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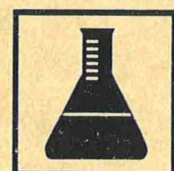
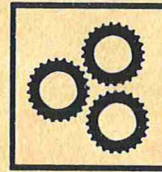
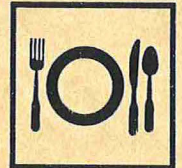

"The mission of IAMFES is to provide food safety professionals worldwide with a forum to exchange information on protecting the food supply."

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ABSTRACT BOOK

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Abstracts

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*Presenter

POSTER SESSIONS

(P1) EFFECTIVENESS OF TRISODIUM PHOSPHATE FOR INACTIVATION OF *E. COLI* O157:H7 ON APPLES

A. Atughonu,* A. F. Mendonca, L. R. Dave and T. L. Jordan, North Carolina A&T State University, 176 Carver Hall, Greensboro, NC 27411-0001, U.S.A.

The purpose of this study was to determine the efficacy of trisodium phosphate (TSP) in wash water for destruction of *E. coli* O157:H7 on the surface and in core tissue (including the stigma and stem scar areas) of apples. Apples (23°C) were immersed in a cell suspension of three strains of *E. coli* O157:H7 for 2 min, air dried and dipped for 2 or 5 min in solutions (37°C) containing 0, 2, 5, 10, or 12% (wt/vol) TSP. The morphology of treated and control cells was evaluated by scanning electron microscopy (SEM). Numbers of *E. coli* O157:H7 on apples and in core tissue were 4.36 $\log_{10}$ CFU/g and 5.20 $\log_{10}$ CFU/g, respectively, before dipping in control (0%) and TSP solutions. *E. coli* O157:H7 cells on apple surface were completely inactivated upon exposure to 10% TSP for 5 min or 12% TSP for 2 or 5 min. Cell numbers in the core tissue were significantly ($P<0.05$) reduced by 1.5 - 2 logs by dipping apples in 5 - 12% TSP for 5 min. When observed by SEM, TSP-treated cells appeared distorted and showed signs of lysis compared to control cells. The use of TSP as a sanitizer in wash water for apples seems to offer a simple, effective approach for destroying *E. coli* O157:H7 on apples.

(P2) COLD SHOCKED *E. COLI* O157:H7: IMPACT ON SURVIVAL AND INJURY FOLLOWING EITHER FREEZING OR HEATING

J. Bollman,* Food Science Dept., University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

To examine the impact of the cold shock response on *E. coli* O157:H7 in food, we examined whether cold shocking could affect survival and/or injury under freezing conditions. The cold shock response was also investigated to determine if it can cause increased heat resistance (55°C) similar to the heat shock response. Seven strains of *E. coli* O157:H7 (animal, human and food isolates) and a generic *E. coli* were cold shocked at 10°C for 3 h and inoculated into irradiation sterilized raw ground beef, ground pork, dinner sausage and whole egg. The products were then maintained at -20°C for up to 28 days. Our previous results demonstrated that the impact of cold shocking on bacterial survival was dependent on the food matrix; in products with a relatively high a_w at the cold shock response was detectable. We subsequently found that cold shocking enhanced the survival of all strains of *E. coli* in whole egg and dinner sausage, but not in ground beef or ground pork. Also, the level of injury between cold and non-cold-shocked cells appeared insignificant. Preliminary studies indicate that cold-shock proteins do not confer greater heat resistance as in the case of heat-shock proteins. This research is important to ensure that pathogen resistance is not enhanced during food processing.

(P3) IRRADIATION INACTIVATION OF *E. COLI* O157:H7 IN APPLE JUICE

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The effectiveness of low dose gamma irradiation for eliminating *Escherichia coli* O157:H7 (932, Ent-C9490, and SEA13B88) from apple juice/cider was evaluated including characterizing the effect of pH-dependent, stationary phase acid resistance on radiation resistance. The strains were grown in tryptic soy broth with or without 1% dextrose for 18 h at 37°C to produce cells that, respectively, were and were not induced to full acid resistance (*rpoS* resistance ± pH-dependent resistance). Cells were transferred to apple juice and irradiated at 2°C with a cesium^{137} irradiator. The non-acid adapted strains had D-values of 0.12-0.21 kGy. D-values increased to 0.22-0.31 kGy for acid adapted cells. Acid adapted SEA13B88 cells were tested in 5 apple juices (Ab=0.04-2.01). Radiation resistance increased with suspended solids; D-values ranged from 0.26 to 0.35 kGy. A dose of 1.8 kGy should be sufficient to achieve the 5-D inactivation of *E. coli* O157:H7 recommended by the National Advisory Committee on Microbiological Criteria for Foods. Low dose irradiation should be an effective means of inac-

tivating *E. coli* in apple juice/cider without subjecting the product to temperatures that may alter its organoleptic characteristics.

