 log CFU/ml higher than in salami slurry (pH 5.23) prepared from salami not showing signs of mold growth. Results indicate that treatment of *E. coli* O157:H7 cells with an alkaline cleaner does not impair their ability to grow in roast beef at 12°C. Cross-protection of cells exposed to an alkaline cleaner against subsequent stress conditions imposed by roast beef and salami at refrigeration temperatures was not evident.

P053 DSC Influence of Inoculum Preparation Procedure and Spoilage Flora on *Escherichia coli* O157:H7 on Fresh Beef Stored Under Anaerobic Conditions at 0, 4, 12, or 25°C

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Hot water and lactic acid are used to reduce the prevalence of *Escherichia coli* O157:H7 on fresh beef; however, the effect of inoculum origin and anaerobic storage on population changes has not been well studied. This study evaluated the use of hot water followed by lactic acid as an intervention for reduction of *E. coli* O157:H7 contamination on beef from inocula prepared in tryptic soy broth (TSB), non-acid (pH 6.0) beef carcass decontamination runoff fluids (WASH), biofilms from stainless steel coupons grown in WASH for 24 h at 35°C (WETB), or WETB followed by drying for 12 h (DRYB). These inocula were applied to fresh beef pieces (40 cm^2) and left untreated or treated with hot water (75°C) followed by 2% lactic acid (55°C) (HW/LA), and subsequently stored in vacuum packages at 0, 4, 12, or 25°C. Initial reductions in *E. coli* O157:H7 originating from WASH and WETB were higher ($P < 0.05$), compared to TSB and DRYB, as a result of decontamination with HW/LA. *E. coli* O157:H7 on untreated or beef treated with HW/LA stored at 0 or 4°C had unchanged ($P > 0.05$) populations during storage. When beef was stored at 12°C, *E. coli* O157:H7 originating from WETB or DRYB grew more rapidly than inocula from TSB or WASH. Beef treated with HW/LA and stored at 0, 4, 12, or 25°C maintained *E. coli* O157:H7, total microbial, yeast and molds, *Pseudomonas* spp., Enterobacteriaceae, and lactic acid bacteria populations lower ($P < 0.05$) than those of untreated beef. These results indicate that at abusive temperatures, *E. coli* O157:H7 from biofilms may have an advantage on fresh beef compared to inocula originating from TSB or WASH. Treatment of beef with HW/LA was effective in retarding or inhibiting growth of both *E. coli* O157:H7 and natural spoilage organisms.

P054 Inhibition of *Shigella sonnei* by UVC Radiation on Agar, Liquid Media and Fresh Produce

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Surface decontamination is achieved using ultraviolet light (UVC) at 254 nm. Other wave lengths of UVC light are also germicidal. Using the combined light from a 185 and 254 nm source, the reduction of *Shigella sonnei* was determined on inoculated agars, radish sprouts, and in distilled water and vegetable broth. The combined UVC lights were very effective in reducing the number of *S. sonnei* on inoculated agar surfaces at a dose of 2040 μW-s-cm^2, resulting in > 8 log reduction. The reduction in liquid samples reached > 5 log at 4080 μW-s-cm^2. When the inoculated radish sprouts (uneven surfaces), were treated with the combined UVC light only a slight reduction was observed. A 2 to 3 log reduction of *S. sonnei* was achieved only when the sprouts were washed with 0.1% H_2O_2 followed by the UVC treatment. The combined light sources were effective in killing the *S. sonnei* on agar and in liquids which can provide pathogen free surfaces and liquids.

P055 Drying Stress-dependent Expression of Proteins of *Shigella sonnei* and *Salmonella enterica* Serovar Enteritidis

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Survival of *Shigella sonnei* and *Salmonella enterica* serovar Enteritidis during drying on hard surfaces is significantly influenced by cell density and carrier medium. The effect of drying stress on cells of *S. sonnei* and *S.* Enteritidis was investigated by distinguishing proteins that are up-regulated when cells were dried. Washed cells of an overnight culture of *S. sonnei* or *S.* Enteritidis suspended in MilliQ water were inoculated (10 μl) onto cover slips at levels of 10^8 CFU. Cover slips were held at 28 ± 1°C for 3 h and cells were removed by vortexing for 20 s in 5 ml of peptone water. Proteins (primarily membrane) were isolated after a combination of enzymatic, ultrasonic and heat treatments of the cells. Proteins were separated using two-dimensional gel electrophoresis at five different pH ranges (pH 3.5 to 4.5, 4.0 to 5.0, 4.5 to 5.5, 5.0 to 6.0, and 5.5 to 6.7). Protein gels of the original culture and 3-h dried cells were compared using "Image Master 2D", Amersham Biosciences, Uppsala, Sweden. In general, a down regulation of proteins was observed for both *S. sonnei* and *S.* Enteritidis. For *S. sonnei*, two and 12 proteins were detected that were up-regulated in the pH range of 4.5 to 5.5 and 5.5 to 6.7, respectively, whereas for *S.* Enteritidis an up-regulation of four and seven proteins was observed in the pH range of 4.5 to 5.5 and 5.5 to 6.7, respectively. Isolation and identification of these proteins may assist in developing strategies to influence the survival of pathogenic bacteria on food and food-contact surfaces.

P056 Synergistic Action of Ozone and Ultraviolet Radiation against *Salmonella enterica* Serovar Enteritidis on Shell Eggs

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Presence of *Salmonella* Enteritidis in shell eggs has serious public health implications. Several treatments have been developed to control *Salmonella* on eggs with mixed success. Currently, there is a need for time-saving, economical, and effective egg sanitization treatments. In this study, shell eggs, externally contaminated with *Salmonella* (8.0×10^5 - 4.0×10^6 CFU/g), were treated with gaseous ozone (O_3) at 0 to 15 psig for 0 to 20 min. In other experiments, contaminated shell eggs were exposed to ultraviolet radiation (UV) at 100 to 2500 mW/cm^2 for 1 to 5 min. Treatment combination included exposing contaminated eggs to UV (1500 to 2500 mW/cm^2) for 1 min, followed by O_3 at 5 psig for 1 min. Eggs that were non-contaminated and untreated, contaminated and untreated, and contaminated and treated with air, were used as controls. Results indicated that treating shell eggs with O_3 and UV separately, or in combination, significantly ($P < 0.05$) reduced *Salmonella* on shell eggs. For example, treatment of contaminated eggs with O_3 at 4 to 8°C and 15 psig for 10 min, or with UV (1500 to 2500 mW/cm^2) at 22 to 25°C for 5 min, produced more than 5.9 or 4.3 log microbial reductions, respectively, when compared to contaminated untreated controls. Combination in sequence using UV followed by O_3 treatment resulted in synergistic inactivation of *Salmonella* by more than 4.6 log in ~ 2 min total treatment time. In conclusion, *Salmonella* was effectively inactivated on shell eggs in a short time, and at low temperatures utilizing gaseous ozone, ultraviolet radiation, and their combination.

P057 Efficiency of Different Sanitizers on the Microbial Control of "Cheiro Verde" Minimally Processed

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Minimally processed vegetables (MPV) are products ready for consumption and must be free of pathogenic microorganisms. Washing using tap water together with sanitizers is considered a critical step for the reduction of microbial load. According to Brazilian legislation, chlorine is the only sanitizer allowed for use on MPV. A survey carried out in 2002 at supermarkets in Campinas/SP pointed out "cheiro verde" (CV), 1:1 mixture of parsley and welsh onion, as the most contaminated MPV among those analyzed. Therefore, the aim of this study was to determine the efficiency of other sanitizers as substitutes for chlorine, using CV as test material. The compounds of CV were submit-

ted to the action of 2% acetic acid, 0.5% citric acid, 120 ppm sodium hypochlorite and 80 ppm peracetic acid solutions for 15 min, including tap water as control. Treatments were repeated in 3 periods at intervals of two months. From each treatment were collected 5 samples for microbial analyses. The efficiency of the sanitizers was evaluated by the number of decimal reductions of population of total and fecal coliforms, molds and yeast and by detection of *Salmonella*. Acetic acid was the most efficient sanitizer with reduction more than 4.8 logarithm cycles in relation to tap water (control). Peracetic acid followed by citric acid and sodium hypochoride produced very similar results (reductions of 2.8, 2.4 and 2.0 logarithm cycles, respectively). *Salmonella* (absence in 25 g of sample) and fecal coliforms (less than 1.0 log) were in accordance with Brazilian legislation in all samples.

P058 Fate of Aerosolized *Listeria monocytogenes* in a Closed Bioaerosol Chamber

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The role of fluid aerosols in transmission of *Listeria monocytogenes* onto ready-to-eat foods is uncertain. The fate of aerosolized *L. monocytogenes* at various levels under different moisture conditions was studied. *L. monocytogenes* cells were released into a bio-aerosol chamber via a commercial nebulizer at relative humidity's (RH) of 40 to 45% and 75 to 80%. A non-selective solid agar (tryptic soy agar) overlay on Modified Oxford medium was also evaluated as a means to recover injured cells. *L. monocytogenes* were released into the chamber at 10^8 CFU/liter air. *L. monocytogenes* cells were essentially undetectable 3 h after release at both humidity levels. Ham slices were exposed to *L. monocytogenes* for 5, 30, 60, 120, 180, and 240 min at 30 to 40; 100 to 1000; 100,000; and 10 million CFU/L air. *L. monocytogenes* were not recovered by enrichment of entire ham slices at 30 to 40 CFU/liter air at both RH conditions. Higher incidence rates occurred with the higher numbers of aerosolized cells released into the chamber. TSAYE and TSAYE/MOX recovered equivalent levels of *Listeria* released into the chamber. However, very low recovery occurred on MOX. Preliminary results from this research suggest that contaminated fluid aerosols are unlikely sources of *L. monocytogenes* contamination of foods in meat processing factories due to high cell injury and apparent death rates in air.

P059 Prevalence of *Campylobacter* and *Salmonella* on Raw Meat Packaging: A Potential Public Health Problem

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During September and October 2002, 3,662 packaged raw meats were sampled with the aim of identifying the extent and nature of microbiological contamination on external surfaces of packaging which could potentially cross-contaminate ready-to-eat foods during and after purchase. *Salmonella* was detected from two (<1%) samples of external packaging (from chicken) and *Campylobacter* from 41 (1.1%) samples of external packaging. The external packaging of game fowl exhibited the highest contamination from *Campylobacter* (3.6%), followed by chicken (3.0%), lamb (1.6%), turkey (0.8%), pork (0.2%), and beef (0.1%), with *C. jejuni* and *C. coli* accounting for 59% and 24% of the contaminating Campylobacters, respectively. *Escherichia coli* (an indicator of fecal contamination) was isolated from the external packaging on 4% of the samples, and at levels in the range of 40 to 100,000 CFU/swab. The external surface of heat sealed packaging was less frequently contaminated with *Campylobacter* and *E. coli* compared to other types of packaging (e.g., overwrapping) ($P < 0.0001$ to $P=0.01$). In addition, external packaging of raw meats was contaminated more frequently with *Campylobacter* and *E. coli* when packaging was not intact, packaging and display areas were not visually clean, the display temperature was above 8°C, and hazard analysis was not in place. The external packaging of raw meats is a vehicle for potential cross-contamination of *Campylobacter*, *Salmonella*, and *E. coli* in retail premises and consumers' kitchens. The food industry and consumers should be made aware of this largely unrecognized risk factor that may have implications for the transmission of *Campylobacter* and *Salmonella*.

P060 DSC Population Diversity of *Pseudomonas* spp. in Bottled Water from Office Coolers

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Pseudomonads are an important and ubiquitous group of Gram negative bacteria many of which are opportunistic plant and animal pathogens. Microbiological standards in the UK state that bottled waters for human consumption should have no *Pseudomonas aeruginosa* in 250 ml at the point of bottling. However, other *Pseudomonas* sp. and related organisms are commonly isolated from bottled water at the point of consumption. The origins of these organisms and the dynamics of their populations is unknown. This paper examines the populations of *Pseudomonas* spp. and related bacteria in bottled water from water coolers in an office environment using traditional microbiology, pulsed field gel-electrophoresis (PFGE), randomly amplified polymorphic DNA (RAPD)-PCR and restriction fragment length polymorphism (RFLP)-PCR. Membrane filtration and culture methods were used to isolate putative *Pseudomonas* spp. from water coolers of the same type with the same source water sampled from 13 different office environments over a four month period. Isolates were biochemically profiled and further characterized using RFLP-PCR, RAPD-PCR and PFGE. *Pseudo-*

monas isolates were predominantly *P. mendocina*, *P. aeruginosa* and *P. putida*. The results indicate that stable *Pseudomonas* populations are present in water coolers over the sampling period and that these populations differ between individual water coolers suggesting that the surrounding environment may play an important role in the contamination of drinking water from such coolers by *Pseudomonas* spp.

P061 DSC Microbiological Contamination of Beverage Dispenser Tips in University Foodservice Operations

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The objective of this research was to reduce high microbial counts on foodservice juice dispenser tips identified previously by our lab. Research was conducted on juice dispensers in university dining halls and in a new juice dispenser in our laboratory. Juices were dispensed at various locations and total plate counts on the dispenser tips were measured at appropriate time intervals. The effect of sanitizer was determined by treatment using sanitizing solution containing 100, 150 or 200 ppm chlorine (at the end of one day of normal use), and then observing the microbial counts throughout the next day's use. Microbial contamination levels were low in the laboratory dispenser when it was new, but increased over time. After several months of use, the level of contamination in the lab dispenser was similar to that seen in most dining halls. Microbial counts tended to be highest immediately after dispensing a beverage, and then declined gradually, until the next time a beverage was dispensed. Swab tests inside the dispenser tips and observations using fluorescent microscopy suggest the possibility of biofilm formation inside the tips. Sanitizing with 150 or 200 ppm chlorine reduced the observed contamination on dispenser tips by two to three-fold (respectively) compared to sanitizing with 100 ppm chlorine, the level which is currently used in the dining halls. These results suggest current juice dispenser design promotes biofilm formation, but using a higher sanitizer concentration (up to the manufacturer-specified limit of 200 ppm) can help control microbial counts in the beverage dispenser tips.

P062 Enumeration of Aerobic Microorganisms, Total Coliforms, Fecal Coliforms, and Fecal *Streptococcus* in Animal and Environmental Samples

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Saprophytic and indicator microorganisms such as aerobic, coliforms, fecal coliforms, and fecal *Streptococcus* may be associated with the prevalence of pathogens on farms. However, data on their concentration in animal and environmental farm samples are limited. The objective of this study was to develop base-line data on densities of microorganisms in various animal and environmental farm samples which may be used in developing risk management models at the farm level. Fecal swab and various environmental samples from beef, dairy, chicken, turkey, and swine farms from 16 locations were collected over 16 months. The samples were analyzed for aerobic plate counts (APC), total coliforms (TC), and fecal coliforms (FC), using SimPlate® methods, and fecal for *Streptococcus* (FS), using KF Streptococcal agar plating. As expected, fecal swabs had the highest concentrations of microorganisms. Concentrations were highest over the spring and summer months: APC (9.5 to 9.7 log CFU/g), TC (9.0 to 9.4 log CFU/g), FC (8.5 to 9.0 log CFU/g), and FS (7.4 log CFU/g). In the fall and winter months, the levels of indicator microorganisms in fecal swabs dropped by approximately a half order of magnitude. The lowest levels of microorganisms were found in samples that had limited environment contact, such as fresh feed, or samples inhospitable to bacteria, such as total mixed ration and dry hay. Samples with significant animal exposure such as grass, soil, bedding, feces, litter, and droppings had log CFU/g as follows: APC (5.6 to 8.7), TC (3.3 to 7.4), FC (2.5 to 7.0), and FS (6.5 to 7.7). These data indicate that there are predictable patterns of indicator and saprophytic microorganisms in environmental farm samples that can be linked to sample condition, environmental exposure, and season. Understanding these patterns and how and why variations occur could be of value in developing risk assessment models for the farm.

P063 Bacterial Survey in Sponges Used in Industrial Restaurant Kitchens from Campinas, São Pãulo, Brazil

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It's known that during the cleaning process of equipment, utensils, sinks, etc. in industrial restaurant kitchens, the pre-washing and washing steps are done with the use of sponges to eliminate food residues. As a consequence of this procedure, part of the food residues adhere to the sponge surfaces. These food residues together with the moisture retained in the sponges offer a favorable environment for bacterial growth. Therefore, the objective of this study was to evaluate, with respect to microbial status, sponges used in 14 industrial restaurant kitchens in Campinas. The sponges were collected after having been used for a 3 day period. From each kitchen were collected 2 sponges (2 samples), which were subjected to microbial analyses for *Salmonella* sp., *Staphylococcus aureus*, total and fecal coliform bacteria, and total count of yeast and molds. Although *Salmonella* and *S. aureus* were not detected, the results showed high contamination of total coliform bacteria (5.5 to 8.4 log CFU/cm^2 of sponge surface), while the counts for fecal coliform bacteria were from 4.0 to 4.8 log CFU/cm^2. Molds and yeast were present in all samples (3.3 to 5.4 log CFU/cm^2). Therefore, sponge handling requires

special attention once this procedure can lead to microbial contamination, since the sponges can serve not only as reservoirs, but also as disseminators of bacterial contamination in the kitchens, a situation that may lead to cross contamination with risks to consumers' health. Consequently, any action for cleaning and disinfecting the sponges will be very helpful for microbial control.

P064 School Food Service Employees' Food Safety Training, Knowledge, and Practices

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The purpose of the study was to investigate relationships of food safety training, knowledge, and practices of school food service employees. A total of 273 Korean school food service employees were surveyed, using a self-administered questionnaire. The questionnaire was developed to identify employees' food safety training, knowledge, and practices on foodborne pathogens, critical control points, and Hazard Analysis Critical Control Point (HACCP) procedures. Statistical analysis was conducted using SPSS for Windows 11. Most of the employees had training sessions on foodborne pathogens and critical control points, whereas approximately 60% had education on the HACCP procedures. Their self-reported practices scores were rated low (1.62 to 2.05 based on a 5-point Likert-type scale: 1-not at all, 5-all the time). An average score of their food safety knowledge was 73.7 (Range: 0 to 100). Less than 70% of the employees knew correctly about relationship of *Salmonella* to poultry and eggs, minimum cooking temperatures, proper storage in refrigerators, chemicals used for utensils and equipment, and corrective actions. Chi-square analysis showed that the employees' actual knowledge did not differ significantly by whether or not they had food safety training (at the level of a = .01). The results will be used to improve effectiveness of food safety training for school food service employees.

P065 Quantitative Microbiological Risks Associated with Food-handling Behaviors Implemented during Domestic Food Preparation

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For many years, poultry has been implicated as a frequent cause of foodborne disease and is acknowledged by many sources as being an important reservoir of foodborne pathogens, particularly *Campylobacter* and *Salmonella*. Consumers regularly bring poultry into the domestic kitchen and the aim of this research was to quantify the microbiological risks associated with cross-contamination behaviors during domestic food preparation. Cross-contamination behaviors of consumers during preparation of raw chicken and salad vegetables were re-enacted in a laboratory. All chicken pieces used for re-enactments were artificially contaminated with *Salmonella* Typhimurium DT104 (marker organism) and the majority of chicken pieces were naturally contaminated by *Campylobacter*. Sampling of contact surfaces / materials immediately after possible contamination maximized recovery of microorganisms. Results showed a significant transfer of *Campylobacter* and *Salmonella* from raw chicken pieces to hand- and food-contact surfaces as well as ready-to-eat foods. Rinsing hands under running, lukewarm water did not result in adequate hand decontamination. Subsequent drying of rinsed hands resulted in 100% contamination of hand towels. Preparation of salad vegetables on rinsed, contaminated chopping boards resulted in a contamination rate of 67%. Preparation behaviors such as touching surfaces, e.g., tap handles, utensil handles and bin lids, with contaminated hands followed by retouching with clean hands resulted in contamination rates of between 10 to 33%. Cumulatively, this study has demonstrated the microbiological risks associated with preparation of raw chicken during domestic food preparation. Results will be considered in terms of modelling cross contamination and implications will be discussed within the context of risk communication.

P066 Protective Effect of Pepsin on Survival of Non-encapsulated and Encapsulated *Bifidobacterium lactis* in Simulated Gastrointestinal Conditions

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Bifidobacteria are probiotic organisms that improve the microbial balance in the human gut, but their viability in acidic environment is limited. Some studies suggest that components of the gastric juices may confer protective effect on bacterial cells. In addition, microencapsulation can be used as a tool to increase the resistance of bacteria to acidic environment. In this work, the in-vitro release of *Bifidobacterium lactis* entrapped in microparticles (alginate, alginate-chitosan, alginate-chitosan-sureteric and alginate-chitosan-acryl-eze) was investigated under conditions simulating gastrointestinal fluids with and without pepsin (3g/L), pancreatin (1g/L) and bile (10g/L), covering the pH range from 1.5 to 7.5. A culture of non-encapsulated cells was used as control. The dissolution media consisted of buffers at pH 1.5 for 2 h, followed by pH 5.6 for 2h, and by pH 7.5 for the last 2h. The tolerance of the cells to gastric juice was greater in the presence of pepsin. Alginate-chitosan-acryl-eze microparticles were the most effective: after transfer from pH 1.5 to pH 5.6 and then to pH 7.5, recovery of 7 and 6 logarithmic cycles was observed in fluids with and without pepsin, respectively. In fluids without pepsin, pH 1.5, the number of non-encapsulated cells was lower than the countable limit ($<10^0$ CFU/mL) in 60 min, while in fluids containing pepsin the number of viable cells (10^8 CFU/mL) was maintained. The

addition of pepsin improved the survival of encapsulated and non-encapsulated cells of *B. lactis* in acidic environment, and the effect was more significant for non-encapsulated cells.

P067 Influence of Up-regulation MUC2 by *Lactobacillus* spp. on Adherence of *Escherichia coli* O157:H7

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Gastrointestinal (GI) tract mucins are major components of the mucus layer of GI tract epithelial surfaces and play a role in protecting them from microbial damages. However, little is known of the interactions between probiotics/pathogens and mucins of epithelial cells. The purpose of this study was to investigate the adherence of *E. coli* O157:H7 to epithelial cells by the up-regulation of MUC2 mucin in *Lactobacillus* spp. The suppression of MUC2 mucin was performed using RNA interference (RNAi) manipulation. The adherence properties of *E. coli* O157:H7 were changed on wild-type HT-29 cells and HT-29 DM2 cells, which inhibited expression of MUC2 mucin. Contents of MUC2 messenger RNA (mRNA) were evaluated by the reverse-transcription polymerase chain reaction (RT-PCR). Inhibition activity of *Lactobacillus* spp. on adherence of *E. coli* O157:H7 was determined by epithelial cell binding assays in vitro. In the case of HT-29 DM2 cells, the expression of MUC2 mucin was decreased up to 60% compared with wild-type HT-29 cells and adherence of *E. coli* O157:H7 on HT-29 DM2 cells was increased. In addition, in *Lactobacillus* spp. (10^9 CFU/ml), the expression of MUC2 mucin was increased, whereas adherence of *E. coli* O157:H7 to epithelial cells was decreased significantly. From these results, MUC2 mucin was an important regulating factor for the adherence of *E. coli* O157:H7 to epithelial cells. The *Lactobacillus* spp. are capable of inducing up-regulation of MUC2 mucin to inhibit the adherence of *E. coli* O157:H7.

P068 Hen Egg White Lysozyme as a Germinant for Spores of *Clostridium sporogenes*

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In this study *Clostridium sporogenes* ATCC 3548 was used as a surrogate for *Clostridium botulinum* to study the role of lysozyme in the spore germination process. Fresh *C. sporogenes* spores from an overnight culture were harvested by heating at 85°C for 15 min and inoculated at 10^6 CFU/ml to 10 ml Luria broth in test tubes supplemented with 100 μg/ml lysozyme. The tubes were incubated anaerobically at 37°C. Both the total clostridial number and the spore number were monitored every 2 to 3 days for up to 15 days by plating on BHI agar. The spores were selectively enumerated using the same heating regime. Compared to the positive control, 100 μg/ml of lysozyme did not significantly affect the total clostridial number. With no lysozyme addition, the spore number did not differ significantly from the total number. However, with 100 μg/ml lysozyme, the spore number was reduced by up to 3 log CFU/ml at day 6, which demonstrated the strong ability of lysozyme to act as a germinant. In another experiment, a combination of 50 μg/ml of lysozyme and 50 μg/ml of L-alanine also produced a similar effect but the time needed for this forced outgrowth was shortened to 2 days. L-alanine alone at 100 μg/ml was not effective in spore germination. The results suggest that lysozyme is a powerful germinant for *C. sporogenes* spores. Synergistic effect was observed between lysozyme and L-alanine in inducing the spore germination.

P069 Acid and Alkaline pH Enhance Spore Adhesion of an Alkaline Tolerant *Bacillus cereus*

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Adhesion of spores of *Bacillus* (*B.*) spp. to food contact surfaces constructed of materials such as stainless steel and polyurethane is a concern for food manufacturers This study determined spore adhesion of *B. cereus* DL5, isolated from alkaline dairy wash solutions, and *B. cereus* NCTC 2599 at pH 4, 7 or 10 to stainless steel, glass and polyurethane surfaces. Spores of each *Bacillus* strain were suspended in sterile distilled water adjusted to pH 4, 7 or 10 and stored at 4°C overnight. Stainless steel, glass and polyurethane surfaces, conditioned with one tenth strength Tryptone Soya Broth, were then exposed to the spore suspensions at each pH value for 1 h. Adhering spores were enumerated by standard plate counts after dislodging with glass beads. The pH had little effect on adhesion of *B. cereus* NCTC 2599 spores to any surfaces. By contrast, *B. cereus* DL5 spores showed enhanced adhesion at pH 4 and pH 10 to polyurethane and glass surfaces. Results showed that acid and alkaline pH may enhance the adhesion potential of spores of alkaline tolerant *B. cereus* strains to surfaces, particularly to glass and polyurethane. Acid or alkaline pH values may occur on food contact surfaces after cleaning in place procedures, for example due to the presence of dilute cleaning solution residues during equipment downtime. Thus, enhanced spore adhesion potential of *B. cereus* strains under such pH conditions may have important spoilage and safety implications, particularly if attached spores outgrow to vegetative cells and subsequently form biofilms.

P070 Spore Formation in *Bacillus subtilis* Biofilms

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This study observed spore formation by a *Bacillus* strain (*B. subtilis* SpoIVFB-GFP) engineered with a green fluorescent protein (GFP) fused to a

polytopic membrane protein (SpoIVF) which fluoresces during sporulation (Dr. D. Z. Rudner and Prof. R. M. Losick, Dept. of Molecular and Cellular Biology, Harvard University, USA). *B. subtilis* SpoIVFB-GFP biofilms (ca. 8 log CFU/ml vegetative cells and spores below the lower detection limit, i.e., 1 log CFU/ml) attached to glass wool were exposed to nutrient-limitation to stimulate sporulation and monitored for fluorescence by confocal scanning laser microscopy (CSLM). Planktonic cells sampled from the growth medium containing the glass wool, i.e., "planktonic in the presence of glass wool" (PGW) were also exposed to nutrient-limitation and observed by CSLM. The PGW *B. subtilis* SpoIVFB-GFP cells fluoresced after 5 h, while the *B. subtilis* SpoIVFB-GFP biofilm cells only fluoresced after 30 h. However, plate counts showed that spores of *B. subtilis* SpoIVFB-GFP were already present in biofilms after 6 h. It may be speculated that these spores originated from PGW cells in the surrounding environment. In the food context, the trend for longer operating runs between cleaning of food processing equipment surfaces may allow both biofilms and spores within biofilms of *Bacillus* to develop on food contact surfaces. Regular cleaning and sanitation of food contact surfaces during shorter processing runs is of paramount importance to prevent attachment of *Bacillus* spores and to ensure early removal of *Bacillus* biofilms.

P071 DSC Mathematical Models for Hot Water, Electron Beam Radiation, and Hydrogen Peroxide Treatments on the Survival of *Fusarium* spp. and Germinative Energy in Malting Barley

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The objective of the study was to pursue mathematical modeling in order to examine the effects of controllable factors on the survival of *Fusarium* spp. and of germinative energy (GE) in malting barley. This was achieved by modeling the survival probabilities of *Fusarium* spp. and GE in malting barley separately as a function of hot water (HWT), hydrogen peroxide (HP), and electron-beam radiation (EBR) treatment parameters. For HWT, the barley was treated with hot water at temperatures of 45, 50, 55, and 60°C for 0, 1, 5, 10, and 15 min, for EBR, the barley was irradiated at 0, 2, 4, 6, 8, 10 kGy, and for HP, the treatments included 0, 5, 10, and 15% concentrations with exposure times for 0, 5, 10, 15, 20, and 30 min. All experiments were repeated three times. Treated samples were evaluated for *Fusarium* infection (FI) and GE. Logistic regression models were developed to predict the probabilities of FI and GE. The models fitted the data with concordance in the range 82 to 86% for FI and 53 to 85% for GE. For HWT, GE probabilities were found to be maximum at 48.8°C for 0.80 min respectively. For EBR, GE probabilities were found to be maximum at 2.6 kGy. For HP, FI probabilities were found to be minimum at 9.8% concentration for 21 min. The predicted and observed probabilities of FI and GE were in satisfactory agreement. The predicted probabilities of FI, when FI was observed and no FI was observed in the treated samples, ranged from 0.30 to 0.90 and <0.01 to 0.01 respectively.

P072 Modeling the Synergistic Effects of High Pressure, Temperature, pH, and Time on the Inactivation of *Bacillus subtilis* ATCC 6633 Spores during High Pressure Processing

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The objective of this study was to model the survival of *Bacillus subtilis* ATCC 6633 spores as a function of pressure, pH, temperature, and time during high pressure processing. *Bacillus subtilis* spores were packed in citrate phosphate buffer at three different pH (3.0, 5.0, and 7.0) values. The packed spores were subjected to a pressure of 690 and 827 MPa at temperatures 60, 65, 70 and 75°C for 0, 3, 5, and 10 min. All treatments were replicated three times. A logistic regression model was developed to predict the probabilities for achieving ≥ 5-log reduction. A response surface model was also developed to evaluate the performance of the fitted logistic regression model. The concordance of the logistic regression model was 95.3. At pressure 827 MPa, the pH, temperature, and time parameters were found to have statistically significant (*P*-value <0.001) impact on the model. The predicted probabilities when ≥ 5-log reduction was observed ranged from 0.92 to 0.99. The predicted probabilities when 5-log reduction was not observed, ranged from <0.01 to 0.30. The optimum values predicted by the response surface model accounted to > 5-log reduction. The predicted probability for achieving ≥ 5-log reduction from the logistic regression model at these optimum values was found to be 0.99. The developed models can aid in understanding the interactive effect of pressure, pH, temperature, and time on the inactivation of *Bacillus subtilis* ATCC 6633 spores. Validation of the developed model against new treatment conditions in model food systems may be of immense value.

P073 Production and Emission of the Long-chain Alcohols Decanol and Dedecanol, Putative Antimicrobial Compounds, by *Escherichia coli*

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Analyses of broth culture using solid phase micro extraction have shown that *Escherichia coli* produces decanol and dodecanol as major volatile compounds. Ten *E. coli* strains tested produced these compounds and the sum of the quantities of the alcohols exceeded quantities of any other compounds released into the vapor phase. Decanol and dodecanol were detected relatively early after the establishment of a tryptic soy broth culture (4 h after 10^7 CFU initial inocula) and level of both compounds increased rapidly and then declined at

24 h. In contrast, indole, the other major volatile compound released by *E. coli*, reached its highest measured level at 24 h after inoculation. Earlier work showed that *E. coli* didn't emit decanol and dodecanol when grown in agar culture, indicating the level of available oxygen triggers the production of long chain alcohols. Modifying broth to contain 2 x glucose prevented the decline of decanol and dodecanol which occur at 1 x glucose. Addition of either decanoic or dodecanoic acid (K+ salt) to the culture at 100 mg/l resulted in a greater than 10-fold increase in the level of the respective alcohol without increasing other volatile compounds. Addition of palmitic acid to the broth (K+ salt at 100 mg/l) virtually eliminated the production of both decanol and dodecanol by *E. coli*. Together, these alterations in broth composition with fatty acids provide means of controlling the levels of decanol and dodecanol produced by the bacteria and provide a tool for studying the biological function of the compounds.

P074 Development of Hybridoma for the Production of Monoclonal Antibody against Sulfamethazine

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Fifteen monoclonal antibodies (MAbs) against the widely used antibiotic sulfamethazine (SMZ) were produced by hybridomas from two fusion of myeloma cells and spleen cells isolated from BALB/c mice immunized with hapten (SMZ-hemisuccinate, SMZ-hemiglutarate) conjugated with keyhole limpet hemocyanin (KLH) and soy bean trypsin inhibitor (STI). To evaluate sensitivity of MAbs, competitive indirect ELISA was used. MAb 1-H11-5 exhibited the highest sensitivity and selectivity toward sulfamethazine. A direct competitive ELISA was established with SMZ coupled to horseradish peroxidase as the labeled antigen. This assay was very sensitive and had a linear range from 0.1 to 10ng SMZ per ml sample. The developed antibody only showed 1.68% cross-reactivity to sulfamerazine but not with other analogues. Recoveries of SMZ added to milk samples at 1, 10, 100 ug/ml ranged from 96 to 119%. This ELISA provided a valuable method to quantify SMZ in various food samples including milk.

P075 Growth/No Growth Interface of *Penicillium digitatum* and *Fusarium culmorum* as a Function of pH, Water Activity and Incubation Temperature

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Using Box-Behnken experimental designs, the combined effects of incubation temperature (T, from 10 to 35°C), pH (from 2.0 to 5.0) and water activity (a_w, from 0.990 to 0.900) were evaluated on *Penicillium digitatum* and *Fusarium culmorum* growth/ no growth response. a_w - pH - adjusted potato dextrose agar was prepared in triplicate for each combination of factors. For each mold, solidified plates were inoculated (2 mL of a 10^6 spore/mL suspension), incubated at the evaluated temperatures and observed during one month. If growth was observed the response was registered as 1; no growth was registered as 0. Backward stepwise logistic regression was used to develop a simplified model able to predict mold probability of growth. *F. culmorum* growth was observed in 33 cases from a total of 51 observations while *P. digitatum* growth was observed in 30 cases. Each model includes the significant ($P < 0.05$) effect of each independent variable as well as a_w-pH-T interaction. Models show that T reduction increased the number of combinations of pH and a_w with probabilities to inhibit growth higher than 0.95. The obtained models predict the probability of growth under a set of conditions and can be used to calculate critical values of pH, T, or a_w inhibiting mold growth for different probabilities. Reduction of pH increased the number of combinations of a_w and temperature with probabilities to inhibit growth higher than 0.95. With a probability of 0.05, minimum growth temperatures were higher as a_w and pH increased, being more noticeable for *F. culmorum* than for *P. digitatum*.

P076 DSC Evaluation of Reduced Toxicity of Zearalenone by Extrusion Processing Using a Human Breast Cancer Cell Line (MCF-7) as Measured by the MTT Bioassay

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Zearalenone (ZEN) is a nonsteroidal estrogenic mycotoxin which is commonly found as a contaminant in cereal grains. ZEN has high binding affinities for the intra-cellular estrogen receptor (ER) and can enhance the proliferation of estrogen responsive tumor cells. Extrusion processing may provide a crucial detoxification strategy to reduce or eliminate toxicity of ZEN in contaminated grains. The objective of this study was to determine loss of toxicity of ZEN in extruded corn grits as measured by the MTT bioassay, using a sensitive mammalian cell line, and to compare the results to results with chemical (HPLC) and biochemical (ELISA) methods. A split-split plot design was used for the extrusion process experiments at temperatures of 150, 175, and 200°C and screw speeds of 70 and 140 rpm. A MCF-7 cell line having estrogen receptors was used to evaluate the estrogenic activity of ZEN. The effect of extrusion temperatures on loss of ZEN was found to be a quadratic response. The percent reductions of ZEN ranged from 78 to 81%, 78 to 79%, and 67 to 69% at 150, 175 and 200°C, respectively, as measured by HPLC. The percent reductions of ZEN at screw speeds of 70 and 140 rpm were mean values of 70 and 73%, respectively. The MTT bioassay results were more closely correlated with HPLC results (r = 0.96) than ELISA results (r = 0.85). The MTT bioassay, using a sensitive mammalian cell line, was proven to be a

useful method for quantification of ZEN, as well as a potential toxicity screening method for contaminated extruded cereal-based products.

P077 Fumonisins in Corn-based Food for Infant Consumption

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A survey of 196 samples of corn-based infant foods from thirteen cities of São Pãulo State, Brazil was carried out in order to investigate the fumonisin contamination in these products. Based on their ingredients, these products were divided into seven groups, namely infant cereal designated as types A, B, C and D, corn meal, corn starch and instant baby food. Although certain infant food samples were free of fumonisin contamination (< 20 μg/kg; corn starch and infant cereals of type A, B and D), contamination levels in the other products (corn meal, instant corn-based baby food and cereal type C) were of concern, particularly those in corn meal. All samples in these categories contained fumonisins. The mean level for total fumonisins (FB1+FB2+FB3) in corn meal was 2242 μg/kg (maximum 8039 μg/kg), in instant corn-based baby food was 437 μg/kg (maximum 1096 μg/kg) and in infant cereal type C was 664 μg/kg (maximum 1753 μg/kg). All samples had adequately low water activities and fungal counts and hence can be classified as safe for consumption based on these criteria. No detectable aflatoxin was found in those products that showed evidence of residual *Aspergillus flavus* contamination.

P078 Monitoring of Aflatoxin B1 on Grains, Peanuts, Foodstuffs and Feeds in Korea

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To systematically screen the aflatoxin B1 contamination in foodstuffs and feeds, monitoring was carried out. For the monitoring of aflatoxin B1, a total of 652 samples including 303 grain samples, 88 peanut samples, 82 cereal samples and 179 feed samples were collected from June, 2003 to October, 2003 throughout the country, in locations such as Seoul, Gyeongin, Gangwon, Chungchang, Chunla and Gyenogsang province. Direct competitive ELISA (DC-ELISA) established in our laboratory was applied. This system was very sensitive and showed a low detectable limit, from 0.01 ppb to 10 ppb levels. Recoveries of aflatoxin B1 spiked in grains, peanuts, cereal and feeds at the level of 1 or 10ng/g were all in the range of 82 to 112% with low coefficients. Total assay time including sample preparation and ELISA procedure was 1.5 h. After ELISA work, HPLC was also applied for redetecting aflatoxin B1 from positive sample on ELISA. On the results of ELISA and HPLC tests, some grain and feed samples showed 10 ppb levels above of aflatoxin B1 contamination. This assay has been validated for application to food samples and demonstrated many advantage over conventional methodology, including the requirement for minimal sample preparation before assay, its technical simplicity, and its potential high sample through-put. In conclusion, more systemical and long term monitoring of aflatoxin B1 from various samples is strongly needed; with those monitoring results, we can do a reliable risk assessment and can suggest more effective guidelines for the protection against the risk of aflatoxin B1.

P079 DSC Detection and Identification of Histamine-producing Bacteria Associated with Harvesting and Primary Processing of Mahi-mahi and Yellowfin Tuna in North Carolina

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Histamine poisoning is one of the most common chemically induced seafood-borne illnesses reported in the US. The causative agent(s) is biogenic, commonly produced by Gram-negative bacteria. The purpose of this study was to isolate and identify histamine-producing bacteria associated with commercial operations for mahi-mahi and yellowfin tuna in North Carolina. Fish and environmental samples were obtained on four separate days at different points during production and processing. Samples were processed for determination of total aerobic bacterial counts, using non-selective media. Representative bacterial isolates were chosen based on morphological differences and screened for potential histamine production, using an indicator media. Presumptively positive histamine producers were further identified at the genus and species level by use of rapid biochemical methods and histamine production verified by an ELISA method. In preliminary screening, 26% of the 513 bacterial isolates were identified as possible histamine producers. However, only 36 of these isolates could be definitively identified by biochemical means, and only 5 of the 36 were confirmed as histamine producers. Three of the five isolates were identified as *Citrobacter freundii*; two were identified as Gram-positive cocci, *Aerococcus urinae*, and *Staphylococcus epidermidis*, contradicting the general view that Gram-negative bacteria are primarily responsible for histamine development in fish. Histamine producers were found at harvest, at first receiver, and during processing. This study confirms that bacteria responsible for histamine poisoning can be introduced at any time from harvest through processing and reinforces the need for adequate temperature control to reduce the risk of histamine production in fish.

P080 DSC Evaluation and Reduction of Biogenic Amines in Korean Traditional Fermented Foods

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This study was carried out to investigate biogenic amine-related health risks with Korean traditional fermented foods and to attempt a solution. It was performed to determine biogenic amine contents, to analyze biogenic amine-producing organisms and to develop the technology for controlling biogenic amine production in the foods, especially Jeotkal (Korean salted and fermented fish products). The amounts of biogenic amines in most Korean traditional fermented foods were within the safe level for human health. However, certain samples which had been fermented for a long period and/or stored poorly contained considerably high concentrations of biogenic amines. Most organisms in the foods showed the activities to produce biogenic amines. Biogenic amine productivity of *Bacillus licheniformis* was the highest, followed by other bacilli, staphylococci and lactic acid bacteria. Food additives, including sucrose, glucose, sorbitol, glycine, lactic acid, citric acid and sorbic acid, and spices, including cinnamon, clove, ginger, green onion and allspice, showed inhibitory effects on biogenic amine-producing bacteria. Also, several isolates with the abilities to degrade biogenic amines and produce bacteriocin-like substances were characterized as *Micrococcus*, *Staphylococcus* and *Bacillus*, and were selected as candidates for starter culture. Garlic, glycine and starter culture of *S. xylosus* isolate No. 0538 were finally applied to the ripening of Myeolchi-jeot (Korean salted and fermented anchovy) in situ, and then biogenic amines were dramatically reduced and degraded by approximately 71%, compared to control. The developed control technology could increase both the safety and shelf life of Jeotkal and other fermented foods.

P081 Advances in Detection of Psychrophilic *Clostridium* spp. Causing "Blown Pack" Spoilage of Chilled Vacuum-packed Meats

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Psychrophilic clostridia, *Clostridium estertheticum* and *C. gasigenes*, have been identified as causative agents of "blown pack" spoilage of chilled meats, during storage at temperatures between -1.5 and 1°C. However, 15 years after "blown pack" spoilage of clostridial aetiology was initially described, isolation of psychrophilic clostridia remains laborious and problematic. The recovery of "blown pack"-causing clostridia is conducted under strict anaerobic conditions and uses fully pre-reduced media followed by incubation at 7 to 10°C, for 3 to 4 weeks. The difficulty with conventional detection of "blown pack"-causing clostridia has led to the development of methods for molecular detection of these micro-organisms. The DNA-based method that is now widely adopted by meat industry and meat testing laboratories uses primer sets and PCR amplification procedures that detect the presence of species-specific 16S rDNA gene fragments of *C. estertheticum* and *C. gasigenes*. Molecular detection of "blown pack"– causing clostridia commonly incorporates a cold enrichment step in anaerobic media. However, various detection strategies can be selected for different purposes, depending on the source and type of samples. This paper will give an overview of strategies available for detection of "blown pack"-causing clostridia in various matrices including dressed carcasses, soil, hide/faeces, meat plant environment and spoiled product.

P082 Recovery and Detection of Microorganisms in Aseptic Low Acid Food Products Using an Automated Microbial Detection System

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The BacT/ALERT® automated microbial detection system detects the presence of microorganisms by the colorimetric monitoring of CO_2 production. Each BacT/ALERT culture bottle contains a colorimetric sensor which is monitored every 10 min by the BacT/Alert instrument. A growth detection algorithm determines the presence of microorganisms. The BacT/ALERT® AST culture bottle is designed to detect the presence of potential spoilage microorganisms in aseptic or processed food products. The bottle accepts up to 10 ml of sample and does not require venting to introduce an aerobic atmosphere. This study was undertaken to verify that the system could recover and detect the growth of microorganisms in the presence of a variety of low acid foods. Food products tested included several flavored coffee creamers, ready to eat puddings and soy milk. Additional products were intentionally contaminated with low levels of microorganisms and incubated at 35°C overnight. These products were then pooled with sterile product (1 ml seeded to 9 ml sterile) and inoculated into bottles. These intentionally contaminated products included milk and coffee based products and individual coffee creamers. *Bacillus subtilis* was recovered in all pooling experiments in <11 h (2 CFU inoculum). Growth of *Staphylococcus aureus* was completely inhibited by coffee based products. *Pseudomonas fragi* and *S. aureus* were delayed in chocolate products and *S. aureus* was delayed in a cinnamon product. The results indicate the system can be used to assess the quality of food products. Additionally the results indicate that proper bacteriostasis controls should be included when challenging the system.

P083 Recovery and Detection of Microorganisms in Aseptic High Acid Food Products Using an Automated Microbial Detection System

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The BacT/ALERT® microbial detection system consists of an instrument and a disposable culture bottle. The instrument is made up of an incubator/shaker unit coupled to a data management system. The disposable culture bottle is made of shatter resistant plastic and contains a sensor responsive to CO_2 and other metabolites from microbial growth. By reflected light, the system automatically monitors changes in the sensor every ten min and determines the presence of growing microorganisms through computer algorithms. The BacT/ALERT® LYM culture bottle is designed to detect the presence of potential spoilage microorganisms in high acid food products. The bottle can accept up to 20 ml of sample. This study was undertaken to verify that the system could recover and detect the growth of microorganisms in the presence of a variety of high acid foods. Various species of bacteria, yeasts and molds were diluted to low levels (<100 CFU) and inoculated into triplicate bottles containing 20 ml of product and bottles containing no product. Genera represented include *Acetobacter*, *Alicyclobacillus*, *Aspergillus*, *Bacillus*, *Byssochlamys*, *Lactobacillus*, *Leuconostoc*, *Paeciliomyces*, *Pediococcus*, *Saccharomyces*, *Zygosaccharomyces* and *Zymomonas*. Foods tested included tomato paste, apple juice, vegetable juice, and pasteurized and aseptic orange juice. At <100 CFU, all bottles tested were positive in less than 72 h with the exception of *Leuconostoc mesenteroides* in apple juice which was positive in 79.1 h. The results indicate that the system can rapidly detect the growth of microorganisms in the presence of a variety of high acid food products.

P084 Comparison of an Automated System (TEMPO) with Aerobic Plate Count for Enumeration of Microorganisms in Ground Beef and Poultry

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Conventional enumeration of microorganisms in foods involves tedious procedures that include preparing serial dilutions, pour plating aliquots of the dilutions, incubating plates for 48 h, and manually counting the resulting colonies that form on the agar plates. The TEMPO system is a new automated methodology that eliminates the need for serial dilutions and pour plates, and automatically reads and records the results. This new technology is based on the most probable number method for enumeration, and includes a card containing multiple wells, as well as a medium specifically formulated to ensure rapid fluorescent detection of microbial growth. The system consists of a data entry station and a reading station. Data management is handled automatically and several report functions are available through the software. The objective of this study was to compare the TEMPO system to the conventional method for determination of total viable count in ground beef and poultry samples. Eighty samples of each food type were tested by each method. Samples were prepared and tested according to the USDA/FSIS Microbiology Laboratory Guidebook for the conventional method. Samples for TEMPO were stomached using a special filter bag, inoculated into the card, incubated at 35°C for 24 h, and read by the automated reader. No significant differences ($P < 0.05$) were observed between the plate count method and the automated method. The new method is more rapid, much less labor intensive, and eliminates the possibility of transcription errors when recording data.

P085 3M™ Petrifilm Plate Reader for the Enumeration of Petrifilm Aerobic, Coliform, and *Escherichia coli*/Coliform Count Plates

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The 3M™ Petrifilm™ Plate Reader has been developed in order to enumerate Petrifilm Aerobic Count Plates, Petrifilm Coliform Count Plates, and Petrifilm *E. coli*/Coliform Count Plates, thus increasing laboratory efficiency and reducing transcription errors and technician fatigue. The performance of the Reader was demonstrated in 3M's laboratory by comparing the counts from food samples containing bacteria using both the Petrifilm Plate Reader and human analysts. Sixty-eight Petrifilm Aerobic Count Plates, 158 Petrifilm Coliform Count Plates, and 235 Petrifilm *E. coli*/Coliform Count Plates were studied. The logarithm to the base 10 counts from the Petrifilm Plate Reader when enumerating Petrifilm Aerobic Count Plates, Petrifilm Coliform Count Plates, and Petrifilm *E. coli*/Coliform Count Plates were within 10% of those logarithm to the base 10 counts obtained manually 93%, 100%, and 96% of the time, respectively. These data indicate that the counting criteria stated in Standard Methods for the Examination of Dairy Products were met. In addition, sets of samples were prepared and enumerated by outside laboratories using multiple Petrifilm Plate Readers and multiple analysts. Analysis of variance indicated no statistical difference between the Petrifilm Plate Reader and human analysts ($P > 0.05$).

P086 Evaluation of the 3M™ Petrifilm Environmental Listeria Plate for Use in Detecting *Listeria monocytogenes*

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New USDA regulations have increased emphasis on environmental testing for *Listeria* spp. and *L. monocytogenes*. Meat processors may prefer to do in-house testing for quicker results; however, they wish to avoid the risk associated with sample enrichment. The 3M™ Petrifilm™ Environmental Listeria plate (EL) method was developed to allow no-enrichment analysis of environmental samples for *Listeria* spp. after a 60 to 90 min repair period in 20 to 30°C Buffered Peptone Water. This study evaluated the ability of stressed and unstressed *L. monocytogenes* cells to repair/grow during the repair step and then form colonies on the EL plate. A 5-strain cocktail of *L. monocytogenes* in Brain

Heart Infusion broth was exposed to either heat (48°C/30 min), sodium hypochlorite (2 ppm total residual chlorine, 15 min, 20°C), osmotic (pH 5.0, 20% sodium chloride, 18 h, 20°C), or heat + freeze/thaw (18 h at -20°C, 18 h at 5°C). Cells were enumerated using the Petrifilm™ EL method before and after each stress and after the repair step, with direct plating on Brain Heart Infusion Agar as a control. Each of the stresses caused significant ($P < 0.05$) cell injury (0.2 to 0.7 log CFU/ml decrease). After a 75 min repair period, numbers of *L. monocytogenes* detected using the Petrifilm™ EL method increased by up to 0.2 log CFU/ml, an increase solely attributable to injury repair. Results suggest that use of the Petrifilm™ EL method may allow detection of *L. monocytogenes* cells from environmental samples without the risks associated with enrichment procedures.

P087 Evaluation of the DOX System for Detection of Acidophilic Bacteria in Juice

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DOX-system is one of the newly developed rapid detection methods for enumeration of viable bacteria in food. In this method, bacterial respiration is monitored continuously in a cartridge cell equipped with a disposable oxygen electrode. This system is easy to handle and is able to measure many samples at the same time using the equipment with multi cartridge channels (maximum 180 samples). A high correlation between the detection time and the total viable cell number by this system has been established in measurement of total bacteria and coliform counts in food samples. DOX system is available for the first sanitary screening test at the food processing facility. We applied this DOX system for the detection of acidophilic spoilage bacteria in fruit juice. *Alicyclobacillus acidterrestris* was used in this study. The concentration of dissolved oxygen was monitored continuously at an optimal temperature of 45°C in Kirin broth containing serial dilutions of *A. acidterrestris* cells. It was found that the sample containing 10^0 – 10^1 cells/ml of this bacterium could be detected by approximately 24 h. Evaluation for the detection limit by the DOX system of *A. acidterrestris*, using spiked juice samples with different numbers of vegetative cells, are in progress.

P088 Qualitative and Quantitative Analysis of Spoilage Microflora on Ready-to-Eat Chicken by Molecular Bioscaping Techniques

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Microbiological quality is the decisive factor in shelf life and consumer acceptance of Ready-to-eat (RTE) chicken. Several microorganisms, either as resident flora or as contaminants during processing, contribute to the spoilage of RTE chicken. Assessment of such contamination load is important to evaluate the processing and to develop any intervention strategies for RTE chicken. A molecular bioscaping technique for qualitative and quantitative analysis of microflora on RTE chicken was developed. Primers were designed based on specific DNA sequences of spoilage bacteria common to RTE chicken. These reference primers were optimized for PCR activity using 14 species-specific ATCC type strains. Microorganisms were isolated from RTE chicken (n=32) by use of standard sampling techniques. Sample rinses were plated on trypticase soy agar for microbial growth after incubation at 26°C for 48 h. Morphologically different colonies (n=21) were isolated and their genomic DNA was extracted. Qualitative analysis with reference primers showed that most isolates from RTE chicken were species of *Pseudomonas*, *Enterobacter*, *Kluyvera*, and *Acinetobacter*. This data was further confirmed by Vitek® biochemical identification system. RTE chicken skin was measured for microbial loads after inoculating with known concentrations of 14 different species of spoilage bacteria including *Pseudomonas putida*, *P. fluorescens*, *Enterobacter cloacea*, *Kluyvera* spp., *Acinetobacter baumanni*, *Edwardsiella tarda*, *Edwardsiella hoshinae*, *Shewanella putrifaciens* and *Serratia liquifaciens*. Quantitative analysis by molecular bioscaping showed ~95% accuracy in measuring the inoculated microbial load on broiler skin. Molecular bioscaping is a rapid and sensitive method for qualitative identification of microorganisms and for quantitation of microbial loads directly on RTE chicken surface.