(P4) EFFECT OF PH-DEPENDENT, STATIONARY PHASE ACID RESISTANCE ON THE THERMAL TOLERANCE OF *E. COLI* O157:H7

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The ability of pH-dependent, stationary phase acid resistance to cross protect *Escherichia coli* O157:H7 against thermal stress was investigated using a submerged coil apparatus. Three strains (Ent-C9490, A9123-C1, and SEA13B88) were grown for 18 h at 37°C in tryptic soy broth with (TSB+G) and without (TSB-G) 1% dextrose to produce cells that, respectively, were and were not induced to full stationary phase acid resistance (*rpo*S resistance ± pH-dependent resistance). These cells were transferred to BHI broth (pH 6.0), heated at 58°C, and survivors enumerated. D-values for TSB-G grown cells ranged from 124 to 223 sec, while those for TSB+G grown cells were 263 to 325 sec. TSB-G grown cells had linear inactivation kinetics, while the TSB+G grown cells had non-linear kinetics, i.e., extended lag before inactivation. Combined, the TSB+G cells required a 2- to 4-fold increase in heating time for a 5-D inactivation. Strain SEA13B88 was used to determine z- values (56°-62°C). Z-values for TSB+G and TSB-G grown cells were similar (4.7° vs. 4.3°C). Induction of pH-dependent acid resistance increased *E. coli* heat resistance in milk and chicken broth, but not apple juice. However, cross protection was restored when the pH of apple juice was increased from pH 3.5 to ≥4.5.

(P5) CONTAMINATION OF INTACT APPLES AFTER IMMERSION IN AN AQUEOUS ENVIRONMENT CONTAINING *E. COLI* O157:H7

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The extent and location of *E. coli* O157:H7 contamination after intact apples were immersed in cold (2°C) peptone water containing approximately 3×10^7 CFU/ml was assessed using four apple varieties (Golden Delicious, McIntosh, Red Delicious, and Braeburn). Room temperature and refrigerated apples were used to determine the effect of a temperature differential on *E. coli* infiltration. The highest levels of *E. coli* were associated with the outer core region of the apple, followed by the skin. Apples were subsequently treated by immersing them for 1 min in 2000 ppm sodium hypochlorite, followed by a 1 min tap water rinse. This treatment reduced pathogen levels by 1 to 3 log cycles, but did not eliminate the microorganism, particularly from the outer core region. While *E. coli* was not detected in the inner core of most apples, occasional warm fruit immersed in cold peptone water internalized the pathogen. The frequency and extent of internalization was less with cold apples. Dye uptake studies with Golden Delicious apples indicated that approximately 6% of warm apples immersed in cold solution accumulated dye via open channels leading from the blossom end into the core region. However, dye uptake did not occur when the dye solution was warmer than the apple.

(P6) FATE OF *E. COLI* O157:H7 IN FOUR VARIETIES OF GROUND APPLES USED IN CIDER PRODUCTION

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Survival of *E. coli* O157:H7 in ground Golden Delicious, Red Delicious, Rome, and Winesap apples stored at 4, 10, and 25°C was determined. A two-strain mixture of *E. coli* O157:H7 was inoculated (log 7 CFU/g) into ground apples, and populations were monitored for up to 18 days by plating samples onto TSA and modified EMB agar. At 25°C, *E. coli* O157:H7 survived better ($P<0.05$) in Red Delicious apples than in the other varieties, followed by Golden Delicious and Rome apples, which were not statistically different ($P>0.05$). *E. coli* O157:H7 survival was poorest ($P<0.05$) at 25°C in Winesap apples. Although recovery was not significantly different ($P>0.05$), *E. coli* O157:H7 populations increased during storage in ground Golden Delicious and Red Delicious apples. At 10°C, survival of *E. coli* O157:H7 was poorest ($P<0.05$) in ground Red Delicious apples, while there was no significant difference in survival of *E. coli* O157:H7 in ground Golden Delicious, Rome, or Winesap varieties ($P>0.05$). When stored at 4°C, survival in ground Golden Delicious and Rome apples was not statistically different ($P>0.05$), and not statistically different in ground Red Delicious, Rome, and Winesap apples ($P>0.05$). In general, apple pH increased during storage and was assoc-

iated with mold growth. Results of this investigation indicate that there is no trend toward a particular apple variety supporting survival of *E. coli* O157:H7, although variation in apple pH during storage can negatively or positively influence *E. coli* O157:H7 survival at 25°C.