P089 Evaluation of VIDAS Staph Enterotoxin II (VIDAS SET 2) Method

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VIDAS Staph enterotoxin II is an automated qualitative test for use on the VIDAS system for the detection of Staphylococcal enterotoxins in food by use of the enzyme-linked fluorescent assay (ELFA) technique. Frozen prepared lasagna, chocolate éclair, canned mushrooms, powdered egg, roast beef, cooked chicken, ham, cheddar cheese, raw milk, and yogurt were spiked with one of the Staphylococcal enterotoxin serotypes A, B, C, D and E. For each food type, twenty samples consisting of 5 controls, 5 samples with 0.25 ng of toxin/g, 5 samples with 1.0 ng/g, and 5 samples with 2.0 ng/g were analyzed by the VIDAS SET II method. Control samples were free of toxin. All 150 samples containing Staphylococcal enterotoxins were positive, resulting in 100% sensitivity for the VIDAS SET II method. All 50 control samples were negative. After a simple extraction, the SET 2 test is fully automated and results are obtained within 80 min, allowing for the rapid detection of staphylococcal enterotoxins in foods.

P090 Comparison of the BAM/FDA and Bax System Methods for *Salmonella* Detection in Food Samples

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Food samples (202) including egg products, confectionery and chocolate products, fruits and vegetables, and lactic products, among others, were analyzed both by the traditional cultural technique (BAM/FDA) and the Bax System, which is based on the polymerase chain reaction. The same results were obtained by the two methods with the exception of two samples, one of honey and the other of cashew nut, which were positive for *Salmonella* when analysed by the Bax System but gave negative results with the BAM/FDA method. Most samples were negative for the presence of *Salmonella* by the two methods applied, with the exception of four samples of meat flour, which gave positive results with both methodologies The false negative rate obtained in our study so far has been 1.0%.

P091 Comparison of an Automated MPN-based System with the USDA/FSIS Microbiology Laboratory Guidebook Method for Enumerating *Escherichia coli* in Ground Beef and Poultry

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The USDA/FSIS Microbiology Laboratory Guidebook (USDA-MLG) method for enumerating *Escherichia coli* specifies a single tube of lauryl sulfate tryptose (LST) broth be used per each ten-fold dilution tested. Inoculated LST tubes are incubated for 24 h. Tubes positive for gas production are transferred to EC broth and incubated at 45.5°C for an additional 24 h. TEMPO is a new system that eliminates the need for serial dilutions and automatically reads and records results. The system is based on a most probable number (MPN) determination, using changes in fluorescence to determine growth. Data management is handled automatically through the data entry station and result reports are available via the reading station software. The objective of this study was to compare the Tempo method for enumerating *E. coli* to the USDA-MLG method. Eighty samples of each food type were tested. Samples were prepared according to the instructions for each method. Tempo cards were incubated at 35°C for 24 h and read automatically by the reader. Growth was determined visually for the USDA-MLG method. No significant differences were observed ($P < 0.05$) between the two methods. Results are obtained in 24 h with the new method versus 48 h for USDA-MLG method. Additionally, results are recorded automatically, eliminating the possibility of transcription errors when recording data.

P092 Comparison of the Automated TEMPO® System with Conventional Plate Counts for the Enumeration of *Enterobacteriaceae* in Food Products

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In the food industry, most quality indicators are determined by time-consuming plate counting methods. The TEMPO system is a new automated method associating an enumerating card with a medium specifically adapted to ensure a rapid fluorescent detection of microorganism growth. It replaces serial dilutions and tedious plate reading with a simple 1/10 dilution and an automated enumeration based on the MPN (Most Probable Number) method. The new method allows a 24-h enumeration for *Enterobacteriaceae* at 35°C without any further oxidase confirmation testing. In this study, the application of this new system to *Enterobacteriaceae* enumeration was compared to the ISO 7402 method by testing 99 different food products including raw and processed meat, dairy food, seafood, vegetables and ready-to-eat meals. Samples were serially diluted up to 10^{-4} and enumerated by inclusion in VRBG incubated 24 h at 35°C followed by an oxidase confirmation test. An aliquot of the same 1/10 dilution was transferred in the TEMPO® EB reagent vial. The system ensured automatic filling of the enumeration card. Card automatic reading was performed after a 24-h incubation at 35°C. No significant differences ($P < 0.05$) were observed between the plate count method and the automated method.

P093 Development of a New Light-cycler Real-time PCR-based Detection Method of *Alicyclobacillus* sp.

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The genus *Alicyclobacillus* was described by Wisotzkey J.D. et al. (Wisotzkey JD, Jurtshuk P Jr, Fox GE, Deinhard G, Poralla K. Int J Syst Bacteriol. 1992 Apr;42(2):263-9.), by reclassification of several thermo-acidophilic *Bacillus* species. This genus was differentiated by 16S rDNA sequencing and by its omega-alicyclic fatty acid possessing. *Alicyclobacillus* species were isolated from acidic beverages like apple juice, orange juice, herbal tea, etc. (2, 3) causing spoilage and off flavor. As these bacteria are thermophilic and spore forming their routine detection and identification by traditional culturing methods takes long time (five days) and is rather unreliable. According to these characteristics, their appearance in the beverage industry means a real problem. We developed a fast, Light-Cycler real-time PCR based system for industrial use. We designed specific primers on an appropriate part of the 16S rDNA. The specificity of the primers allows the specific amplification of the

genus specific sequence. The species identification is carried out by using sequence specific DNA hybridization probes. The species are differentiated by the melting point analysis of these hybridization probes. This system was tested on numerous prokaryote incl. *Bacillus* genus and eukaryote (yeast and mould) species. The testing was carried out on various food samples (e.g., fruit juice, tea) inoculated with both positive (*Alicyclobacillus*) and negative species in various concentrations. The bacterial DNA was isolated directly from the samples. These tests were followed by classical plating methods. The results showed that this method is useful for rapid detection of the *Alicyclobacillus* genus and to differentiate between *Alicyclobacillus* species.

P094 A Molecular Beacon-based Real Time NASBA Assay for *Mycobacterium avium* subsp. *paratuberculosis*

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A molecular beacon-based real-time NASBA assay for detection and identification of *Mycobacterium avium* subsp. *paratuberculosis* has been developed. It targets and amplifies a nucleotide sequence which is specific for this bacterium, and includes an internal amplification control. The assay was tested against 18 isolates of *M. avium* subsp. *paratuberculosis*, 17 other mycobacterial strains and 25 non-mycobacterial strains, and was fully selective. It is capable of detecting 150 to 200 cells per reaction with 99% probability. Using prior centrifugation and nucleic acid extraction, the assay was able to consistently detect 103 *Mycobacterium avium* subsp. *paratuberculosis* cells in 20 ml artificially contaminated drinking water. With a simple detergent and enzymatic sample pretreatment before centrifugation and nucleic acid extraction, the assay was able to consistently detect 104 *Mycobacterium avium* subsp. *paratuberculosis* cells in 20 ml artificially contaminated semi-skimmed milk. The assay will be a useful addition to the range of diagnostic tools available for the study of *Mycobacterium avium* subsp. *paratuberculosis*, and could be used to confirm and complement results of analyses performed with PCR- and culture-based methods.

P095 Development of a DNA Microarray-based Platform for the Simultaneous Detection and Genotyping of Noroviruses

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Norwalk (Norovirus) and Norwalk-like agents account for more than 65% of non-bacterial gastroenteritis in the USA, and they continue to be a major threat to public health as evidenced by the recent outbreaks of gastroenteritis on cruise ships. Noroviruses cannot be cultured, and current detection methods suffer from low sensitivity (i.e., electron microscopy). Further, amplification-based detection methods are subject to strain and genomic diversity, i.e., RT-PCR primers may not amplify nucleic acid from all strains). The objectives of this work are to develop a DNA-based microarray assay that would allow the simultaneous detection and genotyping of Noroviruses in foods. The microarray platform contains 67 features (based on 50-mer oligonucleotides) designed to hybridize to specific sequences of all three open reading frames of Noroviruses. The features were deliberately constructed to allow for confirmation and simultaneous characterization of Genogroups I and II. Noroviruses from stool samples were pre-amplified by RT-PCR prior to labeling and hybridization to the microarray. Preliminary results indicate that the microarray was successful in both the detection and genotyping of Noroviruses, overcoming limitations inherent in other molecular-based methodologies. We are currently developing a non-gene specific amplification method to circumvent the need for specific primers to amplify target nucleic acid. The development of this microarray-based assay should provide a valuable tool for the epidemiological surveillance and genotyping of Noroviruses in clinical, environmental and food matrices. Furthermore, this approach would be applied for the detection and molecular characterization of other enteric foodborne viral agents.

P096 Detection of Attached Bacteria on Food Contact Surfaces Using a Biosensor

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The methods currently used for detection of attached bacteria such as microscopic techniques or fluorescence require the use of expensive equipment, are time consuming and require special skills for analysis. Hence, relatively inexpensive methods with greater speed, sensitivity and capability for on-line and real-time detection are needed. A biosensor could prove to be a suitable device. In this study, a biosensor was used for detecting and quantitating attached bacteria. An overnight culture of *P. aeruginosa* was centrifuged, washed, resuspended and diluted in deionized water to obtain various concentrations of bacterial populations from 10^3 to 10^9 CFU/ml. Coupons of stainless steel, glass, polycarbonate and polyvinyl chloride were inoculated to give 10^8, 10^6, 10^4, and 10^2 CFU/cm^2. Coupons were allowed to dry over night. A drop of deionized water was placed on the surface of the biosensor chip, the coupon surface with bacteria attached was placed on top and the capacitance image captured. The gray scale values of the images were then determined using imaging software. The values decreased with increasing bacterial concentration. With use of a biosensor (ChemArray Chip, STMicroelectronics), microorganisms attached to stainless steel, glass, poly-carbonate, and polyvinyl chloride have been detected. The results of this study indicate that biosensors show capability for detecting bacteria attached to surfaces. The device can be attached to a food contact surface to monitor biofilm formation as needed and may aid in quantitating attached bacteria, allowing a more quantitative risk assessment.

P097 DSC Comparison of Methods for Recovery of *Salmonella* from Cantaloupe Rinds

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Recovery of *Salmonella* from cantaloupe using hand massaging, excision, and the Microbial-Vac™ (M-Vac) was compared. Cantaloupe rinds (100 cm^2) were inoculated with a five strain mixture of *Salmonella* (7.7 or 2.3 log CFU/100 cm^2) and held at 4°C for 24 h. Rind sections were placed in stomacher bags with 100 ml of 0.1% peptone water + 2% Tween 80 and vigorously rubbed through the plastic for two min (hand massaging), pummeled with a stomacher (excision), or vacuum-sampled using the M-Vac system. For the M-Vac, a wet-vacuum sampling device, sterile surface rinse solution (SRS, 100 ml) was applied under low pressure, followed by vacuum collection per manufacture guidelines. High and low inoculum rinsates from the three methods, except the low-inoculum M-Vac method, were serially diluted and surface plated (spiral plater) onto XLT4 agar without tergitol (mXLT4). For the low inoculum M-Vac method, SRS was vacuum concentrated onto a membrane filter, which was aseptically placed onto mXLT4 agar. Media were incubated for 24 h at 37°C, and typical *Salmonella* colonies were enumerated. For the high inoculum, recoveries using the M-Vac and excision were similar (7.5 and 7.1 log CFU/100 cm^2) and better ($P < 0.05$) than with massaging (6.38 log CFU). For the low inoculum, the M-Vac recovered 1.1 log CFU/100 cm^2, while *Salmonella* was not recovered using hand massaging and excision. The M-Vac is a non-destructive and effective method for recovery of *Salmonella* from cantaloupe surfaces. Because of the integrated vacuum concentration option, the M-Vac is superior to traditional hand massaging and excision methods for simple recovery of low numbers of *Salmonella*.

P098 Modeling Growth and Reduction of Microorganisms in Foods as Functions of Temperature and Time

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Food Safety Objectives (FSOs) are established in order to minimize the risk of foodborne illnesses to consumers, but these have not yet been incorporated into regulatory policy. During the process of establishing a FSO, predictions regarding bacterial growth are typically made based on a single addition of contamination during the processing of the food. The calculations presented as part of this research addresses more than one introduction of contamination, each of which can be separated by a substantial amount of time. The advantage of this approach is that it may more accurately and conservatively simulate an actual contamination incident. For example, a comparison of a one-step and a two-step introduction is made where the product is held at 6°C throughout the storage period. The single-addition case involves 20 CFU/g of *Listeria monocytogenes*, which is added all at once, in addition to a 4.65 log reduction kill step. For the single-addition model, the timing of the kill step has no effect on the time to reach the FSO, since the same log reduction amount is subtracted, regardless of when it occurs during processing. For the two-step addition case, two equal steps of 10 CFU/g *L. monocytogenes* each are added 200 h apart, the second addition coming immediately after the 4.65 log kill step. This product is shown to reach the FSO 43 h earlier, when a given amount of contamination is added in two equal portions rather than all at once.

P099 Potential Non-uniform Distribution of *Escherichia coli* O157:H7 in Feces and Underestimation of Prevalence

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Escherichia coli O157:H7 has become one of the most important emerging foodborne pathogens, with many outbreaks being associated with cattle. The objective of this study was to evaluate fecal pat sampling strategies to improve accuracy of *E. coli* O157:H7 prevalence estimates. A total of 120 fresh fecal pats from cattle were used in this study. From each fecal pat five samples were collected systematically going from west to east (positions 1 to 5 respectively) in north to south lines direction to avoid cross contamination and cultured for *E. coli* O157:H7 within 2 h. Samples were pre-enriched in GN-VCC broth and subjected to immunomagnetic separation and confirmation to detect *E. coli* O157:H7. Of the 120 fecal pats, 96 (80%) had no positive samples in any of the 5 samples. One sample was positive in 13 of the pats, 2 in four of the pats, 3 in two of the pats, 4 in three of the pats and only 2 of the pats had all 5 samples positive. Of the 600 total number of samples analyzed, 49 were positive with 14, 9, 8, 8, and 10 on position 1, 2, 3, 4, and 5 respectively. Prevalence estimates may be underestimated as a result of an uneven distribution in fecal material; therefore, sampling procedure plays a critical role in *E. coli* O157:H7 detection in bovine fecal pats.

P100 Contributions of Primary and Secondary Model Uncertainty to the Robustness of a Broth-based Microbial Growth Model for *Listeria monocytogenes* and *Escherichia coli* O157:H7 in Meat and Poultry Products

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Broth-based microbial growth models in the USDA Pathogen Modeling Program (PMP) predict

log counts with 95% confidence intervals. These confidence intervals reflect only the uncertainty in secondary models, thereby neglecting inherent uncertainty in the estimates of the primary model parameters. Quantitative microbial risk assessment requires knowledge of the total uncertainty (primary + secondary) of the model. Therefore, in this study, the total uncertainty of broth-based growth models (PMP) for *Listeria monocytogenes* and *E. coli* O157:H7 was quantified, with specific application to meat and poultry products. Nonlinear regression was performed with the original broth-based data, and the parameters for the Gompertz equation (B and M) were estimated. Response-surface secondary models were generated as a function of pH, temperature, salt and nitrite. Model robustness was then quantified via the Robustness Index (RI), which is defined as the ratio of the standard error of prediction (SEP) to the standard error of calibration (SEC). The SEP and SEC are the root mean square errors (RMSE) of the model against the validation (meat-based) and calibration (broth-based) data, respectively. The maximum, mean, and minimum RMSE of the primary model fit for the broth-based *Listeria monocytogenes* data were 7.78, 0.36, and 0.01 log (CFU/mL), respectively. The R2 of the secondary regressions for B and M were 0.44 and 0.82, respectively. The total uncertainty of the model (SEC) was 1.61 log (CFU/mL); 22% of this was due to the primary model uncertainty, and 78% was due to the secondary model. The RI ranged from 0.87 to 3.99.

P101 Modeling the Effects of Food Handling Practices on the Incidence of Foodborne Illness: Version 2.0 of the Food Handling Practices Model

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This paper presents results of phase two of a research project sponsored by the Food and Drug Administration to develop a quantitative simulation model for estimating the effects of food handling practices and other contributing factors on the incidence of foodborne illness. In 2001 FDA and RTI International developed version 1.0 of the Food Handling Practices Model (FHPM). The FHPM is an operational, stochastic simulation model that provides a quantitative method for estimating the annual benefits (avoided morbidity and mortality) of changes in food handling practices. Because unsafe food handling practices and other contributing factors occur in households, retail food establishments, and institutional establishments, the FHPM explicitly models all three venues. In October, 2003, FDA and RTI began work on version 2.0 of the FHPM to expand the scope and enhance simulation accuracy of the model. Version 2.0 of the FHPM includes an agricultural production stage to allow analysis of contributing factors on the farm. Version 2.0 also substantially expands the number of food categories and contributing factors modeled and explicitly models the level of pathogen contamination ingested. Because version 2.0 tracks food-pathogen pairs from farm to table, the model allows analysts to estimate benefits attributable to changes in food handling practices by food category, by pathogen, and by contributing factor. With appropriate calibration, analysts may also use version 2.0 of the FHPM to estimate the incidence of foodborne illness by food category, by pathogen, and by contributing factor.

P102 Quantitative Risk Assessment for *Salmonella* Brandenburg in Sheep Meat

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An epidemic of *Salmonella* Brandenburg abortions in sheep in the South Island of New Zealand resulted in an increase in human cases and isolation from sheep meat. A quantitative risk assessment (QRA) was initiated to examine the relationship between the occurrence of clinical epidemics of *S.* Brandenburg abortion in sheep flocks and the risk of infection in sheep going to slaughter and humans after consumption of sheep meat. The causative agent was identified as a specific pulsed-field gel electrophoresis (PFGE) clone previously not detected in New Zealand and apparently limited geographically to the epidemic area. The QRA program identified the prevalence and levels of *S.* Brandenburg in the faeces of slaughter stock, and longitudinally through the processing chain: on fresh and chilled carcasses, boned meat and retail cuts. A substantial decrease in prevalence and numbers of *S.* Brandenburg occurred during processing, mediated primarily by surface drying during carcass chilling. Detection of a small number of human cases outside the epidemic area, especially the North Island, attributed to the specific epidemic PFGE clone suggests that a low level of human infection might occur via consumption of sheep meat. However, the presence on other foods and travel to the epidemic area has not been discounted in these cases. Given that case-control studies have not identified any human cases associated with consumption of sheep meat, and the low level of *S.* Brandenburg on retail meats, a sheep meat route of infection is not contributing significantly to the burden of *S.* Brandenburg cases.

P103 National Typing Database for Zoonotic Foodborne Pathogens in New Zealand

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New Zealand is well recognised as having the highest reported incidence of campylobacteriosis among developed countries. Although an effective national disease-notification system may be an

important contributor to the observed incidence, it is nonetheless important that all possible infection routes are clearly identified so that appropriate risk management strategies can be identified and implemented. The foodborne route of infection, while commonly perceived to be the primary route for enteric infections such as campylobacteriosis and salmonellosis, may be secondary to environmental and occupational routes in agricultural countries such as New Zealand. A standardized, national inter-laboratory network and microbial typing database have been implemented in New Zealand to allow more effective detection of clonal linkages between clinical isolates and food and environmental isolates of *Campylobacter* spp., *Salmonella* spp., Shiga-like toxin producing *E. coli* (STEC) and *Listeria* spp. The National Typing Database has been designed to ensure comparability between New Zealand laboratories and with overseas databases such as PulseNet by providing standard pulsed-field gel electrophoresis (PFGE) methods compatible with, although not limited to, international PulseNet methodology. Sub-typing results from standard control isolates have been compared with US-CDC profiles, and the programme certified by PulseNet. The National Typing Database is an important tool, not only for facilitating the extension of foodborne illness outbreak investigations, but adding significant power to government-sponsored inter-agency pathogen pathway surveys enabling better identification of factors that, when controlled, will reduce the burden of human gastroenteritis in New Zealand.

P104 Probabilistic Analysis of Cross Contamination during Cooking

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Cross contamination is one of the major concerns regarding what might occur after preparation or cooking of food both at home and in foodservice facilities. In order to model cross contamination during cooking at home, we have simulated cooking procedures and possible cross contamination scenarios during cooking in a laboratory, using eggs experimentally contaminated with *Salmonella* Enteritidis (SE). Samples were taken from utensils such as bowls and cups, kitchen cloths, sponges that had washed utensils, counter top, and also from previously uncontaminated food such as portions of pudding and soup which had contacted cross-contaminated surfaces, according to simulated contamination pathways. Solid surfaces were swabbed and cloth, sponge or food samples were homogenized for the enumeration of SE. Then SE counts were modeled by applying probabilistic distributions to estimate recovery rates at each sampling point and transmission rates from one point to another, and finally the transferred rate from contaminated egg liquid to another food was calculated using a Monte Carlo simulation. The results were very consistent across trials. In exposure assessment, cross contamination from a contaminated food to uncontaminated foods should be separately analyzed upon possible pathways, and pathways should be designed by considering the type of raw food materials, cooking recipes, and type of kitchen facilities including different materials of surfaces. Our study demonstrates an example of such pathways and probabilistic analyses of cross contamination.

P105 Top Ten Food Safety Problems in the US Food Processing Industry

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The preventive controls at some food processing facilities are less rigorous than those of others, potentially increasing the risk of microbiological and chemical, as well as physical, food safety hazards. We used a four-round Delphi technique to interview nationally recognized food industry experts as a way to identify the ten food safety hazards that are of utmost concern for the food processing industry today, and the preventive controls that should address them. The expert panel members evaluated the frequency and severity of the food safety risk posed for five food processing industry sectors (baked, dairy, frozen, refrigerated, and shelf-stable goods, excluding meat and poultry products) and three plant sizes for each of the ten most important hazards identified. The experts collectively ranked deficient employee training as the top hazard facing food processors today, followed by poor plant and equipment sanitation and contamination of raw materials. Other hazards included poor plant design and construction, post-process contamination, difficult-to-clean equipment, and incorrect labeling and packaging. The expert panel's recommended controls can be used to determine the means to make our food supply safer.

P106 Residues of Antibiotic in Muscle, Kidney and Liver in Pigs

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Guidelines for the use of antimicrobials in animal production should include appropriate withdrawal times to avoid the presence of residues in meat. Residues in meat could contribute to the development of antimicrobial resistance strains and allergic reactions. The presence of residues could also cause economic loss to food producers/processors that use microbiological activity as part of the production process. Frequently, animals are presented to slaughter without observing appropriate withdrawal periods. The objective of this work was to document the presence of antimicrobial residues in porcine tissue by using a 3-plate

inhibitory method and different pH levels. Samples of liver, kidney, and muscle were collected from pigs in the Guadalajara metropolitan city area. Medium consisted of agar plates adjusted to pH 6, pH 1, and pH 8, inoculated with *Bacillus subtilis* ATCC6633. Of the 62 pigs evaluated, 31 (50%) were positive, 17 (27.4%) were negative, and 14 (22.6%) were inconclusive. Twenty-six (41.9%) of kidney samples were positive, 25 (40.3%) were negative, and 11 (17.8%) were inconclusive. Thirty-five (54.8%) of muscle samples were positive, 14 (22.6%) negative, and 14 (22.6%) inconclusive. Forty-one (66.1%) of live samples were positive, 13 (21%) negative and 8 (12.9%) inconclusive.

P107 A PCR-ELISA for Detection of Potential Sterigmatocystin and Aflatoxin Producing Fungi

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A sensitive and specific PCR-enzyme linked immunosorbent assay (PCR-ELISA) was developed to detect potential sterigmatocystin and aflatoxin producing fungi in food. The assay was based on the incorporation of digoxienin-labeled dUTP and a biotin-labeled primer specific for aflatoxin biosynthetic gene omt-A during PCR amplicfication. The labeled PCR products were bound to streptavidin-coated wells of a microtiter plate and detected by an ELISA. The specificity of PCR-ELISA was determined using 21 fungi strains, including aflatoxingenic fungal stains and non-aflatoxingenic fungal stains. All of the aflatoxin-genic fungal stains were positive.The PCR-ELISA, detecting as little as 10 to 4 ng aflatoxingenic fungal genomic DNA, was up to 10-fold more sensitive than conventional gel electrophoresis. Because of its microtiter plate format, PCR-ELISA is particularly suitable for large-scale screening and compatible with future automation.

P108 DSC Production and Characterization of Monoclonal Antibody against Pirimiphos Methyl

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Monoclonal antibody against the organophosphate insecticide pirimiphos methyl (Pir-M) was successfully generated by hybridoma technology and characterized. Myeloma cells were fused with spleen cells of BALB/c mice that were immunized with hapten conjugated via the carboxyl group to keyhole limpet hemocyanin (KLH). To evaluate the obtained Mab (MAb Pir-M-2E10-24, Pir-M-6G5-52), a competitive indirect ELISA procedure was developed. For the purpose of increasing the sensitivity and selectivity of the ELISA, several kinds of coating antigens conjugated with proteins such as bovine serum alumin and ovalbumin were employed. Among them, Pir-M-OVA was the most sensitive one. MAb Pir-M-2E10-24 was specially reacted with Pir-M but much less with its analogs. When Pir-M was spiked in tap water with different concentrations, the average recovery ratio was 100 ± 3%. This immunoassay was potentially a valuable analytical tools for the rapid and sensitive determination of pirimiphos methyl from various samples.

P109 Ranking Microbiological Food Safety Risks in New Zealand

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Risk ranking is driven by the premise that if the relative risks of a range of problems can be established, then risk reduction efforts can be directed at the most significant problems. Risk ranking has been characterized as a five-step process: risk definition and categorization, identification of risk attributes to be used as criteria, description of risks in terms of those attributes, the ranking process, and communication of results. The NZFSA has chosen food/hazard combinations as a risk definition and Risk Profiles for microbiological hazards in particular foods have been produced, which provide the New Zealand context for these combinations. This process is now well established (see: http://www.nzfsa.govt.nz/science-technology/risk-profiles/index.htm) and attention moves to the ranking of food safety issues for risk management. A multi-attribute approach was adopted, with food/hazard combinations being described according to attributes relevant to both public health and trade. Attributes include disease incidence, disease severity (morbidity and mortality), food consumption, frequency of food contamination by the hazard, importance of imports to domestic food consumption and importance of exports to the New Zealand economy. Quantitative information related to these attributes was collated from Risk Profiles into Risk Summary Sheets for each food/hazard combination, along with qualitative information on the importance of the food as a transmission vehicle for the hazard and existing risk management. Information from different Risk Summary Sheets can be used as criteria for Ranking Summary Tables to demonstrate ranking options. These will be used as the basis for a risk ranking process involving stakeholders during 2004.

P110 Mutagenicity and Recombinogenicity of the Unique Radiolytic Compound 2-Dodecylcyclobutanone in Short-term Genetic Toxicology Assays

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Ionizing radiation can induce the formation of a unique class of chemical compounds, called alkylcyclobutanones, in foods that contain fatty

acids, such as red meat and poultry. One of these compounds, 2-dodecylcyclobutanone (2-DCB), formed via the radiolysis of palmitic acid, is found in trace quantities (approximately 1 ppm) in the fat fraction of irradiated ground beef. Recently, it was claimed that 2-DCB produced a weak genotoxic response in a short-term genotoxicity assay, the Comet Assay. These results have been the source of considerable controversy due to the availability of irradiated ground beef in the US National School Lunch Program starting in 2004. Some consumer groups have erroneously claimed results from the Comet Assay as proof that irradiated foods are mutagenic and carcinigenic. However, the Comet Assay, which detects chromosome damage by increased electrophoretic mobility of DNA with strand breaks, has not been validated for detection of weak genotoxins, produces false positive results due to non-genotoxic cell death, and therefore has not been approved by international regulatory agencies. Review of the Comet Assay results by international regulatory agencies have determined the claims of 2-DCB genotoxicity to be questionable due to deficiencies in the experimental design and methodologies used. In order to more accurately assess the genotoxic potential of 2-DCB, it was tested in four different short-term genetic toxicology tests, the *Escherichia coli* TRP Reverse Mutation Assay, the Pro-Tox Gene Expression Profiling Assay, the *Salmonella* Mutagenicity Test, and the Yeast DEL Assay. 2-DCB did not induce mutations in the *E. coli* TRP Reverse Mutation Assay or the *Salmonella* Mutagenicity Test (0, 0.1, 0.5, 1.0, and 2.0 mg/ml 2-DCB), expression of DNA damage inducible genes (recA, umuDC, nfo, ada, dinD) in the Pro-Tox Assay (0, 0.13, 0.25 ,0.5 ,and 1.0 mg/ml 2-DCB), or intrachromosomal recombination in the Yeast DEL Assay (0, 0.63, 1.25, 2.5 and 5.0 mg/ml 2-DCB). The absence of genotoxic (mutagenic or recombinogenic) activity observed in these studies, using purified 2-DCB, are in agreement with the lack of genotoxic and teratogenic activity observed in previously conducted multi-generation feeding studies of laboratory animals (rats, mice, guinea pigs and rabbits) that used radiation-sterilized meat which contained 2-DCB as a unique radiolytic product.

P111 Patulin Reduces Glutathione Level and Enzyme Activities in Rat Liver Slices and Induces Lipid Peroxidation

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Patulin (PAT) reacts chemically with glutathione (GSH), and we have recently elucidated the structures of the major reaction products (Chem. Res. Toxicol. 13, 373, 2000). An attempt was now made to identify GSH adducts of PAT in precision-cut tissue slices from the liver of male Sprague-Dawley rats. After 6 h incubation with 50 to 200 μM, PAT had completely disappeared but none of the GSH adducts could be found. Under these conditions, PAT was virtually non-toxic for the slices as assessed by the release of lactate dehydrogenase. A concentration-dependent decline of the GSH level and the activity of GSH transferase was noted, e.g., slices incubated with 200 μM PAT contained only 25% of the GSH and 70% of the GSH transferase activity of controls. Phase I and phase II enzymes of drug metabolism were also reduced (by 45% with 200 μM PAT), as measured by the hydroxylation and conjugation of testosterone. On the other hand, incubation with PAT markedly increased lipid peroxidation in the slices. The effects of PAT on enzyme activities and lipid peroxidation may be a consequence of the GSH decline, which cannot be due to the direct reaction of PAT with GSH present in the slices at 10 to 13 mM concentration. Supported by Deutsche Forschungsgemeinschaft (Me 524/14-2).

P112 Development of a Consumer Food Safety Communication Strategy Using a Triangulation of Formative Research Methods

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To reduce the risk of food poisoning, food safety education is required to improve consumers' food safety behavior during domestic food preparation. Knowledge of cognitive antecedents that influence food-handling practices and identification of perceptions of food safety interventions are essential for the development of communication strategies. The aim of this research was to use the social marketing approach for development of a consumer food safety communication strategy. A triangulation of quantitative and qualitative formative research methods informed strategy development. Strategy formation was structured using the marketing mix framework and the social marketing approach. Risk based observation data detailing consumer food safety behaviors informed the overall goal of the strategy. Self-complete questionnaires were administered to consumers (n=100) to quantify attitudes and perceptions towards food safety risks in the home and food safety education. A series of focus groups provided an in-depth understanding of a targeted audience. Cross contamination of pathogens from raw chicken during domestic food preparation was the focus of the strategy. Data indicated that the home was an unlikely location for acquiring food poisoning and although quantitative findings indicated a positive consumer attitude towards cross contamination, qualitative findings showed that specific cross-contamination behaviors were misunderstood. Barriers related to the 'price' of implementing desired food safety behaviors included perceptions of optimistic bias and perceived "lack of time and thought". Identification of life-point-paths, preferred promotional types, formats and communication channels informed the "promotion" and "placement" components of the strategy. Examples of quantitative and qualitative findings that complemented each other will be discussed in the context of strategy development, providing valuable information for health educators and further communication initiatives.

P113 Survey Respondents' Attributions about Foodborne Illness: What Leads People to Believe That Certain Meals Made Them Sick?

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The Environmental Health Specialists-Network (EHS-Net), a network of federal and state health agencies, developed questions on beliefs and experiences about restaurants and foodborne illnesses. These questions were administered in a survey of 16,435 randomly-selected people in eight states. The results indicate that of the respondents who had experienced vomiting/diarrhea within the month prior to the survey, 19.7% believed that their illness resulted from a meal eaten outside the home (e.g., in a restaurant). When asked why they attributed their illness to that meal, respondents gave the following reasons: timing of the illness (35%); look or taste of the food (17%); illness of others who ate with them (11%); and meal was the only one they had eaten in that time period (2%). Those who attributed their illness to the meal because of the timing of their symptoms were asked how long after the meal they began to experience symptoms; 60% said that their symptoms began within 5 h of the meal. These results indicate that respondents frequently attribute their illness to restaurants, for a variety of reasons. Although the timing of symptoms was a primary reason for respondents' attribution of illness to the suspect meal, and the majority said that their symptoms began within 5 h of eating the meal, latency periods of 5 h or less are unlikely for most of the known foodborne pathogens. The results of this survey suggest that the respondents may not have a full understanding of foodborne illness.

P114 Status Quo of Food Safety Management in Small to Medium-sized South African Food Processing Companies

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As a result of recently introduced HACCP framework legislation in South Africa, many food processors now need to integrate food safety management systems into their operations at relatively short notice. Traditionally this is particularly problematic for smaller and medium-sized enterprises that often lack the necessary resources. Supplier food safety audits carried out on behalf of a national retailer confirmed that the most common shortfall was the lack of documented and properly managed prerequisite programs. It was determined that lack of management understanding, support and involvement contributed to the above-mentioned situation and food safety programs were frequently branded as an unnecessary expense rather than a tool to continued business success. In addition there was a noticeable failure to correctly assess and interpret the technical requirements of food safety programs which was directly linked to insufficient training at all levels of the companies surveyed. While monitoring of food safety-related processing parameters was evident in many of the businesses, a lack of corrective action in the case of deviations from control parameters was evident. Where monitoring and corrective action procedures were carried out these were generally not documented to acceptable levels. It was shown that in-service training to facilitate documentation and management of prerequisite programs for HACCP and food safety systems could bring about an almost immediate benefit to small and medium-sized food processors. Once compliance was achieved in these disciplines, HACCP-based systems could be successfully set up where appropriate.

P115 Evaluation of a "Train-the-Trainer" Project: HACCP Training for Food Service Managers

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The "Train-the-Trainer: HACCP Training in Foodservices in Illinois" program was developed through a partnership between university faculty and public health officials to assist foodservice managers in anticipation of HACCP implementation. Designed as a five-h session, this training covered two main areas: Part 1. Seven principles of HACCP; and Part 2. How to conduct in-house employee training. Twenty-eight workshops were conducted and 732 foodservice personnel were trained. Evaluation of training by participants indicated that Part 1, the seven principles of HACCP, was easily understood. The exercises, however, proved to be more difficult. They strongly agreed that the materials provided (a video, posters and other job aids) would stand alone as training materials for their workers. When asked when they would plan in-house training sessions, 54% said "within three months," 17% "within six months," and 29% replied that they were not sure. Surprisingly, only 30 managers (5.0%) applied for a cash reward for providing their own in-house employee training. A post-workshop survey after six months (18% response with 133 respondents) pointed out that while institutional foodservices were more likely interested than commercial establishments in HACCP, only a small number of attendees implemented HACCP. Furthermore, only 54 respondents reported performing in-house training using the material received from the workshop. The survey also confirmed that the most serious difficulties in implementing HACCP were insufficient time, employee resistance to change, and employee turnover. This result indicates that HACCP implementation may require further concerted effort within the industry with strong support from research and regulatory communities.

P116 Efficacy of a HACCP-based Foodhandling Training Program for Front-line Food Service Workers

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This article reports a collaborative HACCP-based safe food-handling training project by academicians and regulatory officials. The team developed a three-h training program featuring a 55-min video and several job aids. This training session was evaluated for effectiveness in enhancing the food safety knowledge and food handling practices of front-line workers. The sample trainees for this session consisted of thirty-seven front-line foodservice workers. To determine the degree of learning two measures were used. The first measure consisted of administering pre- and post-training quizzes (n=30), and the second consisted of observing and comparing on-the-job HACCP-based food handling practices by members of the test group both before and after the training session. The result showed a significant improvement in quiz scores ($P < .0001$) and a significant reduction in food handling violations four weeks after the training session ($P < .001$). However, correlation between the post-training quiz scores and the observation scores of individual trainees could not be established ($r = 0.07$, $P = 0.723$). This result indicates that knowledge did not influence the safe food handling behaviors for this sample group. Ironically, among the 12 food handling procedures observed, "washed hands when needed" was the most frequent violation, while "when and how to wash hands" was the best known knowledge as indicated by the largest number of trainees answering this quiz question correctly. In order to reinforce training into behavioral performance, factors such as managerial support for training, continuous supervision, and job environment may be more imperative than merely gaining knowledge.

P117 DSC A Predictive Model to Determine the Effect of Drying Temperature and Marination in Reducing *Listeria monocytogenes* Population during Drying of Beef Jerky

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The objective of this study was to model the effect of drying temperature in combination with pre-drying treatments on inactivation of *Listeria monocytogenes* on beef jerky. Beef slices were inoculated with a 10-strain composite of *L. monocytogenes* and then treated before drying with: i) nothing (C), ii) traditional marinade (M), or iii) 5% acetic acid solution for 10 min followed by M (AM). Samples were exposed to each of three drying temperatures (52, 57 or 63°C). In addition, sequential stresses (10% NaCl, followed by pH 5.0 and 45°C) were applied to the cells before inoculation for drying at 63°C. Survivors were determined on tryptic soy agar supplemented with 0.1% sodium pyruvate (TSAP) and PALCAM agar at 0, 2, 4, 6, 8 and 10 h during drying. Data were modeled using a linear regression (AM) and a logistic-based equation capable of fitting biphasic inactivation curves without initial lag (C and M). Total log CFU/cm^2 reductions of *L. monocytogenes* in control (3.9 to 5.1) and samples treated with M (3.5 - 5.4) were similar, while AM-treated samples had higher (6.1 to 6.8) reductions. Stressed inocula were more sensitive to inactivation in C and M products. All survival curves were characterized by an initial rapid decrease in populations within the first 2 h which was followed by a secondary death phase of a lower rate. No significant ($P \geq 0.05$) differences were observed among drying temperatures. Inactivation differences between stressed and unstressed cells were only minor. The results of this study indicated that the acidified pre-drying treatment increased pathogen inactivation during drying, regardless of drying temperature. The model developed may be useful in improving pathogen inactivation through use of appropriate interventions during beef jerky processing.

P118 Modeling the Effect of Aerobic and Anaerobic Storage on Growth/No Growth Interface of *Listeria monocytogenes* as a Function of Temperature, Sodium Lactate, Sodium Diacetate and NaCl

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There are numerous studies on the antimicrobial effect of sodium lactate (SL) and sodium diacetate (SD) on *Listeria monocytogenes* on cured meat products under vacuum at various temperatures; however, few studies have quantified this effect through predictive modeling. The objective of this study was to model the effect of aerobic and anaerobic storage on growth/no growth boundaries of *L. monocytogenes* as a function of SL, SD and temperature in the presence or absence of NaCl. A 10-strain composite of *L. monocytogenes* was inoculated (10^4 CFU/ml) in microplates containing tryptic soy broth with 0.6% yeast extract (TSBYE) supplemented with antimicrobials. Growth responses were evaluated turbidimetrically every 5 days at 674 aerobic and 674 anaerobic combinations of temperatures (4 to 30°C), SL (0 to 6% v/v), SD (0 to 0.5% w/v) without NaCl and with 2% NaCl, using a microplate reader. Data were modeled with logistic regression to determine the growth/no growth interfaces. Low temperatures (4 and 10°C) reduced the maximum concentrations of SL (0.75 to 1.5%) and SD (0.3 to 0.5%) that allowed growth of *L. monocytogenes*. The minimum inhibitory concentrations of SD

decreased with increasing SL levels. There was no difference in growth boundaries between 0 and 2% NaCl; this suggests that 2% NaCl, which is commonly added to cured meat products, does not necessary enhance the inhibitory effect of SL and SD on *L. monocytogenes*. Anaerobic storage strongly reduced the SL-SD growth limits of *L. monocytogenes* compared to aerobic storage, especially at low temperatures. The developed model showed good performance even with independent data from survival-growth of *L. monocytogenes* on cured meat products. The results provide quantitative data on the antimicrobial effect of SL and SD under anaerobic and aerobic storage, simulating vacuum packaging and retail display, respectively, and hence, address important considerations for risk assessment activities.

P119 Evaluation of Home-preparation Methods for Reduction of *Listeria monocytogenes* on Artificially Inoculated Frankfurters

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Proper storage and preparation methods by consumers represent the ultimate safeguards for minimizing the risks of foodborne illness. The effectiveness of commonly used home preparation methods in reducing *Listeria monocytogenes* on artificially inoculated frankfurters was evaluated. An electronic survey conducted revealed that grilling (29.3%) was the most common cooking method during warmer months, while boiling in water (24.4%) and microwave cooking (26.8%) were the alternatives during winter months. Frankfurters were purchased from a local retail store, inoculated with a five-strain cocktail of *L. monocytogenes*, vacuum-packaged and stored at 10°C for a minimum of 6 weeks. Inoculated frankfurters were removed from the package and prepared following common home preparation methods (grill, boiling water, microwave) for various time periods. The most effective treatment (~8 log reductions) to reduce levels of *L. monocytogenes* on frankfurters was boiling in water for at least 2 min. Grilling for 6 min at high temperatures and microwave heating for more than 1 min in the high setting was required to achieve > 6 log reductions in *L. monocytogenes*. Extended cooking times using these preparation methods compromised the organoleptic properties of frankfurters (burnt or distended frankfurters). Using visual standards of "doneness" as cooking indicators may not be adequate to determine the effectiveness of a particular preparation method to reduce/eliminate *L. monocytogenes* on frankfurters. Effective methods should be adopted for frankfurters and similar ready-to-eat meat products when such products are being prepared for consumption by at-risk populations such as young, elderly and immunocompromised.

P120 DSC Five State Epidemiological Survey of Four Farm Animal Types for *Listeria monocytogenes*

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Listeria monocytogenes (LM) is the leading cause of foodborne mortality. The farm environment may be a source of contamination for meat producing animals. This study compared the isolation of LM from swine, chicken, turkey, dairy cattle, beef cattle and their respective farm environments as affected by temporal, spatial and environmental factors to provide epidemiological information for LM that can be used in developing risk assessment and management strategies. During each season animal and environmental samples were tested for LM from twenty animal rectal swabs and duplicate 25 g environmental samples (soil, bedding, feed) from each farm, using a modified Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM) procedure. Riboprinting® was performed to confirm identity of isolates. Geographic Information Systems (GIS) was utilized for visualizing spatial relationships between the five states surveyed in this study. LM was isolated from 8.25% of all samples. Washington state samples had the highest incidence of LM isolates (12.2%), followed by Tennessee (8%), Alabama (7.6%), North Carolina (6.5%), and California (5.6%). Swine farms exhibited the highest number of LM isolates (27), followed by dairy cattle farms (26), poultry farms (24) and beef cattle farms (18) (n=288 for each farm). The spring sampling exhibited the highest number of LM isolates (37), followed by winter (31), summer (11) and fall (8). Identification of risk factors for LM isolation may provide direction for risk management decisions and will enhance risk management on the farm, thereby improving safety in the farm to table continuum.

P121 DSC Development of Predictive Models for the Survival of *Listeria monocytogenes* on Broth and Sausage as a Function of Temperature, pH and Antimicrobials

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This study was conducted to develop a predictive model on the effect of temperature, pH and antimicrobials against the growth of *Listeria monocytogenes* in broth system and spiked sausage using the Gompertz model. The inhibitory effect of *L. monocytogenes* in broth system and spiked sausage was greatly increased as temperature and concentration of antimicrobials increased,

and the strongest antimicrobial activity was observed in the combined potassium lactate and sodium diacetate, followed by potassium lactate and potassium sorbate. The value of lag time (LT) and specific growth rate (SGR) was obtained using the GraphPad prism program, and the R2 value obtained from the primary Gompertz model showed over 0.99. The values of statistical relative estimates over environmental factors (pH, Temp, antimicrobial) were obtained using the SAS program and then a secondary equation was developed from the values of statistical relative estimates, using Surface response model. The values of LT and SGR on the growth of *L. monocytogenes* in the broth system and spiked sausage were obtained from the developed secondary equation. Results showed that the values of the correlation coefficient (r) of SGR and LT in the broth system were 0.98 and 0.89, respectively, whereas the value of r in the sausage was over 0.99. These results indicate that the developed secondary model could be appllied to predict the growth of *L. monocytogenes* in sasauge containing potassium sorbate, potassium lactate and potassium lactate combined with sodium diacetate.

P122 Cross-contamination of Deli Meat by *Listeria monocytogenes* on a Commercial Slicer

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Listeria monocytogenes contamination of luncheon meat has resulted in outbreaks of listeriosis and major recalls of ready to eat meat products. *Listeria* contamination of processing equipment such as meat slicers is a known source of product contamination. This study was to determine the dynamics of cross contamination of *L. monocytogenes* from the blade of a commercial slicer onto sliced meat products. Three types of deli meats, oven-roasted turkey, salami, and bologna, with growth inhibitors of *L. monocytogenes* were tested. A five-strain mixture of *L. monocytogenes* was inoculated at 10^3 CFU onto the slicer blade. Five meat slices were packed per package, then vacuum-sealed, stored at 4°C, and sampled on 1 and 30 days after slicing. Two sample sizes, 25 g and the entire package, of meat were assayed. *L. monocytogenes* was detected in 1, 5 and >10% of bologna, salami, and turkey slices, respectively. A higher percentage of turkey meat samples were positive when the entire sample rather than a 25-g sample was assayed (27% vs. 13%), but similar percentages of bologna and salami samples were *L. monocytogenes*-positive for both sample sizes. The number of *L. monocytogenes*-positive samples increased during storage of turkey meat but decreased for salami and bologna. More sections of the slicer were *L. monocytogenes*-positive after slicing salami than turkey or bologna. These results indicate *L. monocytogenes* can be transferred from a highly contaminated slicer onto meats and survives or grows better on turkey than on salami or bologna with preservatives. Furthermore, sampling larger amounts of turkey meat results in more *L. monocytogenes*-positive samples; however, this was not observed for salami or bologna slices.

P123 Acquisition of Multi-drug Resistance in the Foodborne Pathogen *Salmonella* Newport

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Salmonella enterica serovar Newport is increasing in importance as a foodborne pathogen, in part due to the rapid emergence of strains resistant to five or more drugs. We mapped 100 clinical and veterinary isolates of *S.* Newport onto an organismal evolutionary tree to better understand the genetic relationships among Newport strains and to track the acquisition of antibiotic resistance in these pathogens. In order to trace the relatedness of Newport to other group I salmonellae, we used 70 strains of the *S. enterica* subspecies I reference collection (SARB), a set of strains representing a homogeneous grouping that includes the human pathogens. Analysis of trees based on sequences in 17 chromosomal genes revealed that Newport strains resistant to one to nine antibiotics divided into two disparate groups. Since multi-drug resistant strains all resided in one group, multi-drug resistance was likely acquired after divergence of the groups. In addition, as some of the isolates resistant to most antibiotics did not contain gene resistance cassettes in class I integrons, some drug resistances may be due to plasmid genes in these strains. Grouping the strains by antibiotic resistances, we found that aminoglycoside-resistant strains contain some isolates sensitive to Cefriaxone and some resistant; the same is true within aminoglycoside-sensitive strains. This indicates that the acquisition of antibiotic resistance did not proceed in a simple stepwise manner. We conclude that multi-drug resistance cannot be attributed to the clonal spread of a single ancestral Newport strain.

P124 A Comparative Study of a New Automated Rapid Test Method and an FDA/BAM Method for the Detection of Staphylococcal Enterotoxins in Food Samples

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Staphylococcal enterotoxins are associated with many food intoxication outbreaks. In one incidence in July 2000 in Japan, as many as 13,420 people were affected. Staphylococcal enterotoxins are also considered as a candidate for bioterrorism. Rapid and sensitive methods to survey foods are essential to ensure the safety of the food for consumption. Such methods allow the testing laboratory to test more samples and free personnel for other tasks. Automation further reduces demands on the time of the laboratory personnel. In this study, 65 food samples including raw and processed meats, salads, fresh fruit, vegetables, cakes and dairy products were screened for the presence of Staphylococcal enterotoxins by the new automated TECRA® UNIQUE™ Staphylococcal

Enterotoxins test method (SETUNQ) and the TECRA Staphylococcal Enterotoxins Visual Immunoassay™ (SETVIA), which is the FDA/BAM preferred test method. (Chapter 13. Staphylococcal enterotoxins, FDA/BAM 8th Edition.) Sample extracts were prepared for testing by the recommended protocols and spiked with Staphylococcal Enterotoxin B (SEB) or Staphylococcal Enterotoxin C (SEC) toxin at 0.0ng/mL, 0.25ng/mL and 1.0ng/mL. The extracts were then tested for the presence of Staphylococcal Enterotoxins. Results showed the SETUNQ automated rapid method to be comparable to the FDA/BAM method for screening for Staphylococcal enterotoxins with 97.8% agreement. All spiked samples returned positive results for both levels by both methods. The SETUNQ test kit provides a simple automated method to screen foods for Staphylococcal enterotoxins.

P125 DSC Evaluation of Gaseous Chlorine Dioxide for Its Effectiveness in Killing *Salmonella* on Blueberries, Raspberries, and Strawberries

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Gaseous chlorine dioxide (ClO_2) was evaluated for its effectiveness in killing *Salmonella* on small fruits. An inoculum (0.1 ml, ca. 5.5 log CFU) containing five serotypes of *Salmonella enterica* was deposited onto the skin, calyx area, or stem scar tissue of blueberries, skin of raspberries, and skin and stem scar of strawberries. Sachets formulated to produce gaseous ClO_2 at concentrations of 4.1, 6.0, and 8.1 mg/liter within 30, 60, and 120 min, respectively, at 23°C were used. The relative humidity of the air was increased by placing 20 ml of hot water (97°C) in a shallow dish in the bottom of the treatment chamber. Air was circulated with fans. Treatment with 8.1 mg of ClO_2/liter of air significantly ($P = 0.0001$) reduced the population of *Salmonella* on blueberries by 2.9 to 4.0 log CFU/g. Lethality to *Salmonella* was significantly higher when the inoculum was placed on the skin, compared to the calyx or stem scar tissue. Populations of *Salmonella* on strawberries treated with 8.1 mg of ClO_2/liter were reduced by 3.8 to 4.4 log CFU/g; significant reductions of 1.6 log CFU/g of raspberries were achieved. Reductions in yeast and mold populations on blueberries, strawberries, and raspberries were 1.2 to 3.0, 1.4 to 4.1, and 2.6 to 3.0 log CFU/g, respectively. Lethality of ClO_2 to *Salmonella*, yeasts, and mold was higher when fruits were treated at 70 to 96% relative humidity rather than at lower relative humidities. Gaseous ClO_2 shows promise as a sanitizer for small fruits.

P126 DSC Fate of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* spp. in Reduced Sodium Beef Jerky

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Interest in low-sodium food products necessitates re-examination of home preservation processes relying in part on salt for antimicrobial effects. The fates of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp. in reduced-sodium home-style beef jerky were determined. Ground or whole beef strips, with different salt levels, were inoculated with the pathogens. Samples were either dried in a 60°C dehydrator or heated to an internal temperature of 71.1°C prior to drying in a 60°C dehydrator. Populations were determined at time 0 and 2 h intervals until dry. Population reductions were greater in ground beef jerky with non-reduced salt levels compared to that with reduced salt levels, and in most cases, greater reduction (1.0 to 1.5 logs) was observed for ground beef strips heated prior to drying. During the drying stage for whole and ground beef jerky, there were differences in the number of viable microorganisms when salt levels were compared, with less lethality in the reduced salt samples. Ground beef strips made with reduced sodium resulted in less pathogen reduction. Preparing ground beef jerky with reduced salt is a potential food safety hazard since the lower salt level did not reduce pathogen populations as much as the regular salt treatment. For dried whole jerky strips, there generally were no significant differences ($P > 0.05$) in pathogen populations between the non-reduced and reduced salt marinade in the end product. The results from this study support the importance of the antimicrobial effect of sodium chloride in particular products on the pathogens used in this experiment.

P127 DSC Variation in Acid Resistance among *Escherichia coli* O157:H7 Strains in a Model Stomach System

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Although some strains of *E. coli* O157:H7 have been shown to be acid resistant (which could account for their low infectious dose and their ability to contaminate acidic foods), there is considerable variation among strains in their ability to survive acidic environments. Previous studies in our laboratory quantified variation in acid resistance among *E. coli* O157:H7 for three separate acid resistance mechanisms. While acid resistance ability was measured individually for each mechanism, the potential exists for the mechanisms to work simultaneously in complex environments, such as the stomach. To characterize variation in acid resistance in complex acidic environments compared to variation using a specific acid resistance mechanism, 8 strains of *E. coli* O157:H7 were selected and tested for survival in a model stomach system (MSS) (pH 2.0). Variation in death rates increased in the MSS, with average death rates using the glutamate mechanism of acid resistance (pH 2.0) and in the MSS of 0.17 ± 0.1 and 0.43 ± 0.2 log decrease per h, respectively. Exposure to mildly acidic environments prior to extreme acid has been shown to increase the acid resistance of *E. coli*. Strains were tested for survival in the MSS after storage in apple juice (pH 3.5) at 22°C and at 4°C.

After 24 h in apple juice at 4°C, the average death rate in the MSS increased and variation decreased, to 0.18 ± 0.08 log decrease per h. These results indicate that storage in an acidic food can reduce variation in acid resistance, and could increase survival in the stomach.

P128 DNA-based Microarray Detection of *Escherichia coli* O157:H7

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Genetic-based microarray technology provides the base for a rapid and reliable foodborne pathogen detection system. This system could be an improvement over conventional methodologies in that exacting cultural conditions will not be required and numerous pathogens can be detected simultaneously. The objective of this research was to select target genes and test a model oligonucleotide microarray that detects key pathogenesis genes from *E. coli* O157:H7 as a tool to improve environmental monitoring of the organism. Target genes were selected from those with the most available functional and bioinformatic information. Experiments were conducted with *E. coli* O157:H7 DNA extracted using the Sigma GenElute kit. Nonpathogenic *E. coli* DNA was used as a control. A direct DNA hybridization protocol using amino allyl-dUTP fluorescence labeling was employed for the microarray assessment. Three levels of DNA were labeled (100, 10, and 1.0 ng) and hybridized to the array. Four genes exhibited a linear quantifiable decrease in sensitivity down to 1.0 ng level. These genes included intimin, adhesin, shiga-like toxin 1, and the translocated intimin receptor, and they will be used for future experiments and in the development of a multiple pathogen array. Several genes, including secreted proteins EspD, EspB, EspA, and integrase, did not provide acceptable levels of repeatability or sensitivity and were eliminated from further experimentation due to this poor response. The results of these experiments provide validation for microarray-based detection systems and serve as a model for the development of a multiple food pathogen microarray.

P129 DSC Occurrence of *Escherichia coli* O157:H7 in Multiple Farm Environments across the United States

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In the United States, foodborne outbreaks caused by *Escherichia coli* O157:H7 have often been linked to the consumption of contaminated undercooked ground beef. However, the occurrence of *E. coli* O157:H7 in other animals has also been reported. The objective of this study was to determine incidence and gather epidemiological information to determine occurrence of *Escherichia coli* O157:H7 in cattle, swine and poultry and the farm environment. Environmental samples (n=648) and fecal swab samples (n=328) from eight locations in the US were collected over 16 months. Overall, in fecal swabs *E. coli* O157:H7 was positive (latex agglutination confirmed) in 6% of beef cattle (n = 328), 5% of dairy cattle (n=328), 9% of swine (n=326), 5% of turkeys (n=80) and 1% of chickens (n= 224). *E. coli* O157:H7 was isolated infrequently from environmental samples (1 to 3%). Washington State had the highest incidence of *E. coli* O157:H7 in beef, dairy cattle and swine followed by Tennessee, California, North Carolina and Alabama, respectively. No trends could be established for poultry. *E. coli* O157:H7 was isolated from feed samples (up to 3 samples) from the states of Tennessee and Washington. Since *E. coli* O157:H7 were seldom isolated from feeds or bedding, sampling for *E. coli* O157 at the farm should focus on the feces or fecal swabs for optimal recovery. The incidence of *E. coli* O157 in swine and turkey was surprisingly high, which indicates that swine and poultry may serve as unexpected vectors for foodborne outbreaks caused by *E. coli* O157.

P130 An Eight-hour Presence/Absence Test for *Escherichia coli* O157:H7 in Ground Beef

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In the food industry, there is a zero tolerance for the presence of *Escherichia coli* O157:H7. Ground beef samples have challenged many of the rapid tests on the market today, because they yield a higher number of false positive results. At Advanced Analytical, we have developed an effective rapid test for *E. coli* O157:H7 that can be completed in less than 8 h. Sixty-five g of freshly ground beef were placed in a sterile stomacher bag and 100 ml of mEC+n was added. Samples were stomached and an additional 485 ml of mEC+n was added. Approximately 20% of the samples tested were spiked with 1-2 CFU *E. coli* O157:H7 (ATCC 35150). The bags were then placed at 37°C and shaken for 7 hours. Samples were 40 μm filtered and centrifuged. The pellet was resuspended in buffer and 3 μm filtered. A blocking agent and *E. coli* O157:H7 antibody conjugated to Alexa 647 were added and the samples were incubated for 15 minutes. The samples were then analyzed on the RBD2100 flow cytometer, and unlabeled material was plated on SMAC-CT. Unlabeled samples were also plated at 24 hours and latex agglutination tests were preformed to verify the presence/absence of *E. coli* O157:H7. In all, 161 samples were tested. A false positive rate of <4% was achieved. A same shift test for *E. coli* O157:H7 would allow processors to ship out meat the day it was ground. This could result in lower numbers of recalls and fewer human casualties caused by this pathogen.

P131 DSC Comparison of Antibiotic Resistance and Genotyping of *Campylobacter jejuni* Isolated from Chickens and Turkeys at Retail

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Campylobacter jejuni has been recognized widely as a foodborne pathogen associated with the consumption of a variety of food products, including poultry meat. To determine genetic variability of *C. jejuni* found on chickens and turkeys at retail and the levels of antibiotic resistance associated with these isolates, one hundred *C. jejuni* isolates were obtained from 25 retail chicken carcasses, 25 retail chicken giblets, 25 retail turkey carcasses, and 25 retail turkey giblets. Following confirmation of each isolate as *C. jejuni*, all isolates were genetically characterized using pulsed-field gel electrophoresis (PFGE) and antibiotic resistance profiles were determined for each. PFGE results indicated a high level of variability between *C. jejuni* isolated from chickens and *C. jejuni* isolated from turkeys. However, PFGE patterns of C. jejuni isolates from retail chicken carcasses and chicken giblets were approximately the same. PFGE patterns of *C. jejuni* isolated from retail turkey carcasses and turkey giblets also were similar but differed from chicken isolates. Antibiotic resistance profiles indicated that *C. jejuni* possessed variable levels of resistance to multiple antibiotics. Antibiotic profiles were similar among isolates obtained from retail chicken carcasses and chicken giblets. *C. jejuni* isolated from retail turkey carcasses and turkey giblets shared similar antibiotics profiles but were different from chicken isolates. The results of this study indicated that different *C. jejuni* strains colonized chickens and turkeys.

P132 The Effect of *Campylobacter jejuni* Population Density on Survival and AI-2 Production at Varying Oxygen Levels

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The microaerophile *Campylobacter jejuni* is a significant foodborne pathogen, which carries the luxS gene responsible for a quorum sensing response involving autoinducer-2 (AI-2). Our results showed that *C. jejuni* strain 81-176 culture supernatants (conditioned media) elicit light in the *Vibrio harveyi* AI-2 bioluminescence assay. The minimal onset time to light induction, which is related to elevated AI-2 levels, was observed when *C. jejuni* population densities reached approximately 10^8 CFU/ml. A *C. jejuni* mutant containing a luxS deletion was constructed and culture supernatants from this mutant resulted in onset times similar to those of the media controls in the bioluminescence assays. Growth and survival under microaerobic and atmospheric oxygen conditions showed no differences between the wild type and luxS mutant. Both strains also survived longer under the aerated conditions at higher cell densities (10^7 to 10^8 CFU/ml) than at lower cell densities (10^6 CFU/ml or lower). Interestingly, conditioned media from either the wild type or luxS mutant prolonged the survival of *Campylobacter* in aerated cultures at lower cell densities (10^6 CFU/ml or lower) compared to survival in fresh media. Such a protective effect was most pronounced in media harvested from 24-h or older cultures and were not directly correlated with the levels of AI-2 production. This finding suggests that *Campylobacter* exudates may contain a non-AI-2 quorum sensing signal that is able to induce protective responses at higher oxygen levels. Alternatively, the exudates may contain secreted proteins that can provide direct beneficial effects related to survival in environments of elevated oxygen levels.

P133 DSC Occurrence and Resistance to Antibiotics of Thermophilic *Campylobacter* spp. in Farm Animals

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Animal and environmental samples from five states (Tennessee, Alabama, North Carolina, California, and Washington) were collected between August 2002 and December 2003 to determine occurrence of *Campylobacter* spp. (*C. jejuni*, *C. coli* and *C. lari*) in cattle (dairy and beef), pigs, and poultry (broilers and turkey) and resistance to antibiotics. A total of 388 *Campylobacter* spp. were isolated from feces collected from rectal swabs, soil samples, and bedding material. Washington state samples were most frequently contaminated with *Campylobacter* isolates (30%), while lower rates of contamination were found in California (22%), North Carolina (21%), Tennessee (18%), and Alabama (9%). *Campylobacter* spp. were detected in 45% of the pigs, 22% of the dairy cattle, 14% of the broilers, 12% of the beef cattle, and 8% of the turkeys examined. Among these, *Campylobacter coli* represented 44% of the confirmed *Campylobacter* spp., compared to 39% and 4% for *C. jejuni* and *C. lari*, respectively. *C. coli* was most frequently isolated from pigs (86%). *C. jejuni* was most frequently isolated from dairy cattle (63%). The resistance patterns of strains of *Campylobacter* isolates were investigated for antibiotic resistance using the agar disk diffusion method in Mueller-Hilton agar supplemented with 5% lysed sheep blood. No species was observed to be consistently resistant to any one antimicrobial agent. *Campylobacter* isolates were frequently resistant to tetracycline, clindamycin and erythromycin, with *C. coli* demonstrating multiple resistances. Of the eight antimicrobial agents investigated, least resistance was observed with chloramphenicol and gentamicin.

P134 DSC Identification of the Cause of Apparent Growth of *Clostridium perfringens* at 4.4°C

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Preliminary experiments in our lab showed an increase in colony forming units (CFU) of *Clostridium perfringens* at 4.4°C after cooling from 54.4 to 4.4°C in 9 h and holding for several h at 4.4°C. Additional experiments (including protein biomass measurements using the Modified Lowry Method, and observation of culture broth under the microscope) were conducted to explain these unusual results. Measurement of cell protein concentrations revealed increasing cell biomass concomitant with CFU increase during the cooling process. While CFU increase halted at 18°C, protein concentration continued to increase until 10°C was reached. Addition of chloramphenicol (a known protein synthesis inhibitor), at 18°C stopped any increase in protein concentration and any subsequent increase in CFU at 4.4°C. Microscopic observation revealed that *C. perfringens* cells appeared as short rods during the early part of the cooling process. Once the incubation temperature reached 19°C, cells elongated until the incubation temperature reached 4.4°C. After several h at 4.4°C, elongated cells were seen to break apart into smaller cells, corresponding with the apparent increase in CFU. We conclude from these results that the "apparent growth" was due to cell elongation as a result of low temperature stress, followed by subsequent division of those elongated cells. This finding is of great practical significance since apparent growth at 4.4°C might cause otherwise acceptable cooling regimes to violate the United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) performance standard, which allows a 1 log colony forming unit (CFU)/g increase of *C. perfringens* during cooling.

P135 Fate of *Enterobacter sakazakii* ATCC 12868 in Temperature-abused Reconstituted Infant Formula Containing Selected Probiotic Cultures

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Enterobacter sakazakii is an emerging foodborne pathogen that has caused fatal cases of neonatal meningitis. This study evaluated the antimicrobial efficacy of probiotic bacteria against *E. sakazakii* ATCC 12868 in reconstituted dried infant formula. Reconstituted infant formula was inoculated with *E. sakazakii* alone (control) or with *Lactobacillus acidophilus* 74-2, *Pediococcus acidilacticii*, or *Enterococcus faecium* M-74, then held at 30°C or 35°C . Microbiological analyses and pH tests were conducted at 4, 8, 12, and 24 h. Probiotic bacteria were enumerated on acidified MRS agar (pH 5.4) following anaerobic incubation (37°C) for 72 h. *E. sakazakii* was enumerated on violet red bile glucose (VRBG) agar after 24 h at 37°C. Initial populations of *E. sakazakii* and each probiotic culture were ~10^2 CFU/ml and 10^9 CFU/ml, respectively. At 12 h, *E. sakazakii* in controls at 30 and 35°C increased to 6.54 and 7.95 log CFU/ml, respectively. Probiotic cultures inhibited growth of *E. sakazakii* at 35°C with *E. faecium* exhibiting the strongest inhibition at both 30 and 35°C. At 12 h, *E. sakazakii* in formula (30°C) with *E. faecium* decreased to 0.59 log CFU/ml and was undetected (< 1.0 CFU/ml) at 35°C. The pH of formula (30°C) with *E. faecium* decreased from 7.06 at 0 h to 5.32, 4.96, and 4.77 at 4, 8, and 12 h, respectively; at 35°C, the pH decreased to 4.93 (4 h), 4.87 (8 h) and 4.52 (12 h). These results demonstrate that *E. faecium* M-74 can inactivate *E. sakazakii* in temperature-abused rehydrated infant formula.

P136 DSC Prevalence of Zoonotic Enteric Bacterial Pathogens in Dogs and Cats with Diarrhea

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With the discovery of the human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS), concerns about dangers of pet ownership have increased considerably. Zoonotic organisms associated with cats and dogs may cause life-threatening infections in immuno-suppressed human beings. The objectives of this project were to determine the prevalence of potential zoonotic enteric pathogens (*Salmonella*, *Listeria*, and *Campylobacter*) from feces of dogs and cats with diarrhea and feces of healthy dogs and cats and to determine the association of diarrhea in dogs and cats with diarrhea in human beings sharing the same household. Feces and fecal swabs were collected from dogs and cats during a chronic or acute episode of diarrhea by their veterinarian, using conventional office practice, and placed into transport tubes. Methods of bacterial isolation and identification followed conventional BAM protocols. Owners of pets participating in the study were interviewed using a questionnaire that focused on identifying association of diarrhea in human beings living in the same household with affected pets. *Salmonella* and *Listeria* spp. were isolated from 1 to 2% of dogs and associated with acute diarrhea. *Campylobacter* was isolated from 1% of dogs and was associated with chronic diarrhea. *E. coli* was isolated from feces in 50% of dogs and cats with diarrhea. The low incidence of enteric pathogens in dogs and cats having acute or chronic diarrhea shows that the risk is low for transmission to humans. However, individuals who are immunocompromised should have animals with acute or chronic diarrhea checked by a veterinarian and consistently follow sound sanitary practices with companion animals.

P137 Critical Parameters in Collaborative Ring Trials of PCR-based Methods for Detection of Foodborne Pathogens

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Lack of validation and standard protocols, as well as variable quality of reagents and equipment, influences the efficient dissemination of PCR methodology from expert research laboratories to end-user laboratories. Moreover, the food industry understandably requires and expects officially approved standards. Recognizing this, in 1999 the European Commission approved the research project, FOOD-PCR (www.PCR.dk), which aimed to validate and standardize the use of diagnostic PCR for detection of pathogenic bacteria in foods. Validation aims at confirming the specificity and reproducibility of the method when used by different laboratories. A common procedure to obtain validation data is an inter-laboratory study, called a "ring-trial", in which the performance of the method is tested using identical material in several laboratories, under control of a supervising laboratory. The Central Science Laboratory coordinated the ring trails within FOOD-PCR, and during their course several steps were identified which are critical to the evaluation of PCR-based methods. These include sample preparation, standard operating procedure formulation, the method of shipment of pathogen-containing samples, thermocycler and consumable requirements of trial participants, quality control of methods, reporting and evaluation of participants' data, and statistical analysis of trial results. These parameters will be detailed, with examples of potential problems and their resolution within FOOD-PCR. In any future validation exercise, careful consideration of each parameter should ensure the successful evaluation of PCR-based methods, thus facilitating the provision of future standard procedures for detection of foodborne pathogens.

P138 3M™ Petrifilm™ Environmental Listeria Plate for the Rapid Enumeration of *Listeria* from Environmental Surfaces

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Accurate and rapid detection of *Listeria* is of significant interest to the food industry. The 3M™ Petrifilm™ Environmental Listeria Plate has been developed in order to provide a *Listeria* result from environmental samples within 26 to 30 h. This study compared the Petrifilm plate method with ISO Method 11290-2 using PALCAM agar for detection and enumeration of *Listeria*. Studies of 88 strains demonstrated that the Petrifilm Environmental Listeria Plate has a sensitivity of 90% and specificity of 100%, while the PALCAM agar method resulted in values of 96% and 97%, respectively. Three different surface types and nine different collection devices were used in the enumeration portion of the study. The surfaces were artificially inoculated with *Listeria* and background microbiota. Both sponges and swabs were used as collection devices. After a 60 to 90 min repair step at 20 to 30°C in buffered peptone water, the samples were plated onto Petrifilm plates and onto PALCAM agar plates. Results showed that, on average, the Petrifilm plate method was not statistically different ($P > 0.05$) from the ISO method for the enumeration of *Listeria*.

P139 Comparison of Polymerase Chain Reaction (PCR) and USDA Culture Procedure to Detect *Listeria monocytogenes* in Deli Meats

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A polymerase chain reaction (PCR) assay (Bax®, DuPont, Wilmington, DE) for *Listeria monocytogenes* in meat products has been approved recently by the US Department of Agriculture (USDA). In addition, USDA has promoted using a larger sampling size such as the entire package of meat for a more sensitive assay. The purpose of this study was to compare the Bax® system and USDA broth enrichment method to detect in deli meat *L. monocytogenes* introduced by a contaminated slicer. Three types of deli meat, oven-roasted turkey, salami, and bologna, were tested. A five-strain mixture of *L. monocytogenes* at 10^2 CFU was inoculated on the blade of a commercial slicer. Five slices were added to each package; packages were vacuum sealed, stored at 4°C, and packages (n=132) were sampled on 1, 30, 60, and 90 days post slicing. Meats sliced before inoculation were used as negative controls. Eight control packages were inoculated with 10^1 or 10^2 CFU *L. monocytogenes* to serve as positive controls. The most probable number (MPN) method was used to enumerate *L. monocytogenes* populations. For salami and bologna, a maximum of two samples were positive on each sampling day. *L. monocytogenes* populations were very low on the positive samples, and gradually decreased during storage. The number of *L. monocytogenes*-positive samples determined by PCR and the broth enrichment method were very similar. Our results indicate that detection efficiency is comparable for PCR and the USDA broth enrichment method, and salami and bologna did not support the growth of *L. monocytogenes* throughout 90 days of refrigerated storage.

P140 Evaluation of a Lateral Flow Immunoassay for the Detection of *Listeria* spp. in a Variety of Foods

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The presence of *Listeria* species on environmental surfaces and ready to eat foods (RTE's) is an important indicator of hygiene in the food industry. In this study, we examined the performance of the RapidChek *Listeria* lateral flow immunoassay with the RapidChek 40 h, one step enrichment procedure to detect *Listeria* spp. in

environmental sponges and 12 types of RTE foods spiked with an assortment of *Listeria* species at 3 log CFU/25g sample in replicates of 20. Five non-inoculated controls were evaluated for each matrix. The samples were cold stressed for 48 h at 4°C, and enriched using the FSIS/MLG or FDA BAM protocol as appropriate, or the RapidChek 40 h protocol followed by screening using the RapidChek test strips. All positive samples were streaked to MOX media and further confirmed using standard biochemical testing. Samples from the FSIS/MLG or FDA BAM enrichment procedures were streaked to MOX media and confirmed using biochemical testing as the primary detection method. The FSIS/MLG and FDA BAM methods reported 194 of the 260 spiked samples positive for *Listeria* spp. The RapidChek 40 h method reported and confirmed 218 of the 260 spiked samples as positive. The results of this study suggest that the RapidChek 40 h methods demonstrate similar performance to the FSIS/MLG and FDA BAM 48 h methods for the detection of *Listeria* spp. in a variety of foods, while the 1 step enrichment method provides an improvement in time and labor for *Listeria* spp. testing.

P141 Detection of *Listeria* spp. in Environmental Samples by a Combination of Wet Composites and a Novel Immunocapture Method

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PATHATRIX is a novel immunocapture method whose unique feature is that the entire sample plus pre-enrichment are re-circulated over antibody-coated paramagnetic beads. Environmental samples (n=40) were taken by the sponge method on various types of equipment in a processing facility, and added to a stomacher bag with 225 ml of Buffered Listeria Enrichment Broth (BLEB). Seven sponge samples were inoculated with either *Listeria monocytogenes* or *Listeria welshimeri* and twenty-eight were left un-inoculated as negative control samples. Five random samples were taken to observe *Listeria* spp. contamination present naturally. Positive broth controls of BLEB spiked with *L. monocytogenes* or *L. welshimeri* without sponge were also included in the sample set. All BLEB + samples were incubated overnight (~19 h) at 30°C. Samples were pooled in a ratio of one inoculated sample to 4 negative samples by taking a 50 ml aliquot from each bag and compositing for a total of 250 ml. Composite samples were placed on the Pathatrix unit for analysis, with re-circulation of the sample over the magnetic capture phase for 30 min at 30°C. The bead/bacteria complex was re-suspended in 200 µl of buffered peptone water and 100 µl was spread onto PALCAM and MOX respectively, and incubated for 18 to 24 h at 30°C. One hundred percent correlation was observed on the plates between the spiked samples and recovery of *Listeria* spp. colonies, with little or no background flora present. By combining wet composites it was possible to achieve economies in time and media without a loss of sensitivity.

P142 Evaluation of CHROMagar™ Listeria with Spiked Hot Dogs

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BBL™ CHROMagar™ Listeria (CL) is intended to isolate and differentiate *Listeria monocytogenes* and is based on the original CHROMagar™ Listeria medium (CHROMagar, Paris, France). This study compares recovery of *L. monocytogenes* and *Listeria* species from spiked hot dog samples on CL to other recommended media: Oxford (OX), Modified Oxford (MOX), PALCAM (PAL), BCM™ *Listeria monocytogenes* (BCM) and RAPID'L.Mono (RLM). Sixteen ATCC™ and clinical/environmental strains of *L. monocytogenes* and nine *Listeria* species were spiked into 225 mL of enrichment broth containing a 25 g hot dog sample. Both low and high-level inocula were tested; spiked samples were subcultured after 24 and 48 h of incubation. Low-level recoveries of *L. monocytogenes* at 24 h were: CL and OX 16/16 (100%), MOX and BCM 15/16 (94%), PAL 14/16 (88%) and RLM 12/16 (75%). After 48 h, PAL recovery improved to 15/16 (94%); results remained the same for other media. High-level recoveries of *L. monocytogenes* at 24 h were: CL, OX, MOX and BCM 16/16 (100%), RLM 15/16 (94%) and PAL 14/16 (88%). Results were unchanged for CL, OX, MOX and BCM with a slight decrease in sensitivity for PAL and RLM at 48 h. Of the nine *Listeria* species tested, two would require additional testing to rule out *L. monocytogenes* when CL and RLM were used, nine when OX and MOX were used, eight when PAL was used and zero when BCM was used. BBL™ CHROMagar™ Listeria offers excellent recovery of *L. monocytogenes* and very good specificity when testing spiked hot dog samples.

P143 Evaluation of a New Chromogenic Medium for the Detection of *Listeria monocytogenes*

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Oxoid Chromogenic Listeria Agar (OCLA) is designed to isolate all *Listeria* species and differentiate pathogenic species from non-pathogenic species. The medium contains the chromogen X-glucoside which is utilized by *Listeria* spp., resulting in blue colonies. It is selective for *Listeria* spp. and differentiates pathogenic species by halo production due to lipase activity. Isolates obtained from pure cultures (n=95), including *L. monocytogenes*, *L. ivanovii*, *L. innocua*, other *Listeria* spp. and non-*Listeria* spp., were examined for colony morphology and recovery rates on OCLA and Oxford Medium (after 24 h and 48 h at 37°C). Naturally contaminated foods, and pre-screened foods spiked with dilutions of 3 *L. monocytogenes* strains, were tested quantitatively and qualitatively according to ISO 11290:1997 using OCLA and Oxford Medium. Recovery rates of both media were within 0.5 log CFU/ml of each other for all sample types tested except for pure cultures of

L. ivanovii that were not recovered on Oxford Medium at 48 h. Based on colony morphology, there were significantly more *L. monocytogenes* recovered on OCLA than on Oxford Medium after 24 h incubation from naturally contaminated foods (McNemars test, 5% level, test value 8.1, table value 3.84). Compared with Oxford Medium, OCLA was more selective, more differential and more rapid and is recommended as a first choice selective medium for the enumeration and detection of *L. monocytogenes*.

P144 Evaluation of a New Rapid Screening Kit for the Differentiation of *Listeria monocytogenes* from Other *Listeria* Species

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Oxoid Biochemical Identification System (O.B.I.S.) mono is a ten min test to differentiate *Listeria monocytogenes* from other *Listeria* spp. The test is based on the ability of all *Listeria* spp., except *L. monocytogenes*, to hydrolyse D-alanyl substrates. If hydrolysis by D-alanyl aminopeptidase has occurred, addition of a solution of p-dimethylaminocinnamaldehyde (DMAC) produces a purple color reaction. This reaction is used in the presumptive differentiation of *L. monocytogenes* from other *Listeria* spp. In this study, a total of 182 pure cultures were used (including *L. monocytogenes*, other *Listeria* spp. and 5 "*Listeria*-like" organisms) that had been fully characterised using conventional methods. Cultures were inoculated onto a Tryptone Soya Agar purity plate and a chromogenic *Listeria* medium. All plates were incubated at 37°C for up to 24 h. Four to five colonies of each culture were then selected and touched onto a test card. Following addition of substrate buffer, the card was incubated at 37°C for 10 min. Developing solution was then added to the test zone, where the reaction developed immediately. All non-*L. monocytogenes* strains (n = 92) produced rapid and strong positive D-ala reactions indicating that they were not *L. monocytogenes*, producing a specificity of 100%. All *L. monocytogenes* strains (n = 90) were negative for D-ala and were, therefore, correctly differentiated using OBIS mono, producing a sensitivity of 100%. OBIS mono is recommended as a simple and rapid tool for the differentiation of *L. monocytogenes* from other *Listeria* spp.

P145 A Rapid Method for Detection of *Escherichia coli* O157:H7 Using Dynabeads® and FT-IR Spectroscopy

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FT-IR spectroscopy is a rapid, non-destructive method that has been used for ident-ifying and classifying microbial pathogens. Current sample preparation methods for FT-IR require 24 to 48 h and identify pathogens from colonies instead of selectively isolating from a complex matrix. Dynabeads® have been successfully used for selective isolation of *E. coli* O157:H7 upon selective enrichment by capture on magnetic beads coated with antibodies for *E. coli* O157:H7. Our objective was to combine the Dynabeads® assay with FT-IR spectroscopy to evaluate a rapid protocol for detecting *E. coli* O157:H7. *E. coli* O157:H7 and *E. coli* K12 strains were used. A commercially available Dynabead® anti- *E. coli* O157:H7 test kit was used for analysis. After capture of the pathogen onto the beads, 35 μL of bead-bacteria complex was air-dried on a gold slide under a bacteriological hood. Spectra were collected by reflectance measurements on a Continuum™ FT-IR microscope (256 scans, 4 cm^{-1} resolution). Spectra of *E. coli* O157:H7 captured on surface of the Dynabeads® were then analyzed using the TQ analyst program. Discriminant analysis was applied to the fatty acid (3000 to 2900 cm^{-1}) and amide regions (1700 to 1620 cm^{-1}). Mahalanobis distances were measured to quantify the spectral differences between the Dynabeads® that captured *E. coli* O157:H7 and those that captured the non-pathogenic strain. Discriminant analysis was able to classify and differentiate *E. coli* O157:H7 from *E. coli* K12 with a performance index of greater than 85%. The use of Dynabeads® in conjunction with FT-IR proved to be a rapid assay (<6 h) for detection of *E. coli* O157:H7.

P146 Automated Microwell-format DNA Hybridization Assays and Immunoassays for Pathogens in Foods

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Two previously described microwell-format DNA hybridization assays for *Salmonella* spp. and *Listeria* spp., and a new immunoassay for *E. coli* O157:H7, have been adapted to an off-the-shelf automated immunoassay analyzer. All steps subsequent to sample preparation (lysis for the DNA hybridization assays, boiling for the immunoassay) are performed by the instrument in a totally walk-away mode. This development provides food testing laboratories with a tool for high throughput pathogen testing with minimal labor. The system is also capable of performing two or more assays simultaneously. Extensive comparisons were made between the automated and manual assays, and between the automated assays and reference culture methods (e.g., BAM/AOAC, USDA-FSIS). Comparison of the colorimetric endpoints (OD450) of manual and automated assays performed on the same samples produced correlation coefficients of 0.92 for the *Salmonella* assay, 0.96 for the *Listeria* assay, and 0.99 for the *E. coli* O157:H7 assay, showing that the manual and automated assays generate comparable results. In the same trials, result interpretations (positive or negative) of the automated assays were compared to results of reference culture methods. Agreement rates obtained were 99.5% for the *Salmonella* methods, 96.3% for the *Listeria* methods, and 99.4% for the *E. coli* O157:H7 methods. An additional finding was that the automated assays

yielded fewer false-positive results than the manual versions, presumably due to the better-controlled and more consistent microwell washing afforded by the instrument.

P147 DSC Comparison of Conventional Culture Methods and FTA® Filtration-nested PCR for the Detection of *Shigella* spp. on Tomato Surfaces

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In this study, the detection of artificially inoculated *Shigella boydii* or *S. sonnei* was evaluated using enrichment protocols of the US Food and Drug Administration's Bacteriological Analytical Manual (FDA BAM; 1998), the Compendium of Methods for the Microbiological Examination of Food (CMMEF; 2001), enrichment in Enterobacteriaceae Enrichment (EE) broth supplemented with 1.0 μg/ml novobiocin and incubated at 42°C, and FTA® filtration/ nested PCR. Conventional culture enrichments were repeated using enrichment media supplemented with 50 μg/ml rifampicin (rif+) to exclude natural tomato microflora and rifampicin-adapted inocula. The lowest detection levels (LDLs) for *S. boydii* and *S. sonnei* were determined for each of the methods investigated. *S. boydii*: >5.3 x 10^5 CFU/tomato (FDA BAM, CMMEF, EE broth), 6.3 x 10^0 CFU/tomato (FDA BAM rif+ and CMMEF rif+) and >5.3 x 10^5 CFU/tomato (EE broth rif+); *S. sonnei*: 1.9 x 10^1 (FDA BAM), 1.5 x 10^3 (CMMEF), 1.1 x 10^1 CFU/tomato (EE broth), 1.9 x 10^1 CFU/tomato (FDA BAM rif+ and CMMEF rif+), and 1.1 x 10^1 CFU/tomato (EE broth rif+). LDLs for the FTA® filtration/ nested PCR were 6.2 x 10^0 CFU/tomato for *S. boydii* and 7.4 x 10^0 CFU/tomato for *S. sonnei*. Logistic regression demonstrated the FTA® filtration/ nested PCR method was significantly better ($P < 0.05$) than all conventional culture enrichment protocols for the detection of *S. sonnei* and all but the FDA BAM rif+ ($P = 0.177$) for *S. boydii*. EE broth was found to be inhibitory to *S. boydii*.

P148 Antibiotic Resistance Patterns of *Yersinia enterocolitica* Farm Isolates

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Virulent serotypes of *Yersinia enterocolitica* carry a plasmid (pYV) that contains several key genes for pathogenesis whose expression is dependent on high temperatures. It is also possible that these plasmids carry antibiotic resistance genes. The objective of this study is to compare antibiotic resistance of *Y. enterocolitica* isolates and to determine if resistance is plasmid or genome based. In addition, antibiotic resistance patterns will be evaluated for potential associations with geographic location or serotype. The antibiotic resistance patterns of 109 virulent *Y. enterocolitica* isolates were determined using standard NARMS Sensititre® panels. The cultures were isolated from farm locations in several geographic regions of the US and characterized for serotype and the presence of the virulence plasmid. A plasmid negative strain was cultured from each plasmid positive isolate. Typical plasmid positive and negative colonies were grown in Mueller Hinton broth to a uniform cell density and distributed onto the NARMS microtiter panels. After an overnight incubation at 37°C, the panels were scored for resistance or susceptibility by observation of growth at the minimum inhibitory concentration for each antibiotic. Preliminary data suggests there are distinct patterns of antibiotic resistance associated with both genomic and plasmid genes and that differing antibiotic resistance patterns can be grouped by geographical region. These results suggest that there may be demographic distribution of antibiotic resistance within *Y. enterocolitica* populations, which leads to differences in subpopulations. Results from this study could lead to insight on emerging antibiotic resistance patterns in bacterial communities.

P149 Serotype, Antimicrobial Resistance Patterns, Genotype and Virulence Characteristics of Pathogenic *Yersinia enterocolitica* Isolated from Swine Feces

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Swine are the only known animal reservoir of *Yersinia enterocolitica* (YE) pathogenic to humans. Since YE is a fecal organism of swine, the primary goal of this study was to evaluate the characteristics of YE from swine feces by serotype, antibiotic susceptibility, presence of virulence plasmid, expression of plasmid-associated virulence determinants, and by determination of the clonal distribution of isolates. A total of 2,793 swine fecal samples collected from September 2000 to March 2001 from 78 production sites in 15 major pork producing states across the United States were tested for the presence of YE in pigs. YE were isolated using a combination of ITC medium for enrichment and CIN agar and were identified by fluorogenic 5' nuclease PCR targeting the chromosomal attachment invasion ail gene. Serotyping and antimicrobial susceptibility testing were performed. Isolates were tested for markers of virulence including carriage of a 70-kbp plasmid, colony morphology, low calcium response, Congo red uptake, crystal violet binding, autoagglutination, hydrophobicity, and presence of a cytotoxicity factor, YopE. Clonal distribution of the isolates was determined by PFGE. Experimentally, 107 ail-positive YE were isolated from the 2,793 samples. One isolate from each positive sample was characterized. The predominant serotype, O:3 (n=81/107), was evenly distributed among isolates from the 15

states. Serotype O:5 (n=26/107) was found in pigs from only three states. Regional and serotype specific differences were observed in antimicrobial susceptibility. All isolates contained the virulence plasmid and expressed virulence-associated phenotypic characteristics. PFGE showed that O:3 and O:5 isolates were highly clonal within a serotype regardless of state of origin. The serotypes, presence of the ail gene, virulence plasmid, and the expression of virulence determinants indicate that these isolates are potentially capable of causing food borne illness. The results from this study will aid in the design of future epidemiological investigations concerning on-farm prevalence of pathogenic YE.

P150 Evaluation of a New Chromogenic Medium for the Isolation of *Bacillus cereus* from Foods

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Oxoid Chromogenic *Bacillus cereus* Medium (CBCM) is a new chromogenic medium for the detection of *Bacillus cereus* from foods. It incorporates the chromogenic substrate 5-Bromo-4-Chloro-3-Indolyl-â-D-Glucopyranoside in a selective base. In this study, 512 naturally contaminated and spiked food samples were analyzed. Samples comprised rice, minced beef, beansprouts, glazed biscuits, pasta and ready-made fish pie. Naturally contaminated foods were homogenized with Maximum Recovery Diluent for 1 min, incubated at 37°C for 2 h and serially diluted to 10^{-3} in sterile distilled water. 100 µl of each dilution was inoculated onto the test media (CBCM, Mannitol-Egg Yolk-Phenol Red Agar (MYP), Polymyxin-Pyruvate-Egg Yolk-Mannitol-Bromothymol Blue Agar (PEMBA) and Biosynth *Bacillus cereus* Medium (BCM)) and incubated at 37°C for 24 h. Presumptive colonies of *B. cereus* were confirmed by Gram stain. For spiking experiments, overnight cultures of *B. cereus* grown in Buffered Peptone Water at 37°C were inoculated into foods at approximately 10^{-2} / 25 g of the food and subsequently processed as detailed for naturally contaminated foods. The rate of recovery of *B. cereus* from naturally contaminated foods was significantly higher on CBCM than on other media. Recovery of *B. cereus* from spiked samples was equivalent on CBCM, MYP and PEMBA, whereas BCM recovered significantly less *B. cereus* than MYP ($P = 0.0009$) and PEMBA ($P = <0.0002$). The ability of CBCM to recover *B. cereus* from both naturally contaminated and artificially contaminated foods was equivalent or better than the other media tested, and CBCM was easier to use.

P151 Antibiograms and Epidemiological Analysis for the *Bacillus cereus* Strains Isolated from Foods and Foodborne Outbreaks or Cases in Taiwan

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Bacillus cereus is one of the major foodborne pathogenic bacteria in Taiwan. This bacteria species may cause foodborne outbreaks and sporadic cases, i.e., the vomiting or diarrhea cases attributed to the enterotoxins produced by *B. cereus*. Thus, to establish the antibiograms and epidemiological data for this bacteria species is important. In this study, the antibiograms, plasmid profiles, randomly amplified polymorphic DNA (RAPD), and pulsed-field gel electrophoresis (PFGE) patterns for *B. cereus* strains isolated from food samples and foodborne outbreaks or cases were used for the investigation. A total of 83 *B. cereus* strains were assayed and they were categorized to 24 antibiograms, 46 plasmid profiles, 57 PFGE patterns, and 59 RAPD patterns. No major subtypes could be found for these isolates. Results obtained from RAPD and PFGE subtyping were similar among 29 epidemiological strains of *B. cereus*. Although the RAPD method was rapid and easier to perform, the PFGE method produced the most discriminative and reproducible results.

P152 Survival and Control of *Escherichia coli* O157:H7 in Drinking Water for Cattle

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Drinking water for cattle is an important vehicle of *Escherichia coli* O157:H7 transmission. Survival of *E. coli* O157:H7 in water contaminated with rumen content at different water:rumen content ratios, *E. coli* O157:H7 cell numbers and temperatures were determined. At 21°C, *E. coli* O157:H7 at a high inoculum survived for 8, 15, 23, >56 and 24 weeks and at a low inoculum survived for 8, 11, 10, 11 and 10 weeks at a water:rumen content ratio of 5:1, 10:1, 25:1, 50:1 and 100:1, respectively. Several treatments, including lactic acid, acidic calcium sulfate, chlorine, chlorine dioxide, hydrogen peroxide, caprylic acid, ozone, and competitive inhibition *E. coli* were tested individually or in combination for inactivation of *E. coli* O157:H7 in the presence of rumen content. Chlorine (5 ppm) and ozone treatment (22 to 24 ppm at 5°C or 8 to 12 ppm at 21°C) of water had minimal effect on killing *E. coli* O157:H7 in the presence of rumen content at ratios of 50:1 and higher. Treatment with competitive inhibition *E. coli* in water with rumen content decreased *E. coli* O157:H7 by 0.2 to 2.5 log CFU/ml by day 15 at 21°C, whereas *E. coli* O157:H7 increased by up to 2.5 log CFU/ml in the control (no competitive inhibition *E. coli*). A combination of lactic acid (0.1 to 0.5%), acidic calcium sulfate (0.5 to 0.9%) and chlorine dioxide (50 to 100 ppm) or lactic acid (0.1 to 0.5%), acidic calcium sulfate (0.5 to 0.9%) and caprylic acid (0.1 to 0.5%) at 21°C effectively killed >5.0 log *E. coli* O157:H7/ml within 2 min in water heavily contaminated with rumen content at a ratio of 10:1.

P153 Comparison of Five *Escherichia coli* O157 Enrichment Media: Growth Rate, Selectivity with Pure Cultures and Recovery in Spiked Beef Samples

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Efficient enrichment of *Escherichia coli* O157 in food samples, including beef, is a critical step for its rapid detection in foods. Most rapid detection methods use cultures from conventional enrichment media, i.e., modified EC or modified TSB. In recent years, several rapid 8 h enrichment media have been commercialized, including Reveal® 8 H *E. coli* Media, RapidChek® 8 H *E. coli* Media, and BAX® 8 H *E. coli* Media. This study compared the performance of the five media mentioned with pure cultures for the growth rate of *E. coli* O157 cells and selectivity against growth competitors. Based on the growth curves, RapidChek® 8 H *E. coli* Media produced the fastest growth, doubling time at ca. 17 min in 8-h period, and mEC the slowest, doubling time longer than 1 h. In a panel of 36 growth competitors, all media showed similar selectivity profiles against some gram-positive strains, such as *Bacillus* and *Lactobacillus*; mEC has strong selectivity against strains like *Enterococcus*, *Staphylococcus*, *Proteus*, and *Vibrio*. A total of 2,000 beef samples (25 g) have been spiked and enriched with either media for 8 h, 18 h, or 20 to 24 h and detected with streaking method or lateral flow device and confirmed by cultural methods. A significantly higher number of positives were recovered with use of RapidChek® 8 H media than with use of mEC or mTSB (by 32% and 10% each). This work suggested that fast growth is more important than selectivity in recovering *E. coli* in beef, and that the use of rapid 8 h media is preferred to mEC and mTSB for better recovery.

P154 Vaccination as an Intervention Strategy for Reduction of *Escherichia coli* O157 during a 45-day Pre-conditioning Period

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Seven hundred fifty-six newly-weaned calves were randomly allotted by gender to six pre-conditioning pens in central Montana. Half of the calves in every pen were vaccinated with an experimental vaccine designed to prevent the attachment of *E. coli* O157 to the intestinal wall, and then co-mingled with controls (non-vaccinated). A 10 g fecal sample was collected from every animal via rectal palpation on day-0 (before vaccination) and day-45 following administration of the vaccine. Fecal samples were evaluated for prevalence of *E. coli* O157 following enrichment, use of immunomagnetic separation, and plating on ctSMAC and Rainbow agars. Morphologically typical colonies were tested for latex agglutination. Overall, irrespective of treatment, 9.4% of fecal samples were positive initially and 3.9% were positive following the 45 d pre-conditioning period. Despite randomization, the initial prevalence of *E. coli* O157 in fecal samples from control calves was lower than the initial prevalence of *E. coli* O157 in fecal samples from calves to be vaccinated (6.8% vs 12.0%; $P = 0.0175$). Prevalence of the pathogen in fecal samples obtained from vaccinated calves tended to be lower than prevalence in fecal samples from the control calves following the 45 d pre-conditioning period (2.7% vs 5.1%; $P = 0.0939$). Results were limited due to low initial *E. coli* O157 prevalence in fecal samples, and low prevalence following vaccination. Additional studies are warranted to determine the potential effectiveness of using the experimental vaccine as an intervention technique for control of pre-harvest *E. coli* O157 during the pre-conditioning phase of beef cattle production.

P155 Role of Curli Fimbriae in Attachment of Enterohemorrhagic *Escherichia coli* Cells to Raw and Cooked, Ready-to-Eat Beef Products

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Both raw and ready-to-eat beef products have been linked to outbreaks of enterohemorrhagic *Escherichia coli* (EHEC) infections. The contamination of these products in beef processing environments could occur at any stage from slaughtering to packaging, and processing surfaces with adhered EHEC cells have been found to be a likely source of the contamination. Research has revealed that a thin, wiry, and aggregative cell surface fiber, known as curli, mediates the attachment of EHEC cells to the abiotic surfaces made of polystyrene, glass, and stainless steel. We evaluated in this study, the role of curli in the attachment of EHEC cells to raw and ready-to-eat beef products. Raw beef from an eye of round roast and beef salami were sliced to a uniform size and were exposed for different lengths of time to six EHEC cultures, the curli$^+$ and curli$^-$ variants of *E. coli* O157:H7 and O103:H2 as well as a curli$^+$ strain of *E. coli* 111:H- and a curli$^-$ strain of *E. coli* O157:H7. The beef slices were rinsed following exposure. The EHEC cells attached to the surfaces of beef were enumerated. Two replications were performed and the date generated was analyzed statistically based on a 95% confidence level. The results indicated that the curli$^+$ variants of EHEC attached to raw beef and beef salami more efficiently than did the curli$^-$ variants. The differences in the efficiency of attachment, however, were minor, with the smallest differences of 0.47 and 0.29 log CFU/cm^2 and the largest differences of 0.86 and 1.17 log CFU/cm^2 on raw beef and beef salami, respectively.

P156 DSC Phenotypic and Genotypic Characterization of Curli-expressing Enterohemorrhagic *Escherichia coli*

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Certain enterohemorrhagic *Escherichia coli* (EHEC) express a thin, wiry, aggregative surface fiber, known as curli, to assist them in attaching to solid surfaces. This study was undertaken to characterize and compare the genotypic and phenotypic differences of curli-expressing and non curli-expressing EHEC cells. Wild type *E. coli* O111-H- 7-57 and O157:H7 5-9, with and without the ability to express curli, respectively, were included in the study. Also included were *E. coli* O103:H2 7-52 and O157:H7 5-11, capable of forming both curli-expressing (C^+) and non curli-expressing (C^-) colonies on tryptone yeast extract agar (TYE) supplemented with Congo red dye. Quantification of curli expressed by EHEC cells was accomplished with a Congo red binding assay. The growth requirements of the cells of both curli phenotypes were compared on casamino acid, and minimal glucose agar (MGA) as well as on the GN2 microtiter plates supplied by Biolog. The rates of curli phenotypic conversion were determined by growing the EHEC cultures on 3 media at 5 incubation temperatures. Polymerase chain reaction (PCR) was performed to determine the prevalence of *csgA* in curli-expressing and non curli-expressing EHEC cells. The results of the Congo red binding assay indicated that the quantities of curli expressed by EHEC cells were inversely proportional to the amounts of free Congo red in the supernatants of the cell suspensions. Two non curli-expressing variants were more fastidious than were their curli-expressing counterparts and failed to grow on MGA although they were capable of growing on casamino acid agar. Both curli-expressing and non curli-expressing cells of EHEC carried *csgA*, the structural gene for a major subunit of curli protein. Curli phenotypic conversion took place spontaneously during growth and was influenced to a certain extent by growth media and incubation temperatures.

P157 Serotypes and Virulence Genes of Ovine Non-O157 Shiga Toxin-producing *Escherichia coli* in Switzerland

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Sixty ovine STEC strains were examined with the aim (i) to serotype the strains, (ii) to characterize virulence factors, and (iii) to assess the potential pathogenicity of these strains for humans. The 60 sorbitol-positive, non-O157 STEC strains belonged to 19 O:H serotypes, whereas 68% were of five serotypes (O87:H16, O91:H-, O103:H2, O128:H2, O176:H4). 52% belonged to serotypes reported in association with HUS. Five serotypes were not previously reported in sheep strains. Of the 47 strains encoding for stx_1 variants, 57% were stx_{1c}-, and of the 45 encoding for stx_2 variants, 80% were stx_{2d}-positive. 82% of the strains showed further virulence factors: 13% were *eae*-, 60% *ehxA*- and 67% *saa*-positive. The associations between harboring (i) *eae* and stx_1, stx_2, *ehxA* or no *saa* and (ii) *saa* and stx_{1c} or stx_{2d} were significant ($P < 0.05$). The strains belonged to 27 seropathotypes (association between serotypes and virulence factors), but 57% belonged to only six and O91:H-stx_1 stx_{2d}*saa* and O128:H2 stx_{1c} stx_{2d} *ehxA saa* were the most common. Seven of the eight intimin-positive strains harbored *eae* epsilon. Four strains of serotype O103:H2 and O121:H10 harboring stx_2, *eae* and *ehxA* showed virulence factors typical for strains associated with severe human disease. However, according to the virulence factors, the majority of the ovine non-O157 STEC strains are assumed low-virulence variants. Nevertheless, as long as the contribution and interaction of virulence factors in milder disease remains unclear, a certain risk for humans cannot be excluded.

P158 Heat Resistance Kinetics Variation among Various Isolates of *Escherichia coli*

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This paper reports an investigation of serotype-specific differences in heat resistance kinetics of clinical and food isolates of *Escherichia coli*. Heat resistance kinetics for 5 serotypes of *E. coli* at 60°C were estimated in beef gravy using a submerged coil heating apparatus. The observed survival curves were sigmoidal and there were significant differences ($P = 0.05$) of the survival curves among the serotypes. Consequently, a model was developed that accounted for the sigmoidal shape of the survival curves and the serotype effects. Specifically, variance components for serotypes and replicates within serotypes were estimated using mixed effect nonlinear modeling. If it is assumed that the studied serotypes represent a random sample from a population of *E. coli* strains or serotypes, then, from the derived estimates, probability intervals of the expected lethality for random selected serotypes can be computed. For example, expected serotype-specific lethalities at 60°C for 10 min are estimated to range between 5 and 9 log with 95% probability. On the other hand, to obtain a 6-log lethality, the expected min range, with 95% probability, is from 6 to 12 min. The results from this study show that serotypes of *E. coli* display a wide range of heat resistance with nonlinear survival curves.

P159 Expression of Caspase-3 during Enterohemorrhagic *Escherichia coli*-induced Apoptosis

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The hallmark of verotoxin-producing *E. coli* (VTEC) is the production of toxins which aid in pathogenesis. The mechanism by which VTEC

produces toxins extracellularly and kills the host cells is largely not understood. In this study we investigated the ability of verotoxin-producing *E. coli* to induce apoptosis in Burkitt's lymphoma (Ramos) cells. Primary cultures of Burkitt's lymphoma cells were inoculated with crude verotoxin extracts and incubated at 37°C for 6 h. To determine the expression of caspase 3, cultures of Ramos cells on slide flaskets were probed with anti-Caspase 3 polyclonal antibodies for 1 h, followed by incubation with anti-Cy 3 mAb antibodies for 1 h, and viewed using an epi-fluorescence microscope. Apoptosis was assessed by a DNA fragmentation assay and the production of histone was measured by an enzyme-linked immunosorbent assay (ELISA). Result indicated that VTEC infection of Ramos cells induced release of significantly higher levels of histone as compared with non-VTEC-producing *E. coli* (1.14 ± 0.2 versus 0.602 ± 0.04, $P \leq 0.5$). DNA fragmentation was very low in the untreated Ramos cells. However, in the verotoxin treated cells, the percentage of DNA fragmentation increased by 75% in the activated Ramos cells. The increase in DNA fragmentation and histone release was associated with an increase in the expression of Caspase 3 positive Ramos cells when viewed by fluorescent microscopy. Thus our data suggest that the increase in sensitivity of Ramos cells to verotoxin-mediated induced apoptosis might be one factor that contributes to the pathogenesis of enterohemorrhagic *E. coli*.

P160 Evaluation of the Genevision™ *Escherichia coli* O157 Real-time PCR Assay

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The Genevision™ *E. coli* O157 real-time PCR assay has been developed for the specific detection of *E. coli* O157 in food commodities. The performance of this assay system was compared with that of the standard culture technique prescribed in the Health Canada Compendium of Analytical Methods (MFLP-80) to validate its use as an approved method for the detection of *E. coli* O157 in food commodities in Canada. Twenty food commodities, including ground beef, pork, dairy products, sausage, seafood, fruits and vegetables, were spiked with three different *E. coli* O157 strains at levels of 0, 0.2, 2 and 20 CFU/g and analyzed by the Genevision assay and cultural method MFLP-80. From a total of 1185 PCR results, 1035 were positive by the Genevision assay. Only one of these positive results could not be confirmed by culture techniques. In addition, a variety of foods and food ingredients were tested for the presence of naturally occurring *E. coli* O157 by both MFLP-80 and the Genevision assay. The individual samples examined were as follows: 24 egg products, 55 dairy products, 176 cooked meats, 45 raw meats, 82 fermented sausages, 156 fruits and vegetables, 45 seafood, and 16 spices. 212 of the 599 samples tested positive by both methods, with no false negative or false positive results observed for the Genevision assay. The results of this study show that the Genevision assay is rapid, and simple and that it performed reliably in detection of *E. coli* O157 in food commodities.

P161 Improved Method for Enrichment of *Escherichia coli* O157:H7 Using Acidification

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A procedure for selective enrichment of *E. coli* O157:H7 was recently developed which utilizes exposure to extremely acidic conditions. *E. coli* O157:H7 (SEA 6396) was enriched by this procedure, as well as by four current standard methods (those used by the US Food and Drug Administration, US Department of Agriculture, Government of Canada, and Government of the U.K.), and by enrichment in buffered peptone water. Resultant population levels were compared by plating on TCSMAC agar, Rainbow agar, Rainbow agar plus tellurite and novobiocin, and TSAYE agar. As measured by all four selective agars, acidification enrichment yielded larger populations of target cells. On TCSMAC, acid enrichment produced more EHEC colonies by factors ranging from 2.7 to 41-fold. On Rainbow agar, acid enrichment produced more EHEC by factors ranging from 4.3 to 28-fold. The acid enrichment procedure was also found to be superior in its ability to eliminate competitors which frequently interfere with analysis for *E. coli* O157:H7 using current methods. The new enrichment procedure also yielded detectible amplicons when spiked enrichment cultures were tested for shiga-like toxin genes stx_1 and stx_2, whereas most of the other methods did not. The improved results obtained with the acid enrichment method may be beneficial in modifying current standard methods used by numerous public health agencies worldwide.