(P7) PERSISTENCE OF *E. COLI* O157:H7 IN DAIRY CATTLE DRINKING WATER

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Dairy cattle have been implicated as a reservior of *E. coli* O157:H7. In recent studies drinking water for dairy cattle has been identified as a possible source of this pathogen. Survival of *E. coli* O157:H7 and wild-type *E. coli* from manure were studied in two cattle feed waters held at 5°C and 15°C. *E. coli* O157:H7 persisted for 8 days at 5°C and for 4 days at 15°C. The indicator organisms were always present when the pathogen was present. These results indicate that *E. coli* O157:H7 can persist in cattle feed water and that assays for fecal indicator organisms should be sufficient for ascertaining the sanitary quality of the water.

(P8) HEAT INACTIVATION OF *E. COLI* O157:H7 IN TURKEY, PORK AND LAMB

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Thermal inactivation of a four-strain mixture of *E. coli* O157:H7 was determined in lean ground turkey, pork and lamb. Inoculated meat was packaged in bags which were completely immersed in a circulating water bath and held at 55, 57.5, 60, 62.5, and 65°C for predetermined lengths of time. The surviving cell population was enumerated by spiral plating meat samples on Tryptic soy agar overlaid with Sorbitol MacConkey agar. D-values, determined by linear regression, in turkey were 11.51, 3.59, 1.89, 0.81 and 0.29 min at 55, 57.5, 60, 62.5 and 65°C, respectively (z = 6.5°C). Using a survival model for non-linear survival curves, D-values in turkey ranged from 11.26 min at 55°C to 0.23 min at 65°C (z = 6.0°C). When the *E. coli* O157:H7 four-strain cocktail was heated in pork or lamb, D-values calculated by both approaches were similar at all temperatures. Thermal-death-time values from this study will assist retail food industry to design cooking regimes that ensure safety against *E. coli* O157:H7 in the ground muscle foods used in the study.

(P9) SURVIVAL AND GROWTH OF *E. COLI* O157:H7 DURING SPROUTING OF INOCULATED ALFALFA SEEDS

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The ability of *E. coli* O157:H7 to survive during storage and sprouting of inoculated alfalfa seeds was determined. Alfalfa seeds were purchased at a natural food store and inoculated with 1.35×10^7 CFU/g *E. coli* O157:H7 (UMD# 263). The seeds were then dried and stored at room temperature for 48 days. The final population of *E. coli* O157:H7 on day 48 was determined to be 2.24×10^3. A group of the inoculated seeds from day 42 were germinated and the population of *E. coli* O157:H7 was examined during the sprouting process. The population of *E. coli* O157:H7 of the pre-germinated seeds was 3.38×10^3 CFU/g and rose sharply to 6.8×10^6 CFU/g during the first 24 hours of germination. The population of *E. coli* O157:H7 remained at a level of 10^7 CFU/g for the duration of the sprouting experiment (7 days). These results suggest that *E. coli* O157:H7 may remain viable in alfalfa seeds for at least six weeks stored at room temperature, and that the population may increase dramatically during the sprouting process.

(P10) INFLUENCE OF ACIDULANT IDENTITY ON THE EFFECTS OF PH AND ACID RESISTANCE ON THE RADIATION RESISTANCE OF *E. COLI* O157:H7

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The effect of acidulant identity on the radiation resistance of *E. coli* O157:H7 (strain Ent-C9490) was determined in BHI containing 0.0 or 5.0 g/L lactic, acetic, citric, or malic acid adjusted to pH 4.0, 4,5, 5.0, and 5.5 using HCl. The pathogen was grown initially in tryptic soy broth with (TSB+G) and without (TSB-G) 1% dextrose for 18 h at 37°C to yield cells that were and were not induced for pH-dependent stationary phase acid resistance. These cells were used to inoculate the acidic BHI tubes which were then irradiated using a cesium[137] source at 2°C with doses up to 1.0 kGY. Radiation D-values were influenced in decreasing order by (a) induction of pH-dependent acid resistance, (b) acidulant identity, and (c) pH. The D-values for TSB+G grown cells were 1.3 to 3.3 times greater

than the corresponding TSB-G grown cells. The study demonstrates that accurate determination of the radiation resistance of *E. coli* O157:H7 requires consideration of the pH and acidulant identity of the food and the prior growth conditions of the pathogen.

(P11) ANTIBIOTIC RESISTANCE OF *E. COLI* O157:H7 ISOLATED FROM ANIMALS, FOODS AND HUMANS

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The antibiotic resistance of 117 *E. coli* O157:H7 and seven O157:NM isolates from animals, foods and humans was determined. Among the 124 isolates, 30 isolates (24%) were resistant to at least one antibiotic and 24 (19%) were resistant to 3 or more antibiotics. Cattle isolates had the highest rate (34%) of antibiotic resistance. The seven resistant food isolates were all from ground beef. The most frequent resistance type of *E. coli* O157:H7 and O157:NM isolates was streptomycin-sulfisoxazole-tetracycline (SSxT) which accounted for over 70% of the resistant strains. Two *E. coli* O157:NM isolates from cattle were found to be resistant to six antibiotics ampicillin, kanamycin, sulfisoxazole, streptomycin, tetracycline and ticarcillin (AKSSx TTi). This study suggests that *E. coli* O157:H7 and O157:NM have developed resistance to antibiotics, which may compromise antibiotic therapy in *E. coli* O157 infections.