P162 Altered Resistance of Acid-adapted *Escherichia coli* O157:H7 and *Listeria monocytogenes* to Hydrogen Peroxide and PRO-SAN™

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A study was conducted to determine if acid-adaptation in *Escherichia coli* O157:H7 and *Listeria monocytogenes* altered their resistance to hydrogen peroxide (H_2O_2) and PRO-SANTM, a food-grade sanitizer for fresh produce. Cells were adapted to acid by growing them overnight (18 h, 35°C) in tryptic soy broth supplemented with 0.6% yeast extract and 1% glucose. Cells grown in TSBYE without glucose served as control. Final pH of acid-adapted and control cultures ranged from 4.7 to 5.0 and 6.9 to 7.1, respectively. A 5-strain mixture of washed cells of each pathogen (acid-adapted or control) was exposed (2 min) to H_2O_2 (0.5 or 1.0%) or PRO-SAN (0.5 or 1.0%) at 23°C. Inoculated solutions were serially diluted in buffered peptone

water and appropriate dilutions were surface-plated on tryptic soy agar with 0.6% yeast extract (TSAYE). Survivors were enumerated by counting bacterial colonies on TSAYE plates at 48 h following incubation (35°C). Acid-adapted pathogens exhibited increased resistance to H_2O_2 (0.5 or 1.0%) compared to controls. H_2O_2 (1.0%) reduced initial populations (~10^8 CFU/ml) of *E. coli* and *L. monocytogenes* controls by 3.29 and 3.05 log, respectively, whereas acid-adapted cells were reduced by 0.95 and 1.07 log, respectively. PRO-SAN (1%) reduced *E. coli* control and acid-adapted cells by 5.64 and 1.0 log, respectively. *L. monocytogenes* were most sensitive to PRO-SAN; both control and acid-adapted cells decreased by >6 log irrespective of PRO-SAN concentration. These results indicate that acid-adaptation can alter microbial resistance to certain sanitizers and may enhance pathogen survival following sanitizer treatment.

P163 Influence of Acid Adaptation on Survival and Injury of *Escherichia coli* O157:H7 in Physiological Saline following Exposure to Ultraviolet Radiation

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The influence of acid adaptation on radiation resistance and injury in *Escherichia coli* O157:H7 following ultraviolet (UV) irradiation in 0.85% saline was evaluated. *E. coli* O157:H7 cells were adapted to acid by growing them overnight (18 h) in tryptic soy broth (TSB, 35°C) containing 1% (w/v) glucose. Cells grown in TSB without glucose served as control. Final pH values for acid-adapted and control cultures were 4.7 and 6.9, respectively. Sterile saline was inoculated with a 5-strain mixture of washed acid-adapted or control cells to give ~10^7 CFU/ml. Aliquots (5-ml) of cell suspension in separate Petri dishes were exposed to UV radiation (260 nm) for 1 min. At 10 s intervals survivors were determined by plating samples on tryptic soy agar and Sorbitol MacConkey agar, and counting bacterial colonies on agar plates following incubation (35°C, 24 h). Survivors of both acid-adapted and control cells decreased with increased exposure to UV radiation; however, acid-adapted cells exhibited greater radiation resistance. Irradiation for 50 s completely inactivated controls (7-log reduction), whereas acid adapted cells were reduced by 6 log. D-values of acid-adapted and control cells were 9.2 and 7.7 s, respectively. For both physiological states of the pathogen, the extent of injury increased with increased exposure to UV radiation. These results indicate that acid adaptation in *E. coli* O157:H7 cross-protects this organism against inactivation by UV radiation in saline and should be considered when determining the microorganism's D-value. Validation of this cross-protection phenomenon during UV irradiation of liquid foods warrants investigation.

P164 Effect of Drying on Survival and Acid Tolerance of *Escherichia coli* O157:H7 Biofilms Formed in Beef Decontamination Runoff Fluids

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Biofilms formed on industrial equipment surfaces are subjected to adverse conditions that may allow for stress adaptation. The objectives of this study were to evaluate the effect of temperature, pH, natural flora and incomplete sanitation on survival and acid tolerance of *Escherichia coli* O157:H7 biofilms, formed on stainless steel coupons immersed into beef decontamination runoff fluids (washings), before (pre-) and after (post-) biofilm drying. Unsterilized water washings (WW; pH 6.9) and lactic acid washings (LW; pH 4.9) containing stainless steel coupons were inoculated with *E. coli* O157:H7 (10^3 CFU/ml). Coupons were subjected to drying (25°C/12 h) after 7 and 24 h at 35°C, or 24 and 48 h at 15°C, then immersed into uninoculated water washings (WW-WW, LW-WW) and lactic acid washings (WW-LW, LW-LW) and stored at 35°C for 24 h, or 15°C for 5 or 10 day. Coupons (35°C / 24 h) were also exposed to 50 ppm peroxyacetic acid for 45 s before drying. Survival and acid tolerance response (ATR; in tryptic soy broth at pH 3.5 with lactic acid) of total bacterial populations and *E. coli* O157:H7 were assessed pre-drying, immediately after drying, and at the end of post-drying storage in the new washings. Higher pre-drying attachment and ATR of *E. coli* O157:H7 was observed in water washings at 35°C for 24 h. Drying caused severe injury and reduced significantly ($P < 0.05$) the attached population of *E. coli* O157:H7, regardless of pH; however post-drying storage of coupons in fresh washings allowed recovery and increases of *E. coli* O157:H7 by 2.5 logs depending on temperature, level of natural flora and pH of washings. Low recovery and ATR were evident for LW-LW. Pre-drying sanitation reduced the recovery and ATR of *E. coli* O157:H7. In conclusion, incomplete removal of biofilms may result in cells of increased ATR.

P165 DSC Acid Tolerance Response of Acid-adapted or Nonadapted *Escherichia coli* O157:H7 Strains Grown as a Mixture or as Individual Strains and Mixed Prior to Inoculation on Beef Tissue or in Beef Decontamination Runoff Fluids

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Escherichia coli O157:H7 contamination in fresh beef plants may consist of single or mixed strains and strain interactions may result in variable responses of cells to subsequent stressful conditions. This study assessed the acid tolerance

response of stationary phase acid-adapted (grown in tryptic soy broth [TSB]+1% glucose) or nonadapted (grown in glucose-free TSB) *E. coli* O157:H7 strains (ATCC43889, ATCC43895, ATCC51658 and EO139) previously grown individually or in a mixed culture and inoculated on untreated or decontaminated (hot water [75°C] followed by lactic acid [2%]) meat or into meat decontamination runoff (washings) fluids (acidic [pH 4.95 with 2% lactic acid] or nonacidic washings [pH 7.01]). Inoculated beef samples and washings were stored at 4 or 15°C for 6 days and at set intervals (0, 2, and 6 days) were exposed (for 0, 60, 120, and 180 min) to pH 3.5 (adjusted with lactic acid) TSB with yeast extract (TSBYE). At day-0 in washings, acid-adapted populations were more resistant to the effects of acid than nonadapted counterparts and remained as such at 4°C but not at 15°C at day-2. Although at day-0 there were no significant ($P < 0.05$) differences in acid resistance between acid-adapted and nonadapted populations on meat; acid-adapted cells displayed consistently higher resistance through day-6. Populations on treated meat were consistently lower than those on nontreated meat during storage and subsequent exposure to acid. Overall, there were no significant ($P \geq 0.05$) differences in growth or survival after acid exposure between total populations prepared as a cocktail and those grown as individual strains and mixed prior to inoculation.

P166 Survival of *Bacillus anthracis* in Foods

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Bacillus anthracis was not previously considered to be a food borne pathogen as is its close relative, *B. cereus*. Thus, little information exists about *B. anthracis* in foods. This study provides information about the survival of *B. anthracis* spores in foods and subsequent growth if the food was temperature abused. Heat activated spore suspensions of the Sterne strain of *B. anthracis* were used to inoculate several types of foods (4 to 5 log spores/g or ml). Inoculated foods were stored at either 4°C (refrigerated products, e.g., beverages, dairy products, deli meats) for 1 to 2 wks or room temperature (shelf-stable products, e.g., beverages, dairy products) for up to 1 month. In general, *B. anthracis* spores did not germinate and grow but they survived in most of the foods tested. However, there was a 2-log decline in spore population of *B. anthracis* in chocolate milk after 1 day of storage at 4°C. Both spore and total population dropped 1-log in a soft drink after 7 days. More than 1-log reduction in spore population was found in deli meat immediately after inoculation; however, the vegetative cells eventually re-sporulated by day 7 and the spores remained stable during the following storage. In contrast, an increase in total population (from ca. 5 to >6.5 log/ml) was found in liquid milk products stored at room temperature; this was the only food in which outgrowth was observed. However, the general stability of *B. anthracis* spores in foods makes the organism a concern for human health because no oral toxic dose has been established.

P167 The Efficiency of Conventional Pasteurization Treatment of Water, Media, and Milk Deliberately Contaminated with *Bacillus anthracis* (Sterne) Spores

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The potential risk from *Bacillus anthracis* as a terrorist weapon in foods has recently increased. Although the likelihood of natural contamination of milk with *B. anthracis* from infected animals is small, there is potential for purposeful addition of spores to bulk milk exceeding 10^6 spores/ml. High temperature-short time (HTST) pasteurization at the legal minimum time and temperature condition (72°C for 15 s), or at higher temperatures and longer times (78°C for 30 s), or at ultra-high temperature pasteurization (130°C for 1 to 2 s) have been successful in reducing levels of vegetative cells of pathogens in milk, but may be ineffective against resilient spore-formers. The decimal reduction times (D-values) for *B. anthracis* (Sterne) spores inoculated to 10^8 spores/ml in dH_2O, brain heart infusion (BHI) broth, or skim milk (SM) were calculated at these pasteurization temperatures. Pasteurization at 72°C was ineffective (< 1 $\log_{10}$ CFU/ml reduction) in reducing spore viability for *B. anthracis* (Sterne) in all 3 test fluids for treatments up to 90 min. The D78-values were estimated at 115.4 + 32.3 min for spores in dH_2O, 612.9 + 431.5 min for spores in BHI, and 640.3 + 513.3 min for spores in SM. As evidenced by D130-values of 0.7 + 0.2 min for spores in dH_2O, 0.9 + 0.3 min for spores in BHI, and 1.1 + 0.1 min for spores in SM, respectively, ultra-high pasteurization conditions were also found to be inadequate for inactivating spores. In related studies, spores from *B. cereus* ATCC strains 14579 and 9818, representative aerobic spore-forming bacteria found in milk, were similar to *B. anthracis* (Sterne) in their thermal resistance.

P168 Capillary Isoelectric Focusing: A Novel Analytical Method for Rapid Detection of the Noroviruses

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The noroviruses continue to emerge as an important cause of viral, foodborne disease. A capillary isoelectric focusing-whole column imaging detection (CIEF-WCID) method was developed and employed to determine the isoelectric point (pI) of norovirus-like particles (VLPs), as a possible method for rapid detection of noroviruses. The VLPs were produced from noroviruses and included Funabashi, Seto, Norwalk, Hawaii, Kashiwa and Narita. Using the imaged CIEF-WCID detection technique, separation and identification of the purified VLPs were accomplished within 6 min, using a short (4 to 5 cm) internally coated capillary

(100 μm diameter) and a whole-column optical absorption imaging detector operated at 280 nm. CIEF-WCID experiments showed the similarity of the pI values of VLPs, with pI values of 5.92, 5.94, 5.95 and 5.96 for Funabashi, Norwalk, Seto and Hawaii, respectively. The two other VLPs displayed pI values of 5.51 (Kashiwa) and 6.89 (Narita). The VLP peaks were shown to be reproducibly resolved. CIEF-WCID shows great promise for norovirus detection in public health, clinical and food safety applications, and it overcomes several limitations associated with genetic and immunological norovirus detection methods.

P169 Long-term Survival of *Enterobacter sakazakii* in Powdered Infant Formula

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As part of an earlier study to characterize the inactivation of *Enterobacter sakazakii* when powdered infant formula was rehydrated with hot water at different temperatures (Edelson-Mammel and Buchanan, 2004), a quantity of powdered formula was prepared to contain approximately 10^6 CFU/ml *E. sakazakii* 607 when rehydrated according to the manufacturer's instruction. The remainder of this contaminated formula was used subsequently to study the long term survival of *E. sakazakii* in powdered infant formula. Over the course of approximately 1.5 years, the infant formula was stored at room temperature in a closed screw cap bottle. Periodically triplicate 4.25 g samples of the formula were rehydrated, and the levels of viable cells were determined by plating in duplicate on tryptic soy agar plates. The identity of recovered microorganisms was confirmed by examination of colonies for characteristic color and morphology, with selected colonies being examined using API 20E strips. During the initial 5 months of storage, the level of viable *E. sakazakii* 607 declined approximately 2.5 log cycles (6.0 log CFU/ml to 3.5 log CFU/ml) at a rate of approximately 0.5 log cycles per month. Over the course of the subsequent year, the level of viable *E. sakazakii* declined an additional 0.5 log cycle to 3.0 log CFU/ml. The results demonstrate clearly that *E. sakazakii* can survive for extended periods in powdered infant formula.

P170 Acid Resistance of Twelve Strains of *Enterobacter sakazakii*

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Enterobacter sakazakii has been linked to several outbreaks of severe illness associated with powdered infant formula fed to immunocompromised and premature neonates. Previous research with 12 strains of *E. sakazakii* indicated that there was substantial diversity in thermal resistance, with half the strains being 10- to 20-fold more resistant that the others (Edelson-Mammel and Buchanan, 2004). In the current study, these strains were further characterized by assessing their ability to survive exposure to an acidic environment. Acid resistance was determined by initially culturing the isolates for 18 h in brain heart infusion broth (BHI) at 37°C and then transferring the cells to culture tubes containing 10 ml of sterile tryptic soy broth (TSB) adjusted to pH 3.0 and 3.5 with HCl. The pH-adjusted TSB culture tubes were then incubated at 37°C over 5 h, with samples being taken hourly and analyzed for viable *E. sakazakii* by plating on duplicate TSB agar plates. At pH 3.5, 10 of the 12 strains showed less than a 1 log cycle decline over the 5-h incubation period, with the most acid sensitive strain showing about 3.5 log cycle decline. At pH 3.0, the decline in population density over the 5 h incubation period ranged from 4.9 to >6.3 log cycles, suggesting similar acid resistance among the isolates. However, the diversity among the 12 *E. sakazakii* isolates was more apparent when the 1-h/pH 3.0 samples were compared; the declines in population density ranged from 0.3 to 5.1 log cycles, with 25% of the strains showing <1 log decline. There was no apparent correlation between the relative acid resistance of the strains based on the 1-h/pH 3.0 results and the previously determined heat resistances.

P171 Novel Selective Medium for Isolation of *Enterobacter sakazakii*

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Enterobacter sakazakii, previously referred to as a yellow-pigmented *Enterobacter cloacae*, was designated as a unique species in 1980. *E. sakazakii* has been implicated in severe forms of neonatal infections such as meningitis and sepsis. FDA has recommended a method for isolation and enumeration of *E. sakazakii* (2002, http://www.cfsan.fda.gov), but specifies media such as VRBG agar that are not sufficiently selective because other microorganisms can grow and produce purple colonies surrounded by a purple halo of precipitated bile salts. In addition, the growth of *E. sakazakii* on TSA agar requires considerable time (48 to 72 h) to produce yellow pigmented colonies. The enzymatic profiles of *E. sakazakii* and related species has shown that the a-glucosidase reaction is a single, simple, and rapid test method to distinguish *E. sakazakii* from other Enterobacter species. 4-methylumbelliferyl-a-D-glucopyranoside (4-MU-a-D-glc) is a substrate for a-glucosidase, and becomes a fluorogenic when degraded. *E. sakazakii* showed extraordinary strong fluorogenic characteristics compared with other microorganisms when grown in 4-MU-a-D-glc containing media. To develop a novel selective medium, basal medium was first selected having low background noise for a-glucosidase activity. Then carbon and nitrogen sources were optimized. Newly developed novel selective media containing 4-MU-a-D-glc as indicator has speed (24 h) and reliability for differentiation and isolation of *E. sakazakii*.

P172 A New Method for Next Day Detection of *Listeria* in Food

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A new, next day, immunoassay, the VIDAS Listeria Express (LSX), for detection of *Listeria* spp. in food has been developed. Samples are culturally enriched for a minimum of 28 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. An independent validation study for the LSX method was conducted at Silliker Microtech Laboratories in Sydney, Australia. The study was performed according to AOAC guidelines and was designed to compare the performance of the new assay with that of the cultural reference methods, AOAC method 993.12 for dairy products and the USDA method for meat products. Fifty samples (10 uninoculated, 20 "low" and 20 "high" level inoculum) were tested for each of the 3 foods (cheese, ice cream and ham). Overall, for the 150 samples tested, the LSX detected 103 confirmed positives for *Listeria*, compared to 90 confirmed positives for the cultural methods. 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 LSX assay compared favorably with traditional methods in sensitivity, specificity and convenience. While the cultural methods required 5 to 6 days for a presumptive positive or negative result, this was achieved within 30 h using the LSX. The ability to give a reliable "next day" result makes the VIDAS LSX a significant advance in *Listeria* testing.

P173 Evaluation of the Vidas *Listeria* Species Xpress (LSX) Test for the Detection of *Listeria* Species in Foods

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One hundred fifty different food types (dairy products, raw meats and poultry, chilled and frozen ready meals and prepared salads) were tested for *Listeria* species using the ISO Reference Method for the detection of *L. monocytogenes* in foods (ISO 11290-1), and the bioMérieux Vidas LSX test. In both cases isolated typical colonies were confirmed using the API Listeria test. Additionally a series of tests were done with *Listeria* cultures to establish the inclusivity and exclusivity of the LSX test. The LSX test is an immunofluorogenic assay that provides the user with a result in under 30 h and can therefore give a result the day after the test is set up. This contrasts with the ISO Reference method that can take up to 5 days to give a presumptive result. Results from the inclusivity / exclusivity tests indicated that all *Listeria* cultures tested were detected by the LSX rest, while all non-*Listeria* cultures were not detected. Results of food testing indicated that all LSX positives were confirmed culturally, and that the LSX was able to detect more confirmed positives than the ISO reference method. Statistical analysis using the McNemar's test indicated that there was no statistically significant difference between the LSX test and the ISO method at the 5% level.

P174 Phenotypic Analyses of the Putative CRP/FNR Family of Transcriptional Regulators of a Serotype 4b Strain of *Listeria monocytogenes*

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A whole-genome sequence analysis of *Listeria monocytogenes* strain F2365 revealed 15 potential members of the Crp/Fnr family of transcriptional regulatory proteins. Each gene and the flanking regions were cloned, subjected to in vitro transpositional mutagenesis, and recombined into strain F2365. Mutant strains, produced for 14 of the family members, were compared to strain F2365 using rapid phenotypic screens to identify functional differences. Differences in carbon, nitrogen, sulfur, and/or phosphorus metabolism were identified for 13 of the mutant strains compared to strain F2365. Mutant strains KO2, KO3, and KO5 showed reductions in swarming motility compared to strain F2365 and each strain was trans-complemented to the parent phenotype by its wild-type gene. Mutant strain KO15 (prfA) showed an increase in motility, which was trans-complemented to the wild-type levels by prfA. Mutant strains KO2 and KO5 also showed reduced oxidative stress tolerance compared to strain F2365. In virulence-related screens, 13 of the mutants showed reduced expression from a plcA promoter fusion. Following an intra-gastric mouse challenge, strains KO2, KO9, KO10, and KO15 showed variations in splenic and hepatic listerial counts when compared to strain F2365. In Caco-2 cell invasion assays, strains KO2 and KO15, but not strains KO9 and KO10, showed variations in invasiveness compared to strain F2365, which correlated with their changes in mouse virulence. While several members of the Crp/Fnr family of *L. monocytogenes* strain F2365 affect various phenotypes studied, orf 00085 (KO2) had the most diverse affects and may represent a major regulatory gene.

P175 Modeling the Interaction of the Physiological State of the Inoculum and CO_2 Atmosphere on the Lag Phase and Growth of *Listeria monocytogenes*

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Several studies have modeled the growth of *L. monocytogenes* under different CO_2 headspace concentrations but they used inoculum cells that were in the stationary phase. In this study, the growth of *L. monocytogenes* under different CO_2

concentrations as affected by physiological state of the cells was investigated. Exponential growth phase, stationary phase, desiccated and starved cells were inoculated into BHI broths at 5°C that were pre-equilibrated under the following atmospheres: 0%, 20%, 40% and 80% CO_2 (balance N_2). Lag phase duration times (LDT) and exponential growth rates (EGR) were determined by enumerating cells at appropriate time intervals and by fitting the data to a two-phase linear function that has a lag period before the initiation of exponential growth. Longer LDTs were observed as the CO_2 concentration increased, with no growth observed at 80% CO_2. With the 40% CO_2 atmosphere, for example, the LDT's for exponential, starved, stationary and desiccated cells were 6.5, 9.8, 18.4 and 19.3 days, respectively. In general, exponential growth cells had the shortest LDTs followed by starved cells and stationary phase cells. Desiccated cells had the longest LDTs. Exponential growth rates decreased as the CO_2 concentrations increased. Once exponential growth was attained, no retained differences among the different initial physiological states of the cells for any of the atmospheres were observed in the EGR's. The EGR's for 0, 20, 40, and 80% CO_2 averaged 0.39, 0.37, 0.19 and 0.0 logs/day, respectively.

P176 Colonization of Various Sprouts by *Listeria monocytogenes*

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In nature, the foodborne pathogen *Listeria monocytogenes* lives as a saprophyte, and is found in the soil associated with plants and decaying plant material. Despite this known niche, little is known of the physiology of *L. monocytogenes* when it is in association with plants. To begin to understand this interaction, we have studied the colonization of various sprouts with different strains of *L. monocytogenes*. The bacterium colonized and grew on alfalfa, radish, broccoli, and turnip sprouts to different levels. Radish sprouts harbored *L. monocytogenes* to levels of 10^6 CFU/sprout after 2 to 3 days of growth. After 2 to 3 days, broccoli sprouts contained *L. monocytogenes* in a range of 10^4 to 10^5 CFU/sprout. Sprouting turnip contained approximately 10^4 to 10^5 CFU/sprout after 3 days of growth. There were variations in alfalfa colonization from $<10^2$ to 10^6 CFU/sprout depending on the strain tested. Some strains that colonized alfalfa very poorly colonized the other sprouts quite well, indicating that different mechanisms may be used to colonize the different plants. Microscopic observation of GFP-containing *L. monocytogenes* cells indicated that the bacterium preferred to colonize the roots of the sprouts. In other plant-microbe interaction systems, motility and chemotaxis are known to play a role. Mutants of *L. monocytogenes* targeting the systems of chemotaxis, motility, and flagellar development are being generated to assess the role of these systems in the *L. monocytogenes* sprout colonization process.

P177 Effect of Competitive Microflora on the Growth and Cold Shock Response of *Listeria monocytogenes*

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Stress response facilitates survival of *Listeria monocytogenes* (Lm) in the cold food environment, where Lm also competes with the background microflora. The objective of this research was to determine if the presence of competing bacteria affected the growth and cold stress response of clinical and food Lm isolates. Lm Scott A (LA, clinical isolate, serotype 4b) and Lm G (LG, food isolate, serotype b) were prepared at 27°C and subsequently incubated at 4°C in tryptic soy broth (TSB) for 37 days, alone or in co-cultures with the Gram-negative spoilage bacteria *Serratia liquefaciens* 2R4 (Sl) or *Shewanella putrefaciens* A2 (Sp). Control cultures were incubated at 27°C for 72 h. Lm and competitors were enumerated on Oxford agar and TSA. The growth characteristics of LA and LG in pure cultures at 4 and 27°C were identical. Presence of Sl decreased the maximum population density of LA and LG regardless of incubation temperature, but had no effect on the generation time. Growth of Sl was not inhibited by the presence of Lm. Presence of Sp had no inhibitory effect on LA or LG, whereas Sp was inhibited by Lm. The protein expression of LA during cold shock conditions, alone or with competitors, is presently being investigated to determine if LA's stress response differs in the presence of Sl and Sp. In conclusion, no differences were found between the growth characteristics of food and clinical Lm isolates alone or in the presence of competitors, which either inhibited Lm (Sl) or became inhibited by Lm (Sp).

P178 Heat and Acid Tolerance Response of *Listeria monocytogenes* as Affected by Sequential Exposure to Hurdles during Growth

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Short exposure (<1.5 h) of exponential or stationary phase cultures of *Listeria monocytogenes* to sublethal stresses may induce cross protection to other stresses. However, it is not clear whether it is the sequence, the number, or the type of hurdles that increase the adaptive response of the pathogen to lethal stresses. The objective of this study was to evaluate the effect of sequence and level of hurdles applied during growth on the subsequent heat and acid tolerance of *L. monocytogenes*. A 10-strain composite of *L. monocytogenes* grown in glucose-free tryptic soy

broth with 0.6% yeast extract (TSBYE), was inoculated (10^4 CFU/ml) in TSBYE supplemented with NaCl or pH-adjusted with lactic acid to prepare cultures exposed to the following single hurdles: NaCl (3%) and pH 7.2 (C), 6.0 or 5.0 for 24 h at 30°C, as well as combinations of two sequential hurdles (the second hurdle was applied after 12 h of incubation at 30°C): NaCl-pH 6.0, NaCl-pH 5.0, pH 7.2-NaCl, pH 5.0-NaCl, pH 6.0-NaCl. After 24 h of incubation at 30°C, the heat tolerance (HT) and acid tolerance (AT) of *L. monocytogenes* was assessed in TSBYE at 57°C (for 2 h) and at pH 3.5 (for 7 h), respectively. Exposure to heat resulted in dramatic death during the initial 60 min, which was followed by survival of approximately 2 logs. However, exposure to acid resulted in continued death through 7 h. The single treatment of pH 5.0, and sequential treatment of pH 7.2-NaCl, reduced the HT but not the AT of *L. monocytogenes* compared to C. The sequential treatment of NaCl-pH 6.0 sensitized cells to both heat and acid, in contrast to the reverse sequential treatment (pH 6.0-NaCl). The HT of the other treatments was pH 6.0=NaCl>NaCl-pH 5.0>pH 6.0-NaCl>pH 5.0-NaCl>C, and the AT pH 6.0=NaCl>NaCl-pH 5.0>pH 6.0-NaCl=pH 5.0>pH 5.0-NaCl>pH 7.2-NaCl>C. The results suggest that prolonged exposure of *L. monocytogenes* to NaCl and low pH may increase its acid and heat tolerance, depending on the sequence of the processing steps.

P179 Acid Tolerance Response of Inoculated *Listeria monocytogenes* during Storage in Vacuum Packages at 10°C of Pork Sausage or Frankfurters Treated with Antimicrobials

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New strategies including antimicrobials in product formulations as well as post-processing application of antimicrobials are currently used or under study to help control *L. monocytogenes* (LM) in ready-to-eat meat products; however, the effect of such treatments on the acid tolerance response (ATR) of the pathogen have not been well studied. In this study we evaluated the ATR of LM in pH 1.0 simulated gastric fluid (GF) during storage (10°C) of commercial frankfurters (97% fat free) (F) and polish sausage (PS) with or without antimicrobials in the formulation. The LM inocula were prepared in tryptic soy broth plus yeast extract (TSB), or in PS slurry as planktonic (SUS) or biofilm (BIO) cells. Inoculated products were left untreated or dipped in 0.5% Nisaplin (N), 2.5% acetic acid (AA), 2.5% lactic acid (LA), or 5% potassium benzoate (PB) for 2 min at 25°C and stored in vacuum packages for 48 days at 10°C. The ATR of LM was determined during storage by enumeration (PALCAM agar) of survivors after exposure (60 min) to GF. Regardless of treatment, a higher ($P < 0.05$) ATR of LM was observed in PS compared to F, in which populations were reduced to the detection limit. The ATR of LM on untreated and N treated PS was not affected by inoculum origin. When antimicrobials were present in the formulation of PS, survival of LM in GF was population dependent. However, when PS did not have antimicrobials in the formulation, the pathogen expressed the highest ATR at 12 and 28 days of storage. LM on untreated F or PS maintained a higher or equivalent ATR compared to other treatments. These results indicate that treatment of polish sausage or frankfurters with antimicrobials did not result in an increased ATR of LM.

P180 Regulation of *Vibrio vulnificus* CadBA Operon Required for Acid Tolerance by CadC and Leucine-Responsive Regulatory Protein

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The *Vibrio vulnificus* cadBA genes encode a lysine/cadaverine antiporter and a lysine decarboxylase, whose combined activity leads to the synthesis and excretion of cadaverine to counteract external acidification. Lysine decarboxylase activity of *V. vulnificus* was induced at a low pH (pH 5.8), and the induction of lysine decarboxylase was regulated at the level of transcription. A primer extension analysis revealed that cadBA genes are organized as a single transcriptional unit, and that the transcription of cadBA begins at a specific site, consisting of a putative promoter Pcad. An open reading frame, cadC, consisting of 526 amino acids, was identified upstream of cadBA, and the activity of Pcad is under the positive control of CadC. The present study revealed that Lrp, a leucine-responsive regulatory protein, is also involved in the regulation of cadBA transcription by activating Pcad. Western blot analyses demonstrated that the cellular levels of CadC and Lrp were not significantly affected by each other, indicating that CadC and Lrp function cooperatively to activate the Pcad rather than sequentially in a regulatory cascade. Direct binding of CadC and Lrp to the upstream region of Pcad was demonstrated by chromatin immune precipitation assays and gel-mobility shift analyses, respectively. Accordingly, the present results revealed that CadC and Lrp coactivate the expression of cadBA, and that the activators exert their effect by directly binding to DNA upstream of Pcad.

P181 Identification of the *Vibrio vulnificus* putAP Operon and Evaluation of Its Role in Survival under Osmotic Stress

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Transcriptional orientations of the *Vibrio vulnificus* putAP genes encoding a proline dehydrogenase and a proline permease are in the same

direction. Proline dehydrogenase activity and the level of putA transcript were determined to reach a maximum in exponential phase and were then repressed when growth slowed down. Northern blot and primer extension analyses revealed that transcription of putAP genes results in two different transcripts, transcript A (putA transcript) and transcript AP (putAP transcript). Expression of putAP genes was directed by two promoters, promoter PputA and promoter PputAP. A crp null mutation decreased proline dehydrogenase activity and the level of the put transcripts, indicating transcription of putAP is under the positive control of CRP (cyclic AMP receptor protein). Proline dehydrogenase and the level of both put transcripts were increased by proline, but repressed by glutamate. In contrast to this, the level of transcript A, not transcript AP, increased when proline dehydrogenase was induced by NaCl. It is apparent that since PputA activity, not PputAP activity, was increased by NaCl, transcript A and transcript AP are transcribed through PputA and PputAP, respectively. Cells challenged with NaCl and various hyperosmotic stresses accumulated higher levels of glutamate, indicating that glutamate is a compatible solute in *V. vulnificus*.

P182 Comparison of BBL CHROMagar *Vibrio* and TCBS for the Recovery of *Vibrio cholerae* and *Vibrio parahaemolyticus* from Spiked Oyster Samples

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This study compares the recovery of *Vibrio cholerae* and *V. parahaemolyticus* from oysters on TCBS and a new medium, BBL™ CHROMagar™ *Vibrio* (CV). Homogenized oysters were added to flasks containing Alkaline Peptone Water. Ten *V. cholerae*, 8 *V. parahaemolyticus*, 3 *Vibrio* species and 3 non-*Vibrio* strains were added to the oyster suspension to achieve either 1 to 5 CFU/flask or 10 to 50 CFU/flask. Oyster suspensions were incubated at 35°C and 42°C, subcultured at 6 h and, if negative, at 24 h onto CV, TCBS and a nonselective control. Following 24 h incubation at 35°C, CV plates demonstrated 100% recovery of colonies characteristic of *V. cholerae* from the 6-h subculture at both low and high dilutions; at 42°C, recovery was 70% for the low dilution and 100% for the high dilution. At 35°C, TCBS plates recovered 90% and 100% of colonies characteristic of *V. cholera* at the low and high dilutions, respectively; at 42°C, recovery was 40% and 90%. At 35°C, CV plates recovered 100% of colonies suggestive of *V. parahaemolyticus* at the low and high dilutions; at 42°C, CV recovered 88% at both dilutions. At 35°C, TCBS recovered 38% and 50% of colonies suggestive of *V. parahaemolyticus* at the low and high dilutions; at 42°C TCBS recovered 0% and 38% of the low and high dilutions. Of the 3 non-*Vibrio* strains, 1 *Aeromonas* and 1 *Pseudomonas* strain produced colonies indistinguishable from *Vibrio* species on both media.

P183 Study on Detection and Identification of Foodborne Pathogens by Gene Chip Assay

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A rapid and accurate assay for detecting and identifying food borne pathogens was established. The eight food borne pathogens include *Escherichia coli* O157:H7; *Vibrio parahaemolyticus*; *Salmonella* Typhimurium; *Vibrio cholerae*; *Listeria monocytogenes*; *Campylobacter jejuni*; *Shigella flexneri*; *Yersinia enterocolitica*. It omitted the trivial steps (electrophoresis etc.) and decreased the time (< 3 h) tremendously. This method was an innovation in detection and characterization of pathogens. The DNAs of pathogens were amplified by 16S rDNA and 23S rDNA universal primers under the same conditions respectively (94°C, 2 min, 35 cycles of 20 s at 94°C, 20 s at 56°C, 20 s at 72°C, followed by a final extension for 5 min at 72°C). The PCR products were then applied to the gene chip for hybridization. The results showed the selected oligonucleotide probes were very specific, the stability and repeatability of gene chip were fit. The limit of detection was approximately 1.55×10^2 CFU/ml. The results were accurate, reliable, and feasible. Eight food borne pathogens were detected by the method simultaneously. The whole process takes 3 h. The establishment of this method provided a firm basis for further research in the field of multiple pathogen detection and identification.

P184 Fecal Bacterial Pathogens and Indicators in Commercially Available Compost

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Compost is a valuable soil amendment used by organic and conventional growers to improve the physical, chemical, and biological properties of soil. In the US, compost is produced from a variety of feedstocks that are sources of potentially pathogenic microbes, e.g., landscape trimmings, animal or poultry manure, food residuals, and biosolids from municipal or industrial wastewater treatment facilities. Aerobic, thermophilic compost production processes are designed to achieve significant reductions in fecal coliforms and salmonellae through time-temperature exposures. Currently, only biosolids compost must meet time-temperature process standards according to federal statute (40 CFR Part503), and few states have pathogen or pathogen indicator standards for marketable compost. Thus, product quality could vary widely and if inadequately composted the product could introduce pathogens into systems producing fresh fruits, vegetables, and herbs that may be consumed raw. We conducted a study of the microbial quality (total heterotrophs, total and fecal coliforms,

E. coli, *Salmonella*, and *Enterococci*) and seasonal variability, of commercially available compost from 11 facilities across the US. Feedstock compositions represented the wide range of materials commonly used. Results show that nearly all composts had fecal coliform counts above 1000 CFU/g and at least three contained salmonellae above 4 MPN/g (limits acceptable for distribution to the general public according to USEPA). This indicates the need for adherence to time-temperature process standards and product quality testing. Fruit and vegetable producers need to ensure that they use high quality composts, as not all currently available commercial products are disinfected.

P185 The Survival of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* in Angelica Keiskei and Sliced Carrot after Electron Beam Irradiation

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The consumption of fresh juices of Angelica keiskei (Shinsun-cho) and carrot for health has increased considerably in Korea. So the survival of *E. coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* after electron beam irradiation was studied in fresh vegetable samples such as Angelica keiskei and sliced carrot. Samples were artificially inoculated with each pathogenic bacteria to 10^8 to 10^9 cells/g and were irradiated with 2.5 and 5.0 KGy doses. The presence of pathogens after irradiation was observed during cold storage at 4°C for 5 days. Most pathogens were not detected after irradiation and during cold storage except *Listeria*-inoculated samples that had been treated with 2.5 KGy dose, on the third storage day. None of the three pathogens were detected at 5.0 KGy dose. Therefore 5.0 KGy dose of electron beam can be used to destroy *E. coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* in fresh Angelica keiskei and sliced carrot.

P186 Development of a Multiplex PCR Assay for the Detection of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Campylobacter jejuni* in Artificially Contaminated Food Samples

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Escherichia coli O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Campylobacter jejuni* have been identified as the major foodborne pathogens. These bacteria have been isolated from a wide range of food products, including dairy, meats, ready-to-eat vegetables and fruits. Detection of these pathogens in contaminated foods by a single assay would be useful. Polymerase chain reaction (PCR) assays have been used for rapid and specific detection of each of these pathogens separately. In this research, a multiplex PCR assay was developed and evaluated for detecting the four pathogens at one time from 11 different food samples. All food samples, including meat (ground beef, ground turkey, and chicken wash solutions), ready-to-eat vegetables (fresh-cut lettuce, broccoli, and mushrooms), fruits (watermelon and grapes), and dairy products (milk, cheddar cheese, and yogurt), were inoculated with the pathogens at various concentrations, allowed to attach for 1 h and then stomached with phosphate-buffered saline solution. Fifty milliliter of wash solution was centrifuged and the bacterial DNA was extracted. Primers for the four pathogens were added to a single PCR super-mix, the DNA was amplified at selected conditions (35 cycles, 50°C for annealing temperature which fit all primers), and the products were visualized by ethidium bromide staining. Results indicated that all four pathogens in food samples tested could be detected in one reaction tube with a total test time of 5 h. The sensitivity of the multiplex PCR assay in detection of all four bacteria in food samples is 10^4 CFU/PCR reaction. The multiplex PCR method is rapid and cost-effective for foodborne pathogen detection and can detect these target pathogens in any combination by using only one assay.

P187 Preliminary Study: Effectiveness of Ferrioxamine E as a Supplement to Isolate *Salmonella* Enteritidis from Shell Eggs

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The use and effectiveness of the hydroxamate siderophore Ferrioxamine E as a supplement to isolate *Salmonella* Enteritidis from white shell eggs was tested. Ferrioxamine E is a siderophoric compound biotechnologically produced by Streptomyces pilosus. As a growth factor of *S. enterica* serotypes, FE can reduce the pre-enrichment and detection time to less than 20 h, compared with conventional methods that require a pre-enrichment of 24 h to isolate *Salmonella* spp. To determine the minimum concentration of FE needed to enhance SE growth, egg samples were pre-enriched with different FE concentrations (0, 2, 200, 2000, 20,000 ng/g), and inoculated with low levels of SE (0.2 CFU/g). Samples were incubated at 37°C for 24 h. The optimum concentration of FE was determined to be 200 ng/g of liquid egg. Growth curves were performed with 200 ng/g of FE, and without 0 ng/g of FE to calculate the lag phase and the exponential growth rates of SE in eggs. After 24 h of incubation at 37°C, the SE population increased markedly, from 2 log CFU/g to 8 log CFU/g from eggs enriched with 0 and 200 ng/g of FE, respectively. Using a linear growth model, the lag phase for SE with 0 ng/g of FE was 12.743 versus 1.371 h with 200 ng/g of FE. The exponential growth rate for the control SE with

0 ng/g of FE was 0.20642 log CFU/h versus 0.40279 for 200 ng/g. Ferrioxamine E improved the efficiency of detecting SE in eggs by accelerating the growth of a bacterium that is usually present in low numbers in shell eggs.

P188 Selective Isolation of *Salmonella* Enteritidis from Mixed Cultures in Eggs, Using Ferrioxamine E Supplementation

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Direct plating method for detection of *Salmonella* Enteritidis (SE) in raw eggs has been hindered due to the antimicrobial and iron-restricting compounds in albumen. Studies show that utilization of ferrioxamine E as sole source of iron distinguishes *Salmonella enterica* serotypes Typhimurium and Enteritidis from a number of related species, including *Escherichia coli* (*E. coli*). However, the samples used for these studies were artificially contaminated with exclusively SE, so no interference with a number of related species could have taken place. The effectiveness of ferrioxamine E as a selective supplement was evaluated by inoculating whole eggs or egg white with proportionally mixed cultures of *Salmonella* Enteritidis phage type 13a (0.1 ml of 10^2 CFU/ml) and *E. coli* K12 (0.1 ml of 10^9 to 10^2 CFU/ml each). After 24 h of incubation at 37°C, the SE populations recovered from whole eggs supplemented with ferrioxamine E were 10^3 to 10^7 CFU/ml. However, 0 to 10^3 CFU/ml SE were isolated from samples without ferrioxamine E. In egg white, the SE population for samples supplemented with ferrioxamine E was 10^3 to 10^6 CFU/ml, whereas no SE was detected for samples without supplementation. The growth of *E. coli* in whole egg supplemented with ferrioxamine E was inhibited when the proportion of the mixed culture was under 1:100 (SE versus *E. coli*). In egg white, whether the samples were supplemented or not, the growth of *E. coli* was not significant. Our data indicate that ferrioxamine E could be used in the direct plating method for detection of *Salmonella* Enteritidis in raw eggs to increase SE growth rate while selectivity is maintained.

P189 DSC Differentiation and Classification of the Crude Lipopolysaccharides from *Salmonella* Species Using Fourier Transform Infrared Spectroscopy and Canonical Variate Analysis

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Fourier transform infrared (FTIR) spectroscopy and multivariate statistical analysis have been used to differentiate various whole-cell microorganisms based on differences in the spectra of microbial cell walls. *Salmonella* species contain lipopolysaccharides (LPS) in their outer membrane, and these LPS are composed of an O-polysaccharide, a core oligosaccharide, and lipid moieties. LPS have a strain-specific molecular structure, and spectra of these LPS show differences in the fingerprint region. The objective of this study was to differentiate and classify seven *Salmonella* LPS, using FTIR spectra and multivariate statistical procedures. *Salmonella* strains used included: *S.* Typhimurium, *S.* Enteritidis, *S.* Thomasville, *S.* Brandenburg, *S.* Hadar, *S.* Seftenberg, and *S.* Havana. The crude LPS were isolated from the 18 h-culture of each strain using the hot phenol-water extraction procedure. After 10 μL of a suspended sample (ca. 5 mg/ml) was placed on a gold slide and dried, spectra from 4,000 to 750 cm^{-1} were collected using a Continuum IR microscope, 128 scans, 4 cm^{-1} resolution, reflection mode, and units of log (1/R). Canonical variate analysis (CVA) was performed using the carbohydrate spectral region (1,200-900 cm^{-1}) after data compression by principal component analysis (PCA). The Mahalanobis distances were measured to quantify the spectral differences between the LPS from *Salmonella* species. FTIR in combination with CVA-PCA was able to successfully classify the seven *Salmonella* LPS; therefore, this approach could be used to identify these *Salmonella* species based on LPS spectra.

P190 Evaluation of Competitive Exclusion and Water Acidification on *Salmonella* in Live Turkey Operations

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Salmonella is a common bacterium in poultry production environments. A survey of several locations within one commercial live turkey operation demonstrated *Salmonella* in 41.1% of the samples. Samples included hatchery box pads, fecal samples before and after moving the flocks to the finish farms, before slaughter and swabs of transport cages. Isolates recovered from the survey were serotyped and tracked throughout the production environment. *S.* Heidelberg was the most prevalent isolate early in the turkey grow-out process. As the flocks matured, *S.* Typhimurium, which was previously undetected, became the most prevalent isolate. To better control *Salmonella* contamination, a commercially available competitive exclusion (CE) culture was administered to the poults. Results indicated a 2.7% reduction in *Salmonella* level in CE-culture treated poults after six weeks of grow-out. Because of the limited effectiveness of the CE culture, a water acidification agent was tested. Water acidification before slaughter caused a 40% reduction in *Salmonella* positives on fecal samples as compared to levels found on box pads. This study indicates that water acidification is an effective control strategy to reduce the levels of *Salmonella* before slaughter. Therefore, this treatment could potentially increase the microbial safety and quality of any resulting raw poultry products.

P191 *Salmonella* Typhimurium DT104 and Non-DT104 in Ontario, 2000

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The first *Salmonella* Typhimurium definitive type 104 (ST DT104) isolate was identified in Canada in 1970 and was sensitive to all antibiotics tested. ST DT104 has become of increased concern because now it is frequently resistant to multiple antibiotics. Because antibiotic resistant bacterial infections may compromise treatment in debilitated individuals, this study was undertaken to better understand this organism. In the year 2000, all isolates of ST in Ontario were analyzed for phage type and antimicrobial sensitivity. Additionally, every other case of ST was eligible for inclusion into a case-control study to identify demographic characteristics and symptomatology. In the laboratory portion of the study, 573 isolates of ST were identified. Of these, 271 isolates were ST DT104 (47.3%). In absolute numbers this was less than the 299 ST DT104 isolates identified in 1997/1998, but an increase in percentage, that is, ST DT104 of total ST (39.3% vs. 47.3%). Of the 271 ST DT104 isolates, 249 displayed the ACSSuT resistance pattern. In the case-control study, symptoms were found no more severe for ST DT104 cases than ST non-DT 104 cases.

P192 Significant Reduction in Incidence and Numbers of Total *Campylobacter* and Ciprofloxacin-resistant *Campylobacter* in Rinses from Retail Raw Chicken Carcasses in 2003

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Over a 103-week period from July 2001 through November 2003, a total of 412 retail raw chicken carcasses were tested at the rate of 4 per week for total *Campylobacter* CFU and ciprofloxacin-resistant *Campylobacter* CFU using a direct plating method. Significant reductions in total *Campylobacter* CFU and ciprofloxacin-resistant *Campylobacter* CFU were observed for samples in 2003 versus 2002 or 2001. Percentage of carcasses yielding no detectable *Campylobacter* CFU (detection limit = >0.90 log CFU) were 40% in 2003, 3% in 2002 and 14% in 2001. For carcasses with detectable cells, the numbers of total *Campylobacter* CFU per carcass were in the range of 0.90 to 4.77, 0.90 to 4.58 and 0.90 to 4.21 log CFU/carcass in 2003, 2002, and 2001, respectively. Percentage of carcasses yielding detectable ciprofloxacin-resistant *Campylobacter* CFU (detection limit = >0.90 log CFU) were just 17.5% in 2003 versus 59% in 2002 and 60% in 2001. The numbers of ciprofloxacin-resistant *Campylobacter* CFU per positive carcass were in the range of 0.90 to 2.86, 0.90 to 3.88, and 0.90 to 4.06 log CFU/carcass in 2003, 2002 and 2001, respectively. Weekly historical quantitative data trends from 2001 through 2003 confirm that the incidence and numbers of ciprofloxacin-resistant *Campylobacter* on retail raw chicken has been reduced in 2003 in correlation with the FDA withdrawal of approval for use of fluoroquinolones on poultry farms.

P193 Use of a Hybridoma Cell Model to Assess the Virulence of *Campylobacter jejuni* Isolates from Broiler Samples

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The objectives of this study were to assess the suitability of a hydridoma cell model to assess virulence of *C. jejuni* isolates from broiler samples. Fifty-four samples were analyzed for *Listeria*, using the USDA/FSIS procedure. Isolates were tested for catalase, oxidase production, genotypic profile, and ability to react with the specific monoclonal antibody 10-C89, in an ELISA assay. Virulence potential of *C. jejuni* isolates was tested by an in vitro 6-h hybridoma cell assay with lactate dehydrogenase (LDH) release assay at different concentrations (10 to 100%) of *C. jejuni* toxin (CJT). Three samples (5.5%) were positive for *Campylobacter*. A total of 180 isolates was obtained, of which 7 (4%) were confirmed to be *C. jejuni*. These isolates were all oxidase and catalase positive (100%), had strong reaction to 10-C89 (71.4%) and were positive for flagellin (fla A) gene by PCR analysis. All *C. jejuni* isolates were virulent and caused 67% hybridoma cell death, similar to positive control *C. jejuni* ATCC 24922 (80%). The hybridoma cell death values were compared to primary cell lines (Ramos) and Vero cells. In the LDH assay, *C. jejuni* isolates caused an average 62% of LDH release in hybridoma cells, similar to positive control, ATCC 24928 (76%). Similarly, *C. jejuni* treated Ramos cells caused an average 65% compared to 25% LDH release in Vero cells. This data will help to develop a rapid cytotoxicity assay to screen the virulence of *C. jejuni* isolates, and to further understand the interaction between *C. jejuni* and hybridoma cells.

P194 Development of a Novel Therapeutic Treatment of Chickens to Control *Campylobacter jejuni* Colonization

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Campylobacter jejuni is an important foodborne agent of human gastroenteritis. This organism is thought to be transmitted most frequently through exposure to poultry products. In an attempt to control the pathogen, we screened 365 *Bacillus/Paenibacillus* spp. isolates from poultry production to identify potentials for anti-*C. jejuni* activity. Zones of *C. jejuni* inhibition surrounding 56 isolates piqued our interest. One novel antagonistic *Bacillus circulans* (NRRL B-30644)

and two *Paenibacillus polymyxa* (NRRL B-30507 & NRRL B-30509) strains were identified and deposited under provisions of the Budapest Treaty. The cell-free, ammonium sulfate precipitate from each candidate culture also created zones of *C. jejuni* inhibition in spot tests. Exposure of the crude antimicrobial preparation to protease enzymes removed *Campylobacter* inhibition, thus demonstrating a peptide characteristic consistent with bacteriocin definition. The pure peptides were obtained by gel-filtration, ion-exchange chromatography and hydrophobic interaction. The peptides were characterized by SDS-PAGE electrophoresis; isoelectric focusing; and amino acid sequencing. In 15 separate experiments, one or two days-post-hatch chicks were colonized with challenges of ~2 x 6 log CFU *C. jejuni*, and placed in isolation units. Three days before sampling, therapeutic feeds were provided ad-libitum. This feed consisted of purified bacteriocin (0.25 or 0.5 g) micro-encapsulated in polyvinyl-pyrrolidone and incorporated into 1 kg of chicken feed. Therapeutic treatment consistently reduced *C. jejuni* colonization in birds ranging from 8 to 24 days of age by at least 100,000 fold over the untreated chicks. Therapeutic bacteriocin treatment of mature chickens prior to slaughter may substantially reduce public exposure to this organism.

P195 Prevalence and Types of *Campylobacter jejuni* in Potential Reservoirs and Transmission Routes of Human Campylobacteriosis

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New Zealand has the highest rate of campylobacteriosis notifications in the developed world. Currently the incidence is 369.0 cases per 100,000 population. Our group is involved in a number of projects aiming to identify transmission routes relevant to New Zealand, and to explain why the incidence is above that reported elsewhere. As part of a larger study into transmission routes of campylobacteriosis, a focused study of the types of *Campylobacter* in a number of reservoirs and transmission routes was carried out in a defined geographical rural area. 1450 samples of river water, pig, sheep and beef liver, composite beef cattle, sheep, duck and dairy cow feces, individual possum and rabbit feces, raw whole chickens and human case feces were tested for the presence of *C. jejuni* by enrichment and PCR. Prevalences were; composite cow feces 97.8%, composite cattle feces 83.9%, composite duck feces 65.2%, composite sheep feces 59.8%, river water 55.3%, sheep liver 38.9%, chicken carcasses 27.5%, beef liver 9.0%, pork liver 4.8%, rabbit and possum feces, both 0%. Positive samples were cultured to yield isolates for typing and data for 616 *C. jejuni* isolates were obtained. Penner serotypes 2, 4 complex and 23,26 predominated. The combination of serotyping and *SmaI*-MRP-PFGE typing revealed a high diversity of isolates within each matrix, and types which were indistinguishable from those obtained from human cases were detected in all of them. Some types were only, or predominantly, isolated from one source and it is possible that they rarely cause disease in humans.

P196 Genetic Characterization of Multi-drug Resistant Strains of *Campylobacter coli* from Turkeys

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Recent studies have shown that turkeys in North Carolina are frequently colonized by *Campylobacter coli*. Many of the turkey-derived *C. coli* isolates were found to be resistant to several antibiotics (tetracycline, streptomycin, ampicillin, erythromycin, kanamycin, nalidixic acid and ciprofloxacin) and have been designated multi-resistant (MR). The ecology and potentially unique physiological and genetic attributes of these MR strains remain poorly understood. To characterize the population genetic structure of this group of *C. coli* isolates, we employed two different molecular genotyping tools: restriction fragment length polymorphism (RFLP) of the flaA gene (fla typing) and pulsed field gel electrophoresis (PFGE). A total of 100 MR strains were characterized, representing isolates from different farms, from birds of different ages, and from different times within a three-year period. Even though ca. 20 fla types were identified among these MR strains, two of these types accounted for the majority of the isolates. PFGE revealed that strains of the same fla type could have different PFGE profiles. However, we also identified numerous strains which had indistinguishable PFGE profiles but different fla type, suggesting selection for specific flagellin sequences in strains of otherwise closely related genomic backgrounds. The findings suggest that the majority of the *C. coli* MR strains from turkeys constitute a clonal lineage which may be especially adept in colonizing the birds, and that selection for specific flagellin sequences may account for part of the observed diversity on fla types within this lineage.

P197 Withdrawn

P198 DSC Electrochemical Sensor Method for Rapid Detection of Coliforms in Milk

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The coliform group is routinely used as an indicator of potential enteric pathogen contamination of foods. Several rapid methods for monitoring coliforms in foods have been developed; however, most methods have limitations such as duration of incubation, antagonistic organism interference, lack of specificity and poor detection. Therefore, this study was conducted to develop a rapid and simple method for the estimation of

coliforms in food products. An electrochemical CO_2 sensor was developed to detect CO_2 produced through lactose fermentation by coliforms in laboratory media and milk. Modified selective medium and sample were mixed and incubated at 37°C. Concentrations of CO_2 were automatically measured by CO_2 electrodes set on the top of reaction tubes. Detection time, the minimum required for detection of CO_2 by the sensor method, was calculated and compared with the population of coliforms. There was a strong relationship between the population of coliforms and detection time in both laboratory medium and milk ($R^2 > 0.97$). The recovery and estimation of cold-injured coliforms by the sensor method strongly agree with those of the conventional plating method ($R^2 = 0.98$). The sensor method was applied to ascertain levels of natural coliforms in non-inoculated raw milk samples and the correlation between both methods was acceptable ($R^2 = 0.81$). These results indicate that coliforms in milk can be easily and automatically estimated within about 13 h. The sensor method is a rapid, simple, and accurate way to monitor total coliforms in milk.

P199 Phenotypic and Genotypic Traits of *Staphylococcus aureus* Strains Isolated from Raw Bulk-tank Milk Samples of Small Ruminants

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Two hundred ninety-three isolates of *Staphylococcus aureus* obtained from 127 bulk-tank milk samples of small ruminants collected throughout Switzerland were characterized by pheno- and genotypic traits. Of the 293 isolates, 193 (65.9%) were egg yolk-negative and 15 (5.1%) were negative for clumping factor and/or protein A determined by a latex agglutinating test system. For 285 *S. aureus* isolates PCR amplification of the 3' end of the *coa* gene showed a single amplicon. Five different sized PCR products of 500, 580, 660, 740 and 820 bp were distinguished. In 191 isolates (n=293) toxin genes were detected: 123 isolates tested positive for *sec*, 31 isolates for *seg*, 28 isolates for *sea*, 26 isolates for *sej*, 24 isolates for *sei* and 4 isolates for *seb* and 4 isolates for *sed*. Furthermore, 126 isolates were positive for *tst*, the gene encoding the toxic shock syndrome toxin 1. Coagulase gene restriction profile (CRP) analysis of the 145 isolates harbouring *sea* or *sec* genes revealed 6 different patterns using *AluI* and 5 different patterns using *Hae*III. In summary, within these two groups, high genotypic uniformity within the different sized *coa* gene amplicons was proved. This is the first study providing comprehensive characterization data of *S. aureus* strains originating from bulk-tank milk samples of small ruminants. Compared to data on bovine milk, *S. aureus* originating from small ruminants milk showed remarkable differences in phenotypic traits. Moreover, the high prevalence of toxin-producing *S. aureus* is an issue requiring consideration as it relates to food hygiene.

P200 DSC Efficacy of Ultraviolet Light Treatment for the Reduction of *Listeria monocytogenes* in Raw Fluid Goat Milk

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Traditional goat cheeses are produced using unpasteurized milk, which increases the food safety concerns for these types of products. Goat milk products have seen an increase in popularity and consumption per capita, and the niche market includes gourmet goat cheeses that may be found only at natural food stores, farmer's markets and high-end restaurants. The US Code of Federal Regulations and the Pasteurized Milk Ordinance both address the possibility for processing alternatives to heat treatment and the use of ultraviolet processing may be a viable alternative with potentially less detrimental effects on flavor while still ensuring the safety of the product. The CiderSure 3500 UV apparatus was designed with a series of eight germicidal UV lamps and computer monitored UV sensors to adjust the flow rate to ensure enough UV exposure for a consistently greater than 5-log reduction of the pertinent pathogen. Fresh goat milk from New York was inoculated to 10^7 CFU/ml with *Listeria monocytogenes* (L-2289) and exposed to UV light by use of the CiderSure apparatus. Inoculated milk was exposed to an ultraviolet dose range between 2 and 20 mJ/cm^2 to determine the optimal UV dose. A greater than 5-log reduction was achieved ($P < 0.0001$) when the milk was passed through the machine 12 times for a cumulative exposure time of roughly 18 sec and a cumulative UV dose of 15.8 +/- 1.6 mJ/cm^2. The results of this study indicate that UV irradiation could be used as an alternative to thermal pasteurization for the reduction of *Listeria monocytogenes* in goat milk.

P201 Evaluation of the Microbiological Safety in Three Ultra-pasteurized Milk Companies

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We randomly analyzed over 173 ultra-pasteurized tetra brick milk samples, coming from three different companies in Xalapa, Veracruz, Mexico. Although the Mexican Health Department Secretary (SSA) establishes a zero CFU/ml Standard Plate Count (SPC) of aerobic and anaerobic mesophilic and thermophylic bacteria, the three tested companies displayed high percentages of contamination, with maximum numbers of CFU

above 87,000. Significant differences were found between companies in regard to the kind of microorganism, except for thermophylic anaerobic bacteria. Company labeled 2 was less contaminated with those bacteria. These results suggest inadequate practices of handling as well as a deficient pasteurization process. Nowadays, latex gives assessment to these companies as well as to their main buyers, in order to diminish the risk from the base of the chain, and maintain it within a HACCP system.

P202 Effectiveness of *Enterococcus faecium* M-74 for Controlling *Listeria monocytogenes* in Re-hydrated Dried Milk during Temperature Abuse

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The persistence of *Listeria* spp. in the dairy plant environment poses a risk for contamination of dried dairy products with *Listeria monocytogenes*. This study evaluated the effectiveness of a probiotic strain of *Enterococcus faecium* for controlling *L. monocytogenes* in re-hydrated dried milk. Samples of re-hydrated milk were inoculated with a 5-strain mixture of *L. monocytogenes* alone (control) or with *E. faecium* M-74 and held at 25°C or 30°C for 24 h. Microbiological and pH tests were conducted at 0, 4, 8, 12 and 24 h. Numbers of *L. monocytogenes* and *E. faecium* were determined by plating diluted samples onto Modified Oxford (MOX) agar and acidified MRS agar, respectively, and counting bacterial colonies on agar plates after 72 h at 35°C. Initial populations of *L. monocytogenes* and *E. faecium* in the milk were ~ 3.20 and 8.57 log CFU/ml, respectively. *L. monocytogenes* in controls increased to 6.18 log CFU/ml (25°C) and 6.85 log CFU/ml (30°C) in 12 h. In contrast, the pathogen was strongly suppressed in milk with *E. faecium*; numbers of *L. monocytogenes* were 3.24, 3.49, and 3.56 log CFU/ml (25°C) and 3.33, 3.36, and 2.85 log CFU/ml (30°C) at 8, 12, and 24 h, respectively. Populations of *E. faecium* remained relatively unchanged at 25°C or 30°C for 24 h; however, pH values of milk samples with this organism were significantly ($P < 0.05$) lower than those of controls. Based on the results of this study, E. faecium M-74 seems to have good potential for inhibiting growth of *L. monocytogenes* in temperature-abused re-hydrated dried milk.

P203 Survival of *Mycobacterium avium* subsp. *paratuberculosis* during Manufacture and Ripening of Laboratory-produced Cheddar Cheese

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Mycobacterium avium subsp. *paratuberculosis* (Map) is the known causative agent of Johne's disease in dairy cattle and other ruminants. Some evidence has implicated Map as an etiologic agent of Crohn's disease in humans and several studies have shown that Map may not be completely inactivated by high-temperature short-time pasteurization. Consequently, the possibility exists for viable Map cells to be in milk used for dairy products, for example cheese, where the milk may undergo a lesser heat treatment than pasteurization. A laboratory-scale method was developed to produce model Cheddar cheese, simulating commercial product. Cheeses were prepared from pasteurized milk, artificially contaminated with high (10^4 to 10^5 CFU/ml) and low (10^1 to 10^2 CFU/ml) levels of a reference type strain, and two field isolates. Homogenized cheese samples were inoculated onto Herrold's egg yolk medium, Middlebrook 7H10 medium and radiometric BACTEC medium – each supplemented with antibiotics and mycobactin J. For all cheeses manufactured, a slow gradual decrease in Map CFUs/g cheese was observed over the 6-month ripening period. In all cases where high levels (> 3.6 log) of Map were present in 1-day cheeses, the organism was culturable after the 6-month ripening period. The D values calculated for the three strains were 107, 96 and 90 days. Map cells were recovered from the whey fraction in 10 of the 12 cheeses manufactured. While Map persists throughout the manufacture and early ripening stages of Cheddar manufacture, the number of viable Map cells in commercial Cheddar will depend on the source of the cheese milk, the effectiveness of the milk heat treatment and the "health status" of any Map cells surviving the applied heat treatment.

P204 Fate of *Salmonella* spp. in Oaxaca Cheese during Refrigeration and Abusive-temperature Storage

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Oaxaca cheese is a traditional Mexican dairy product. It is frequently produced with raw milk under poor sanitary conditions, but its role in foodborne illnesses remains unknown. The behavior of *Salmonella* on Oaxaca cheese was evaluated during storage at refrigeration and under abusive temperature. Oaxaca cheese was prepared with raw or pasteurized milk in the laboratory and then, portions of 10 g were spot-inoculated (1.7 and 3.0 log CFU/g) with a four strain rifampicin-resistant serotype cocktail. The cheese was stored for 7 cycles of 4 to 7°C/12 h, 22°C/8 h, and 30°C/4 h (to simulate temperature abuse); and at 4 to 7°C for up to 7 days. *Salmonella* was enumerated on tryptic soy agar with rifampicin (150 ppm) at the lowest inoculum level; numbers did not change during temperature abuse conditions on both products. In contrast, with the highest inoculum, the numbers increased to 5.2 and 6.3 log CFU/g on raw and pasteurized cheese, respectively. During refrigeration, the population of the pathogen remained unchanged regardless

of the inoculum size and type of milk used to prepare the cheese. Althought natural antimicrobial mechanisms in raw milk may have been inactivated by pasteurization, competitive microflora might have interfered with the *Salmonella* growth. Both of these factors might explain the higher numbers of *Salmonella* observed on pasteurized cheese relative to those on raw cheese associated with use of abusive temperatures. The survival of *Salmonella* in Oaxaca cheese might constitute a health risk and a concern to public health authorities.

P205 Survival of *Escherichia coli* O157:H7 in Galotyri, a Traditional Greek Soft Acid-curd Cheese

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Post-process contamination of fresh acid-curd cheeses with *Escherichia coli* O157:H7 may pose a risk. To evaluate this, portions (0.5 kg) of two commercial types of fresh Galotyri, one artisan (pH 3.9 ± 0.1) and the other industrial (pH 3.7 ± 0.1), were inoculated with ca. 3.0 or 6.5 log CFU/g of a five-strain cocktail of *E. coli* O157:H7, including rifampicin-resistant derivatives of the strains ATCC 43895 (acid-resistant) and ATCC 51657, and stored aerobically at 4 and 12°C. Survival was monitored for 28 days by plating cheese samples on tryptic soy agar with 100 mg/l rifampicin (TSA+rif), SMAC and Fluorocult *E. coli* O157:H7 agar media. The pathogen declined much faster ($P < 0.05$) in the industrial as compared to the artisan cheeses at both temperatures. Thus, while *E. coli* O157:H7 became undetectable by culture enrichment after 14 days at 4°C in industrial samples, irrespective of the inoculation level, populations of 1.4 to 1.9 and 4.2 to 5.1 log CFU/g survived after 28 days in the corresponding artisan cheeses with the low and high inocula, respectively. Survival was longer and greater ($P < 0.05$) on TSA+rif than on SMAC and Fluorocult, indicating the presence of acid-injured cells. Interestingly, survival of *E. coli* O157:H7 after 14 to 28 days in cheeses was better at 12°C than at 4°C, probably due to yeasts grown on the surface of temperature-abused cheeses. The dramatic difference in the pathogen's inactivation between the industrial and artisan cheeses could not be associated with differences in pH or type/concentration of organic acids, suggesting another anti-*E. coli* O157:H7 activity by the industrial starter.

P206 DSC Ambient Storage of Aged Hard Cheese at Retail: A Food Safety Assessment

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Ambient display of aged hard cheeses in retail food establishments is a common practice, yet little research information exists that supports the food safety and quality implications of such practices. A variety of published studies indicate that certain cheeses do not support the growth of bacterial pathogens, including *Listeria*, *Salmonella* and *E. coli*, because of factors such as preservatives, pH, a_w and salt content. Examples of such cheeses include hard and semi-soft cheeses. We examined the impact of ambient storage practices on the quality and safety of five cheese varieties: Parmesan, Romano, Cheddar, Swiss and fresh Mozzarella. We compared storage of these cheeses at ambient (72°F) versus refrigerated temperatures (37°F) in a retail food establishment setting. Safety and quality implications of each storage temperature was assessed through microbiological analysis for Aerobic plate count, Coliform count, *Staphylococcus aureus*, *Lactobacillus* and yeasts and molds, using 3M Petrifilms™. For each experimental trial, 13 blocks of each cheese were placed at each temperature. Temperature was monitored with thermocouples linked to data loggers for continuous measurement. The cheeses were collected for microbiological analysis at time zero and at 24 h intervals for 10 days. All cheese samples were negative for growth of Coliforms, *S. aureus* and yeasts and molds, except Cheddar, which had visual mold at 7 days. *Lactobacillus* counts for all cheeses were consistent for starter culture activity except the fresh Mozzarella, which showed rapid increases in numbers. This assessment found that ambient storage of aged cheeses negatively impacts quality but not safety.

P207 Survival of *Listeria monocytogenes* in Cow and Goat Milk as Well as in Cottage Cheese Made from Cow and Goat Milk during Storage at Various Temperatures

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The ability of *Listeria monocytogenes* to survive in cow and goat milk as well as cottage cheese made from both milks during storage at different temperatures was studied. Pasteurized cow milk and ultra pasteurized goat milk, purchased from retail outlets in Georgia, were inoculated with a 5-strain mixture of *L. monocytogenes* at a level of 10^2 or 10^4 colony forming units per milliliter (CFU/ml). The inoculated milk and uninoculated controls were used to manufacture cottage cheese following a procedure described by El-Shenawy and Marth, with modifications. Both milk and cheese were stored at 4, 10, or 22°C for 14 days. Samples were drawn twice a week and serially diluted with buffered peptone water. Appropriate dilutions were surface plated on modified Oxford agar with antimicrobial supplements. Colonies with the typical characteristics of *Listeria* were enumerated. The growth of *L. monocytogenes* was slow at 4°C in the cow and goat milk as well as the cheese. However, the samples stored at 10°C and 22°C showed a steady increase in *Listeria* populations throughout the storage period. Salting of the cheese with 1% NaCl did not have an inhibitory effect on the pathogen. *L. monocytogenes* was able to survive the manufacturing process of both cow and goat milk cheese and proliferated to potentially dangerous levels during the storage period.

P208 DSC An Artificial Neural Network Approach to Predict *Clostridium botulinum* Toxin Production in Process Cheese Spreads

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Experiments to determine the outgrowth and toxin production of *Clostridium botulinum* in process cheese were conducted by Nobumasa Tanaka, Kathleen Glass and Eric Johnson. The formulation factors considered were moisture level, pH, water activity, sodium chloride, sodium phosphate and other additives. The back-propagation approach was used to learn weights in the neural network. Sigmoid functions were used to fit the model. Tuning set and cross-validation methods were applied to improve the model performance. This artificial neural network was evaluated by comparison with the decision tree approach and statistical modeling by logistic regression. Our results showed that the artificial neural network provided satisfactory prediction accuracy in classifying the potential hazardous and safe formulations. This neural network was robust with respect to noisy and incomplete data. The antibotulinal properties of formulation factors were evaluated. Graphical plots were made to assist with formulation adjustment, as well as to compare different model building approaches. These results suggest that the artificial neural network approach can be a reliable, efficient and convenient tool to predict microorganisms' response in terms of environmental conditions. It can be applied to assist manufacturers in developing safe process cheese formulations.

P209 Laboratory Investigation of a *Listeria monocytogenes* Outbreak Associated with Consumption of Soft Cheese

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Listeriosis is a severe foodborne illness affecting primarily neonates, pregnant women, and other immunocompromised persons. Common source outbreaks of listeriosis have been associated with various foods, including ready-to-eat meats and soft cheeses. PulseNet, the national molecular subtyping network for foodborne disease surveillance, detects foodborne disease clusters and helps to identify or confirm outbreak sources. PulseNet laboratories routinely subtype isolates of foodborne pathogenic bacteria, using standardized pulsed-field gel electrophoresis (PFGE) protocols, and electronically submit the patterns to the PulseNet national database. In May 2003, the Texas Department of Health reported three cases of listeriosis with indistinguishable PFGE patterns (PulseNet pattern numbers: GX6A16.0017 and GX6A12.0671) on the WebBoard (the PulseNet listserv). The WebBoard is used to alert epidemiologists and PulseNet laboratories of possible outbreaks. Subsequently, three additional cases with identical or very similar PFGE patterns were identified. Investigation of case-patients revealed that 5/6 reported consumption of unpasteurized queso fresco—a soft Mexican-style cheese—that was imported legally but sold illegally in the United States. Imported cheese was obtained for laboratory testing from area markets. Multiple PFGE types of *Listeria monocytogenes* were isolated from two samples, including one isolate that was indistinguishable from the outbreak pattern. Quantification studies showed that *L. monocytogenes* strains isolated from the queso fresco were three-fold more likely to have the outbreak pattern than isolates with differing patterns. Information from this outbreak will facilitate targeted education of pregnant women regarding the risks posed by consumption of unpasteurized soft cheeses not approved for sale in the United States.

P210 Survival of *Listeria monocytogenes* in Commercial and Laboratory-scale Prepared Galotyri, a Traditional Greek Soft Acid-curd Cheese, Stored Aerobically at 4 and 12°C

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Galotyri is a traditional Greek soft acid-curd cheese. Because its technology may allow *Listeria monocytogenes* post-process contamination, this study evaluated survival of the pathogen in fresh cheese during storage. Portions (0.5 kg) of two commercial types (< 2% salt) of Galotyri, one artisan (pH 4.0 ± 0.1) and the other industrial (pH 3.8 ± 0.1), were inoculated with ca. 3 or 7 log CFU/g of a five-strain cocktail of *L. monocytogenes* and stored aerobically at 4 and 12°C. After 3 days, average declines of pathogen's populations (PALCAM agar) were 1.3 to 1.6 and 3.7 to 4.6 log CFU/g in samples for the low and high inocula, respectively. These declines were independent ($P > 0.05$) of the cheese type or the storage temperature. From day 3, however, declines shifted to small or minimal to result in 1.4 to 1.8 log CFU/g of survivors at 28 days of storage at 4°C, indicating a strong "tailing" independent of initial level of contamination. Low (1.2 to 1.7 log CFU/g) survival of *L. monocytogenes* also occurred in cheeses at 12°C for 14 days. When ca. 3 log CFU/g of *L. monocytogenes* were inoculated in laboratory-scale prepared Galotyri of pH @ 4.4 and @ 3% salt, the pathogen died off at 14 and 21 days at 12 and 4°C, respectively, in artisan-type cheeses fermented with the natural starter. In contrast, the pathogen survived for 28 days in cheeses fermented with the industrial starter. These results indicate that *L. monocytogenes* can not grow but may survive during retail storage of Galotyri, even when post-process contamination is low.

P211 A Comparative Study of a New Next Day Method and the ISO 11290-1 Method for the Detection of *Listeria* sp. in Meat and Dairy Products

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This study was conducted to evaluate the efficiency of a new rapid method for the detection of *Listeria* sp. in meat and dairy products, the VIDAS Listeria species Xpress method (VIDAS LSX), in comparison with the ISO 11290-1 reference method. 289 naturally contaminated and spiked food samples, of which 141 were meat products and 148 dairy products, were tested by the two methods. For the LSX method, samples were pre-enriched for 22 h at 30°C in a proprietary enrichment broth, the LX broth. 3 ml of this pre-enrichment were then sub-cultured into 6 ml of LX broth for 6 h at 30°C, before testing in the VIDAS instrument. Presumptive positive samples were further confirmed by classical identification tests after isolation on a chromogenic plate medium. The LSX method detected 201 positive samples (102 meat and 99 dairy products) and the ISO method 203 positive samples (103 meat and 100 dairy products), with an agreement of 95.2%. The difference observed was not statistically significant, especially as the pre-enrichment was different for the two methods. This study showed that the VIDAS LSX method is comparable to the ISO 11290-1 method for the recovery of *Listeria* sp. in meat and dairy samples. In addition it allows release of negative products within 30 h compared with 5 days for the standard cultural method. Furthermore, the combination of the VIDAS LSX screening method with the use of a chromogenic plate medium can lead to a rapid identification of *Listeria monocytogenes*.

P212 The Detection and Survival of *Salmonella, Escherichia coli,* and *Listeria monocytogenes* in Selected Pesticide Sprays for Use on Fresh Produce

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In several produce-associated outbreaks it was hypothesized that topical sprays were a causative factor. This study was conducted to determine if pathogens such as *Salmonella, Escherichia coli* O157:H7 and *Listeria monocytogenes* are present in pesticide sprays and whether pathogens can survive and grow in the spray mixes over time. Four commodities and sprays typically applied to these commodities were chosen for this investigation. The pesticides tested were Imidan®, Captan, Warrior®, Dimethoate, Dual®, Spintor™, Elevate® and Switch™; the commodities involved were cabbage, apple, strawberry and tomato. The pesticides were mixed with the on-farm water source normally used for spray applications. The spray-mixture (pesticides and water), the pesticides, and the on-farm water sources were plated on selective media to determine presence of selected organisms. The spray-mixes and concentrated pesticides were enriched to determine if any pathogens were present at extremely low levels. Initial results indicate that *Salmonella*, *E. coli* and *Listeria* were present in the on-farm water sources, especially surface water, but they were not usually present in spray-mixes. None of the selected organisms were detected in the pesticides tested. As an additional control to determine pathogen growth and survival, the pesticides were diluted with sterile water to application concentrations and inoculated separately with a cocktail of three strains of *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes*. The inoculations consisted of: *Salmonella gammanera*, *hartford*, and *rubisawl*; *E. coli* O157:H7 43889, 43895, and 933; *L. monocytogenes* L2289, L-99, and 104025. Inoculated spray-mixes showed a general decline in organisms over time.

P213 Pre-harvest Survival of Viruses on the Surface of Produce

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The goal of this study was to compare the effects of different environmental conditions on the survival of viruses on cantaloupe, lettuce, and green peppers. The organisms used in this study included hepatitis A virus (HAV), feline calicivirus (FCV) and coliphage PRD1. The study took place in a controlled environment chamber that allowed for the control of temperature and relative humidity. Survival under high and low relative humidity was compared. The edible surface of each plant was inoculated with the study viruses. Samples were collected at the beginning of each experiment and at various time intervals over the course of two weeks. The viruses were eluted from the plant surfaces using 3% beef extract. PRD1 survived at least 14 days under all conditions, with the exception of cantaloupe under humid conditions. HAV survived in excess of 14 days under all conditions, with the exception of lettuce under humid conditions. FCV survived less than 5 days under all conditions, with the exception of cantaloupe under dry conditions. The longest survival of HAV occurred on cantaloupe in dry conditions and lettuce in humid conditions. In dry conditions, the longest survival of PRD1 occurred on cantaloupe. The longest survival of FCV occurred on lettuce in humid conditions and cantaloupe in dry conditions. The results suggest that survival of viruses on the surface of produce is not uniformly affected by relative humidity.

P214 Comparison of Cetylpyridinium Chloride-Ethanol and Lauryl Sulfate-Chlorine Disinfection of Ready-to-Eat Vegetables Artificially Contaminated with *Escherichia coli* and *Salmonella*

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This study compared the effectiveness of cetylpyridinium chloride (CPC)-ethanol (EtOH) with lauryl sulfate (LS)-chlorine disinfection of broccoli, celery, lettuce, mung bean sprouts, and scallions artificially contaminated with *Escherichia coli*, *Salmonella* Gaminara, and *S.* Minnesota. Test portions (25 g) were sonicated in aqueous solutions of CPC (0.5%) for 5 min at 25°C, followed by EtOH (10%) for 1 min at 50°C. *E. coli* was enumerated on CHROMagar™ *E. coli*, and *Salmonella*, on Rambach™ agar media. *E. coli* and *Salmonella* populations in untreated produce remained relatively unchanged at spiking levels of 6 to 7 log CFU/g throughout the 14-day storage period at 5°C. Surviving *E. coli* and *Salmonella* in CPC/EtOH-treated produce showed a 2 log reduction compared to the control. In our earlier work, *Listeria* and *Campylobacter* showed a >5 log reduction with similar treatment. Treatment with chlorine (200, 400, 600, and 1200 ppm), or with LS (0.5%)-chlorine at 25°C did not improve the logR value for *E. coli* and *Salmonella*. The 2 log reduction indicated the limited efficacy of the disinfectants against *E. coli* and *Salmonella*, possibly due to the resilient and compact nature of biofilms formed by these organisms on the surfaces and crevices of fresh produce. Neither CPC-EtOH nor LS-chlorine appears to be an effective approach in controlling the viability of *E. coli* and *Salmonella* at contamination rates of 6 to 7 log CFU/g. The persistent survival of these microorganisms at 5°C in the five produce studied reinforces the need for good agricultural practices and special handling of ready-to-eat vegetables before consumption.

P215 Efficacy of Acidified Sodium Chlorite Treatments in Reducing Pathogens on the Surface of Leafy Vegetable

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Efficacy of acidified sodium chlorite for reducing the population of pathogenic bacteria on Chinese cabbage leaves was evaluated. Washing leaves with distilled water could reduce the population of *E. coli* O157:H7, *Salmonella* and *Listeria monocytogenes* by approximately 1.0 log CFU/g or less depending on the microorganisms artificially inoculated on the surface. There were no significant differences of efficacy found among water, alkaline chlorite solution and citric acid solution used for washing. However, treating with acidified chlorite solution could reduce the population of *E. coli* O157:H7 by 3.0 log CFU/g level on Chinese cabbage leaves, and the efficacy was not significantly influenced by washing with sodium chlorite solution containing various organic acids. Acidified sodium chlorite in combination with mild heat treatment could reduce the population by 4.0 log CFU/g without affecting the color but it softened the leaves. However, a similar level of efficacy was achieved by this treatment even at low temperature, indicating that acidified sodium chloride solution could offer a benefit as a sanitizer for surface washing of fresh produce. The application of this treatment for producing lightly fermented vegetables was also examined.

P216 Growth of *Listeria monocytogenes* in Stored Red Delicious Apple Tissues

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Reported survival and growth of *Listeria monocytogenes* in apple tissues suggests that this human pathogen may pose a risk in the manufacture and distribution of fresh-cut apple slices. Accurate assessment of the risk requires an understanding of factors which affect behavior of the bacterium in apple tissues. The present investigation was undertaken to determine wether prolonged storage and consequent physiological changes in the apple influence the survival of *L. monocytogenes*. Red Delicious apples stored under controlled atmosphere at 1°C for 5, 6, 8 and 10 months after harvest were periodically withdrawn from storage. Discs (2 cm diameter) of apple tissue were inoculated (10^4 CFU/cm^2) with a washed cell suspension composed of five *L. monocytogenes* strains. The disks were stored at 5°C and 15°C for 7 days under aerobic and anaerobic conditions. Populations estimates were obtained by plating onto PALCAM agar. *L. monocytogenes* grew in apple tissues stored under all test conditions and there were no apparent differences due to age of the apples. The implications of this finding on the mitigation of risks incurred in the manufacture of fresh apple products will be discussed.

P217 Inactivation of *Salmonella* during Drying and Storage of Nantes Carrot Slices Treated with Steam, Water or Acid Blanching before Dehydration

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Salmonellae may pose a food safety risk from home-dried products such as carrot slices. This study evaluated the influence of pre-drying treatments on inactivation of *Salmonella* during preparation, home-type dehydration (60°C, 6 h) and storage of carrot slices. Inoculated (five-strains, 7.8 log CFU/g) slices were subjected to the following treatments: 1) untreated control, 2) steam blanching (88°C, 10 min), 3) water blanching (88°C, 4 min), 4) 0.105% citric acid blanching (88°C, 4 min), or 5) 0.210% citric acid blanching (88°C, 4 min), dried for 6 h at 60°C (140°F), and stored aerobically

for up to 30 days. Samples were plated on Tryptic Soy Agar with 0.1% pyruvate (TSAP) and xylose lysine deoxycholate (XLD) agar for bacterial enumeration. Bacterial populations were reduced by 3.8 to 4.1, 4.6 to 5.1 and 4.2 to 4.6 log CFU/g immediately following steam, water, or acid blanching, respectively. After 6 h of dehydration, reductions were 1.6 to 1.7 (control), 4.0 to 5.0 (steam blanched), 4.0 to 4.6 (water blanched) and 4.9 to 5.4 (acid blanched) log CFU/g. Populations were higher ($P < 0.05$) on control samples than with other treatments after 6 h of dehydration and throughout 30 days of storage. Populations declined gradually with storage, but were still detectable by direct plating at 30 days on all samples except those blanched in 0.210% citric acid, which were detectable only by enrichment at 15 and 30 days storage. Results suggest that water and steam blanching, particularly blanching in a 0.210% citric acid solution, before dehydration are critical steps in the control of *Salmonella* during home-type dehydration of carrot slices.

P218 Fate of *Escherichia coli* O157:H7 and *Salmonella* during Osmotic Dehydration and Subsequent Storage of Apples

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The fate of selected foodborne pathogens during osmotic dehydration of apple slices was determined at different processing temperatures, times and calcium chloride ($CaCl_2$) concentrations. Apple slices were inoculated to achieve ~8 log CFU/apple slice of five strain mixtures of nalidixic-acid resistant *E. coli* O157:H7 or *Salmonella* and were dehydrated using concentrated sucrose solutions (60% w/w). In the first study, apple slices were subjected to osmotic dehydration at different temperatures (20, 45 and 60°C) and $CaCl_2$ concentrations (0, 2, 4 and 8%). In a second study pathogen survival was determined during dehydration (20°C, 72 h) and short-term, refrigerated storage (7 days, 4°C). At appropriate intervals, samples were withdrawn, diluted with 0.1% peptone water and surface plated onto Tryptic Soy Agar + 50 ppm nalidixic acid (TSAN) followed by incubation at 35°C for 48 h. Survival of *E. coli* O157:H7 and *Salmonella* was influenced by processing temperature, $CaCl_2$ concentration, and length of refrigerated storage ($P < 0.05$). On average, *E. coli* O157:H7 and *Salmonella* decreased by approximately 1.0 and 5.0 log CFU/apple slice at 20 and 60°C, respectively. At 45°C, *E. coli* O157:H7 and *Salmonella* populations decreased by 1.7 and 3.0 log CFU/apple slice, respectively. Addition of $CaCl_2$ during processing resulted in greater reduction of both pathogens than in non-$CaCl_2$ treated slices ($P < 0.05$). During refrigerated storage, *E. coli* O157:H7 and *Salmonella* in apple slices decreased by approximately 4.5 log CFU/apple slice, but were still recoverable via direct plating at Day 7. Therefore, this study demonstrates the survival of certain foodborne bacterial pathogens, as well as the influence of processing parameters on survival, during osmotic dehydration and subsequent storage of apple slices.

P219 DSC Inactivation of Foodborne Pathogens on Produce Surfaces with Atmospheric Plasma

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A study was conducted to determine the effect of a one atmosphere uniform glow discharge plasma (OAUGDP) on inactivation of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* on apples, cantaloupe, and lettuce, respectively. A five strain mixture of cultured test organisms was washed, suspended in phosphate buffer, and spot inoculated onto produce (7 log CFU/sample). Samples were exposed inside a chamber affixed to the OAUGDP blower unit, operated at a power of 9 kV and frequency of 6 kHz. This configuration allows the sample to be placed outside of the plasma generation unit, while allowing airflow to carry the antimicrobial active species, including ozone and nitric oxide, onto the food sample. Cantaloupe and lettuce samples were exposed for 1, 3, and 5 min, while apple samples were exposed for 30 s, 1, and 2 min. After exposure, samples were pummeled in 0.1% peptone water/ 2% Tween 80, diluted, and plated in duplicate onto selective media, and TSA incubated as follows: [(*E. coli* O157:H7 (modified EMB) and *Salmonella* (XLT4); 48 h, 37°C) (*L. monocytogenes* (MOX); 48 h, 32°C)]. *E. coli* O157:H7 populations were reduced by >1 log after 30 s and 1 min exposure and >2 log after 2 min exposure. *Salmonella* populations were reduced by >2 log after 1 min. Three- and 5-min exposure times resulted in a >3-log reduction. *L. monocytogenes* populations were reduced by 1 log after 1 min exposure. Three- and 5-min exposure times resulted in >3- and >5-log reduction, respectively. This process has the capability of serving as a novel, non-thermal processing technology to be used for reducing microbial populations on produce surfaces.

P220 DSC Effect of Electron Beam Irradiation on the Reduction of *Salmonella* Poona and Native Microbial Flora of Fresh-cut Cantaloupe

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The objective of this study was to determine the effect of electron beam irradiation on the survival of *Salmonella* Poona and native microflora of fresh-cut cantaloupe over 21 days of storage at 5°C. Cantaloupes were cored into cylindrical pieces and dipped in 8.6 log CFU/ml suspension of *Salmonella* Poona. Fruit were packed in polyethyl-

ene bags and three replicates were irradiated at 0.7 kGy and 1.5 kGy respectively. A non-irradiated set of packets served as the control. Samples were analyzed for pH as well as surviving *Salmonella*, lactic acid bacteria and yeast and molds over 0, 3, 6, 9, 12, 15, 18 and 21 days. Irradiation resulted in reduction in *Salmonella* counts of 1.1 log CFU/g between the control and 0.7 kGy and 3.5 log CFU/g between the control and 1.5 kGy on day 0. The difference in *Salmonella* counts on day 21 was 2.6 log CFU/g between the control and 0.7 kGy and 3.9 log CFU/g between the control and 1.5 kGy. Lactic acid bacteria were reduced to a lesser extent by irradiation with a difference of 0.2 log CFU/g between the control and 0.7 kGy and 2.8 log CFU/g between the control and 1.5 kGy on day 0. Counts for yeasts on day 0 for the control, 0.7 and 1.5 kGy were 2.09, 1.63 and 1.85 log CFU/g respectively and those for mold were 2.2, 1.75 and 0.5 log CFU/g. pH of fruit for the control was 5.4 followed by 5.8 for 0.7 kGy and 6.1 for 1.5 kGy.

P221 Factors Affecting Survival, Growth, and Retrieval of *Salmonella* Poona on Intact and Wounded Cantaloupe Rind and Stem Scar Tissue

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Studies were done to determine the survival and recovery of *Salmonella* Poona from cantaloupe rind as affected by environmental conditions between the time of contamination and analysis. Detection and enumeration of the pathogen as influenced by analytical methods were also investigated. Combinations of preenrichment broth (lactose broth or universal preenrichment broth), enrichment broth (Rappaport Vassiliadis broth or tetrathionate broth), and selective agar medium (bismuth sulfite agar or xylose lysine desoxycholate agar) for detecting *S.* Poona on inoculated cantaloupes stored at 4°C for 7 days or 21°C for 3 days were equivalent in performance. The composition of the carrier (water or 5% horse serum, a high-organic matrix) used to prepare inocula did not influence the number of *S.* Poona recovered from the intact rind surface, wounds in the surface, or the stem scar tissue. Regardless of inoculation site or composition of the carrier, populations on spot inoculated melons stored at 4°C remained constant between 2 and 24 h after inoculation. The pathogen grew within 24 h in wounds of spot and dip inoculated cantaloupes stored at 21 and 37°C. The addition of up to 1.0% Tween 80 to 0.1% peptone used to remove *S.* Poona from the rind surface did not adversely affect viability and may have enhanced detachment. Consideration of these observations is recommended when developing a method to test the efficacy of sanitizers in killing salmonellae on the rind surface of inoculated cantaloupes and to detect or enumerate salmonellae that may be natural contaminants.

P222 Examination of Yeasts for Antagonistic Activity against *Salmonella* Poona in Cantaloupe Juice and Wounds in Rinds Co-infected with Phytopathogenic Molds

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Application of yeasts as biocontrol agents to prevent mold decay of fruits and vegetables has been described. Ten yeasts were screened for potential antagonistic activity against survival and growth of *Salmonella* Poona in cantaloupe juice and decay by *Cladosporium cladosporioides* and *Geotrichum candidum* in wounds on cantaloupe rind. Cantaloupe juice was inoculated using five inoculation schemes: *S.* Poona only (1.10 log CFU/ml), high (3.93 to 5.21 log CFU/ml) or low populations (1.79 to 3.26 log CFU/ml) of yeasts only, and *S.* Poona combined with high or low populations of yeasts. High initial populations of *Debaryomyces hansenii*, *Pichia guilliermondii*, and *Pseudozyma* sp. were antagonistic to *S.* Poona in cantaloupe juice stored at 20°C for 48 h. Wounds in cantaloupe rinds were inoculated with yeast and mold or yeast, mold, and *S.* Poona and incubated at 4°C for 14 days or 20°C for 7 days. The pH of rind tissue inoculated with *C. cladosporioides* and yeasts increased significantly ($P \leq 0.05$) at 20°C. At 20°C, populations of *S.* Poona (6.40, 7.26, and 7.98 log CFU/sample) were lower in wounds co-inoculated with *G. candidum* and three of the test yeasts (*D. hansenii*, *P. guilliermondii*, and *Cryptococcus albidus*, respectively) compared to co-inoculation with *G. candidum* and the other yeasts. While *Candida oleophila* and *Rhodotorula glutinis* showed most promise for reducing the population of *S.* Poona in the wounds in cantaloupes co-inoculated with *G. candidum* and stored at 4°C, *Cr. albidus*, *D. hansenii*, and *P. guilliermondii* were most effective in controlling growth at 20°C.

P223 A Method for Detection of Enteric Viruses in Soft Fruit

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A robust and effective method has been developed for detection of enteric viruses in soft fruit. The method is applicable for detection of norovirus, hepatitis A virus, and enterovirus (poliovirus). Initial sample treatment is performed by a simple homogenization procedure with 1 M sodium bicarbonate. Subsequent removal of pectin and gross food solids is performed by incubation with pectinase, during centrifugation in the presence of a flocculant. Viruses are left in the supernatant, from which they are then sedimented by ultracentrifugation. Virus concentration is achieved by resuspending the sedimented virus particles in a small volume of medium, after which they can be detected by cell culture or nucleic acid amplification. The extraction procedure, which can be performed within a working day, can mediate

recovery of at least 10% infectious virus in 100 g strawberries or 60 g raspberries. This recovery is consistent, i.e., can be achieved with over 95% reliability. The procedure can deliver extracted viruses to a reverse transcription PCR (necessary especially for detection of norovirus, which cannot grow on cultured cells), in a suspension which contains minimal inhibitory substances. Internal amplification controls have been constructed for each virus type, to allow confirmation that the RTPCRs are not inhibited.

P224 Irradiation and Modified Atmosphere Packaging of Endive Influences Survival and Regrowth of *Listeria monocytogenes* and Product Sensory Qualities

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Salad vegetables such as endive can be contaminated with human pathogens such as *Listeria monocytogenes*. Modified atmosphere packaging and ionizing radiation are means to control the growth of pathogens on produce. To determine how these interventions could be combined synergistically, cut pieces of endive were inoculated with *L. monocytogenes*, packaged in gas-impermeable bags in air, 5/5/90 or 10/10/80 percent CO_2, O_2 and N_2 (AAir-0@, A5/5 and A10/10 respectively) and irradiated to 0.0 (control), 0.3 or 0.6 kGy. At various times during refrigerated storage, samples were taken and a determination made of a) total microflora, b) *L. monocytogenes*, c) headspace gas composition, d) color and e) texture. Irradiation reduced initial microbial counts. Bacteria grew during storage on Air-0 samples, but not on 5/5 or 10/10 samples. In each of the three atmospheres, O_2 declined and CO_2 increased, irrespective of radiation dose. Irradiated leaf material in Air-0 tended to retain color attributes during storage better than non-irradiated; color retention was more variable under 5/5 and 10/10 packaging. After 8 days, maximum shear force relative to the initial level was significantly reduced in 5/5 at all radiation doses, was not significantly changed in Air-0, and was dose-dependent in 10/10. By 14 days, the texture of all samples had degraded significantly. These results indicate that irradiation and MAP can be combined to prevent the growth of *L. monocytogenes* during post-irradiation refrigerated storage, but at the cost of produce quality.

P225 DSC Increased Resistance to Chemical Inactivation of *Salmonella* Montevideo during Biofilm Formation on Tomatoes

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Reducing pathogens in produce is essential to decrease the risk of foodborne disease associated with fruits and vegetables. A study was conducted to compare the efficacy of chlorine and Citricidin 2000®, a commercial sanitizer, in reducing *Salmonella* Montevideo populations during colonization on the surface of tomatoes. Tomatoes (*Lycopersicon esculentum*) were spot-inoculated with ca. 10^8 CFU of *S.* Montevideo. The inoculated fruits were stored at 22°C for 90 min (attachment step) and then washed and stored at 30°C for up to 10 days (colonization step). Both steps were carried out at 97% relative humidity. In previous studies, *S.* Montevideo showed ability to produce biofilms on tomato cuticles stored under the same conditions. At intervals during storage, groups of tomatoes were separated and treated with chlorine (1000 and 200 mg/L), Citricidin 2000® (200 mg/L) or water (control). At the beginning of the storage, treatment with chlorine at 1000 or 200 mg/L, Citricidin 2000®, and water reduced the pathogen by 5.0, 4.5, 1.9, and 0.4 log CFU/tomato, respectively. The log reduction obtained with all sanitizers decreased as the storage time increased. By the seventh day, the recovery of *S.* Montevideo was not different between treatments ($P > 0.5$). This suggests that microorganisms colonizing surfaces with biofilm production became more resistant to germicides than their non-biofilm counterparts, that biofilms might have produced protected sites, or both. These findings underscore the importance of maintaining appropriate storage conditions to prevent biofilm formation on tomato surfaces.

P226 DSC HPLC Method for Multiple *Fusarium graminearum* Mycotoxins

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Fusarium head blight (FHB) in North America is mainly caused by *Fusarium graminearum*. The predominate mycotoxin in FHB infected grain is deoxynivalenol (DON); other mycotoxins may be produced as well, including nivalenol (NIV), 3-acetyldeoxynivalenol (3-AcDON), 15-acetyldeoxynivalenol (15-AcDON) and zearalenone (ZEN). No HPLC method that can simultaneously detect the five mycotoxins has been reported so far due to the inability to baseline separate 3-AcDON and 15-AcDON, different polarity between B trichothecenes and ZEN, and interference from sample matrix under UV light. The objective of this study was to develop an HPLC method which overcomes these problems in detecting these mycotoxins in grain cultures. The first part of the study evaluated four analytical columns. Both gradient and isocratic elution methods were evaluated. Detection range for all 5 mycotoxins were 1 to 200 μg/g. The next step involved clean-up using Carbograph-4 with an initial elution using methanol for recovering the trichothecenes and a final elution with chloroform or tetrahydrofuran to recover ZEN. Recoveries were determined from mycotoxin spiked wheat and corn. A step isocratic procedure with Phenomenex SYNERGI Hydro-RP 80A column was found to baseline separate the five mycotoxins. The retention time for NIV, DON,

15-cADON, 3-AcDON and ZEN were 2.4, 3.7, 10.0, 10.4, and 15.3 min respectively. Acetonitrile and water were mobile phase with a flow rate of 1mL/min. All 5 mycoxins were separated and quantified, and the recoveries ranged from 50 to 90%. The HPLC method developed in this study is suitable for analyzing multiple mycotoxins in inoculated grains.

P227 Efficacy of Acidic Electrolyzed Water Ice for Pathogen Control on Lettuce

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Acidic electrolyzed water (AcEW), which is produced by the electrolysis of an aqueous sodium chloride solution in an anode cell, has been reported to have a strong bactericidal effect on most pathogenic bacteria. AcEW has been used as frozen AcEW (or AcEW-ice) for inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on lettuce. The AcEW-ice was placed into styrene-foam containers with lettuce samples at 20°C for 24 h. Although AcEW-ice which generated 30 ppm Cl_2 had no effect on *L. monocytogenes* cell counts, AcEW-ice generating 70 to 240 ppm of Cl_2 significantly ($P < 0.05$) reduced *L. monocytogenes* by ca. 1.5 log CFU/g. *E. coli* O157:H7 cell counts were reduced by 1.0 log CFU/g with AcEW-ice generating 30 ppm of Cl_2. AcEW-ice generating 70 and 150 ppm of Cl_2 reduced *E. coli* O157:H7 by 2.0 log CFU/g. Further significant reduction of *E. coli* O157:H7 (2.5 log CFU/g) was demonstrated by treatment with AcEW-ice generating 240 ppm of Cl_2. However, treatment with AcEW-ice generating 240 ppm of Cl_2 resulted in a leaf burn-like physiological disorder. AcEW-ice that generated less than 150 ppm of Cl_2 had no effect on the surface color of the lettuce. AcEW-ice, regardless of the magnitude of the emission of Cl_2, had no effect on the ascorbic acid (ASA) content in the lettuce. The weight ratio of lettuce to AcEW-ice required was determined to be over 1:10. The bactericidal effect of AcEW-ice appeared within the first 2 h. The use of AcEW-ice provides simultaneously for low temperature storage and for the inactivation of the bacteria.

P228 Microbial Quality of Fresh-cut Cantaloupe and Honeydew Melon as Estimated by Bioluminescence ATP Assay

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The level of sanitation and microbiological quality are of primary importance to the shelf stability and safety of fresh-cut melons. Research was undertaken to investigate use of a bioluminescence ATP assay to monitor the population of microbial contaminants in inoculated fresh-cut melon. Aerobic mesophilic bacteria and yeast and mold were isolated from the cantaloupe surface by plating dilutions of homogenized melon rind on PCA and CMA, respectively, and colonies from these individual plates were grown in nutrient broth for use in inoculating fresh-cut melon. The fresh-cut pieces were inoculated at 10^3 CFU/g of aerobic mesophilic bacteria or yeast and mold by submersion for 1 min. Following inoculation with aerobic mesophilic bacteria, the ATP of fresh-cut melon increased from 5.40 to 6.83 log ATP fg/g for cantaloupe and from 3.94 to 5.17 log ATP fg/g for honeydew. ATP of fresh-cut pieces inoculated with yeast and mold increased from 5.40 to 7.47 log ATP fg/g for cantaloupe and from 3.94 to 6.37 log ATP fg/g for honeydew. Fresh-cut melon inoculated with yeast and mold had higher ATP values than those inoculated with aerobic mesophilic bacteria at the same inoculation level. ATP was linearly related to the microbial populations on fresh-cut melons that had been inoculated with aerobic mesophiles and stored at 5°C for 15 days. Results of this study indicate that the bioluminescence ATP assay can be used to monitor microbial populations of fresh-cut melons for quality control purposes during production and storage.

P229 Cantaloupe Surfaces Affect Bacterial Attachment and Detachment by Sanitizing Treatments

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The surface of cantaloupe is comprised of a meshwork of tissue commonly referred to as the "net" which gives the surface an inherent roughness that may favor microbial attachment and hinder detachment. Research was undertaken to survey surfaces of cantaloupe for possible areas of microbial attachment, and to determine the effects of sanitizer treatments on attached microbial populations. Cantaloupe surfaces were examined, using scanning electron microscopy (SEM), before and after inoculation with *Escherichia coli* ATCC 25922 and after application of sanitizer treatments. Cantaloupes were submerged in 10^8 CFU/ml *E. coli* ATCC 25922 for 10 min, placed inside a biosafety cabinet for 24 h, and then sanitized with 1000 ppm chlorine or 5% hydrogen peroxide solution for 3 min or hot water (96°C) for 1 min. SEM observation of cantaloupe rind showed the presence of attached bacteria mostly on the netting rather than on the cuticle. It also showed bacterial attachment within the cantaloupe netting. All sanitizer treatments tested removed debris from the surface of the cantaloupe. Bacterial populations and yeast and mold on the surface of the cantaloupes were significantly ($P < 0.05$) reduced as revealed by SEM and plate counts. However, bacterial populations attached within the netting appeared to be only slightly reduced by the sanitizer treatments compared to those on the netting. In addition, the hydrogen peroxide treatment altered the cell surface morphology of attached bacteria, suggesting that hydrogen peroxide has a mode of bacterial inactivation that differs from that of chlorine or hot water.

P230 Role of Irrigation and Microbial Survival in Wastewater Reuse

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This study aimed to compare as well as evaluate microbial survival under arid and humid conditions, using two irrigation types — subsurface drip and furrow irrigation methods. Water that was seeded with *Escherichia coli* ATCC 25922, *Clostridium perfringens*, and coliphage PRD1 was used in irrigation of sixteen soil plots growing cantaloupe, lettuce, and bell pepper in the greenhouse. Eight of these received subsurface drip irrigated (SDI) water at a depth of 20 cm below the soil surface, while the remaining plots were furrow irrigated (FI). Relative humidity varied from 15 to 65% in dry condition experiments, whereas humidity fluctuated between 55 and 80% in humid condition experiments. Temperature was maintained at 15 to 33°C. For each of six sampling events during two weeks following injection, six samples of each type of produce as well as samples of surface soil and subsurface soil at a depth of 10 cm were collected and assayed for the study microorganisms. Soil samples were taken from the furrow in FI plots and from the seed bed in SDI plots. The levels of crop contamination were ranked from the highest to the lowest: cantaloupe, lettuce, and bell pepper. Greater initial contamination took place on furrow-irrigated crops under both humid and dry conditions as compared to subsurface drip irrigated plots. Overall, *E. coli* and PRD1 microorganisms survived for longer periods of time in dry conditions than in humid conditions. *Clostridium perfringens*' survival did not appear to be influenced by humidity.

P231 Comparing Survival of a Pathogenic and a Non-pathogenic *Salmonella* Strains in Manure Compost Applied to Soil to Grow Green Onions in an Environmentally Controlled Growth Chamber

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A green fluorescent protein (GFP)-expressing virulent *Salmonella* serovar Typhimurium strain was prepared and inoculated at 10^7 CFU/g into a commercial dairy manure compost. Similarly, a non-virulent *Salmonella* Typhimurium strain was inoculated into a different batch of the same compost. The inoculated composts were mixed separately with Tifton soil at a ratio of 1:100. Sixty horticultural pots were filled with ca. 5000 g of each of the inoculated and fertilized soil (30 pots each) and 15 pots with uninoculated and fertilized soil. Three healthy transplants of green onion plant were planted 100 mm apart into each pot. The pots were placed in environmentally controlled chambers. At weekly intervals 4 soil samples from each quadrant of 4 treated (2 pots from each treatment) and 1 untreated pot (soil samples) were analyzed for the recovered organisms. Similarly, 2 onion bulbs from each pot (Plant samples) were also analyzed for survival of organisms on the surface of the bulb as well as inside the bulb. Results showed that over a period of 84 days in soil samples, the populations of the avirulent strain of *Salmonella* was steadily reduced only by 3 log, whereas the population of the virulent strain of GFP-expressing *Salmonella* was reduced to undetectable level in 77 days. On the onion bulb surface, the avirulent strain could be detected for 56 days but the virulent strain survived only 35 days. None of the organisms were detected inside the onion bulb.

P232 Uptake and Translocation of *Escherichia coli* O157:H7 in Lettuce Grown in a Hydroponic System

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The purpose of this study was to evaluate the uptake and transmission of *E. coli* O157:H7 in lettuce plants. Lettuce seedlings were transplanted to a hydroponic system with *E. coli* O157:H7/pGFP at different concentrations of 10^3, 10^5, and 10^7 CFU/ml and grown for 3, 7, and 14 days. Confocal microscopy was employed to detect internalized and root surface adhered *E. coli* O157:H7 cells. Leaves and stem surfaces were harvested and disinfected with 80% ethanol and then immersed in 0.1% mercuric chloride. Samples were homogenized by stomaching for 2 min in Butterfields Phosphate Buffer and spread plated on Luria agar plates containing ampicillin (100 μg/ml). Stems were sliced longitudinally, placed with inner and outer surfaces on Luria agar plates and incubated for one h. Stems were removed from the agar and the plates were further incubated. All incubations were done at 37°C for 24 h. Green fluorescent protein expressing colonies were visualized under UV light and confirmed with Latex agglutination. Leaves from fourteen of 71 plants (20%) tested positive for *E. coli* O157:H7/pGFP. Stem sections tested for surface contamination were negative for *E. coli* O157:H7/pGFP and of the ones tested for internal contamination, 13% tested positive for *E. coli* O157:H7/pGFP. The association of *E. coli* O157:H7/pGFP with lettuce roots was dose dependent. Our results suggest that *E. coli* O157:H7/pGFP was transmitted through the vascular system from the contaminated hydroponic system to the edible portions of lettuce. This research was done at Auburn University.

P233 DSC Effectiveness of Cleaners and Sanitizers in Killing *Salmonella* Newport in the Gut of a Free-living Nematode, *Caenorhabditis elegans*

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Caenorhabditis elegans, a free-living nematode found in soil, has been shown to ingest human enteric pathogens, thereby potentially serving as a vector for preharvest contamination of fruits and vegetables. A study was undertaken to evaluate the efficacy of cleaners and sanitizers in killing *Salmonella* Newport in the gut of *C. elegans*. Adult worms were fed nalidixic acid-adapted cells of *Escherichia coli* OP50 (control) or *S.* Newport for 24 h, washed, placed on paper discs, and incubated at 4 or 20°C and relative humidities of 33, 75, or 98% for 24 h. Two commercial cleaners (Enforce® and K Foam Lo®) and four sanitizers (2% acetic acid, 2% lactic acid, Sanova®, and chlorine [50 and 200 μg/ml]) were applied to worms for 0, 2, or 10 min. Populations of *E. coli* and *S.* Newport (CFU/worm) in untreated and treated worms were determined by sonicating worms in 0.1% peptone and surface plating suspensions of released cells on tryptic soy agar containing nalidixic acid. Populations of *S.* Newport in worms exposed to 33 or 75% relative humidity at 4°C or to 33% relative humidity at 20°C were significantly ($P \leq 0.05$) lower than the number surviving exposure to higher relative humidities at respective temperatures. In general, treatment of desiccated worms with cleaners and sanitizers was effective in significantly ($P \leq 0.05$) reducing the population of *S.* Newport. Results indicate that temperature and relative humidity influence the survival of *S.* Newport in the gut of *C. elegans* but cleaners and sanitizers may not eliminate the pathogen.

P234 Bacterial Contamination Risk Associated with Application of Non-composted Bovine Manure to Soils in Low-chemical Input Vegetable Gardening

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This study tested the validity of the National Organic Program (NOP) requirement for a ≥120-day interval between application of non-composted manure and harvesting of vegetables grown in manure-fertilized soil. During spring 2003, fresh (≤ 3 days) non-composted bovine manure was applied on 100 ft² plots at three Wisconsin sites at various times prior to spring/summer vegetable planting. Manure was applied with or without the addition of oat straw, and was tilled into the soil either immediately, at planting, or not at all. Carrots, radishes, and lettuce were grown using typical gardening practices with no chemical inputs other than during site preparation the preceding fall. Soil and water-washed vegetables were analyzed for indigenous *Escherichia coli* (proven surrogate for *Salmonella* spp. and *E. coli* O157:H7) using direct plating or a two-step enrichment procedure. Indigenous *E. coli* in manure-fertilized soil decreased by about 3 log CFU/g from mean initial levels of 4.2 to 4.5 log CFU/g within 63 to 90 days after manure application, depending on soil type. Neither tilling nor addition of oat straw had a clear effect on *E. coli* survival. *E. coli* was detected in enriched soil from all three sites ≥154 days after manure application. Generally, only low levels (enrichment-positive, direct plating-negative) of *E. coli* were detected on washed vegetables harvested 90 (radish, lettuce) or 100 days (carrot) after manure application. The absolute absence of *E. coli* from manure-fertilized soils is not achievable, but the safety of vegetables would apparently not be jeopardized by reducing the NOP requirement to 90 days.

P235 Comparison of Growth Kinetics and Dynamics of Adherence of *Escherichia coli* O157:H7 Isolates and *Salmonella* Serotypes on Greenhouse and Field-grown Romaine Lettuce

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A study was undertaken to evaluate the growth kinetics and dynamics of adherence of *Escherichia coli* O157:H7 and *Salmonella* serotypes to the surface of Romaine lettuce. Three different isolates of *E. coli* O157:H7 and *Salmonella* Michigan, Montevideo, and Agona were inoculated on greenhouse-grown and field-grown Romaine lettuce at approximately 5.5 log CFU/leaf and allowed to surface-dry in a biohazard hood. These inoculated samples were placed in clear containers and incubated at room temperature (approximately 21°C) with high humidity (>95%). Bacterial recoveries were initiated at inoculation (-0.5 h) and at 0, 24, 48, and 72 h. At each time point, 3 replicate sets of leaves were weighed and viable CFU were recovered, using a standard protocol designed to correct for carry-over of tightly adhering or internalized wash water. Recovery of bacteria from inoculated leaves was conducted using a sequential rub-shake-rub (1°) followed by a separate stomaching (2°) procedure. Significant differences ($P < 0.05$) were found between the carrying capacity (D 2.0 log) and peak population density (D 1.7 log) among isolates and between greenhouse- and field-grown lettuce. An apparent differential decrease in adherence over time was observed with only one *E. coli* O157:H7 strain at T48 and T72 (D 2.0 and 2.5 log, respectively; $P < 0.05$) between the 1° and 2° recovery. This variance in recovery may be due to increased adherence, internalization, or increased removal over time. Differences observed between greenhouse- and field-grown lettuce clearly show the need for caution in extrapolating from laboratory models to field conditions.

P236 Evaluation of a Fiber-optic Biosensor for Detection of *Escherichia coli* O157:H7 in Fresh Produce

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Rapid detection of pathogens in food is critical to prevent foodborne illnesses. Biosensors show great potential for real-time pathogen monitoring. In this work, a fiber-optic biosensor (Analyte 2000TM) was evaluated for detection of *E. coli* O157:H7 in buffer solutions, lettuce extract, and alfalfa sprout irrigation water. The sensor operates as a fluorometer that detects fluorescence generated by evanescent excitation on the surface of tapered polystyrene optical probes. A sandwich immunoassay consisting of strepavidin-biotin linked anti-*E. coli* O157:H7 antibodies coated on the probe surface and cyanine 5 dye-labeled anti-*E. coli* O157:H7 antibodies was used for the detection. The sensor performance was evaluated based on two detection formats, the ramping-up test and the individual test. The results showed that the sensor was able to detect *E. coli* O157:H7 at levels as low as 1 to 10 CFU/ml in all sample matrices tested, using both detection formats. However, signal strength varied greatly among probes tested with the same samples. Consistent, positive reactions only occurred in samples containing *E. coli* O157:H7 at levels above 10^6 CFU/ml. False positives were frequently observed among the uninoculated lettuce and sprout irrigation water samples. This great fiber-to-fiber variability makes the reliability of the sensor questionable. Integration of the Analyte 2000™ with an automatic flow-through system was evaluated. The feasibility of integrating biosensor detection with continuous sampling for pathogen detection in sprout water was demonstrated.

P237 Surface Area Measurement and Recovery of *Escherichia coli* O157:H7 from Raw Strawberries and Mushrooms

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The microbial qualities of food surfaces are usually determined with a rinse or a sponging or swabbing procedure. Isolated and enumerated microorganisms are generally reported as counts per ml of rinse fluid or counts per square centimeter of swabbed surface. If a statistical relationship exists between the surface area of a raw fruit or vegetable and the weight of the same unit, then concentrations of organisms per surface area may be reported and compared, when the sample has been weighed but the surface has not been directly measured. A new optical imaging system was used to measure and calculate the surface area and volume of strawberries and white button mushrooms. Results for strawberries and mushrooms using simple linear regression showed good statistical correlation between surface area and unit weight. From the regression analysis, the following equations were developed to predict surface area from weight measurements: for strawberries, surface area (sq. cm.) = 10.67 + 1.391 W (g); and for mushrooms, surface area (sq. cm.) = 19.91 + 1.94 W (g). Concentrations of microorganisms recovered from surface inoculations were reported on a per volume diluent and per surface area basis. Comparisons of CFU/mL and CFU/sq cm showed that the concentrations typically varied by 0.1 to 0.2 log units. Our research indicates that surface area measurement with microbiological surface sampling can be used as an alternative analytical technique to determine pathogen concentrations on some produce.

P238 Comparison of Inoculation Method and Drying Time for Their Effects on Survival and Recovery of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* Inoculated onto Apples and Strawberries

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A study was undertaken to evaluate the effects of method of inoculation and drying time on survival and recovery, before and after a wash treatment, of foodborne pathogens inoculated onto the surface of apples and strawberries. Five-strain mixtures of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica* were separately applied to apples and strawberries by dip, spot, and spray inoculation methods. Inoculated fruits were dried for 1 or 24 h at 24 ± 2°C before treating for 1 min with water (control) or chlorine (200 ppm, pH 6.8 ± 0.2). Significantly higher populations of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* were recovered from apples and strawberries inoculated by dipping compared to spot and spray inoculation. Populations of the three pathogens recovered from apples and strawberries inoculated by spot and spray methods were not significantly different, with the exception of *E. coli* O157:H7 inoculated on apples (spray > spot). Populations were significantly higher by 0.5 to 2 log CFU/fruit when dried for 1 h compared to 24 h. Significant differences were observed in populations of all pathogens recovered from treated apples and strawberries (water > chlorine). Results indicate that inoculation method and drying time should be considered when designing experiments to evaluate antimicrobial treatments for produce. In addition, safety considerations for handling large volumes of inoculum (dip method) or aerosol generation (spray method) should be carefully addressed.

P239 Inactivation *Escherichia coli* O157:H7 and *Salmonella* spp. on Whole Tomatoes Following Immersion in Selected Chemical Sanitizers

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The Bioregenerative Planetary Life Support Systems Test Complex (BIO-Plex) is a proposed habitat for long duration space missions. In BIO-

Plex, sanitization of vegetables to ensure microbial safety without generating chemical wastes is crucial. This study evaluated 5% (w/v) hydrogen peroxide (H2O2), 500 ppm hypochlorite, and 1% (w/v) PRO-SANTM, a biodegradable foodgrade sanitizer, for inactivating *Escherichia coli* O157:H7 and *Salmonella* spp. on whole tomatoes. Tomatoes, spot-inoculated with a 5-strain mixture of nalidixic acid-resistant *E. coli* O157:H7 or *Salmonella* spp., were immersed for 4 min in distilled water (control) or in each sanitizer at 23°C and then rinsed for 5 s in fresh distilled water. Some of the treated tomatoes (non-rinsed) were stored at 25°C for 8 days. Survivors were enumerated by washing tomatoes in buffered peptone water (BPW), plating aliquots of BPW onto sorbitol MacKonkey agar or bismuth sulfite agar (each agar containing 50 ug/ml nalidixic acid) and counting colonies on agar plates after incubation (37°C, 24 h). Initial populations of *E. coli* or *Salmonella* on tomatoes were ~ 7.0 log CFU/tomato. PRO-SAN consistently inactivated >3.0 log of each pathogen. Log reductions on tomatoes immersed in water, H2O2, hypochlorite, and PRO-SAN were 1.25, 2.0, 2.31 and 3.51 (*E. coli*), and 1.08, 2.47, 2.43, and 3.22 (*Salmonella*), respectively. A significant decrease in *E. coli* survivors occurred only on PRO-SAN-treated tomatoes at day 8 of storage ($P < 0.05$). The use of 1% PRO-SAN at 23°C seems to have good potential as a sanitizer for fresh produce on BIO-Plex because of its antibacterial efficacy and its biodegradable characteristic.

P240 DSC Reduction of Foodborne Pathogens on Almonds Using Gaseous Propylene Oxide

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Propylene oxide (PPO) is commonly used to reduce microbial populations in domestic raw almonds but the process has not been validated for reduction of foodborne pathogens. Almonds were inoculated with *Salmonella* Enteritidis or with five-strain cocktails of *Salmonella*, *E. coli* O157:H7 or *L. monocytogenes* to approximately 8.0 log CFU/g after drying. Pathogen-inoculated almonds were separately placed in bags designed for gaseous sterilization (Steris) and tempered from 4 to 35°C for 14 to 24 h. Tempered almonds were placed in polyfoam containers predetermined to maintain a target temperature range and transported to a commercial facility to receive a standard PPO treatment (0.5 L/m3, 4 h). When almonds were held at 4°C, no significant decrease in *Salmonella* Enteriditis was observed during treatment. When treatment temperatures of approximately 15, 25, and 35°C were used, reductions of approximately 1, 3, and 5-log CFU/almond, respectively, were observed during the PPO treatment. Greater than 5-log reductions of pathogen cocktails were observed when almonds were tempered to 35°C. PPO efficacy was further evaluated by placing treatment bags in the center of polyethylene-lined fiber bulk shippers containing approximately 1000 kg of almonds. When almonds were tempered to at least 30°C before PPO exposure, populations of *Salmonella* Enteritidis were reduced by >5-log CFU/almond. However, a significant part of this reduction was observed up to 5 days post-PPO treatment during the time that PPO residues declined. Temperature significantly influences the ability of PPO to reduce foodborne pathogens in almonds and should be considered when establishing critical limits for PPO treatments.

P241 Survival, Growth, and Thermal Resistance of *Listeria monocytogenes* in Peanut and Chocolate Matrices

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Recent outbreaks of listeriosis associated with the consumption of ready-to-eat foods have raised interest in determining growth and survival characteristics of the pathogen in a wide range of products. A study was undertaken to determine the thermotolerance of *Listeria monocytogenes* in a peanut-based beverage, whole-fat milk, full-fat and reduced-fat chocolate milk, a chocolate-peanut spread, and peanut butter. The $D_{60°C}$ value in peanut beverage (3.2 min) was not significantly different ($P > 0.05$) from the $D_{60°C}$ value in whole-fat milk (3.3 min) or full-fat chocolate milk (4.5 min) but significantly lower ($P \leq 0.05$) than the $D_{60°C}$ value in reduced-fat chocolate milk (5.9 min). The pathogen was significantly more resistant to heat when enmeshed in chocolate-peanut spread (a_w 0.46) ($D_{60°C}$ = 37.5 min) and peanut butter (a_w 0.32) ($D_{60°C}$ = 26.0 min) than in liquid products. At 10°C, the pathogen grew most rapidly in full-fat chocolate milk and least rapidly in peanut beverage. At 22°C, populations increased significantly within 12 h in whole-fat milk and reduced-fat chocolate milk and within 4 and 8 h in full-fat chocolate milk and peanut beverage, respectively. Initial populations (3.3 to 5.0 log CFU/g) of *L. monocytogenes* in chocolate-peanut spread and peanut butter at a_w 0.33 and 0.65 declined but the pathogen was not eliminated over a 24-week period at 20°C. Survival was enhanced at reduced a_w. Results indicate that a pasteurization process similar to that used for full-fat milk would be adequate to ensure the destruction of *L. monocytogenes* in peanut beverage.

P242 DSC Survival of *Salmonella* Enteritidis PT 30 on Almond Hulls and Kernels

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From late 2000 through spring 2001, an outbreak of *Salmonella* Enteritidis Phage Type 30 (SEPT30) was linked to consumption of raw almonds. Unusually high rainfall occurred during the time that approximately one third of the outbreak-associated almonds were drying on the ground. This raised the question of whether SEPT30 could multiply under these environmental conditions and survive subsequent drying on hulls and kernels. Almond hulls were covered in water, inoculated with 10^4 CFU/ml SEPT30, and incubated

at 23°C. Populations of SEPT30 increased from 10^4 to 10^8 CFU/ml within 24 h and then remained constant over the next 6 days. Hulls were removed at daily intervals and dried for 24 h at 15 and 37°C. Populations of SEPT30 fell by 2.9 log CFU/hull (day 2) or 1.4 log CFU/hull (day 7) during drying at both 15 and 37°C. Kernels were inoculated with SEPT30 at 10^9, 10^7,10^5, and 10^3 CFU/almond and stored for 162 days at 24°C or inoculated at approximately 10^8 CFU/almond and stored for 171 days at 4, 24, and 35°C. When stored at 24°C, SEPT30 populations slowly declined by approximately 2.2 log CFU/almond over 162 days regardless of initial inoculum level. Reductions of 0.5, 2.1, and 2.5 log CFU/almond were observed over 171 days of storage at 4°C, 24°C, or 35°C, respectively. Inclement weather and prolonged survival of SEPT30 on almond hulls during drying and kernels during storage may have contributed to an outbreak of salmonellosis associated with consuming almonds.

P243 Evaluation of Dry-plant Sanitation Procedures Used in Almond Huller/Sheller Facilities for Reducing Microbial Loads on Almond-contact Surfaces

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After harvest, almond hulls and shells are separated from the kernal in specialized huller/sheller (HS) facilities. HS facilities have not traditionally employed cleaning and sanitizing programs beyond general housekeeping and little data exists on appropriate and effective approaches for this unique dry environment. The objective of this study was to evaluate the typical practice of removing dust with air blowing and application of an alcohol-based quaternary ammonium sanitizer (QUAT, Alpet D2, Best Sanitizers Inc.) for reducing the microbial load on kernal-contact surfaces typical of HS facilities. Dust (1 g) collected from HS facilities (a mixture of soil, and hull and shell particulate) was used to artificially contaminate 225-cm^2 surfaces of galvanized steel, painted galvanized steel, and worn and new smooth and scalloped rubber belting. Surfaces (100 cm^2) were sampled using a standard swabbing technique before and after combinations of blowing with air for 1 min and/or wetting with QUAT for 1 min. Control (untreated) surfaces had aerobic plate counts (APC) of 5 log CFU/100 cm^2. Air blowing alone reduced the APC by 10- (either worn or new rubber belting) to 100-fold (galvanized or painted steel). Applying an alcohol-based QUAT after air blowing reduced microbial counts by an additional 10-fold (rubber belting) or 100-fold (steel surfaces). However, in a commercial HS facility, APC were typically reduced by less than 10-fold after sanitation regardless of surface type. Current HS cleaning and sanitation practices reduce microbial populations on kernal-contact surfaces but additional or alternative strategies may need to be evaluated.

P244 Bacterial Populations Associated with Extended Storage of Commercially Manufactured Yeast

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The effect of storage temperature on bacterial counts of commercially manufactured yeast was investigated in three replicate surveys. Cream, compressed and vacuum-packaged dry yeast samples were stored at 4, 10, 25 or 37°C for 21 days. Aerobic plate counts (APC), *Enterococcus*, coliform and *E. coli* counts were performed every three days and pH changes recorded. During the first survey, 318 colonies were isolated from the highest dilution of APC plates showing growth, purified and characterized. In general, APC were higher than *Enterococcus*, coliform and *E. coli* counts, respectively, and APC and *Enterococcus* counts of cream yeast samples were consistently the highest (ca. 6 to 8 log CFU/ml) compared to compressed and dry yeast samples. Counts of cream and compressed yeast remained fairly constant at 4 and 10°C; however, APC and *Enterococcus* counts increased to ca. 8 log CFU/g or ml after 9 days when stored at 25 and 37°C. Conversely, APC and *Enterococcus* counts for dry yeast samples remained lower (ca. 5 log CFU/g) than for cream and compressed yeast samples irrespective of storage temperature. Coliforms and *E. coli* were present in all three yeast products at 4 and 10°C, but decreased to non-detectable levels in cream and compressed yeast samples stored at 37 and 25°C, after 3 and 9 days, respectively. The pH of cream and compressed yeast samples increased over time with increasing storage temperature, while the pH of dry yeast samples remained unchanged. Of the 318 isolates, 73% were characterized as lactic acid bacteria and 22% as *Enterococcus* spp.

P245 DSC Survey of a Yeast Manufacturing Process for Sources of Spoilage and Potentially Pathogenic Bacteria

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Seed, dry, cream and compressed yeast samples emanating from the same batch, as well as corresponding raw materials were analyzed before and after preliminary incubation at 37°C for 24 h over three replicate surveys. Aerobic plate counts (APC) on WL Nutrient Agar with cyclohexamide, coliform and *E. coli* counts on Rapid' *E. coli* 2 Agar, and *Enterococcus* counts on KF Streptococcus Agar supplemented with 1% (v/v) TTC were determined. Bacterial spore counts were performed on cream, compressed and dry yeast finished products on Tryptone Soya Agar with 1.5% Bacteriological Agar. The presence of *S. aureus* on 3M™ Petrifilm™ Rapid *S. aureus* count plates, *Listeria* spp. on Rapid' L. mono Agar and *Salmonella* spp. on XLD Agar were also determined. Raw material counts were below the lower detection limit (0.7 log CFU/g or

ml), except those of soda ash, which contained counts of ca. 2 log CFU/ml on KF *Streptococcus* Agar. APC and *Enterococcus* counts increased during the scale up of yeast biomass. APC also increased by 2 to 3 log CFU/ml after cold storage of yeast cream during cream, compressed and dry yeast production. Coliform and *E. coli* counts implicated the storage tanks as initial sites of this contamination and/ or its proliferation. Spore counts were detected only in the dry yeast finished product (ca. 1.5 log CFU/g). Although *Salmonella* spp. and *S.* aureus were not detected, presumptive *Listeria* spp. were isolated from cream, compressed and dry yeast finished products.

P246 Survival of *Listeria monocytogenes* and *Escherichia coli* O157:H7 during Sauerkraut Fermentation

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Sauerkraut was produced from shredded cabbage, as is typical in the United States, and from whole head cabbages, which is a traditional process in parts of Eastern Europe. The sauerkraut was inoculated with five strain mixtures of *Escherichia coli* O157:H7 and *Listeria monocytogenes*, and the populations of these bacteria, as well as lactic acid bacteria, pH and titratable acidity, were monitored over the course of the fermentation. The fermentation variables were temperature (18°C and 22°C) and salt concentration (1.8%, 2.25% and 3%). For most of the analyses, the type of cabbage was a significant factor, although within cabbage type neither salt nor fermentation temperature had significant effects. The final pH of the whole head sauerkraut was lower than the shredded sauerkraut, but the titratable acidity was significantly higher in the shredded sauerkraut. *E. coli* O157:H7 and *L. monocytogenes* persisted in the brines for most of the fermentation, although at the end of the fermentations (15 days for shredded, 28 days for whole head), neither pathogen had detectable populations. *E. coli* populations decreased more rapidly in the shredded sauerkraut even though the pH was higher, because of the higher total acidity in the shredded sauerkraut. Acid tolerant strains of *E. coli* and *L. monocytogenes* were isolated from both shredded and whole head sauerkraut at different salt concentrations and temperatures, after 15 days of fermentation, and could be detected at 35 days in the whole head sauerkraut.

P247 Microbiological Quality of Ready-to-Eat Foods — The Results of a Long-term Survey

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A collaboration of organizations involved with food safety in Wales led to the development in 1995 of a long-term, coordinated and structured food-sampling program that was designed to monitor over a continuing timescale the microbiological quality and safety of specific retail ready-to-eat products. Local authorities randomly sample the same food types within a specified time frame, collecting a significant amount of data that accurately reflect the quality of food available to nearly three million consumers. There is now a substantial database of results collected since 1995. Randomization has removed an element of bias associated with targeted programs and has produced a unique resource. There are currently around 16,000 results, each with seven or eight bacterial analytes, with information on when and where sampled. The data indicates that ready-to-eat foods available in Wales are generally of very high microbiological quality, both in terms of safety and quality. No foods have been found to be positive for *Campylobacter* and only one food was found to be positive for *Salmonella*. In terms of microbiological quality, very few of the foods tested were either unacceptable or potentially hazardous at point of sale (based upon current guidelines). The overall conclusion that can be made at present is that ready-to-eat foods in Wales tend to be of very good microbiological quality and, at point of sale, cannot be considered to be a major source of foodborne illness.

P248 The Effect of Structured Technical Audits on the Development of Traceability Systems in SMEs in the Further Processing Sector

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The development of a more structured approach to technical auditing in the UK and the EU has forced SME food companies to develop stronger technical control. Following the BSE and Foot and Mouth outbreaks in the UK, primary processors were legally obliged to operate traceability systems on their livestock processes. This study analyzes the traceability systems developed by secondary processors and their efficiency within the manufacturing environment. A semi-structured interview with technical representatives of 15 companies, followed by an audit of the traceability systems, was used to establish an understanding of the companies' expertise in this key area. Only 30% of companies could demonstrate a full traceability during a live exercise forming a part of this study. However, 83% of companies that were accredited by a recognised auditing body were able to demonstrate complete traceability. Eighty-six percent of companies also improved their traceability systems as a result of the aforementioned problems in the primary sector. The study also evaluated the complexity of the product range and raw materials used on the efficacy of the traceability systems. The systems developed to

validate and verify the traceability of company operations were also audited. These will be discussed at the presentation.

P249 Recovery of *Salmonella* from Commercial Shell Eggs by Shell Rinse and Shell Crush Methodologies

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Salmonella is the most important human pathogen associated with shell eggs. *Salmonella* Enteritidis is the serotype most often implicated in outbreaks. However, other serotypes have been recovered from eggs and from the commercial shell egg washing environment. Many sample methods are used to recover microorganisms from eggshells. This work was completed to explore the use of shell rinse and a modified crush method for recovery of *Salmonella*. Eggs were collected from three commercial shell washing facilities during three visits. Ten eggs were collected from 10 to 12 locations on the egg processing. After being transported back to the laboratory, each egg was placed in a sterile whirl-pak bag and sampled by rinsing the shell surface with 10 ml of phosphate buffered saline for 1 min. Each egg was then aseptically cracked, contents discarded, and shells crushed and rubbed in 20 ml of phosphate buffered saline. This was accomplished using a conical centrifuge tube and a sterile glass rod. For each technique (rinse or crush/rub), two five-egg pools per location (A-L) sampled were selectively enriched for the recovery of *Salmonella*. Samples were pre-enriched in buffered peptone water and selectively enriched in TT and RV broths. Presumptive colonies from selective agar plates (BGS and XLT-4) were inoculated onto LIA and TSI agar slants. Presumptive positives were confirmed serologically. Overall, there were 10.6% (42/396) *Salmonella*-positive pooled samples. *Salmonella* were recovered by the shell rinse and by the crush and rub technique (11.6% v. 9.6%). Plant X yielded 21.5% *Salmonella* positives, while less than 5% of samples from plants Y and Z were found to be contaminated with the organism (4.2% and 4.5%, respectively). *Salmonella* was recovered more often from dirty eggs (15.8%) than from clean eggs (8.3%). B was the most commonly encountered serotype. None of the *Salmonella* recovered were from serogroup D, which includes the serotype Enteritidis. For some eggs, *Salmonella* was recovered by only one of the methods. Using both approaches increased sampling sensitivity.

P250 Thermal Resistance Parameters for Pathogens in Juice Concentrates

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The objective of this study is to obtain data for establishing critical limits necessary to achieve 5-log reduction of pathogens in juice concentrates. The heat resistance of *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* was determined in various juice concentrates. Stationary-phase, acid adapted, and cells exposed to high osmotic pressure were evaluated in white grape juice concentrate (58 Brix, pH 3.3) and apple juice concentrate (70 Brix, pH 3.6). D-values were determined at four different temperatures from 58 to 66°C to calculate z-values and D-values at and above 71°C. For all three pathogens, acid adapted cells were less heat resistant. Under similar conditions, the pathogens were less heat resistant in apple juice concentrate than in white grape juice concentrate. *E. coli* O157:H7 exposed to high osmotic pressure was most heat resistant in white grape and apple juice concentrate, with a D-value at 71°C of 13.2 and 5.4 s, respectively. At temperatures below 86°C, *E. coli* O157:H7 exposed to high osmotic pressure may be considered as the conservative target organism, because its heat resistance was higher than that of *L. monocytogenes* and *Salmonella*. However, in white grape juice concentrate, at temperatures above 86°C, *L. monocytogenes* exposed to high osmotic pressure was most heat resistant due to the differences in z-values. A validation experiment was conducted which validated the calculated D-value of *E. coli* O157:H7 at 71°C.

Technical Abstracts

T01 Reduction of *Campylobacter* on Commercial Broiler Carcasses by Postchill-dip Application of Acidified Sodium Chlorite

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Two trials were performed to assess the reduction of *Campylobacter* spp. in commercial carcasses after post-chill dip applications of acidified sodium chlorite (ASC). Samples were collected before the inside-outside-bird washer (IOBW, Site I), after the IOBW (Site II), after the chiller (Site III), and after the post-chill application of ASC (Site IV). In the first trial, 20 samples were collected from each site per day (two days). In the second trial, ten samples were collected per day (four days). Bacterial counts were converted to log CFU/ml for statistical analysis. In the first trial, the mean *Campylobacter* counts (and percentage positive) were 2.83 (100%), 2.13 (100%), 1.04 (100%), and 0.12 (12.5%), for Sites I, II, III and IV, respectively. In the second trial, *Campylobacter* counts (and percentage positive) were 2.86 (95%), 2.52 (95%), 1.22 (77.5%) and 0.02 (2.5%), for Sites I, II, III and IV, respectively. The reduction in counts by the IOBW was significant only in one experiment, but the chiller reduced *Campylobacter* counts significantly in both experiments. No major reduction in positive carcasses after enrichment for *Campylobacter* spp. was seen post-IOBW or post-chill. However, a significant reduction in *Campylobacter* counts and incidence was seen after the application of ASC. These results demonstrate that the antimicrobial effect of ASC applied post-chill may have a better impact in reducing *Campylobacter* spp. in commercial broiler carcasses. Post-chill applications may eventually evolve into different systems, such as mist, spray or bath, which could be applied closer to the final stages in processing.

T02 DSC Inhibition of *Clostridium perfringens* Spore Germination and Outgrowth by Salts of Organic Acids in Roast Beef during Chilling and Refrigerated Storage

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Spores of foodborne pathogens can survive traditional thermal processing schedules used in the manufacturing of meat products. They can germinate and grow to hazardous levels if chilled slowly. Inhibition of germination and outgrowth of *Clostridium perfringens* spores by organic acid salts in roast beef during chilling and storage at refrigeration temperatures was evaluated. Beef top rounds were injected with a marinade (1% salt, 0.2% potassium tetrapyrophosphate and 0.2% starch), ground and mixed with antimicrobials [sodium lactate (SL) and SL plus sodium diacetate (SD), 2.5%; buffered sodium citrate (BSC) and BSC plus SD, 1.3%]. The product was inoculated with *C. perfringens* spores, mixed, vacuum packaged, heat shocked for 20 min at 75°C and chilled exponentially from 54.5 to 7.2°C in 9, 12, 15, 18 or 21 h. *C. perfringens* populations (vegetative and spore) were enumerated after heat shock, during chilling and storage up to 60 days. *C. perfringens* was able to grow in control samples from an initial population of ca. 2.8 log CFU/g by 0.9, 2.1, 2.7, 4.5 and 5.3 log CFU/g subsequent to 9, 12, 15, 18 and 21 h exponential chill times, respectively. Addition of antimicrobials prevented germination and outgrowth of *C. perfringens*, regardless of the chill times. *C. perfringens* spores were recovered from samples containing organic acid salts that were stored up to 60 days at 10°C. Extension of roast beef chilling time beyond 9 h resulted in >1 log CFU/g growth of *C. perfringens* under anaerobic conditions. Organic acid salts inhibited outgrowth of *C. perfringens* spores during chilling of roast beef.

T03 Antimicrobial Blend to Control *Listeria* in Deli Salads

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A proprietary blend of GRAS ingredients (NovaGARD™ CB-1) composed of maltodextrin, cultured dextrose, sodium diacetate, egg white lysozyme and nisin has been reported to reduce the risk of *Listeria monocytogenes* growth in a variety of ready-to-eat (RTE) food products. CB-1 was evaluated in model systems of commercially prepared deli salads for its ability to produce a 2 log reduction in viable *Listeria monocytogenes* and maintenance of the reduction through out the shelf life of the product. To test efficacy, deli salads made with or without varying levels of CB-1 were inoculated with a three strain *Listeria* cocktail and held at 4°C (39 to 40°F). Samples were enumerated for *L. monocytogenes* and Standard Plate Counts over their normal shelf life. Samples with *L. monocytogenes* below the detection limit were enriched, retested and reported as positive or negative with detection limit of 0.04 CFU/gram. The data indicated that NovaGARD™ CB-1 is an effective anti-*Listeria* treatment in deli foods such as chicken salad. A decreased pH appears to

enhance the effect. For example, chicken salad with a pH of 4.8 required a final CB-1 concentration of 0.5% (w/w) to achieve and maintain a 2 log reduction. At pH 5.7 at least 1.5% (w/w) was needed to achieve comparable results.

T04 Natamycin: An Effective Preservative for the Control of Wine Spoilage Yeasts

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The polyene macrolide natamycin is used worldwide as a natural food preservative, mainly as a surface treatment of cheese and dried sausages. It can also be used in wine prior to bottling to prevent unwanted secondary fermentations. In the present study yeast strains implicated in wine spoilage were shown to be sensitive to low levels of natamycin. In preliminary experiments with wine (pH 3.4, 13 mg/liter free sulfur dioxide), growth of *Candida krusei*, *Saccharomyces bayanus* and *Zygosaccharomyces bailii* was prevented by 5 to 10 mg/liter natamycin. Natamycin efficacy was then demonstrated in a larger scale experiment where the growth of *S. bayanus* was monitored by HPLC assay of glucose and fructose levels, visual observation of turbidity and microbiological analysis. This strain has become adapted to growth in wine, tolerating conditions of low pH, high acid, low sugar, high alcohol, pressure, and chemical preservatives. Natamycin at 5 to 10 mg/liter completely inhibited yeast growth for the experiment's duration (117 days) whereas control samples not containing natamycin were visibly spoiled after 7 days. Natamycin had a bacteriocidal effect on yeast cells growing in the wine. Degradation of natamycin in the wine was more rapid at ambient temperature. After addition of 10 mg/liter natamycin, none was detected after 3 weeks at 25°C and after 19 weeks at 8°C. It is presumed that, before the levels become significantly reduced, natamycin preserves the wine by killing any spoilage yeast cells. Natamycin could be used to reduce levels of chemical preservatives; this has health and safety advantages as well as taste benefits.

T05 The Effect of Ozone against *Pseudomonas* sp. and *Bacillus globigii* Spores

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Contaminated surfaces and atmospheres within the food production environment can contribute to cross contamination, increase microbial load and reduce food safety. To improve plant sanitation, food manufacturers are using a range of decontamination treatments, including ozone. Data on the efficacy of ozone is available for a limited number of organisms, and results of trials against *Pseudomonas* sp. and *Bacillus globigii* spores, both of which can be persistent environmental isolates, are presented. Aerosolized organisms were introduced into a bioaerosol test chamber using a collison nebulizer and survivors after ozone treatment were isolated using AGI impingers. The survival of organisms attached to horizontal, vertical and inverted stainless steel coupons was also tested. Ozone at 0.05 ppm was ineffective against surface attached *Pseudomonas* but gave a 2.5 log reduction in aerosolized bacteria. Ozone at 0.1 ppm gave a 3.5 log reduction for aerosolized *Pseudomonas* and >1 log reduction for attached organisms. Ozone at 2 ppm achieved the greatest log reduction (3.5 aerosolized organisms and 2 log reduction surface attached) and the results were unaffected by the orientation of the coupons. Ozone at 2 pm had little effect on *Bacillus globigii* spores (aerosol or attached). There was no significant difference between surface attached spores and control samples, with only a 0.1 log reduction against aerosolized spores. Ozone at levels of 2 ppm was effective against vegetative spoilage organisms but relatively ineffective against bacterial endospores. Results are discussed within the context of ozone as a decontamination procedure with food production.

T06 Disinfection Failures Associated with Cotton Cloth Wipers when Compared to Non-Woven Wiper Products

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Cloth wipers and sponges when used in food service tend to accumulate food soils, compromising sanitary integrity. Even in the presence of quaternary ammonium chloride sanitizers (QACS) microbial surveys have shown disinfectant failures. In order to identify possible underlying causes of failure, testing was performed regarding microbial status (when new), utility and cleaning/sanitizing effectiveness of cotton cloth wipers (CCW) compared to a hydro-entangled non-woven wiper (NWW). The data provided shows that based on microbial indicators, the NWW has significantly ($P \leq 0.05$) lower levels of viable aerobic/anaerobic vegetative cells and spores than the 5 commonly available CCW tested. In surface cleaning tests to compare the ability of wipers to clean up organic soil (egg white and yolk) containing marker bacteria (*Serratia marcescens*), both wipers were shown to be statistically equivalent ($P > 0.05$) in their ability to clean/sanitize surfaces, eliminating over 7.0 log of bacteria embedded in organic soil. Neither size, weight or condition of use influenced utility of either wiper type. When used with QACS (200 ppm) the NWW significantly ($P \leq 0.05$) outperformed CCW in capacity to become sanitized (< 5 log reduction). When saturated with QAC, inoculated with soil containing marker bacteria

and then cleaned in a wide variety of treatments, including washing machine cycles, the NWW came consistently and significantly ($P \leq 0.05$) cleaner than the CCW treated under identical conditions. While the NWW was shown to be compatible with QAC, sanitizer quenching or overload appears to occur with the standard CCW.

T07 Efficacy of Volatiles Produced by *Muscodor albus* in the Disinfection of Edible Horticultural Commodities

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Muscodor albus is a novel endophytic fungus that is being developed as a registered commercial biological formulation for the control and disinfection of both postharvest plant pathogens and bacterial pathogens of concern in foodborne contamination and illness associated with fresh consumed horticultural products. During growth on complex organic substrate, *M. albus* releases a complex of volatiles that have been shown to be biocidal or biostatic to a broad variety of fungi and bacteria. We have demonstrated the sensitivity of multiple strains of *E. coli* O157:H7, *Salmonella*, *Shigella*, and *Listeria monocytogenes*, both in vitro and en planta. Bactericidal effects, in vitro, were shown to be temperature dependent, with the effective range for volatile elaboration occurring between at least 12.5 and up to 28°C. Above 28°C, the production of volatiles is altered in a manner that results in insensitivity or biostatic inhibition rather than a biocidal effect, depending on the species of pathogen and a stress-repair enrichment recovery method. Above 32°C, *M. albus* ceases growth under test conditions. Efficacy tests, primarily with melons, tomato, and bell pepper have demonstrated a reduction of at least 3.5 log CFU on the surface of non-wounded fruit. Disinfection-efficacy dependent variables identified thus far include exposure temperature and duration, rate of volatiles accumulation, initial pathogen inoculum density, pre-treatment temperature and temporal dynamics of post-inoculation storage, and produce surface characteristics. The appeal of biofumigation with *M. albus* includes a non-aqueous application, ease of scalability and the absence of direct contact between the viable microbial pesticide and the edible produce.

T08 DSC Attachment and Infiltration of *Salmonella* Poona to Surface Tissues of Cantaloupe as Affected by Temperature Differential, and Migration into Edible Tissues as Affected by Co-infection with Phytopathogens

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The effect of temperature differential between Eastern or Western (shipper) type cantaloupes and suspensions of *Salmonella* Poona, each at 4°C and 30°C, on changes in cantaloupe weight and populations of the pathogen recovered from rinds and stem scar tissues was assessed. The percent of weight increase in Western type cantaloupes was significantly greater ($P < 0.05$) than that of Eastern cantaloupes for all cantaloupe / inoculum temperature combinations. *S.* Poona attachment to or infiltration of Eastern but not Western type cantaloupe rind was enhanced when the fruit is at 4°C compared to 30°C, regardless of the temperature of immersion suspension. Populations of *S.* Poona recovered from stem scar tissues of both types of cantaloupes were not significantly affected by temperature differentials. Populations of cells adhering to or infiltrating cantaloupe tissues were influenced by temperature differential between fruits and immersion suspensions and by structures unique to surface tissues. Migration of *S.* Poona into sub-surface mesocarp tissues as affected by co-infection with *Cladosporium cladosporioides* and *Penicillium expansum* was investigated. Wounds in cantaloupe rind were inoculated with *S.* Poona only, *S.* Poona and mold simultaneously, or mold followed by *S.* Poona 3 days later. Recovery of *S.* Poona from edible tissues 3 to 4 cm below the site of infection up to 10 days after inoculation supports the hypothesis that the pathogen can migrate into mesocarp tissues. Survival and migration of *S.* Poona into the internal tissues of cantaloupes were enhanced by co-inoculation with *C. cladosporioides* and, to a lesser extent, *P. expansum*.

T09 Critical Steps in Development of Effective Methods for Detection of Foodborne Viruses in Soft Fruit

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Enteric viral pathogens have caused outbreaks of disease in which soft fruit was the vehicle of transmission. An effective method for detecting viruses in soft fruit would be one of the ways to address this problem, by facilitating monitoring, and mediating studies of disinfection efficiency. In a general method, there exist several basic stages, i.e., initial sample treatment (homogenization/ washing), removal of food solids (e.g., by filtration), virus concentration (e.g., by flocculation), and ultimately application of a detection system such as nucleic acid amplification or cell culture. Within these stages, there are elements critical to the final efficiency of the procedure. These comprise steps that can be optimized to ensure maximum and consistent recovery and detectability of viruses. For example, the medium used for homogenization or washing should promote the liberation of virus particles from food solids or surfaces, while not being detrimental to virus infectivity. In another example, the method used for removal of food solids from a sample should be one that does not remove viruses or that removes only a minimal number of them. During optimization, the efficiency of the recovery of artificially introduced viruses must be compared statistically between treatments. This presentation will list the critical elements in a method to detect viruses in

soft fruit, and present practical examples of their testing and optimization. The information presented will be useful to all workers who currently possess or are considering development of similar methods.

T10 Detection of *Cryptosporidium parvum* Oocysts on Fresh Produce Using a Parasite Viral Protein

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Over the past few years foodborne transmission of *Cryptosporidium parvum* oocysts has been implicated in outbreaks of severe gastroenteritis associated with fresh produce. *C. parvum* oocysts have been found on green onions, various herbs, lettuces, apples, and berries. Detection of foodborne pathogens has gained renowned attention along with the need for rapid detection methods. Current rapid detection methods involve the use of high-priced molecular tools and highly-skilled technicians using DNA analysis or microscopy. Even with advanced tools it remains difficult to find oocysts in large amounts of potentially contaminated food. This work proposes a novel detection method for fresh produce using a virus-like double-stranded RNA particle found within *C. parvum* sporozoites. Using viral RNA increases the chance for positive detection, as there are more than 600 structures per oocyst. In this study, two selected produce items, green onions and cilantro, were seeded with oocysts ranging from 10^5 to 10^1 oocysts per 5 g sample of green onions and per 10 g sample of cilantro. Homogenized samples and wash water were analyzed for the presence of viral capsid protein using a dot-blot assay with our antibody directed to a recombinant viral capsid protein. Viral presence was detected on all samples of wash water from cilantro and all wash water and homogenized samples of green onions. The detection of parasite viral particles appears to be a possible means of evaluation of *C. parvum* on fresh produce items, with the potential for use on other food items as well.

T11 DSC Managing Food Safety and Environmental Risk on Ontario Fruit and Vegetable Farms: Implications for Nutrient Management Regulation

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Increased nutrient management regulation in North America has implications for food safety and water quality in agriculture, including fruit and vegetable farms. The use of manure, irrigation water, and wash water, coupled with the growing number of reported cases of foodborne illness linked to the consumption of fresh produce, has highlighted the need for more stringent nutrient and food safety management at the farm level. Adoption of best management practices and risk management programs by fruit and vegetable producers in Ontario, Canada was investigated to understand how nutrient regulations could be most efficiently met on these farms. Producers' needs for information and training, as well as practices and current perceptions of risk management, were assessed using triangulation of research methods. Surveys and interviews were conducted with organic and conventional fruit and vegetable producers, and interviews were conducted with agricultural professionals. Results indicated that there was inconsistent knowledge of best management agricultural practices and on-farm food safety, but producers expected nutrient regulations to impact their farm in some way. There were differences in how organic and conventional producers obtained information on food safety and environmental quality and in how they managed these aspects on the farm. Additional costs, paperwork or perceived lack of relevance to their industry have resulted in a high level of concern for impending nutrient management regulations within these groups. Recommendations for meeting the needs of fruit and vegetable producers for nutrient, environmental and food safety management were made, following the suggestions and information provided by producers.

T12 DSC Improving On-farm Food Safety through the Development and Evaluation of an Agricultural Worker Training Video

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Fresh fruits and vegetables are generally perceived as safe to eat by many North Americans. However, several high profile food-related outbreaks have been associated with such commodities. Research has demonstrated that the practices of food workers can contribute significantly to such outbreaks. These findings suggest that improving food safety knowledge on the farm is a necessary step in improving on-farm food safety overall. In the summer of 2003, members of the Food Safety Network developed a training video intended to raise awareness of safe food handling of agricultural workers. The video was shown to 50 workers and its effectiveness was evaluated using a pre- and post-survey, assessing worker food safety knowledge before, and after, viewing the video. Focus groups were also used to help the researchers achieve an understanding of how the video was received amongst workers and what improvements should be made. Although the training video only improved overall worker knowledge by 3%, a significant (based on a Wilcoxon signed rank test) increase in knowledge occurred for specific topics covered within the video. After viewing the video, workers demonstrated a significantly improved understanding of germs and where they come from, hand sanitizers, and unsafe food handling. The focus groups demonstrated that the video was

easy to understand, was relevant to the intended audience, and was a good reminder of the proper way to handle food. The information learned from this study can not only be used to improve the current training video but also be applied for the production of other effective food safety videos.

T13 An Evaluation of New Microbiological Surface Sampling Kits

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Inadequately cleaned surfaces can lead to cross contamination and a reduction in food shelf life and safety. Traditionally, microbiological methods have been used to assess surface cleanliness. Rapid chemical methods can be used, although swabbing is the most frequently used method. However, there is no standardized protocol and the technique is known to lack sensitivity. Poor overall recovery from swabbing could be due to lack of ability to remove organisms or to release collected organisms, or it could be due to the inability of organisms removed from the surface to multiply. The swabbing efficiency of a traditional Dacron tipped swab (control) was compared to a range of prototype swabs using sprayed nylon flock with velvet or brush textures. Swabbing replicates were performed on standard bacterial inocula, using a previously published method to compare pickup, release and overall sensitivity. The ability of the new swabs to remove bacteria (*E. coli* and *S. aureus*) from stainless steel surfaces was no greater than that of the control. However, all the nylon swabs released more of the bacteria removed compared to the control (19 to 57% of *E. coli* compared to 11% for the control). The superior release was even greater for *S. aureus* and translated into improved overall swabbing efficacy, which could be up to 1 log value higher and ranged from 13 to 29% for the new swabs for *E. coli* compared to 7% for the control. The performance of the control was inferior to that of all the new prototype swabs. Implications of the findings will be discussed within the context of cleaning and setting microbiological standards for food contact surfaces after cleaning.

T14 DSC Transfer of *Listeria monocytogenes* from a Delicatessen Slicer to Ready-to-Eat Meat Products

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Listeria contamination of cooked/ready-to-eat delicatessen products has become a major concern over the last six years, as evidenced by at least two major outbreaks and over 80 recalls involving 130 million pounds of product. While quantitative transfer of *Listeria* in delicatessens was identified as a key informational gap in the 2003 FDA *Listeria* Risk Assessment, no published data currently exists on quantification of *Listeria* during mechanical slicing of delicatessen products. A mechanical slicer blade was inoculated to contain *L. monocytogenes* (~10^8, 10^5, 10^3 CFU/blade), using meat slurries containing a 6-strain cocktail of weak, medium, and strong biofilm formers. Whole blocks of commercially available ready-to-eat delicatessen bologna, salami, and turkey products (3 replicates) were sliced (27 to 35 slices) to entirety at ~4°C using the inoculated blade. Sliced products were plated on Modified Oxford Agar and enriched in University of Vermont Media to determine transfer rates. Transfer from the slicer blade inoculated at 10^8 CFU/blade was logarithmic as determined using the Weibull model with 10^2 CFU/slice after 29 slices. Meat slices sampled from slicer blades inoculated at 10^5 CFU/blade contained 10^2 CFU/slice after 5 slices with enriched samples typically positive after 27 slices. Low-level blade inoculation (10^3 CFU/blade) was non-quantifiable by direct plating and typically negative after enrichment. These findings on *Listeria* transfer suggest additional precautions may be needed when dealing with *Listeria*-related recalls of ready-to-eat products.

T15 DSC Impact of Biofilm-forming Ability on Transfer of Surface-dried *Listeria monocytogenes* Cells from Knife Blades to Smoked Turkey Breast

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Listeria contamination of food contact surfaces can lead to cross contamination of ready-to-eat foods in delicatessens. In the present study, six previously identified strong and weak biofilm-forming strains of *L. monocytogenes* were grown at 22°C for 48 h on Trypticase soy agar containing 0.6% yeast extract and harvested in 0.1% peptone. The strains were combined into two 3-strain cocktails and resuspended in turkey slurry to inoculate flame-sterilized grade 304 stainless steel knife blades at concentrations of 10^8 and 10^6 CFU/blade. After incubation at ~78% relative humidity for 6 and 24 h, retail smoked turkey breast was cut into 16 slices using the knives mounted on an Instron Universal Testing Machine. Using the strong biofilm cocktail at 10^8 CFU/blade after 6 h, slices 1 and 16 yielded 7.1 and 4.1, and 4.5 and < 2.2 log CFU/slice when inoculated at 10^6 CFU/blade, respectively. Using the high and low inoculum after 24 h, slices 1 and 16 yielded 5.8 and 2.8, and 4.3 and < 2.4 log CFU/slice, respectively. Using the weak biofilm cocktail at 10^8 CFU/blade 6 h, slices 1 and 16 yielded 6.5 and 4.0 log CFU/slice and 4.1 and < 2.3 when inoculated at 10^6 CFU/blade, respectively. Using the high inoculum after 24 h, slices 1 and 16 yielded 5.0 and 3.0 log CFU/slice, while most samples were negative by direct plating when the low inoculum was used. Greater transfer observed for the strong biofilm cocktail suggests that these strains are better adapted to survive the stressful conditions when dried on knife blades.

T16 Reducing the Risk of Microbial Cross Contamination Using Conveyor Belts Containing a Microbial Inhibitor

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Many factors contribute to foodborne disease outbreaks, including product to contact surface cross contamination occurring at the processing plant, in commercial kitchens, and in the home. To address this factor, studies were conducted in our laboratory that demonstrated the efficacy of commercial HabaGUARD® conveyor belts containing an inhibitor to reduce the populations of several foodborne bacterial pathogens. The objective of this study was to evaluate the effectiveness of HabaGUARD® conveyor belts of varying composition to inhibit *Salmonella* spp., *C. jejuni*, *L. monocytogenes*, and *E. coli* O157:H7 on both treated and control flexible and rigid belting materials fabricated from either polyethylene, polypropylene, or polyacetal. Using a quantitative direct inoculation test where the test organisms were deposited directly onto the surface of the belt samples, covered with a film to prevent drying, and then incubated for 24 h at 35°C, the range of inhibition achieved over three replicate trials was from 3.6 to 7.7 logs. Moreover, HabaGUARD® conveyor belts that had been used in commercial meat and poultry processing facilities for 12 to 16 months yielded bacterial inhibitions ranging from 0 to more than 6 logs depending on the test organism. To estimate the shelf life of the HabaGUARD® belts, the belts were immersed for 8 weeks at room temperature in several commercial cleaners and disinfectants at normal use concentrations and then evaluated for inhibitory activity against *L. monocytogenes* and *E. coli* O157:H7 as outlined above. Inhibition ranged from 3.95 to 6.8 and 0.6 to 6.6 logs for *Listeria* and *E. coli*, respectively.

T17 DSC Protocol for Evaluating Relative Performance of Footwear Materials Used in Food Processing Environments Based on the Efficacy of Cleaning/Sanitation Compounds for Elimination of *Listeria monocytogenes*

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There are few published studies on protocols for cleaning and sanitizing footwear used in food processing environments. In our study, seven footwear materials were evaluated for their performance based on the efficacy of 14 different cleaning and sanitation compounds to eliminate artificially applied coatings of 4 different food materials containing approximately 3 x 10^8 CFU of *Listeria monocytogenes* CWD F5069 per gram. The 7 footwear materials were also evaluated for their ability to withstand heat treatment at 121°C for 15 min and for integrity of the material over time under conditions similar to operating conditions in food processing environments, i.e., the materials were coated with food materials and left to stand for 8 h at 4°C, simulating 8 h work shifts. These materials were then evaluated for *Listeria* load, cleaned and sanitized, and re-evaluated for *Listeria* load to check for the effectiveness of various compounds. All the cleaning/sanitizing compounds we used were found to be consistently effective against *L. monocytogenes* CWD F5069. The absence of *Listeria* on the cleaned/sanitized footwear materials was confirmed by enumeration and scanning microscopy. Except for material 1 (Thermosplastic Polyurathane), all other footwear materials tested were found to withstand sterilization temperatures (121°C for 15 min). Because *Listeria* is a ubiquitous foodborne pathogen, this protocol is imperative to ensure elimination of *Listeria* from food processing environments, especially the ready-to-eat industry. Moreover, most of the footwear materials analyzed in the current study allow for effective decontamination.

T18 The Cleaning Efficacy and Particle Spread of Dry Cleaning Techniques

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Novel dry food products are now being marketed that are less microbiologically stable and wet cleaning techniques used during production in ready-to-eat products are now being replaced by dry cleaning techniques, wherever possible, to control pathogens, e.g., *Listeria*. The efficacy and microbiological hazards of dry cleaning techniques is thus under industry consideration. The cleaning efficacy of compressed air, brushing and vacuuming was assessed using a cleaning rig that varied cleaning sweep number, speed and application pressure. Cleaning to visual and ATP levels was examined, using flour, rice and corn dust soils, and ultimately compared to standard industry wet cleaning. The 2-dimensional spread of soil particles was assessed visually and the 3-dimensional distribution of particles was assessed in an aerobiology containment room using a particle counter. Particle counts in the air (0.5 to 25μ) were recorded for 16 min following cleaning. When cleaning to "visual cleanliness", neither manual brushing, vacuuming or compressed air cleaned to total soil removal as measured by ATP. The repeating of brushing or vacuum sweeps is the critical factor in soil removal and achieved cleaning efficacy comparable to wet cleaning. The 2-dimensional spread of particles by compressed air was far greater than with brushing or vacuuming and was the only technique to significantly increase the number of airborne particles following cleaning. Many of these particles were still airborne after 16 min. The results have implications on the choice and optimization of a dry cleaning system to be used during food production periods in terms of potential pathogen dispersal and cleaning efficiency.

T19 English Butchers' Beliefs and Perceptions about HACCP

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A requirement for food safety management systems based on HACCP is currently being incorporated into the legislation of many countries (that sell both unwrapped raw meat and ready to eat foods). In England legislation requires all relevant butchers/those that sell, to be licensed, and in order to obtain a licence or be re-licensed, a documented HACCP plan is required. Support was provided in the form of an accelerated HACCP initiative including training and consultancy. An evaluation of butchers' beliefs and perceptions, following the initiative and subsequent HACCP implementation, is presented. After piloting, a questionnaire was distributed to 2,700 butchers obtained from environmental health departments (response rate was 16%). Butchers did not believe their shops presented a high risk to food safety and self reported they implemented high levels of food safety. A minority (23%) believed that possession of a HACCP plan would help attract new business. Over 70% felt that hygiene was now under greater control. Fifty-four percent felt that introducing HACCP had made little change to their working practices, with only 11% believing it had been difficult to apply. Fifty-three percent believed they could find time for HACCP monitoring and 59% believed it helped them run their business more efficiently. Increased costs associated with implementing HACCP were less than estimated. Sixty-three percent believed that introducing HACCP would not have been possible without support. The results are of value in understanding the food industry's perception of HACCP and may be of benefit in designing and evaluating initiatives to increase HACCP use within other sectors and countries.

T20 Standards of Surface Cleanliness in English Butchers' Shops

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Raw meat and poultry are known to be good sources of microogranisms which may ultimately soil equipment. Minimizing this is important as cross contamination has been identified as an important contributory factor in food poisoning. The aim of the present work was to assess the contamination levels of a range of food, hand contact and other surfaces in English butchers' shops serving both raw and cooked meats. Shops from an opportunistic sample of randomly recruited butchers were assessed for levels of surface contamination by use of a standard proforma, although precise surfaces sampled depended upon design and layout of premises. Surfaces were assessed visually, by use of ATP bioluminescence, and microbiologically. Surface condition and wetness were also recorded. Overall, 575 surfaces in 63 butchers' shops were tested; 31% of hand contact surfaces were moist, and this has implications for the zig-zag transfer of organisms. Visual assessment of contamination, although widely used, was an unreliable indicator. ATP levels on surfaces were too high, especially on hand contact surfaces, although this may have decreased subsequent to the UK licensing and HACCP initiative. Microbial surface counts were also variable, with display and preparation surfaces having particularly high values: 46% and 61% of sites respectively were in excess of 12 CFU/cm^2 and 8% and 11% had counts of 10^0 CFU/cm^2. Some food contact sites (display surfaces, slicer blades and preparation surfaces, 6%, 2% and 4% respectively) had enterobacterial counts up to 10^0 CFU/cm^2. The results are discussed within the context of possible standards for surface cleanliness, the potential for cross contamination and managing food safety.

T21 DSC Comparison in the Recovery of *Vibrio vulnificus* under Different Microbial Stresses with the Use of Sodium Pyruvate

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Vibrio vulnificus, a naturally occurring marine bacterium, causes severe disease in at-risk individuals consuming contaminated raw shellfish. The organism has been challenging because of its propensity to injury and entry into the so-called "viable but non-culturable" (VBNC). It has been suggested that injury and perhaps VBNC may be manifested by increased sensitivity to hydrogen peroxide. The objective of this study was to evaluate sodium pyruvate supplementation as a means to improve the recovery of sublethally injured *V. vulnificus*. *V. vulnificus* ATCC 27562 was submitted to various processing related stresses, including freeze/thaw tolerance (-20°), starvation, cold storage (5°C), cold temperature adaptation (15°C for 4 h then 5°C) and acid tolerance (pH 4.0 and 3.5). Cells were recovered on non-selective media [tryptic soy agar-2% NaCl] with and without sodium pyruvate supplementation. Survivor curves were plotted and D-values calculated and compared using ANOVA. There were statistically significant differences in the recovery of *V. vulnificus* on supplemented versus non-supplemented media, with higher D-values found using supplemented media. For instance, under starvation conditions, D-values were 11.64 + 1.54 days vs. 7.52 + 0.86 days using supplemented and non-supplemented media, respectively. Likewise for cold storage (D-values 4.91 + 0.13 vs. 2.15 + 0.18 days, respectively), cold adaptation (D-values 9.74 + 1.44 vs. 2.84 + 0.28 days, respectively), and pH 3.5 (D-values 18.83 + 6.25 vs. 5.20 + 0.24 min, respectively). The data suggests that media supplementation with sodium pyruvate may aid in the recovery of *V. vulnificus* cells sublethally injured by exposure to food processing-related stresses.

T22 Detection of *Listeria monocytogenes* in Foodstuffs by a Validated PCR-based Method

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A PCR assay was developed for *Listeria monocytogenes*. It has a high diagnostic accuracy, being 100% inclusive (detection of target *L. monocytogenes* strains) and 100% exclusive (non-detection of non-*L. monocytogenes*), and having a 99% probability of limit of approximately 7 cells per reaction. With a very simple sample pretreatment, the PCR is capable of detecting *L. monocytogenes* in a variety of foodstuffs. The pretreatment involves a 24 h enrichment in ISO-recommended half-Fraser broth, followed by 16 h enrichment in a medium which can be added directly into the PCR, therefore avoiding the need for complex nucleic acid extraction steps. Both the assay and the complete detection method involving sample pretreatment have been validated in international collaborative trials. The PCR-based method offers the possibility of rapid and effective screening of foodstuffs; *L. monocytogenes*-free samples can be identified within 48 h, while PCR-positive samples may be confirmed by analysing the half-Fraser culture according to the ISO procedures (EN ISO 11290-1; ISO 10560) for culture-based detection of *L. monocytogenes*. The performance characteristics (e.g., sensitivity and specificity) of both the PCR assay and the PCR-based method will be presented, and the effectiveness of the method for detection of *L. monocytogenes* in milk, cheese, pate, fish and salad vegetables will be described. The detection of viable *L. monocytogenes* cells by means of the method will be discussed.

T23 Internal Amplification Controls in PCR-based Detection Methods

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A major drawback of most published PCRs for foodborne pathogen detection is that they do not contain an internal amplification control (IAC). An IAC is a non-target DNA sequence present in the same sample reaction tube, which is co-amplified simultaneously with the target sequence. In a PCR without an IAC, a negative response (no band or signal) can mean that there was no target sequence present in the reaction. But it could also mean that the reaction was inhibited because of several circumstances, not least of which is the presence of inhibitory substances in the sample matrix. Conversely, in a PCR with an IAC, a control signal will be produced. When neither IAC signal nor target signal is produced, the PCR reaction has failed. Thus, when using a PCR-based method in routine analysis, an IAC will indicate false-negative results. Since the power of PCR in routine use will lie in its ability to rapidly identify pathogen-negative samples, incorporation of an IAC is a very significant quality control measure. We have constructed IACs for (RT)PCR-based detection of *Listeria monocytogenes*, *Mycobacterium avium* subsp. *paratuberculosis*, and norovirus. We describe the strategy for construction of each, and give details of their use in both conventional and real-time format reactions, and examples of (RT)PCR-based food sample analysis in which their function is illustrated.

T24 DSC Multi-virulence-locus Sequence Typing: A Portable and Discriminatory Tool for Molecular Subtyping of *Listeria monocytogenes*

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Use of multilocus sequence typing (MLST) to subtype foodborne pathogens has been increasingly popular during the past five years. MLST directly targets the DNA sequence variations in six or more housekeeping genes and defines strains by their unique allelic profiles (or sequence types). MLST provides portable DNA sequence data and has great potential for automation and standardization. However, due to the sequence conservation in housekeeping genes, MLST sometimes lacks the discriminatory ability to differentiate bacterial strains, which limits its use in epidemiological investigations. To improve the discriminatory ability of MLST, a multi-virulence-locus sequence typing (MVLST) approach was developed, using *Listeria monocytogenes* as a model foodborne pathogen. MVLST extends the benefits of MLST but targets more polymorphic virulence or virulence-associated genes. Results of the MVLST study on *L. monocytogenes* showed that: (i) Virulence or virulence-associated genes (prfA, inlB, inlC, dal, lisR, and clpP) provided higher percentages of nucleotide polymorphism than housekeeping genes; (ii) MVLST was able to differentiate epidemiologically-unrelated *L. monocytogenes* strains that were indistinguishable by EcoRI-ribotyping, ApaI- pulsed-field gel electrophoresis or MLST; (iii) Neighbor-joining analysis based on MVLST indicated a strain phylogeny closely related to the serotypes and genetic lineages of *L. monocytogenes*. In conclusion, MVLST improves the discriminatory ability of MLST for subtyping *L. monocytogenes* and provides a portable and highly discriminatory tool for tracking *L. monocytogenes* in the food chain. The MVLST approach can also be used for subtyping other bacterial genera and species and may prove useful for studying the evolution of bacterial virulence genes and pathogenesis.

T25 DSC A Real-time PCR Assay for *Mycobacterium avium* subsp. *paratuberculosis*

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A real-time PCR assay for quantitative detection of *Mycobacterium avium* subsp. *paratuberculosis* has been developed. It co-amplifies a specific nucleotide sequence for this bacterium and an

internal amplification control (IAC) for the assessment of PCR results. The assay was tested against 18 isolates of *M. avium* subsp. *paratuberculosis*, 17 other mycobacterial strains and 25 non-mycobacterial strains, and was fully selective in that it detected all target strains and did not detect any non-targets. It is capable of detecting 12 cells per reaction with 99% probability. Using prior centrifugation and nucleic acid extraction, the assay was able to consistently quantify down to 100 *M. avium* subsp. *paratuberculosis* cells in 20-ml artificially contaminated drinking water. Furthermore, with a simple detergent and enzymatic sample pretreatment before centrifugation and nucleic acid extraction, the assay consistently detected down to 100 *M. avium* subsp. *paratuberculosis* cells in 20-ml artificially contaminated semi-skimmed milk. Thus, the assay will be a useful addition to the range of diagnostic tools available for the study of *M. avium* subsp. *paratuberculosis*, and could be used to confirm and complement the results of culture-based analyses of food and environmental samples.

T26 A Novel and Sensitive PCR-based Method for the Specific Identification of Cattle in Foodstuffs and Specific High-risk Materials

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Bovine spongiform encephalopathy (BSE) can spread through the consumption of contaminated animal feed by healthy bovines. This critical situation has forced the European Commission to enforce a ban on feeding ruminants with any material of animal origin. In this work we report on a novel set of cattle-specific primers: CYTbos1 (forward) and CYTbos2 (reverse), which allow the amplification of a 115 bp fragment of the cytochrome b gene between nt 844 (mitochondrial site: 15,590) and nt 958 (mitochondrial site: 15,704). The proposed PCR method enabled the direct identification of bovine DNA, with no cross-reaction observed with DNA from twelve other commercial meat species with bovine DNA only. The PCR product obtained was also cleaved specifically by endonucleases ScaI (leading to restriction fragments of 86 and 29 bp) and TspE1 (leading to two restriction fragments of 62 and 53 bp). The sensitivity of the method was estimated to be, at least, of 0.025%. Bovine DNA was also successfully detected in samples heated for 2 h at 121°C or 10 min at 133°C, in commercial meat products and in specific high-risk materials processed at 133°C. This confirms the usefulness of the proposed primers for detecting the presence of bovine DNA in foods destined to human or animal consumption.

T27 The Effect of Fecal Contamination and Immersion Chilling on *Escherichia coli*, Coliform, *Campylobacter*, and *Salmonella* Counts of Broiler Carcasses

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The effect of pre-chill fecal contamination on bacterial counts of immersion chilled carcasses was tested in each of three replicate trials. In each trial, 16 eviscerated broiler carcasses were split into 32 halves, which were tagged for identification. One hundred mg of cecal contents (inoculated with 10^5 cells *Campylobacter* and naladixic acid resistant *Salmonella*) was applied to each of eight halves, which were placed into one (contaminated) of two pilot scale immersion chillers filled with ice and tap water. The contralateral halves were placed in the other (control) chiller. Eight other uncontaminated halves were also placed in the contaminated chiller and contralateral halves then placed in the control chiller. After chilling for 1 h at 0.5°C, all carcass halves were sampled by a one-min rinse in sterile water. Rinsate was collected and cultured; results are reported as log CFU/ml rinsate. There were no significant statistical differences (paired t test, $P < 0.05$) between control and contaminated paired halves (in different chillers), respectively, for *E. coli* (2.6 vs. 2.7), coliforms (2.9 vs. 3.0), or *Campylobacter* (1.5 vs. 2.1). Within the same chiller (contaminated) there were no statistical differences (analysis of variance, $P < 0.05$) between control and contaminated halves, respectively, for *E. coli* (2.6 vs. 2.7), coliforms (3.0 vs. 3.0), *Campylobacter* (2.0 vs. 2.1), or marked strain *Salmonella* (0.7 vs. 0.8). Immersion chilling appears to equilibrate counts between contaminated and control halves in the same chiller, and to minimize differences in counts on carcasses between control and contaminated chillers.

T28 *Salmonella* spp. and *Listeria monocytogenes* in Raw Liquid Egg Products in Federally Inspected Processing Establishments

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The US Department of Agriculture Food Safety and Inspection Service (FSIS) conducted a baseline study of raw liquid egg products collected at federally inspected processing establishments. Over a 16-month period in 2002 to 2003, 375 raw whole liquid egg, 340 raw liquid egg whites, and 319 raw liquid egg yolk product samples were analyzed for *Salmonella* spp. and *Listeria monocytogenes*. For the liquid whole egg products, 80.0% were positive for *Salmonella* spp. and 2.1% were positive for *L. monocytogenes*. For liquid egg whites, 73.5% were positive for *Salmonella* spp., but no samples were positive for *L. monocytogenes*. For liquid egg yolks, 67.4% of samples were positive for *Salmonella* spp. and 2.5% were positive for *L. monocytogenes*. For all liquid egg products positive for *Salmonella* spp., the predominant serotypes were *S.* Heidelberg and *S.* Enteritidis, found in 48.9% and 40.5% of the positive samples, respectively.

T29 DSC Effects of Pre- and Post-cooling Treatments on CO_2 Cryogenically Cooled Table Eggs Inoculated with *Salmonella* Enteritidis Held at 10°C for 56 Days

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CO_2 cryogenic cooling has proven successful in reducing *Salmonella* Enteritidis (SE) growth in inoculated eggs, possibly from the addition of CO_2 to the egg contents. A study was undertaken to determine the effects of CO_2 cryogenic cooling on CO_2 levels of egg contents, as well as SE levels from high inoculum (5×10^5 CFU/egg) under various egg treatments. Eggs were inoculated at ca. 1×10^4 CFU/ml. Eggs were then cooled with one-fourth of eggs being oiled before cooling (Pr) and one-fourth after cooling (Po). Another 25% of eggs were controls (C) while the final one-fourth of eggs were placed in GasPak 150® jars and held under anaerobic conditions (G). All eggs were held at 10°C. CO_2 levels in eggs were tested every day until Day 4 and then weekly thereafter up to Day 56. SE levels were sampled at Days 0, and 3 and then weekly up to Day 56. Control eggs had a significantly greater mean pH than all other treatments, with a 56-day average of 8.51, in contrast to pH 6.85 in anaerobically held eggs. No significant differences in SE growth were noted among the treatments. CO_2 levels were ca. 0.5 mg CO_2/ml lower in control eggs than in the Pr and Po treated eggs for the duration of the project, while G-eggs were ca. 0.75 to 1.0 mg CO_2/ml greater than that of the control eggs. This data suggests that no type of currently practiced egg processing methods should encourage SE growth in CO_2 cryogenically cooled eggs above the growth in unprocessed, untreated eggs.

T30 Comparison of Hygiene Performance of Two Cattle Abattoirs Based on Relationship between Microbial Counts on Hides and on Dressed Carcasses

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It is difficult to expect comparable microbial levels on carcasses at different abattoirs, even at those of equal process hygiene, if microbial loads on hides of incoming animals are not comparable, i.e., if they significantly differ between abattoirs. Total Viable Counts (TVC) and *Enterobactericeae* counts per square cm of (both) hide and dressed carcasses were determined on 62 bovines over 3 days at two abattoirs (A and B). A simple parameter "bacterial input-bacterial output factor" (BOIF) was derived as follows: the sum of microbial loads on all carcasses ("microbial output") divided by the sum of microbial loads on all hides ("microbial input") and multiplied by 100. Hygiene performance of the two abattoirs were simultaneously assessed through: a) the BOIF parameter, b) the EU quantitative microbiological criteria for carcasses only, and c) the UK Hygiene Assessment System (HAS) scoring based on subjective observation of the whole abattoir operation. The results showed that when the hygiene performance was assessed via BOIF, abattoir A was better than abattoir B. Similarly, hygiene performance of abattoir A was better than that of abattoir B when measured through subjective visual observation (the HAS scoring). In contrast, when the performance was assessed via EU criteria based on bacterial counts on carcasses only, abattoir B was better than abattoir A. Therefore, this pilot study indicated that between-abattoir comparison of hygiene performance assessment through a parameter based on relationship between microbial populations on hides and the resultant carcasses (BOIF) may be more appropriate than between-abattoir comparison based on bacterial counts on carcasses only.

T31 Effects of Feeding Whole Cottonseed on the Prevalence of *Escherichia coli* O157 among Finishing Beef Steers

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Observational studies have yielded conflicting results on the association between feeding whole cottonseed to cattle and the prevalence of *Escherichia coli* O157. Our objective was to conduct a feeding trial designed to determine the effects of feeding whole cottonseed on the prevalence of *E. coli* O157. One hundred twenty steers were assigned randomly to 24 pens (five steers/pen). Cattle received one of three treatment diets throughout the feeding period: (1) a standard steam-flaked corn-based finishing diet (STD); (2) a finishing diet in which 15% whole cottonseed (WCS) replaced steam-flaked corn; and (3) a whole cottonseed equivalent diet (EQU) based on components of whole cottonseed. Fecal samples were collected from the rectum of each animal and analyzed for the presence of *E. coli* O157 using immunomagnetic separation on the first day of the feeding period, after 56 days on feed, and on the day of harvest. Hide swab samples were also collected from the perineum area of each animal on the day of harvest. No differences in the prevalence of *E. coli* O157 among treatments were detected ($P \geq 0.229$) at any sampling point. The harvest fecal prevalence of *E. coli* O157 was 33.3, 27.5, and 35.0%, and the hide prevalence was 28.2, 30.0, and 30.0%, among those receiving the STD, WCS, and EQU diets respectively. In contrast to some previous data, our results do not provide evidence that feeding whole cottonseed at 15% of the dietary dry matter lowers *E. coli* O157 prevalence among finishing beef cattle.

T32 Prevalence of *Escherichia coli* O157 among Finishing Beef Cattle Supplemented with a *Lactobacillus*-based Direct-fed Microbial

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The objective was to describe the prevalence of *Escherichia coli* O157 among finishing beef cattle receiving a *Lactobacillus*-based direct-fed microbial (DFM). Three hundred sixty steers, housed at the Clayton Livestock Research Center in Clayton, NM, were blocked by weight and randomly assigned to 13 pens (20 steers/pen). Steers received the control diet or the DFM treatment diet throughout the feeding period. The DFM supplement contained *Lactobacillus acidophilus* strains NP 51 (10^9 CFU/steer daily) and NP 45 (10^6 CFU/steer daily) and *Propionibacterium freudenreichii* (10^9 CFU/steer daily). *E. coli* O157 prevalence estimates were based on fecal grab samples collected from half of the animals in each pen (10 steers/pen, 90 steers/treatment) at arrival, day 56 (re-implant), and at harvest. Hide swab samples were collected on the day of harvest. Samples were analyzed for the presence of *E. coli* O157 using immunomagnetic separation-based methods. At arrival, one animal was positive for fecal shedding of *E. coli* O157 in each treatment group (1.1%). On day 56, the prevalence was 14.4 and 1.1% among the control and DFM groups, respectively. The prevalence of fecal shedding of *E. coli* O157 was 3.3% among those receiving DFM and 11.1% among the control animals on the day of harvest. The percentage of positive hide samples was 21.2% among the DFM group and 32.2% among the controls. These data agree with the results of other research indicating that cattle receiving *Lactobacillus acidophilus* strain NP 51 at 10^9 CFU/steer daily are less likely to shed *E. coli* O157.

T33 DSC Reduction of *Escherichia coli* O157 in Finishing Beef Cattle by Various Doses of *Lactobacillus acidophilus* in Direct-fed Microbials

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Lactobacillus acidophilus NP 51-based direct fed microbials (DFM) have been shown to decrease the prevalence of *Escherichia coli* O157 in cattle. Our objective was to evaluate the effects of three different doses of NP 51 on the prevalence of *E. coli* O157 and feedlot performance. Two hundred forty steers were assigned randomly to 48 pens (five steers/pen) receiving one of four treatments: (1) control – no added DFM; (2) HNP51 – high dose of NP 51 at 10^9 CFU/steer daily; (3) MNP51 – NP51 at 10^8 CFU/steer daily; or (4) LNP51 – low dose of NP 51 at 10^7 CFU/steer daily. All DFM treatments included *Propionibacterium freudenreichii* at 10^9 CFU/steer. Individual rectal fecal samples were collected upon arrival and every 28 days throughout the feeding period. Fecal and hide samples were collected on the day of harvest. Samples were analyzed for presence of *E. coli* O157 by use of immunomagnetic separation-based methods. Cattle receiving the HNP51 dose had significantly lower prevalence of *E. coli* O157 throughout the feeding period ($P < 0.01$), whereas the lower doses did not result in significant decreases until harvest. Harvest fecal prevalence of *E. coli* O157 was 35.0, 16.7, 21.7, and 11.7% among cattle receiving control diet, LNP51, MNP51, and HNP51 respectively. The percentage of positive hide samples was 23.3, 6.7, 6.7, and 13.3% among cattle receiving control diet, LNP51, MNP51, and HNP 51 respectively. Feedlot performance did not differ across treatments. Although lower doses of the NP 51 resulted in significant decreases in *E. coli* O157 at slaughter, the greatest decreases over time were achieved with the highest (10^9 CFU/steer) dose.

T34 DSC Potential Legal Ramifications of the Development of Pre-harvest Food Safety Interventions in the Beef Industry

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Because live cattle are recognized as reservoirs for several foodborne pathogens, researchers have been examining methods to control these pathogens on the farm. However, producers have concerns about whether the development of pre-harvest interventions will lead to increased liability or regulation if validated interventions become available on a large scale. Our objective was to analyze the potential for increased liability, regulation, or both from increases in the knowledge and technology associated with pre-harvest food safety in the beef industry. To achieve this objective, we surveyed current laws and regulations associated with pre-harvest food safety and analyzed several proposed measures for future regulation. Second, utilizing traditional tort law principles and current legal cases, we assessed the likelihood that producers will be required to implement pre-harvest food safety interventions in order to reduce the threat of personal injury liability from foodborne illnesses. While several new measures have been proposed, the potential for their adoption by federal regulators is limited by concerns with implementation and enforcement. The potential for producers to incur liability for negligently failing to implement pre-harvest interventions is also limited. The cost and effectiveness of pre-harvest interventions, the validity of such lawsuits under federal law, and difficulties in proving a causal link to the plaintiff's injuries are factors that may limit the success of any such claims. Based upon our observations and analysis,

it does not appear that the development of pre-harvest food safety interventions will have a major impact on the potential for increased regulation or liability for producers.

T35 Tissue Distribution, Elimination, and Metabolism of Dietary Sodium (36Cl) Chlorate in Beef Cattle

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Treatment of livestock species with dietary sodium chlorate during the period immediately prior to slaughter selectively reduces the prevalence of *E. coli* O157:H7, *Salmonella* and Clostridial species. Pre-slaughter reduction of such pathogens decreases the risk of carcass contamination during animal slaughter and subsequently reduces risks to consumers of animal products. Safe use of sodium chlorate in food animals requires that its absorption, distribution, metabolism, and excretion (ADME) be described in target species. The objectives of this preliminary study were to describe the ADME of sodium [36Cl]chlorate after dietary administration to cattle. Two Loala cattle (200 kg) were each orally dosed with 63 or 126 mg Na[36Cl]O3 (114 dpm/μg; 94.4% Na[36Cl]O3, 5.6% Na[36Cl]) per kg bw per day for three consecutive days. All feces and urine were collected during the dosing periods. Approximately 8 h after the last dose, animals were slaughtered by captive bolt and exsanguination, tissues removed, and tissues analyzed for total radiochlorine content. Approximately 33 to 47% of each dose was excreted in the urine, while only 0.4 to 1.7% was recovered in excreted feces. The low dose carcass contained 30% of the total dose. Total residues were 70 to |81, 226 to 236, 53 to 47, and 38 to 29 ppm in liver, kidney, skeletal muscle, and adipose tissue, of the low and high dosed animals, respectively. Sodium chlorate may be a viable tool for pre-harvest pathogen elimination provided that the chloride ion is the major chlorate metabolite present in edible tissues. Studies are in progress to investigate the metabolic fate of chlorate in ruminants.

T36 Comparison of Rapid Test Methods and Validation of Composite Sampling for Detection of *Escherichia coli* O157:H7 in Raw Beef Trims and Raw Ground Beef

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The beef industry responded to recent FSIS regulatory requirements by designing sampling and testing protocols to detect *E. coli* O157:H7 (O157) in raw ground beef and raw beef trim. The effects of sample compositing sizes, shortened 8-h incubation time, and choice of rapid test kit on protocol test results were unknown. This research was the first comparative study to determine the limits of sample compositing sizes on detecting low levels of O157 in ground beef and beef trims, with the use of new, rapid (8-h) test kits. Sample compositing sizes were evaluated for reliable detection of O157 by the new 8-h test kits: Neogen Reveal, Strategic Diagnostic's Rapid Check, BioControl's VIP, and ABAX. Test kits were challenged with low numbers of O157 in composite sample sizes commonly used in industry and ICMSF sampling plans: 75 g, 125 g and 375 g. Composite samples were compared against the ability of each kit to detect O157 in a 25 g sample (x 2 analysis). Three lots of ground beef and beef trim were evaluated. Test kit enrichment broths were evaluated for the ability to detect O157 after 8 h, 12 h, and 16 h of incubation. Sensitivity of detection of O157 in composited samples depended on the method used. Industry can use results to correctly choose a rapid test kit, incubation time, and composite size to reliably detect O157 in beef products and to design testing protocols to meet desired Food Safety Objectives.

T37 Managing Food Safety: USFDA HACCP Guides for Operators and Regulators of Retail and Food Service Establishments

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In its advisory role, USFDA, in cooperation with other federal, state, and local regulators, industry, academia, and consumer groups, has drafted two guidance documents to assist retail and food service operators and regulators in reducing the occurrence of foodborne illness risk factors. "Managing Food Safety: A Guide for the Voluntary Use of HACCP Principles for Operators of Food Service and Retail Establishments" offers the retail and food service industries a simple yet effective scheme for voluntarily developing and implementing food safety management systems to achieve active managerial control of foodborne illness risk factors. Its companion document, "Managing Food Safety: A Regulator's Guide for Applying HACCP Principles to Risk-based Retail and Food Service Inspections and Evaluating Voluntary Food Safety Management Systems," provides regulators with a risk-based inspection methodology to identify out-of-control foodborne illness risk factors. It also offers regulators voluntary enforcement options designed to assist industry with preventing foodborne illness risk factor occurrence. At the end of this presentation, you will be able to: (1) incorporate the process approach to HACCP to develop and implement food safety management systems to achieve active managerial control of foodborne illness risk factors; (2) use risk-based inspections and methods to identify foodborne illness risk factors; and (3) assist industry with developing intervention strategies designed to prevent foodborne illness risk factors from occurring.

T38 Evaluation of Novel Information Resources to Assist SMEs and Microbusinesses with Hazard Analysis

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Small and medium sized food manufacturers, especially microbusinesses, implement HACCP to a lesser extent than large companies and have problems understanding the concepts and legislation. Previous research has shown that microbusinesses scored significantly lower than SMEs in all aspects of food safety management on a specifically designed benchmarking audit. Novel, sector-specific information resources were designed to assist these businesses to engage more fully with hazard analysis. They specifically avoided HACCP jargon and concentrated on allowing the manufacturer to identify the steps in their process that make the food safe. The resources were piloted and evaluated in a small number of SME and microbusinesses. The food safety management systems were benchmarked and the knowledge of the person responsible for food safety was assessed. The resource was used for one month and a reassessment was undertaken. Data were analysed qualitatively and quantitatively. The resource was well received by all companies although the larger SMEs considered that the lack of jargon could provide a barrier to acceptance by enforcement officers. The smaller companies found the resource useful and appreciated its simplicity. All companies requested that they be allowed to keep the resource. Conversely, the quantitative data showed that there was no demonstrable increase in either the benchmarking audit score or the knowledge base following implementation of the resource. It was found, however, that the questions were more rapidly answered and the answers were more specific and had greater depth. Jargon-free, sector-specific resources show potential for further development to improve food safety management in small businesses.

T39 Grower and Farm Worker Surveys Highlight the Need for Personal Hygiene Training Programs

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Proper use of toilets and hand washing facilities is imperative in the production of safe fresh fruits and vegetables. Achieving this goal requires two things; the presence of facilities and proper training of workers. In 2002 to 2003, the National GAPs Program conducted comprehensive surveys of farm workers and growers. Significant portions of the surveys focused on the availability of toilet and hand washing facilities, and the type of worker training programs, if any, that were being conducted. The two separate surveys revealed similar results and highlighted specific details that will allow the National GAPs Program to tailor its educational materials to meet both farm worker and grower needs. Major obstacles to implementing GAPs from the grower's perspective were cost and uncertainty about which GAPs are of the highest priority. Growers responded that they provide toilets for farm workers in the field most or all of the time (~87%), but over half (~57%) of the respondents do not offer training programs that specifically address the importance of hand washing and good personal hygiene. Forty two percent of the farm workers responded that their employers do not have rules about hand washing and ~16% of farm workers said toilets are never available in the field. These data highlight the need to continue developing farm worker personal hygiene training programs and suggest that there is still a need to strongly encourage growers to provide toilets and hand washing facilities in the field for all workers at all times.

T40 DSC Secret Shopper: Grocery Store Employee Food Handling Practices from a Customer's Perspective

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Food is always at risk of microbial contamination along the farm-to-fork continuum. However, food safety is especially critical in grocery stores because this may be the last opportunity to control potential hazards leading to foodborne illness. An increased responsibility has been placed on the newer supermarkets, which offer a variety of additional food services and products. This research reports on food handling trends discovered by observing the food handling practices of grocery store employees, and by inquiring about specific food safety related topics in supermarkets across Southern Ontario. Ten researchers of various age and appearance, trained to portray customers, visited 14 randomly selected supermarkets in Southern Ontario. Each store was visited 3 times by different researchers. Observations and information were evaluated against the content of supermarket training programs and current literature. The triangulation of results was used to establish and confirm the observed trends. Despite the increase in the number of grocery store employees receiving food handling training, a number of poor food handling practices were observed. These include: improper glove use; cross contamination between raw and ready to eat meats and poultry; improper food storage; and poor personal hygiene. And while many grocery store employees appeared confident in their food safety knowledge, when asked for storage and handling advice, many were unaware of the proper methods within their department, and were willing to offer incorrect advice. This advice often conflicted with the food handling information posted throughout the grocery store. This research highlights the need for more interactive training specific to individual departments within a supermarket and will help in the improvement of training resources for grocery store food handlers.

T41 DSC An Evaluation of Food Safety Information Transfer to Employees: One-page Media Summary Sheets in Food Service and Agriculture

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Ongoing training is an integral element of a complete food safety training program for food handlers. Recent outbreaks have increased media coverage of food safety issues and increased the profile of foodborne pathogens. The Food Safety Network (FSN) reviews websites and newspapers on a daily basis, seeking out food safety media stories, and redistributes them through the FSnet listserv. Information packages containing articles culled from FSnet were circulated in English and Spanish to managers in agricultural and food-service settings, where they were posted for employees to view. These information packages were distributed with the goal of linking specific global food safety issues with individuals' food handling practices. Information packages were one page and contained a full article or article summaries related to food handling, foodborne pathogens, outbreaks and new research as well as FSN commentaries. The articles were written at a consumer audience level of understanding. Triangulation of research methods, including surveys and stakeholder interviews, was used to obtain qualitative evidence. It was found that passively providing compelling reading material on food safety resulted in greater influence and retention of the information than if the active training sessions were to stand alone. The use of information packages resulted in a change in individual employee's knowledge and attitudes towards food safety and safe food handling. Anecdotal comments provided a template for researchers to improve the information packages and delivery service. The information gathered through this research provides a model for other, similar food safety training programs to follow.

T42 Food Safety Education in the United Arab Emirates

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International food trade and foreign travel to the United Arab Emirates are increasing, bringing important social and economic benefits. As new toxic agents are identified and new toxic effects recognized, the health and trade consequences of toxic chemicals in food will also have implications for the U.A.E. In addition, many of the reemerging or newly recognized pathogens are foodborne or have the potential of being transmitted by food and/or drinking water. Meeting these food safety challenges in the 21st century requires the local food control agencies to address the specific training needs of their food inspectors and to make laboratory analysis a high priority. The first United Arab Emirates International Food Safety and Sanitation Professional Development Program was established through a partnership between Zayed University and Dubai Municipality, Food Control Section, United Arab Emirates. The collaboration began by training a cohort of Emirati employees in food safety and sanitation, technical writing, and research and presentation skills for Dubai's food and hospitality sectors.

T43 DSC The Design and Evaluation of Food Safety Messages and Media for Canadian Restaurant Take-out Consumers

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As consumer food purchase decisions move toward convenience, restaurant take-out food sales are increasing. Recent surveys have shown that after leaving the restaurant, consumers are unaware of the food safety risks associated with take-out foods. Consumers require effective information so they understand potential risks and need to be provided with practical guidelines on the safe handling of take-out foods. Qualitative research methods were used to design a set of effective messages and evaluate the most compelling medium for delivering the message to consumers. Surveys were used to assess consumer attitudes of the safety of take-out foods as well as their post-restaurant handling of the items. Using triangulation, survey results were combined with evidence-based guidelines and results from focus groups of consumers and restaurant professionals to develop a food safety sticker that was affixed to take-out bags and boxes. The sticker was placed on take-out containers in 15 restaurants of varying size and fare. A URL and Toll-free number for further information were included on the stickers. Tracking of website hits and phone calls demonstrated that consumers read the stickers and searched for further information. The stickers were piloted for three months and consumers were surveyed after being exposed to the stickers. Survey results demonstrated that the food safety stickers had an impact on changing awareness of safe food handling practices.

T44 Handling and Storage Practices for Frankfurters, Deli Meats, and Deli Salads: Results of a Consumer Survey

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Consumption of food contaminated with *Listeria monocytogenes* (Lm) can cause listeriosis, an uncommon but potentially fatal disease. A quantitative assessment of the relative risk to public health from foodborne Lm among selected categories of ready-to-eat (RTE) foods showed that keeping refrigerated foods at 40 degrees Fahren-

heit or lower and using perishable precooked or RTE foods as soon as possible can reduce the risk of illness from Lm by more than 50%. Thus, proper handling and storage of RTE foods by consumers can reduce the risk of listeriosis. To characterize consumers' knowledge of Lm and their handling and storage practices for frankfurters, deli meats, and deli salads, a survey was conducted of 1,212 US adults randomly selected from a nationally representative Web-enabled panel. The survey findings suggest that consumers are not very knowledgeable about Lm. Less than half of consumers reported awareness of Lm (whereas more than 90% of consumers are aware of the pathogens *E. coli* and *Salmonella*) and nearly 40% of those who had heard of Lm were unable to identify implicated food vehicles. Most consumers safely store frankfurters and deli salads; however, some consumers exhibit risky storage practices for deli meats—34% store freshly sliced deli meats and 42% store opened packages of vacuum packed deli meats longer than recommended. Analysis is currently being conducted to identify the characteristics and attitudes of consumers who engage in high-risk practices. The findings from this research will be used to develop risk communication materials targeted to these consumers.

T45 *Bacillus cereus*: A Quantitative Risk Assessment-based Approach to the Development of Infant Formula Safety Standards

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Bacillus cereus is a pathogen with limits specified in national and international infant formula standards because of its potential to cause foodborne illness. *B. cereus* produces diarrheal or emetic toxins and, despite its disease-causing potential, the number of cases of *B. cereus*-based food poisoning reported internationally is extremely small. Given this low rate of illness, it is important that the standards set for *B. cereus* in infant foods are realistic and scientifically based. In this paper quantitative risk assessment has been used as a basis for developing an appropriate *B. cereus* standard for infant formula. The risk assessment used the Codex methodology, which incorporates the following elements: hazard identification: data were collected on *B. cereus* and *B. cereus* mediated food poisoning events; exposure assessment: growth of *B. cereus* was modelled in rehydrated infant formula, allowing for a range of possible handling and storage conditions. Different initial levels of *B. cereus* in the powdered formula (including real industry data) were evaluated in the model; hazard characterization: an estimated dose-response relationship was developed for *B. cereus* and infants consuming rehydrated formula. This relationship was based on evaluation of literature data and expert opinion; Risk characterization: the predicted levels of *B. cereus* in the rehydrated infant formula were compared with the estimated dose-response relationship to produce quantitative data on the likelihood of illness from consuming rehydrated formula.

T46 DSC Application of Decision Analysis Tools to the Decision of Whether to Test for the Presence of *Listeria monocytogenes* in Smoked Fish

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Food processors have many options to minimize the risk of contamination, including finished product testing for pathogens. The purpose of this study was to develop a model addressing the issues encountered when making a testing decision. *L. monocytogenes* contamination in smoked fish and a commercial PCR-based method were used in the model. Three potential consequences of contamination were considered as elements of the decision, e.g., no consequences, regulatory recall without disease, and disease outbreak. Accordingly, the inputs of the model were (1) costs associated with foodborne contamination (business and health related costs); (2) reliability of testing; and (iii) prevalence of contamination. Prevalence of contamination, test sensitivity, and test specificity values were estimated in distribution form from the literature. Information on costs associated with contamination was obtained in consultation with food safety experts. The elements of the decision were structured into a logical format using decision trees (DecisionTools®, Palisade Corp., Newfield, NY). Each branch of the decision tree was solved using the corresponding estimates for probability of contamination, costs incurred, and positive predictive value. Sensitivity analysis indicated that the decision to test or not to test for *L. monocytogenes* in smoked fish is most influenced by the positive predictive value. Product value inputs were also important to the decision. In general, the model indicated that testing for highly prevalent pathogens may provide an increase in food safety, but end product testing for pathogens of low prevalence should be carefully considered and may not be justified in view of the limited return on investment.

T47 The Food Safety Universe Risk Assessment and Risk Ranking Database

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Systematically assessing and ranking food safety risks is an essential step to optimizing the finite resources available to risk managers. Unfortunately, this is not easily achieved, given the complexity of the food system. Food safety risk assessment involves studying the likelihood and consequences of failures to food safety. Risk assessments may vary greatly in their level of

detail, ranging from qualitative opinions (low, medium or high risks) to complex, detailed, quantitative mathematical models that include Monte-Carlo simulation and quantification of uncertainty. There is a need for a "middle ground" risk assessment tool that facilitates systematic ranking of many risks, without the detail and resources required for developing full mathematical models. Since different foods may be contaminated with various hazards at different locations, from production to consumption, each food-hazard-location-of-entry combination could be defined and assessed in terms of its risk to the health of consumers. This theoretical set of all possible combinations could be thought of as the "universe" of food safety data. The Food Safety Universe Data Base (FSUDB) was developed into a semi-quantitative food safety risk assessment and ranking tool, providing a systematic method of assigning, recording and comparing scores for the risks from various hazards in various foods. This has facilitated systematic and repeated data capture, storage, searching, summarizing, counting, averaging, sorting, re-sorting, ranking and graphing, functions required to assess, prioritize and communicate food safety risks. This dynamic database has been developed to be used in conjunction with other relevant information to help optimize allocation of food safety resources.

T48 Deploying Risk Assessment Modeling to Determine Safe Shelf Life

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The aim of this study was to model the effects of extending the chilled shelf life of food products on *Bacillus cereus* levels. The output was an estimate of the percentage of packs in which the predicted number of *B. cereus* organisms at the end of modelled shelf life would be over 10^4 CFU/g, assumed to represent the upper limit of a safe level. The modelling tools used were a combination of predictive growth models and Monte Carlo probabilistic modelling. The chilled shelf lives chosen for modelling ranged from 14 to 42 days, over which time the temperature of the product was varied depending on the predicted conditions of distribution and storage at the manufacturer, retail and consumer stages. Different assumptions for the starting proportion of psychrotrophic and mesophilic *B. cereus* present at the end of cooking were modelled. In all scenarios, the percentage of packs predicted to exceed 10^4 CFU/g was judged to be unacceptable. However, laboratory studies performed at selected static temperatures suggest that our model is likely to be conservative. A sensitivity analysis showed that variability of consumer fridge temperatures and length of time the product takes to sell at retail have the greatest effects on final levels. These are factors outside the direct control of manufacturers. Refinements to the model could be made by using predictive microbiological models based on growth of *B. cereus* in solid foods after heating and by gathering further information on the prevalence, concentration and growth characteristics of *B. cereus* organisms remaining after heating.

T49 Modeling the Frequency and Duration of Microbial Contamination Events: Considering Uncertainty and Variability in Censored Data

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The frequency and duration of microbial contamination events in the environment to which ready-to-eat (RTE) foods are exposed for processing and packaging is subject to uncertainty and variability. Two empirical examples are presented to illustrate how variability, within-model parameter uncertainty, and uncertainty regarding model selection may be formally considered in modeling the frequency and duration of such microbial contamination events. Maximum likelihood estimation methods are used to account for censored temporal data. The estimated duration of contamination events represents a case where variability dominates with relatively little uncertainty about parameter values or model form. The estimated frequency of contamination events represents a case where there is not only substantial variability but also considerable within-model parameter uncertainty, as well as some uncertainty regarding model selection. The Bayesian Information Criterion provides a formal way of taking into account model uncertainty. The statistical methods presented are generalizable to the analysis of continuous data where some values are censored (e.g., chemical concentrations below analytical detection limits) but do not address uncertainties about the representativeness of the available data. Adjustments made to take into account a lack of representativeness may be warranted on a case-by-case basis and require expert domain knowledge about the relevance of the available data to the problem at hand.

T50 Verification of a Tertiary Model for Growth of *Salmonella*

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Incorporation of predictive models for pathogen growth into user-friendly computer software applications (tertiary modeling) is important for their routine use in the food industry to assess food safety. Kinetic data for high-density (4.8 log CFU/g) growth of *Salmonella* Typhimurium ATCC 14028 on sterile cooked ground chicken breast meat portions (1 g) incubated at 8 to 47°C were fit to a logistic with delay primary model to determine lag time (LT), maximum specific growth rate (SGR) and maximum population density (MPD). Secondary models for LT, SGR and MPD were combined in a computer spreadsheet with the primary model to create a tertiary model that predicted changes in density of *S.* Typhimurium as a function of time and temperature. Ability of the tertiary model to reverse the modeling process and predict the data used to develop it (i.e., verification) was evaluated, using a safe prediction zone

method in which the log cycle difference (Delta) between observed density and density predicted by the tertiary model was determined for 433 prediction cases obtained from 30 growth curves. The proportion of Delta (pDelta) in a safe prediction zone from a Delta of -0.5 (fail-safe) to 0.25 (fail-dangerous) log CFU per g was used as the index of model performance. Models with pDelta greater than 0.7 were found to provide unbiased and accurate predictions of *Salmonella* growth. An acceptable pDelta of 0.79 was obtained for the tertiary model predictions of the kinetic data used to develop it and thus its predictions were successfully verified.

T51 Rapid Pathogen Quantification in Meat Swabs and Rinsates Using Real-time PCR

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Current methods for pathogen detection in food samples are very time consuming due to the requirement for culture enrichment prior to measurement. Thus, rapid techniques for direct detection of pathogens without enrichment are greatly needed. We have developed a method for direct detection and quantification of bacterial pathogens on meat swabs and rinsates, using quantitative real-time PCR (qPCR) and Taqman probes. Bacteria in the samples were concentrated onto 0.2 μm filters, using vacuum filtration. DNA was extracted directly from the filters and quantification of the target pathogens was performed by use of qPCR. The target organisms and their corresponding target sequences that were amplified in qPCR included: coliforms (LacZ gene), *E. coli* O157:H7 (eaeA gene) and *Salmonella* spp. (invA gene). Spiking experiments demonstrated greater than 85% recovery of bacteria spiked onto sponges, with a sensitivity of detection of $5x10^1$ CFU/sample. Using this approach, we have detected coliforms and *Salmonella* on swabs taken from beef, veal, pork and sheep and chicken rinsates. Results obtained using qPCR were comparable to those obtained with culture methodology. A method to destroy DNA from dead bacteria, so as to achieve quantification of only viable cells on the meat samples, is being developed and will be incorporated into the bacteria concentration step of the assay. This procedure does not require pre-enrichment of the samples, is very sensitive, and yields results in under 4 h.

T52 Survival of Pathogenic Bacteria on Contaminated Gloves

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Several studies have been conducted on contamination and transfer of bacteria between hands, gloves, and kitchen surfaces in a foodservice setting; however, microbial survival on gloves has been minimally documented. The objective of this research was to examine the ability of bacteria to survive on commonly used glove materials, to better assess the potential for transfer of foodborne pathogens from contaminated gloves. In the first phase, the survival of four common foodborne pathogens (*Escherichia coli*, *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Vibrio vulnificus*) on latex or polyethylene gloves was evaluated over a 2 h period at four different contamination levels such that the inocula stayed visibly wet over the 2 h period. Under these conditions, the test microbes survived well on both latex and polyethylene glove surfaces, with *L. monocytogenes* and *V. vulnificus* displaying decreases in viability of less than one log on either glove after 2 h and *E. coli* and *S.* Typhimurium displaying decreases in viability of greater than one log after 2 h at only one inoculum level and only on the latex gloves. In the second phase of the study, the inocula were applied to a larger surface area so that they were visibly dry within min. These results will be presented and compared to results from the first phase of testing. Our findings demonstrate that bacteria can survive for hours on foodservice gloves and further support the standard precaution of changing gloves and handwashing when gloves have been exposed to potential sources of microbial contamination.

T53 DSC Comparison of Bacteria Identified from Dairy Cattle, Swine, and Poultry Confinement Facilities

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The air in animal confinement facilities is of concern because of possible health effects on animals and animal caretakers. This research was designed to recover and identify organisms in air from dairy cattle, swine, and poultry confinement facilities. Eleven agar media were used in BBL RodacTM plates: Tryptic Soy agar (TSA), MacConkey Sorbitol agar (MSA), Thin Agar Layer (TAL)-MSA, Baird-Parker agar (BP), TAL-BP, Modified Oxford agar (MOX), TAL-MOX, Xylose Lysine Sodium Desoxycholate agar (XLD), TAL-XLD, *Yersinia* Selective agar (CIN), and TAL-CIN. All isolates were Gram stained with the 3-Step Gram staining method (Becton Dickinson, Sparks, MD). Gram-negative colonies were identified by use of API 20E (bioMérieux, Inc., Hazelwood, MO) and BBL Enterotube test kits (Becton Dickinson, Sparks, MD). Gram-positive colonies were identified by use of BBL Crystal™ Gram-Positive test kits. Samples were taken at indoor dairy, swine, and poultry facilities, using a BioScience SAS air sampler. Depending on the confinement unit, 10 to 60 L of air was sampled. Three replications of the experiment were performed for all facilities. A minimum of 150 isolates were selected from each unit and identified, resulting in a total of 508 isolates. The most recovered genus from the dairy facility was

Staphylococcus, from the swine unit was *Aerococcus*, and from the poultry unit was *Corynebacterium*. Overall, the most common organism recovered was *Corynebacterium*. The majority of identified organisms were found to be Gram-positive. Some Gram-negative organisms identified in this study include *Escherichia*, *Klebsiella*, *Proteus*, *Shigella*, and *Yersinia*, suggesting that air in confinement facilities may be considered a source of pathogens.

T54 *Escherichia coli* and Shiga Toxin-producing *E. coli* in Livestock Forages

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Livestock feeds have been suggested as a possible source of Shiga toxin-producing *E. coli* (STEC), particularly *E. coli* O157, in animals destined for human consumption. *E. coli* O157 have been isolated from dry or concentrate feeds. In the UK, fresh grass or silage are common livestock feeds for cattle and sheep. Numbers of *E. coli* and STEC were determined in grasses collected from pastures during late summer and autumn, and in silages collected from on-farm feeding troughs. None of the feeds contained visible fecal material, even though they were collected from fields or troughs where animals (cattle or sheep) had grazed within the previous 24 h. Fresh grasses (n = 50) contained an average of 5.03 log CFU *E. coli* per gram. Silages (n = 31) contained, on average, 6.19 log CFU *E. coli* per gram. The presence of STEC was determined by hybridizing DIG-labelled probes complementary to *stx*1 and *stx*2 to blots prepared from *E. coli* count plates. Five of the 50 (10%) fresh grasses harboured STEC, but none were serotype O157. STEC were not detected in any of the silages examined. This study shows that forages used for livestock feed can harbour *E. coli*, including STEC. Grass may be a vector that allows *E. coli* to be recycled amongst livestock, or *E. coli* clones "new" to particular livestock herds or flocks to be introduced. This transmission route may contribute to the risk of pathogenic *E. coli* clones passing further along the food chain.

T55 Association between the Microbial Profile of Freshly Dressed Beef Carcasses and Regulatory Compliance Ratings of Ontario Abattoirs

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The prevalence and levels of indicator and pathogenic bacteria on beef carcasses were monitored in a baseline study at Ontario's provincially regulated abattoirs. The provincial regulation stipulates a licensing requirement for a rigorous annual audit, whereby the plants' regulatory compliance in food safety, animal welfare and occupational health are assessed objectively by independent auditors. The baseline survey was designed to collect swab samples from randomly selected carcasses, which were analyzed for indicator organisms: aerobic colony count (ACC), total coliforms (TCC) and *Escherichia coli* (ECC); and for pathogens: *Campylobacter jejuni/coli*. (Cjc), *Listeria monocytogenes* (Lm), *Salmonella* spp. (S) and VTEC. Overall, the bacterial contamination rates were 100.0%, 27.8%, 18.6%, 1.5%, 9.9%, 1.6% and 0.3% for ACC, TCC, ECC, Cjc, Lm, S and VTEC, respectively. The analysis has detected an inverse relationship: as the overall food safety compliance level decreased, incidences of coliform-positive carcasses have increased ($P = .02$). Higher food handling compliance scores have also been associated with 43% lower incidences of coliform-positive and *Escherichia coli*-positive carcasses ($P = .04$). Higher food handling compliance scores are associated with significantly lower TCC levels (0.83 vs. 1.02 log CFU/cm^2; $P = .03$). Approximately eighty percent of the Lm-positive samples collected either from low volume strata or from region "B" originated from plants which scored below average in safe food handling compliance ($P < .001$). There were significant differences among the three regions in food safety compliance and water quality and quantity scores ($P < .01$ & $P < .05$; respectively).

T56 Supercritical Carbon Dioxide Inactivation of Microorganisms in Liquid Food

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Studies were conducted to assess the use of supercritical carbon dioxide for controlling spoilage and pathogenic microorganisms in liquid food. Orange juice inoculated with generic *Escherichia coli*, *Escherichia coli* K-12, yeasts, lactic acid bacteria, *E. coli* O157:H7, *Salmonella* spp., or *Listeria monocytogenes* was processed with carbon dioxide at 7.9 MPa at 40°C for 2 or 4 min. Among microorganisms tested, *E. coli* species, *E. coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* were sensitive to the supercritical carbon dioxide process and were readily inactivated even at lower percent saturation levels (greater than 5 log reductions). Yeasts and lactic acid bacteria were more resistant to the process than those microorganisms. Their inactivation was affected by the percent saturation, and it required 100% saturation to assure the inactivation of greater than 5 logs. When the saturation reached 100%, the process time could be reduced from 4 min to 2 min to achieve the same levels of inactivation. In addition, the initial microbial loads did not affect the degree of inactivation of lactic acid bacteria in orange juice. Supercritical carbon dioxide can be used to effectively inactivate undesirable microorganisms in liquid foods without applying significant amount of heat, which could affect the quality of foods.

T57 Sensory Evaluation of Irradiated Watercress (*Nasturtium officinallis*)

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Consumer attitudes toward foods have changed in the last two decades, increasing the demand for freshlike products. Consequently, less extreme treatments or additives are being required. Minimally processed (MP) foods have freshlike characteristics that satisfy this new consumer demand. This study was performed in order to evaluate the sensory shelf life of MP watercress processed by use of gamma radiation with doses of 1.0; 3.0; 4.0 kGy. Simultaneous to sensory analysis, the most probable number of fecal coliforms and total counts of mesophiles and psycrothrophics, lactic acid bacteria and *Enterobacteriaceae* as well as the presence of *Salmonella* spp. were determined. Irradiated (1, 3 and 4 kGy) and nonirradiated MP watercress maintained under refrigeration (7°C) were submitted to a sensory acceptance evaluation performed on days 0, 2, 5, 7, 9 and 12 after treatment. About 25 panelists rated each sample for overall liking on a hybrid 10-cm hedonic scale (0 = dislike extremely; 10 = like extremely) by use of a balanced complete blocks design for serving order. Data were submitted to ANOVA and Tukey's means comparison test ($P < 0.05$). Compared to the control (non-irradiated sample), 1 kGy irradiated watercress had increased its sensory shelf life by one day (16 days). On the other hand, the samples exposed to higher doses had their shelf lives reduced to 9 days (3 kGy) and 6 days (4 kGy) due to changes in appearance. Microbiological quality of watercress samples was good throughout the experiment.

T58 Changes in *Listeria monocytogenes* Heat Resistance during Cold Storage

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Listeria monocytogenes is a psychrotrophic bacterium that is often found in cold storage foods and that causes listeriosis. The high lethality of listeriosis has caused great concern and has led to many research works. Recent food safety-related studies suggest that bacterial characteristics change as the result of environmental shocks such as cold shock or acid shock. The focus of this study was to study changes in *L. monocytogenes* heat resistance during storage at 4°C. Stationary phase *L. monocytogenes* ATCC 19114 and ATCC 7644 were stored at 4°C for 1, 3, 7, and 14 days and D-values at 60°C were calculated by enumerating surviving colonies on Modified Oxford Agar. Results indicate that the D-values of *L. monocytogenes* increase as cold storage time increases. D-values at 0 h of 4°C storage for ATCC 19114 and ATCC 7644 were 1.8 and 1.5 min, respectively. After 14 d of cold storage, D-values of both strains increased to more than 3 min. This study confirms that heat resistance of *L. monocytogenes* changes with the length of cold storage, and can provide crucial information for risk analysis of *L. monocytogenes* in refrigerated foods.

T59 Growth Characteristics and Guaiacol Production Ability of *Alicyclobacillus* spp.

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Alicyclobacillus spp. are well recognized as strictly aerobic bacteria, because they are unable to grow under anaerobic conditions. However, our preliminary experiment suggests that while most *Alicyclobacillus* spp. cannot grow under anaerobic conditions, some are capable of growth under microaerophilic conditions. Since *Alicyclobacillus* spp. spoils apple juice by producing guaiacol, the ability of *Alicyclobacillus* spp. capable of growth under microaerophilic conditions and their ability to produce guaiacol should be studied. This study was conducted to investigate the possible relationship between the guaiacol producing ability and microaerophilic growth ability of *Alicyclobacillus* spp. Fifty-five *Alicyclobacillus* spp. isolates were individually tested for microaerophilic growth and guaiacol production. Of the tested isolates, 41 isolates (80%) could grow under microaerophilic conditions, and 29 isolates (52.7%) could produce guaiacol. All guaiacol producing *Alicyclobacillus* spp. were able to grow under microaerophilic conditions, with the sole exception of one isolate. This finding indicates that most guaiacol producing *Alicyclobacillus* spp. can grow under limited oxygen environments; thus microaerophilic growth may be used to screen for potential spoilage *Alicyclobacillus* spp. This report is the first to document the relationship between microaerophilic growth and guaiacol production. More studies on the enzymatic and metabolic reactions of *Alicyclobacillus* spp. can provide insights into new methods for the control of *Alicyclobacillus* spp. spoilage in apple juices.

T60 DSC Simple Detection Method for Guaiacol Producing *Alicyclobacillus* spp.

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Alicyclobacillus spp. can survive high temperatures and acidic environments and are important spoilage microorganisms in the apple juice industry. *Alicyclobacillus* spp. cause apple juice spoilage by producing guaiacol, resulting in a medicinal off-flavor. Several analytical methods, such as high pressure liquid chromatography (HPLC) and gas chromatography (GC), are available for the detection of guaiacol. However, these

accurate analysis require additional equipment and personnel training, thus limiting their use in the industry. Most apple juice companies currently screen for guaiacol by utilizing organoleptic presence/absence evaluation. This method is simple and economic, but results may differ according to the environment and physical conditions of the panelists. This study was conducted to develop a simple colorimetric method to detect guaiacol-producing *Alicyclobacillus* spp. Two mechanisms were used: (1) the conversion of vanillic acid to guaiacol in the presence of *Alicyclobacillus* spp.; and (2) the reaction between peroxidase and guaiacol to produce brown pigments. Our results indicate that K broth supplemented with 100 ppm vanillic acid, termed KV broth, was optimum for color differentiation. Fifty-five *Alicyclobacillus* spp. isolates were evaluated for their ability to produce guaiacol by use of both conventional organoleptic evaluation and KV broth. KV broth results not only correlated with organoleptic evaluation results, but were also capable of identifying false negative isolates. Our results indicate that KV broth provides a simple, economic and applicable method to differentiate guaiacol producing *Alicyclobacillus* spp. from non-guaiacol producing *Alicyclobacillus* spp.

T61 DSC Flow Cytometric Assessment of Dead, Viable and Injured *Listeria* Cells during Heat Injury

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In foods, *Listeria* can become injured when subjected to physical and chemical stresses. Injured *Listeria* have the ability to repair and regain their pathogenicity; thus detection of injured *Listeria* in foods is consequential. Traditional methods for detecting and identifying injured *Listeria* have proved to be inadequate in precision, speed and accuracy. We investigated the usefulness of flow cytometry (FC) for assessment of dead, viable and injured *Listeria* cells after their exposure to heat stress. Carboxyfluorescein diacetate (cFDA), propidium iodide (PI) and oxonol (DiBAC4 (3)) were used to analyze esterase activity, membrane integrity, and membrane potential as indicators of bacterial viability. Untreated and heat treated *Listeria monocytogenes* F5069 cells (70°C for 30 min) were used as control samples. A 99.9% injury was achieved by subjecting the cells to 56°C heat for 30 min. Samples were analyzed with an EPICS® XL / XL-MCL Flow Cytometry System with 488 nm Argon laser that is capable of simultaneously collecting data from up to eleven parameters in parallel. The results of the study demonstrate that the use of differential dyes and molecular probes affords a powerful and sensitive tool for assessment of relative injury and physiological heterogeneity of *Listeria* populations in high risk foods. The speed and precision of this approach may offer application to routine food screening and safety assessment.

T62 Comparative Analysis of Modified Ecolite Method and MPN Method for Detecting *Escherichia coli* in Orange Juice

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The Ecolite formulation, a promulgated presence/absence detection method for coliforms and *E. coli* in 100 ml potable water, was modified, 1g bicarbonate/1.7 g media, to accommodate a 10 ml juice sample diluted into 90 ml of Butterfield's buffer. Samples are added to test bags pre-dispensed with detection media and are then incubated at 35°C for a 24 to 48 h incubation period. Fluorescent-blue bags are positive for glucuronidase and galactosidase producing microorganisms specifically, indicative of over 95% of *E. coli* strains. The method was compared to the FDA/BAM standard most probable number (MPN) method in artificially contaminated orange juices. Two strains of *E. coli*, ATCC 11775 and ATCC 25922, were added to juices at levels ranging from 0.5 to 6.8 CFU/ml. Enteric mixes, *Enterobacter/Klebsiella*, *Citrobacter/Proteus*, or *Hafnia/Citrobacter/Enterobacter*, at levels ranging from 340 to 500 CFU/ml, were added to simulate background flora. Pasteurized no-pulp, pasteurized high-pulp and freshly squeezed un-pasteurized juices were evaluated. Ten 10 ml replicates with each enteric mix were tested with and without the *E. coli* strains (60 negative and 60 positive samples). Ecolite produced 60 positive (blue-fluorescent) results with *E. coli* spiked juices and 60 negative (not fluorescent) results with un-spiked juices. By MPN the *E. coli* values ranged from <0.36 to 9.3 CFU/ml, with 100% agreement between methods. Isolated colonies recovered from positive samples were confirmed *E. coli*. The Ecolite method requires no pre-enrichment or transfers, making it a simple protocol that can be applied by juice producers. Double testing meets 20ml sample volume requirements of the Fresh Juice HACCP regulations.

T63 Microfluidics-based Optical Immunosensor for Detection of Foodborne Pathogens

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Microfluidic channels were designed and fabricated on silicon substrate with a depth of 50 μm and a length of 5 cm for use in an optical immunosensor to rapidly detect foodborne pathogens. The inlet and outlet of a microfluidic channel were connected through tubing to the injection pump and the optical detector, respectively. Antibodies were immobilized on the surface of the microfluidic channel as the capture antibody, using a self-assembled monolayer technique. Meat and vegetable samples artificially inoculated with *Escherichia coli* O157:H7 or *Salmonella* Typhimurium were filtered and then pumped

through the microfluidic channel, using an external injection pump, and the target bacteria were captured. The secondary antibody labeled with alkaline phosphatase was pumped into the channel, binding to the captured target bacteria. After free secondary antibodies were washed out, an enzymatic substrate, p-nitrophenyl phosphate, was pumped into the channel to be hydrolyzed into p-nitrophenol, which has a maximum optical absorbance at 404 nm. A CCD-array optical detector was connected to the outlet of the microfluidic channel for measurement of the absorbance, which was correlated to the concentration of the target pathogen. The data from absorbance measurements were collected, processed and displayed by use of a laptop computer connected to the optical detector. The results showed that the microfluidics-based optical immunosensor could detect *E. coli* O157:H7 and *S.* Typhimurium in a food sample with a detection limit of 50 CFU/ml in 1.5 h. The microfluidic channel showed its advantages in improved immunoassay kinetics, minimized reagent consumption, enhanced automated operation and more practical reusable sensor.

T64 Rapid and Quantitative *Campylobacter* Detection Using an Interferometric Biosensor

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Demand is growing for rapid, reliable, specific and cost-effective pathogen detection methods in food processing, quality control and food safety. Conventional microbiological practices are time consuming and may require highly skilled labor or expensive equipment. We have developed an optical interferometric biosensor for fast and direct detection of foodborne pathogens such as *Campylobacter* and *Salmonella*, based on whole-cell capture by antibodies coupled to a planar silicon nitride waveguide. An evanescent field is generated by light propagating through the waveguide, which is sensitive to refractive index changes above the waveguide surface. By combining a guided sensing beam and a reference beam in the interferometer, the change in refractive index caused by antibody-antigen binding can be measured. Several commercially available antibodies specific to *Campylobacter jejuni* were tested in this study. The lower limit of detection for *C. jejuni* in phosphate buffered saline solution is 100° CFU/ml within 30 min, using 200 ng of monoclonal antibody. No antibody labeling or extended incubation steps are required. The performance of the biosensor, including quantitation, selectivity and regeneration, were characterized. Samples of poultry processing water were examined in initial field trials of an on-line sensor prototype. Development of a multi-assay sensor format is currently under way. This technology promises detection sensitivity greater than that of commercially available rapid methods, with a simple and rugged design to allow its use in online process control.

T65 Direct Detection of *Listeria monocytogenes* from Artificially Contaminated Frankfurters

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A method for the direct detection (without cultural enrichment) of *Listeria monocytogenes* from 11 g samples of frankfurters, with particular attention to alternative nucleic acid preparation methods prior to amplification, is described. Frankfurter samples were artificially inoculated with *L. monocytogenes* at levels of 10^1 to 10^6 CFU/11g and processed for initial bacterial concentration using centrifugation, with recoveries ranging from 58 to >100%. Nucleic acids in centrifugal pellets were extracted by use of a commercial guanidinium isothiocyanate system, further purified by column chromatography and processed for total DNA, total RNA, and specific bacterial rRNA isolation, using two magnetic bead-based technologies, i.e., MICROBEnrich® and MICROBExpress®. Overall, 11-g samples were concentrated 100-fold to 100 μl with recovery of target nucleic acids. Using primers targeting rRNA sequences, the nucleic acids were amplified by PCR and RT-PCR, allowing for a direct comparison of detection limits. PCR detection limits were 10^5 CFU/11g sample and RT-PCR detection limits were 10^3 CFU/11g. Detection limits were improved an additional 10-fold (to 10^2 CFU/11g) when extracted RNA was further purified using MICROBExpress®. Results from this study show that for this particular matrix / pathogen / primer set combination, amplification via RT-PCR yielded more sensitive detection than DNA based amplification, and further purification of RNA extracts was beneficial for improving RT-PCR detection limits.

T66 A Solid Agar Overlay Method for Recovery of *Listeria monocytogenes*

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A solid agar overlay method was developed for recovery of heat-injured *Listeria monocytogenes*. A pre-solidified non-selective medium, tryptic soy agar with 0.6% yeast extract (TSAYE, 2% agar), was overlaid onto a solidified selective medium, modified oxford agar (MOX), in Petri dishes. Cells were injured at 58°C for 6 min in TSB broth. Controls were plated onto TSAYE, MOX and TSAYE/MOX overlaid plates. No significant differences ($P > 0.05$) were found among the three media for recovery of control cells. Recovery of heat-injured cells on TSAYE/MOX overlaid plates was equivalent to that obtained on non-selective TSAYE. However, MOX recovered significantly ($P < 0.05$) fewer cells compared to TSAYE and the overlaid plates. There were no significant differences among the overlaid plates prepared 0, 2, 4, 6, 8, 16, and 24 h prior to plating heat-injured of *L. monocytogenes*. Inhibition associated with diffusion of selective agents in MOX into the non-

selective overlay (TSAYE) apparently occurred between 24 and 48 h. *L. monocytogenes* could be differentiated from *Salmonella* spp., *Yersina enterocolitica* strains, *E. coli* and *Bacillus cereus* on the TSAYE/MOX overlaid plates. The solid agar overlay method for recovery of heat-injured *L. monocytogenes* cells requires less time and is less complicated than the traditional fluid selective-over-nonselective agar technique and allows for precise determination of contact time between selective and non-selective agars under controlled temperature conditions.

T67 Thermal Resistance of *Salmonella* spp., *Listeria monocytogenes* and *Staphylococcus aureus* in High Solids Egg Mixes

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Decimal reduction times (D values) were determined for *Salmonella* spp., *Listeria monocytogenes*, and *Staphylococcus aureus* (four pooled strains per pathogen) in two high solids egg mix products C95 and C55 (a_w = 0.76 and 0.82; pH = 5.09 and 5.29; solids = 53.12% and 52.63%, respectively) using a capillary-tube procedure. Heating temperature ranged from 64.0 to 70.0°C. For *Salmonella*, D values ranged from 0.05 min (at 70°C) to 0.29 min (at 64°C) in product C95 and from 0.06 to 0.22 min in product C55. For *Listeria*, D values ranged from 0.16 min (at 70°C) to 0.46 min (at 64°C) in product C95 and from 0.08 to 0.36 min in product C55. For *Staphylococcus*, D values ranged from 0.37 min (at 70°C) to 1.31 min (at 64°C) in product C95 and from 0.50 to 1.85 min in product C55. For *Listeria*, the D values derived from all four processing temperatures were significantly higher ($P < 0.001$) in the higher solids egg mix (product C95). This same trend was also observed for *Salmonella* and *Staphylococcus* but only at the lower temperatures (64 and 68°C for *Salmonella* and 64°C for *Staphylococcus*). Mean Z values for *Salmonella* were 7.65 and 10.76°C for products C95 and C55; for *Listeria*, Z values were 10.4 and 8.6°C for products C95 and C55; for *Staphylococcus*, Z values were 11.78 and 11.20°C for products C95 and C55. Of the three organisms evaluated, *S. aureus* was the most heat resistant.

T68 DSC Survival of *Escherichia coli* O157:H7 in Manure under Different Storage Conditions

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Although *E. coli* O157:H7 prevalence estimates in cattle have increased over time as the result of improvements in detection methodologies, fecal sample transport conditions from farm to microbiological laboratories for their further analysis may be a factor in prevalence underestimation for this pathogen. In this study, comparison of survival of *E. coli* O157:H7 in feces of cattle under various experimental conditions was determined. Bovine fecal samples were inoculated with two different levels of a cocktail of 4 different *E. coli* O157:H7 strains. A high level (H) contained 1 x 10^4 CFU/g initially while the low level (L) contained 1 x 10^1 CFU/g. Each inoculated sample was subdivided and a subsample placed in each of the 4 following conditions: 37°C, room temperature (23°C), refrigeration temperature (4.4°C) and in plastic coolers with refrigerant packs. Samples from each of the temperature conditions were taken at 0 h, 24 h, 48 h, 120 h, and 144 h and subjected to detection of *E. coli* O157:H7. At 5°C, *E. coli* O157:H7 was recovered from both the L and H treatments for the duration of the study. Holding in an ice cooler resulted in detectable populations in both the H and L treatments for up to 120 h. At room temperature, L samples were positive for up to 48 h while H samples were positive through the entire study. At 37°C, both the H and L samples were negative after 48 h. While further research is needed to quantify the amount of reduction during holding, it may be possible to hold samples for 2 to 4 days under certain conditions prior to processing.

T69 Factors Affecting Survival/Growth of *Escherichia coli* O157:H7 in Fresh Beef Decontamination Runoff Waste Fluids and the Resistance of Pathogen Cells to a Subsequent Lactic Acid (pH 3.5) Stress

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Survival and potential resistance development by *Escherichia coli* O157:H7 in meat plants applying decontamination technologies may be an important safety concern. This study evaluated survival/growth of inoculated (10^5 CFU/ml) acid-adapted and nonadapted *E. coli* O157:H7 ATCC 43895 in acetate-containing (0.2%; pH 3.6 ± 0.1) or in plain water (pH 7.2 ± 0.2) fresh beef decontamination runoff fluids (washings) stored at 4, 10, 15 or 25°C for 14 days, and the resistance of pathogen cells recovered from the washings after 2 or 7 days of storage to a subsequent lactic acid (pH 3.5) stress. Corresponding cultures in sterile saline or in heat-sterilized water washings were used as controls. In acetate washings, acid-adapted survived better than nonadapted populations, with survival being greatest at 4°C and lowest at 25°C. In contrast, survival in saline was lowest at 4°C, irrespective of acid adaptation, while it increased at 10 to 25°C, mainly in nonadapted cultures. The pathogen survived without growth in water meat washings at 4 and 10°C, while it grew by 0.8 to 2.7 logs at 15 and 25°C. Growth was lowest in non-sterilized water washings at 15°C due to growth (>8 logs) of natural flora. Growth-arrested *E. coli* O157:H7 from water washings at 4 or 10°C, and acid-adapted survivors from acetate washings at 4°C, were the most sensitive to pH 3.5. Conversely, pathogen

populations grown for 7 days in water washings at 15 or 25°C were relatively the most resistant. These results should be considered for monitoring decontamination programs and post-decontamination conditions in the meat industry.

T70 Phage Instability in *Escherichia coli* O157:H7: Implications for Both Phage-directed and Culture-based Methods of Identification

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We have investigated the reliability of phage-derived targets within the *E. coli* O157:H7 genome as markers for the presence of *E. coli* O157:H7. We examined the distribution of various prophage markers among a variety of isolates as well as the loss of these markers during routine growth and when cells are stressed by temperature, pH or antimicrobials. Frequent loss of one or both *Stx* genes is seen even in the absence of induced stress. In addition, many isolates lack the phage carrying the telluride resistance locus. Both of these findings have important implications for food safety testing. Many culture-based systems (e.g., Rainbow agar, CT-SMAC) require telluride resistance for identification of *E. coli* O157:H7. Similarly, genetics based approaches that require the presence of a *Stx* bearing phage are at risk of missing *Stx* negative O157:H7. This is not a trivial observation, as *Stx* production is not essential for *E. coli* O157:H7 virulence in animal models (Tzipori et al. 1987) and *Stx* negative *E. coli* O157:H7 strains have been isolated from patients with hemolytic-uremic syndrome, the most severe manifestation of *E. coli* O157:H7 infection in man (Schmidt et al., 1999). Our analysis indicates that more stable targets in the genome provide more reliable markers for the detection of *E. coli* O157:H7.

T71 DSC An Integrated Mathematical Model of Heat Transfer and Dynamic Growth of *Clostridium perfringens* during the Cooling of Ready-to-Eat Meat Products: Combining Engineering and Microbiological Modeling

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Numerous small meat processors have difficulties complying with the stabilization performance standards for preventing growth of *Clostridium perfringens* by 1 log cycle during cooling of ready-to-eat (RTE) products. In recent years, several attempts have been made to develop predictive models for growth of *C. perfringens* within the range of cooling temperatures. These studies mainly focus on microbiological aspects, using hypothesized cooling rates. Conversely, studies dealing with heat transfer models to predict cooling rates in meat products do not address microbial growth. Integration of heat transfer relationships with *C. perfringens* growth relationships during cooling of meat products has been very limited. A computer simulation scheme was therefore developed to analyze heat transfer phenomena and temperature-dependent *C. perfringens* growth during cooling of RTE meats. The temperature history of meat products was predicted by use of a finite element heat diffusion model. Validation used experimental data collected in commercial meat-processing facilities. For *C. perfringens* growth, a dynamic model was constructed, using Baranyi's non-autonomous differential equation. The bacterium's growth model was integrated into the computer program by using predicted temperature histories as input values. Predicted growth curves obtained from dynamic modeling showed good agreement with validated results for three different cooling scenarios. Maximum difference between predicted and experimental cell counts was within 5%. This study introduces combined engineering and microbiological modeling as a powerful tool applicable in food safety programs. The integrated model presented can be used to provide valuable insights into air chilling of RTE meats for risk assessment and HACCP plans.

T72 DSC A Longitudinal Study of Genetic Diversity of *Campylobacter jejuni* Isolates from Turkeys

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Campylobacter jejuni is the leading bacterial cause of human gastroenteritis in the United States and other industrialized nations, with poultry considered to be an important source of infection. Even though numerous studies have investigated colonization of broilers with this pathogen, similar studies with turkeys are relatively limited. In this study, two molecular typing methods, Restriction Fragment Length Polymorphism of the flaA gene (fla typing) and Pulsed Field Gel Electrophoresis (PFGE), were employed to investigate the genetic diversity of *C. jejuni* from turkeys pre-harvest. Isolates were obtained at several time points (5, 7, 10, 13, and 18 weeks) during the lifetime of the flock in 3 different brooder (5-week)/grow-out (7 to 18 weeks) operations. When brooders from one farm were used to stock two different grow-out operations, both of the latter were surveyed. In one of the three brooder/grow-out operations we failed to identify *C. jejuni* isolates of the same fla type in brooders and in grow-out birds. At least five different fla types were identified among isolates from the same operation, with some fla types present throughout the lifetime of the flock. These apparently persistent strains with the same fla type were further analyzed by PFGE, which indicated that certain of these strains also shared the same PFGE type. The findings suggest

that, even though each turkey flock was colonized by several strain types of *C. jejuni*, in certain brooder/grow-out operations, strains colonizing the young birds (brooders) may persist throughout the lifetime of the flock.

T73 DSC Survival of Bacterial Foodborne Pathogens in Chorizos

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Chorizos, Mexican-style raw meat sausages, are a concern in California because of their unregulated production in small ethnic food markets, and they can appear cooked to consumers, who may eat them raw, leading to potential foodborne illness. Survivabilities of *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* were evaluated under different storage conditions following a survey of non-USDA-inspected chorizos. Sausages were formulated to five initial a_w levels (0.85, 0.90, 0.93, 0.95, 0.97), stored under four conditions ("Ref", "RT", and "Inc" at 6 to 8°C, 24 to 26°C, and 30 to 31°C, respectively, with convective air circulation and "Hd" at 24 to 26°C with forced air circulation), and sampled after 1, 2, 4 and 7 days of storage. The pH, initially 4.8, remained near 5.0 throughout the storage period. Inoculated-pack studies using a five-strain cocktail per pathogen were repeated twice for each a_w. Results indicated that, for all three pathogens, the three lowest initial a_w and the Hd and Inc conditions were significantly ($P \leq 0.05$) more effective in reducing bacterial levels than the two highest initial a_w and the Ref condition, irrespective of storage time. However, *E. coli* O157:H7 appeared to be most resistant to a low-pH, low-a_w, elevated-temperature condition, as bacterial reductions were observed between 2 and 7 days of storage, compared to significant reductions in *Salmonella* spp. and *L. monocytogenes* observed only between 1 and 2 days of storage under similar conditions. These results can be useful to non-USDA-inspected chorizo producers to reduce probability of foodborne illness.

T74 Survival, Attachment and Internalization of *Salmonella* on Oranges

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Oranges can become contaminated with *Salmonella* in the field during picking, and at the various unit operations in the packing and processing sheds. The contamination can originate from surfaces such as conveyor belts and boxes, from contaminated processing water, and from the hands of workers. To develop effective strategies to prevent and decontaminate such contaminated oranges, the ecology (persistence, attachment and internalization) of the pathogen on the fruit surface needs to be understood. Oranges obtained from the field and market were used in studies to understand the persistence, attachment and internalization of *Salmonella* Gaminara and *Salmonella* Agona. The pathogens were found to persist in significantly high numbers (in the laboratory) for up to 5 days irrespective of whether the oranges were maintained at 4°C, 25°C and 37°C and irrespective of whether the oranges were washed or unwashed. *Salmonella* Agona exhibited a stronger attachment to orange surfaces as compared to *Salmonella* Gaminara, with only a 40 to 60% recovery from inoculated surfaces compared to 60 to 80% recovery exhibited by *Salmonella* Gaminara. Bacterial attachment to orange surfaces appears to be mediated by electrostatic forces (between the bacterial cell and the orange surface), based on the recovery of inoculated *Salmonella* cells and when the orange surface was washed with pH 4.0, pH 7.0, and pH 9.0 buffer. *Salmonella* strains were found to infiltrate (up to 2-log units) into the field-obtained oranges. This was confirmed by use of organic iodine as a tracer and with X-ray imaging. There was negligible infiltration into the market-obtained oranges. The studies suggest that there are significant differences in the ecology of enteric bacteria such as *Salmonella* on oranges and that these differences need to be considered during quantitative microbial risk modeling.

T75 Genetic Basis of Dry Stress Resistance of *Enterobacter sakazakii*

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In recent decades, *Enterobacter sakazakii* has been implicated in several incidents as the cause of meningitis and *enterocolitis* in premature infants. In a number of cases, there were strong indications that powdered milk formulas were the source of *E. sakazakii*. Recently, we found that *E. sakazakii* survives desiccation better than other *Enterobacteriaceae* and may therefore persist longer in dry products such as powdered infant formula. The objective of this study was to understand the mechanisms by which *E. sakazakii* can survive desiccation. Expression profiling by means of the cDNA-AFLP technique was used to obtain information on gene expression upon desiccation at an a_w of 0.23. The results showed an induction of 7 genes from the heat shock regulon, 4 genes from the CRP (cyclic AMP receptor protein) regulon, 6 genes involved in the stringent response, and a number of genes involved in trehalose synthesis and cell wall functions such as lipid A and LPS biosynthesis. Furthermore, several genes induced upon drying and without a known function were identified. The results supported the notion that the response to dry stress involves a genome-wide expression of functionally different groups of genes. In further studies we have focussed on the importance of the trehalose metabolism for survival, and on the role of LPS during desiccation.

T76 Growth of Foodborne Pathogens during Production of Compost Tea

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Compost tea (CT) is increasingly used in various cropping systems as a non-chemical foliar spray or soil drench to promote plant growth and suppress disease. We examined the potential for foodborne pathogen bacteria to grow during certain tea production practices. This study addressed the influence of pre-packaged, commercial additives, such as unsulphured molasses, soluble kelp, humic materials and proprietary "nutrient solution" preparations on the survival and growth of *Escherichia coli* O157:H7, *Salmonella enterica* serovar Typhimurium and *Enterococci faecalis* during the tea production. Commercially available, mature compost with undetectable numbers of target bacteria was used to produce CT aerobically (dissolved oxygen > 7 ppm) in 18.9L containers in 24 h. Aliquots of compost were inoculated or not with small amounts of target strains (pre-conditioned for growth on compost) and placed into mesh retainers, which were immersed in the brewing bucket with dechlorinated tap water. Test batches included varying amounts of inoculum and commercial additives. In general, target foodborne pathogens grew in CT that contained additives, but did not grow in the absence of additives. Results show that soluble carbon additives in CT production promote growth from even small concentrations of pathogens present in a matrix containing large concentrations of heterotrophic bacteria. The concept that the diversity of heterotrophs in compost is sufficient to inhibit growth of the target pathogens was not supported by this data when soluble carbon additives were supplied. The benefits of soluble carbon supplements in CT warrants further study.

S10 Food Safety for Immunocompromised Populations

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In the evolving field of food safety, there has been the growing recognition that immunocompromised populations constitute an at-risk group for foodborne illness due to their increased susceptibility. Many individuals may not know or recognize that they are a member of a high risk group, and therefore do not take steps to protect themselves by decreasing their exposure. While there is no clearcut definition of an immunocompromised host, several categories of host characteristics, both intrinsic and extrinsic, are known which have specific immune defects that in turn predispose to either increased susceptibility to infection if exposed, or to increased severity of illness if infected. Examples include pregnancy, aging, malignancy, solid organ or hematopoietic transplant, HIV infection, particular medications and immunosuppressive therapy. This symposium will delineate current knowledge in the area of food safety and immunocompromised hosts. The various categories of immunocompromise will be described and data on the magnitude of these populations and their trends in the near future will be reviewed. Normal immune function and the immunologic basis for the more common immune compromising conditions will be described. Whether the burden of foodborne disease is disproportionately borne by the immunocompromised population is not clear, and data from surveillance systems will be analyzed to address this question. The rationale for, and effectiveness of, selected educational efforts to decrease risk in immunocompromised populations will be analyzed. Finally, a perspective on risk assessment which incorporates the immunocompromised population will be discussed.

S11 Chatterbugs: Quorum Sensing and Food Safety

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Quorum sensing is the term used to describe the process of cell-to-cell communication via the production of small extracellular signaling molecules (autoinducers) that accumulate in proportion to cell population density. The extracellular signals can act upon specific sets of target genes by a variety of mechanisms resulting in the modulation of a number of cellular functions, including antibiotic production, biofilm development, sporulation, and toxin production. Knowledge is scarce, however, concerning the role of cell-to-cell signaling in food spoilage or on growth, survival, and virulence expression of pathogens in food environments. In efforts to enhance food safety, it may be feasible to design strategies to alter quorum sensing-regulated behaviors of pathogens in foods. This symposium will cover topics on different quorum-sensing mechanisms and regulators that function in bacteria, in particular, in pathogens of concern to the food industry, including *Escherichia coli* O157:H7, *Listeria monocytogenes*, and spore-formers. Emphasis will be directed toward understanding factors in food environments that may have an effect on cell-to-cell signaling and on the development of potential interventions that can be employed to control or inhibit quorum sensing, ultimately impacting food safety.

S12 Transfer and Spread of Pathogens in Food Environments

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Raw foods, e.g., raw meat and poultry, may be naturally contaminated with pathogens during their handling/processing, and can be spread in food production and preparation environments by contaminated equipment, surfaces or workers. Reports of cross contamination emphasize the

importance of cleaning, work organization and worker personal hygiene. The transfer and spread of pathogens within the work environment depends on food contamination frequency, levels, and viability over time, and dose ingested. This symposium reviews how pathogens are spread in a work environment from ingredients to contact surfaces to foods to people and from people to contact surfaces and foods. The first part of the symposium reviews those risk factors influencing the spread of pathogens in food environments. The first three speakers review transmission routes and the food handler behaviors that increase and decrease pathogen spread within the context of food safety management systems; the dynamics and modeling of pathogen transfer between surface and the influence of surface cleansing; and the persistence and survival of pathogens in food and food environments. The second part of the symposium is a series of case studies from specific links within the food chain. These consider (1) the transfer of *Listeria* from turkey to slicers and other foods; (2) the transmission of bacteria in an integrated food system from farm animals to humans; and (3) the likelihood of workers developing infections from handling animals and carcasses in slaughterhouses. Insights gained can develop more effective risk reduction strategies in food environments for both workers and consumers.

S13 Indicator Organisms and Testing — Where's the Value?

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Approximately 84% of all microbiological testing performed by the food industry is for indicator organisms. For as long as this testing has been going on, there has been debate as to whether or not it is value added. This symposium will take a close look into the area of indicator organism testing and the issues surrounding it. Speakers from a number of food sectors; meat and poultry, dairy, and dry products, will discuss which indicators are used for verifying the absence of safety and spoilage organisms in various products and processing environments. The symposium will also encompass how specifications are set for the specified indicator organisms by the different food sectors. Current methodology for detection of indicator organisms will be discussed, as well as an in-depth look into alternatives to indicator testing (ATP, biosensors, protein residues, etc). To round out the discussion, a cost-benefit analysis of the value of indicator organism testing to the food industry will be presented. This one is sure to invoke plenty of lively discussion!

S14 Update on Foodborne Disease Outbreaks

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Foodborne outbreak investigations are often extremely complex, require extensive resource commitments, and frequently may not reveal the "smoking gun". However, thorough, complete, and standardized epidemiological and environmental investigations are critical if we are to learn what factors may have contributed to the introduction, growth, and/or survival of pathogens in foods implicated in foodborne outbreaks. In this symposium, presenters will review two recent foodborne outbreak investigations; Hepatitis A in green onions and *Salmonella* Typhimurium DT104 in ground beef. The epidemiological phase and environmental phase of both investigations will be presented. Additionally, we will provide a summary of several recent lettuce associated outbreaks in the United States.

S15 Everything You Wanted to Know about Adopting New Methods . . . But Were Afraid to Ask!

RUTH FIRSTENBERG-EDEN, BioSys, 3810 Packard Road, Suite 100A, Ann Arbor, MI 48108, USA; JAY ELLINGSON, Marshfield Clinic Laboratories, Food Safety Services, 1000 N. Oak Ave., ORB, Marshfield, WI 54449, USA; SHARON BRUNELLE, Brunelle Biotech Consulting, 14104 194th Ave. NE, Woodinville, WA 98077, USA; ROY BETTS, Campden & Chorleywood Food Research Association, Station Road, Chipping Campden, Gloucestershire, GL55 6LD, UK; MARK CARTER, Kraft Foods, Inc., 801 Waukegan Road, Glenview, IL 60025, USA; TIM JACKSON, Nestlé Research Center, Microbiological Safety Group, Vers-Chez-Les-Blanc, Lausanne 26, 1000, Switzerland

With the advancement of science, new methods in microbiology are being developed at a rapid pace. The goal of this symposium is to follow the development process and review the necessary steps to implement and validate new methods. The symposium will follow the process of method development, how new methods need to be challenged, their official approval and finally their implementation by the end users. In the process, one will learn about the development of a new idea into a valuable product on the market. How methods are prepared by their inventors to

withstand all the requirement of the marketplace, making them rugged and reproducible. The symposium will address the US and international approval process, the types of tests that methods have to pass in order to prepare them for acceptance such as inclusivity and exclusivity studies, comparative studies against a reference method, examination of the limits of detection/quantification, linearity studies for quantitative tests, ruggedness trials, etc. All of this will give potential users a high degree of confidence that a new method will work and give them the results they require in order to adopt the method in their laboratory. Finally, case studies of two major food processing companies adopting new methods will be discussed. The presentations will be followed by a panel discussion.

S16 Food Toxicology 101: Basics for the Food Safety Professional

TBA

S17 *Salmonella* Control in Broiler Chickens: What Can We Learn from the Scandanavian Experience

JOHAN LINDBLAD, The Swedish Poultry Meat Association, Sibyllegatan 17, Box 556 33, Stockholm, S-102 14, Sweden; J. STAN BAILEY, USDA-ARS, P.O. Box 5677, Athens, GA 30604, USA; TANYA ROBERTS, USDA-ERS, 1800 M. St. NW, Washington, D.C. 20036, USA

Reports from Sweden from the early 1990s and from Denmark from the late 1990s suggest that their respective poultry industries have been able to almost totally eliminate *Salmonella* from broiler chickens. This symposium is designed to elucidate the steps taken in these countries to control *Salmonella* and to see if similar approaches would be applicable and economically feasible to the much larger US poultry industry. The control programs including intervention procedures and testing programs of Sweden and Denmark will be discussed. Alternative approaches which might achieve similar results but which might be more feasible for the much larger US industry will be proposed. Finally, the economics of each of these scenarios will be discussed to show what might be economically feasible for the US industry.

S18 Credibility in Science

SYLVIA ROWE, International Food Information Council, 1100 Connecticut Ave., NW, Suite 430, Washington, D.C. 20036, USA; ROBERT B. GRAVANI, Cornell University, 11 Stocking Hall, Ithaca, NY 14853, USA; DAVID M. THENO, Jack-in-the-Box, 9330 Balboa Ave., San Diego, CA 92123, USA; BOB BRACKETT, FDA-CFSAN, 5100 Paint Branch Pkwy., Room 3B-004, HFS-32, College Park, MD 20740-3835, USA

In the past several years, research scientists and science communicators have become the focus of public concern in regard to the integrity of the scientific research and review process, and the credibility of those who advise opinion leaders or speak on science to the public. A scattering of publicized incidents involving fraudulent research, undisclosed conflicts-of-interest among researchers or the peer-reviewers, and scientific studies manipulated or suppressed by their sponsors has created a small but growing challenge to the credibility of science generally, and to private sector-funded projects in particular. Some critics of industry-sponsored research, for example, have argued that the mere presence of private funds compromises scientific integrity or at least casts a shadow of suspicion on a research project. This challenges the role of IAFP science professionals, science communicators, and others who are dedicated to providing solid science-based information for the public to use in understanding complex food safety issues. A panel of experts will explore this issue in detail: the state of "quality assurance" in scientific research; the effect on public credulity of conflicts of interest — disclosed or undisclosed; the effect on public attitudes in general or a loss of confidence in science. The forum will consider and discuss, particular measures that science organizations, research scientists, and science communicators might take to address the challenges to science credibility.

S19 Risk and Control of *Enterobacter sakazakii*

MARIA NAZAROWEC-WHITE, Agriculture and Agri-Food Canada, 13 Maki Place, Nepean, ON K2H 9R5, Canada; STEVE FORSYTHE, Nottingham Trent University, Dept. of Life Sciences, Cliffton Lane, Nottingham, NG11 8NS, UK; LES SMOOT, Nestlé USA, 6625 Eiterman Road, Dublin, OH 43017, USA; JATINDER BHATIA, Medical College of Georgia, Dept. of Pediatrics, 1120 15th St., BI W-8078, Augusta, GA 30912, USA; MARCEL ZWIETERING, Wageningen University, Bode 117, Postbus 8129, 6700 EV Wageningen, The Netherlands; DON ZINK, FDA-CFSAN, Harvey W. Wiley Federal Bldg., 5100 Paint Branch Pkwy., College Park, MD 20740, USA

Enterobacter sakazakii is an opportunistic pathogen that has been associated with cases of meningitis, bacteremia and necrotizing enterocolitis in premature and immunocompromised infants. The purpose of the symposium is to provide current knowledge on the risk, control and monitoring of the organism in infant formula production and preparation. Speakers will discuss the background and current understanding of *E. sakazakii* ecology, development of methods to recover the organism from foods and the environment, and control of the organism in food production and clinical settings. An overview of the safety and economic risks posed by the organism will also be discussed as well as the perspective of regulatory agencies in the control of *E. sakazakii* and other opportunistic pathogens of infants.

S20 Impact of Environmental Viral and Parasitic Contamination on Food Safety

ALBERT BOSCH, Virus Enterics, Microbiologia UB, Diagonal 645, Barcelona, 08028, Spain; WIM VAN DER POEL, National Institute of Public Health and the Environment, Microbiological Laboratory for Health Protection, P.O. Box 1, A. van Leeuwenhoeklaan 9, Bilthoven, 3720BA, Netherlands; SABAH BIDAWID, Health Canada, Microbiology Research Div., Sir F.G Banting Research Centre, Tunney's Pasture, P.L. 2204 A2, Ottawa, ON K1A 0L2, Canada; DONNA WOLK, University of Arizona, College of Medicine, Dept. of Clinical Pathology, 259 E. Shadow Bluff Place, Tucson, AZ 85704, USA; HUW SMITH, Scottish Parasite Diagnostic Laboratory, Stobhill Hospital, Glasgow, G21 3UW, Scotland; JAMES TROUT, USDA-ARS, Environmental Microbial Safety Lab, Bldg. 173, BARC-East, 10300 Baltimore Ave., Beltsville, MD 20705, USA

Pathogenic viruses and parasites can enter the environment in waste materials from infected persons or animals. Fresh and marine waters may be contaminated through sewage discharge or run-off from agricultural land, and soils may be contaminated through application of organic waste or via deposition of animal excreta. Transmission of viral and parasitic agents through the environment to foodstuffs forms a significant part of the cycle of disease. Outside a host, neither viruses nor parasites will multiply, but they can survive in an infectious state until contact with a new host, and they have been shown to be capable of prolonged survival in various environments such as water and soil. Several outbreaks of foodborne viral and parasitic disease have been implicated to the growth of shellfish in contaminated waters, irrigation of crops with contaminated water, and growth of crops in contaminated soils. Subsequent to contamination, shellfish, soft fruit and salad vegetables are the foods which are at most risk of mediating transmission of viruses and parasites, as they are either minimally processed before consumption or eaten raw. The risk of contamination of such foodstuffs can be controlled during growth or harvesting, especially through good agricultural practice and HACCP, or by appropriate disinfection procedures during processing. This symposium will review the impact of environmental viral and parasitic contamination on food safety, detailing current knowledge, describing areas where more information should be acquired, and highlighting the on-going efforts to address the issue effectively. There will be a panel discussion session following the presentations.

S21 Safety of Raw Milk Cheeses — The State of the Science

JOHN C. BRUHN, University of California-Davis, Dept. of Food Science and Tech., 101 B Cruess Hall, Davis, CA 95616, USA; LINDA J. HARRIS, University of California-Davis, Dept. of Food Science and Tech., Davis, CA 95616, USA; KATHRYN J. BOOR, Cornell University, Dept. of Food Science, 413 Stocking Hall, Ithaca, NY 14853, USA; ELLIOT T. RYSER, Michigan State University, Dept. of Food Science and Human Nutrition, 2108 S. Anthony Hall, East Lansing, MI 48824, USA; CATHERINE W. DONNELLY, University of Vermont, 200 Carrigan Hall, 536 Main St., Burlington, VT 05405, USA; DEBRA DICKERSON, 3-D Cheeses, P.O. Box 22127, Oakland, CA 94623, 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) has been increasingly questioned by health authorities and researchers. Furthermore, questions have arisen whether the required aging of these raw-milk cheeses for 60 days or longer at a temperature of not less than 1.7°C is sufficient to eliminate any pathogens present. This symposium will examine and evaluate the science behind the 60-day aging requirement, and the research that has tested the effectiveness of the aging requirement with current-day pathogens including *Listeria monocytogenes*, *Salmonella* sp., *Escherichia coli* O157:H7. Speakers will also address foodborne outbreaks ascribed to raw milk cheese and the latest risk assessments for raw milk cheese. Finally, we will place the science into perspective by addressing what the consumers expect from cheese manufacturers in terms of safety and quality.

S22 Packaging Innovations, Safety Concerns and Seafood

DOUGLAS L. MARSHALL, Mississippi State University, Dept. of Food Science & Tech., P.O. Box 9805, Mississippi State, MS 39762, USA; JON BELL, Louisiana State University, Dept. of Food Science, 111 Food Science Bldg., Baton Rouge, LA 70803, USA; JEFFERY RHODEHAMEL, Cryovac/Sealed Air Corp., 100 Rogers Bridge Road, Duncan, SC 29334, USA; JAMES COX, All AQ Products LLC, 71 McAdenville Road, Belmont, NC 28012, USA; MARLENE E. JANES, Louisiana State University, Dept. of Food Science, 111 Food Science Bldg., Baton Rouge, LA 70803, USA; MARY LOSIKOFF, FDA-CFSAN, Office of Seafood, 5100 Paint Brush Pkwy., College Park, MD 20740, USA

The seafood industry looks to achieve superior product safety through packaging technology. This symposium will explore advances in new technologies related to seafood packaging that can control *Listeria monocytogenes* and *Clostridium botulinum*

Abstract Book Addendum

as of **August 2, 2004**

MONDAY MORNING – AUGUST 9, 2004

REVISED SCHEDULE

Late Breaking Session – *Mycobacterium paratuberculosis* – The Latest on a Potential Foodborne Disease

Sponsored by: Kraft Foods North America and IAFP Foundation Fund

Convenors: Joseph Frank and Paul A. Hall

8:30 Detection of Viable *Mycobacterium avium* subsp. *paratuberculosis* in Retail Pasteurized Whole Milk from California, Minnesota, and Wisconsin by Two Culture Methods and PCR — JAY ELLINGSON, Marshfield Clinic Laboratories, Marshfield, WI, USA

9:05 Heat Sensitivity of *Mycobacterium avian* subsp. *paratuberculosis* in Dairy Products — JOSEPH ODUMERU, University of Guelph, Guelph, ON, Canada

9:40 Kinetic Studies on the Heat Inactivation of *Mycobacterium avium* subsp. *paratuberculosis* in Milk — LINDSAY PEARCE, Fonterra Research Center, Palmerston North, New Zealand

10:15 Heat sensitivity of *Mycobacterium* avium *paratuberculosis* — KIERAN JORDAN, Teagasc, Dairy Products Research Centre, Fermoy, Co. Cork, Ireland

10:50 Association of *Mycobacterium avium* subsp. *paratuberculosis* and Human Crohn's Disease — SALEH NASER, University of Central Florida, Orlando, FL, USA

11:25 Roundtable Discussion

REVISED SCHEDULE

S01 – Molecular Subtyping of Foodborne Pathogens: Tying It All Together

8:30 PulseNet, 2004: Where We Are and Where Are We Going — TIMOTHY J. BARRETT, CDC, Atlanta, GA, USA

9:00 The Who, What and Why of USDA VetNet — PAULA J. FEDORKA-CRAY, USDA-ARS-RRC, Athens, GA, USA

9:30 Developments in PCR Diagnostics and DNA/RNA Arrays for Detection of Foodborne Viruses and Bacteria — JOHN M. COVENTRY, Food Science Australia, Werribee, Victoria, Australia

10:00 Break

10:30 Application of Molecular Subtyping Techniques in Outbreak Investigations — CRAIG W. HEDBERG, University of Minnesota, Minneapolis, MN, USA

11:00 The Future of Molecular Subtyping — MARTIN WIEDMANN, Cornell University, Ithaca, NY, USA

11:30 Panel Discussion

PRESENTATION TIME CHANGES

S03 – Validation and Verification of Pathogen Interventions in Meat and Poultry Processing

9:30 Initial and Continued Verification and Data Collection — ANN MARIE MCNAMARA, Silliker, Inc., Homewood, IL, USA

11:00 Validation of Pathogen Control in Ready-to-Eat Products — RANDALL PHEBUS, Kansas State University, Manhattan, KS, USA

T09 Critical Steps in Development of Effective Methods for Detection of Foodborne Viruses in Soft Fruit – DEFRA Central Science Laboratory is located in Sand Hutton, York, UK

P01 Session Convenors are Wendy Maduff and Carrie Hew

P021 Withdrawn

P029 Antimicrobial Activities of *Zanthoxylum schinifolium* Extract against *Vibrio parahaemolyticus* — will be presented by Kim Jeong Soon, Samsung Everland Inc., Yongin-si, Kyunggi-do, Korea

P035 FSIS *Salmonella* Pathogen Reduction/Hazard Analysis Critical Control Point Data 1998 — will be presented by Kristina Barlow, USDA-OPHS-FSIS, Washington, D.C., USA

P039 A Novel Continuous Intervention System for the Reduction of *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in Ground Beef — will be presented by Hasan Yurttas, University of Minnesota, St. Paul, MN, USA

MONDAY AFTERNOON – AUGUST 9, 2004

S06 Water's Role in Food Contamination

Sponsored by Quality Flow Inc.

3:40 Dean C. Davidson, FDA-CFSAN, College Park, MD, USA will replace Douglas Park in the Panel Discussion

S08 Integrating Genomic Data into Quantitative Risk Assessment

Sponsored by National Food Processors Association and IAFP Foundation Fund

1:30 Title Change – *Salmonella* Subtyping as a Means for Hazard Identification in Quantitative Risk — Tine Hald, Danish Institute for Food and Veterinary Research, Soborg, Denmark

P061 Title Change to Microbiological Contamination of Beverage Machines in Foodservice Operations

P065 Quantitative Microbiological Risks Associated with Foodhandling Behaviors Implemented during Domestic Food Preparation — will be presented by Christopher J. Griffith, University of Wales Institute-Cardiff, Cardiff, Wales, UK

P074 Authors Updated — Development of Hybridoma for the Production of Monoclonal Antibody against Sulfamethazine — WON-BO SHIM, Zheong-You Yang, Jung-Sook Kim, Baik-Sang Nam, Se-Ri Kim, and Duck-Hwa Chung, Graduate School of Gyeongsang National University, Chinju, Gyeongnam, Korea

P077 Withdrawn – Replaced with T64 Rapid and Quantitative *Campylobacter* Detection Using an Interferometric Biosensor — Jie Xu and David S. Gottfried, Georgia Tech Research Institute, Atlanta, GA, USA

P078 Authors Updated – Monitoring of Aflatoxin B1 on Grains, Peanuts, Foodstuffs and Feeds in Korea — WON-BO SHIM, Zheong-You Yang, Baik-Sang Nam, Jung-Sook Kim, Se-Ri Kim, Seon-Ja Park, and Duck-Hwa Chung, Graduate School of Gyeongsang National University, Chinju, Gyeongnam, Korea

P080 Withdrawn

P084 Withdrawn

P090 Withdrawn

P093 Withdrawn

P096 Detection of Attached Bacteria on Food Contact Surfaces Using a Biosensor — will be presented by Sadhana Ravisnkar, Illinois Institute of Technology, Summit-Argo, IL, USA

TUESDAY MORNING – AUGUST 10, 2004

S12 Transfer and Spread of Pathogens in Food Environments

Bert Bartleson and Ewen Todd will convene the session.

Last speaker withdrawn – replacement:

11:30 Sources of *Escherichia coli* on Meat at a Beef Packing Plant – Mueen Aslam, Agriculture and Agri-Food Canada, Lacombe Research Centre, Lacombe, Alberta, Canada

P101 Modeling the Effects of Food Handling Practices on the Incidence of Foodborne Illness: Version 2.0 of the Food Handling Practices Model — will be presented by Katherine Vierk, FDA-CFSAN, College Park, MD, USA

P107 Authors Updated – A PCR-ELISA for Detection of Potential Sterigmatocystin and Aflatoxin Producing Fungi — ZHENG-YOU YANG, Won-Bo Shim, Baik-Sang Nam, and Duck-Hwa Chung, Graduate School of Gyeongsang National University, Chinju, Gyeongnam, Korea

P108 Authors Updated – Production and Characterization of Monoclonal Antibody against Pirimiphos Methyl — ZHENG-YOU YANG, Won-Bo Shim, Jung-Sook Kim, Se-Li Kim, and Duck-Hwa Chung, Graduate School of Gyeongsang National University, Chinju, Gyeongnam, Korea

P112 Development of a Consumer Food Safety Communication Strategy Using a Triangulation of Formative Research Methods — will be presented by Christopher J. Griffith, University of Wales Institute-Cardiff, Cardiff, Wales, UK

P121 Authors Updated – Development of Predictive Models for the Survival of *Listeria monocytogenes* on Broth and Sausage as a Function of Temperature, pH and Antimicrobials — S. S. JIN, B. K. Park, D. H. Oh, and Ki-Sun Yoon, Kangwon National University, Chunchon, Kangwon, Korea

P133 Occurrence and Resistance to Antibiotics of Thermophilic *Campylobacter* spp. in Farm Animals — will be presented by F. Ann Draughon, University of Tennessee, Knoxville, TN, USA

P140 Evaluation of a Lateral Flow Immunoassay for the Detection of *Listeria* spp. in a Variety of Foods — will be presented by J. Li, Strategic Diagnostics Inc., Newark, DE, USA

P141 Detection of *Listeria* spp. in Environmental Samples by a Combination of Wet Composites and a Novel Immunocapture Method — will be presented by Phil Coombs, Matrix MicroScience, Golden, CO, USA

TUESDAY AFTERNOON – AUGUST 10, 2004

S14 Update on Foodborne Disease Outbreaks

2:40 The FSIS Investigation and Perspective on the *S.* Typhimurium DT104 Outbreak – will be presented by Emilio Esteban, USDA-FSIS-OPHS, Alameda, CA, USA

S16 Food Toxicology 101: Basics for the Food Safety Professional

2:00 The US Food and Drug Administration's Approach to Chemical Contaminants in Food — Terry C. Troxell, FDA, College Park, MD, USA

3:00 "Hot" Issues in Food Toxicology — Joseph H. Hotchkiss, Cornell University, Ithaca, NY, USA

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P183 Withdrawn

WEDNESDAY MORNING – AUGUST 11, 2004

PRESENTATION TIME CHANGES

S18 Credibility in Science

10:30 FDA-CFSAN Approach BOB BRACKETT, FDA-CFSAN, College Park, MD, USA

11:00 Panel Discussion

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P151 Withdrawn

P160 Evaluation of the Genevision™ *Escherichia coli* O157 Real-time PCR Assay – will be presented by D. Plante, Warnex Research Inc., Laval, Quebec, Canada

WEDNESDAY AFTERNOON – AUGUST 11, 2004

S26 Optimizing Data and Minimizing Risk

1:40 Using Data Management and Trend Analysis Technique to Drive Continuous Improvement – will be presented by Les Smoot, Nestlé USA, Dublin, OH, USA

2:25 Microbial Control: Where and How Raw Ingredient and Finished Product Testing Fit into the Big Picture — will be presented by Jeff Kornacki, Kornacki Food Safety Associates, LLC, Madison, WI, USA

3:30 Microbial Control: Where and How Environmental/ Investigation Sampling Fit into the Big Picture — will be presented by Robert Behling, Kornacki Food Safety Associates, Madison, WI, USA

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T55 Association between the Microbial Profile of Freshly Dressed Beef Carcasses and Regulatory Compliance Ratings of Ontario Abattoirs — will be presented by Pat Johnson, Joseph Odumeru, and Tom Baker, Ontario Ministry of Agriculture and Food, Guelph, ON, Canada

T64 Moved to P077

P201 Withdrawn

P207 Survival of *Listeria monocytogenes* in Cow and Goat Milk as Well as in Cottage Cheese Made from Cow and Goat Milk during Storage at Various Temperatures — will be presented by Dharmendrasingh Pawar, University of Georgia, Griffin, GA, USA

P213 Pre-harvest Survival of Viruses on the Surface of Produce — will be presented by Charles P. Gerba, University of Arizona, Tucson, AZ, USA

P223 A Method for Detection of Enteric Viruses in Soft Fruit — DEFRA Central Science Laboratory is located in Sand Hutton

P231 Withdrawn