(P12) GROWTH AND RECOVERY OF *E. COLI* O157:H7 IN RECONDITIONED WASTE-WATER

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The pathogen, *E. coli* O157:H7, can be recovered from various water and food samples. The growth potential of these bacteria was determined in nutrient-limited reconditioned wastewater over the temperature range of 4 to 46°C. Bioassays for the assimilable organic carbon and coliform growth response indicate that sufficient nutrients were present to support growth over temperature ranges of 15 to 28 ± 1°C even though the BOD was <2. Recovery was statistically higher ($P<0.05$) using non-selective agar (TSA) over the selective agar (Sorbitol-MacConkey). These results indicate that the bioassays are better indicators of the nutrient content of water to support pathogen growth, that *E. coli* O157:H7 grew in the nutrient limited reconditioned wastewater, that growth occurred below the optimum temperature reported for *E. coli*, and that recovery is lower using selective agar when grown under nutrient limiting conditions.

(P13) ATTACHMENT OF *E. COLI* O157:H7 TO LETTUCE LEAF SURFACES

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Confocal scanning laser microscopy (CSLM) was used to observe the location of *E. coli* O157:H7 on and within lettuce leaves. Leaves (ca. 0.5 × 0.5 cm sections) were inoculated by submersion in a suspension of *E. coli* O157:H7 (ca. 10^7 to 10^8 CFU/ml) overnight at 7°C. FITC-labeled antibody was used to visualize the attached bacteria. *E. coli* O157:H7 were found attached to the surface, trichomes, stomata and the cut edges. Three-dimensional volume reconstruction of interior portions of leaves showed that *E. coli* O157:H7 were entrapped 20 to 100 µm below the surface in stomata and cut edges. Agar plate culturing and microscopic observation indicated that *E. coli* O157:H7 preferentially attached to cut edges as opposed to the intact leaf surface. Dual staining with FITC-labeled antibody and propidium iodide was used to determine viability of cells on artificially contaminated lettuce leaves after treatment with 10 mg/L chlorine solution for 5 min. Many live cells were found in stomata and on cut edges following chlorine treatment. *E. coli* O157:H7 did not preferentially adhere to biofilm produced by *Pseudomonas fluorescens* on the leave surface. In contrast to *E. coli* O157:H7, *Pseudomonas* adhered to and grew mainly on the intact leaf surface rather than cut edges.

(P14) ENUMERATION OF VEROTOXIGENIC *E. COLI* IN GROUND BEEF

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Verotoxigenic *E. coli* (VTEC), notably *E. coli* O157:H7, have been implicated in numerous outbreaks of foodborne disease associated with the consumption of ground beef. Other serotypes of bovine origin have also caused disease in humans and have been isolated from ground beef. Quan-

titative data for VTEC are needed for the development of risk assessment models and to determine their significance in foods. The objective of this study was to quantify VTEC in retail ground beef samples. Preliminary screening suggested that these organisms were present in fresh ground beef in very low numbers, and therefore a Most Probable Number (MPN) procedure was developed. Two-hundred samples were initially tested by overnight enrichment of 25 g in MacConkey Broth at 42°C. The enrichments were screened for verotoxin (VT) production in a VT-ELISA using monoclonal antibodies specific for VT1 and VT2. Fifty-three samples positive for VT were then tested with an MPN format, consisting of enrichment in MacConkey Broth, screening for toxin by VT-ELISA, and isolation of colonies from the positive broth cultures using a VT-immunoblot procedure. Isolates were confirmed for VT production, biochemically verified and serotyped. Of the samples from which VTEC were recovered using the qualitative method, 44% were negative by the quantitative MPN method (<0.03 MPN/g). Populations of VTEC recovered by the MPN method ranged from 0.03 to 4.6 MPN per gram of ground beef.

(P15) REDUCTION OF *E. COLI* O157:H7 ON APPLES USING ACETIC ACID, HYDROGEN PEROXIDE, AND PHOSPHORIC ACID WASH TREATMENTS

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Fresh unpasteurized apple cider has been implicated in several outbreaks involving *E. coli* O157:H7. The apples used to make the unpasteurized apple cider were most likely contaminated by falling to the ground and coming in contact with *E. coli* O157:H7 in animal feces. The objective of this study was to provide a scientific basis for determining if wash treatments can reduce or eliminate *E. coli* O157:H7 on apples before they are used to make unpasteurized apple cider. Raw apples, uniform in size, shape and condition purchased from a commercial Virginia orchard were subjected to four different wash treatments. The four treatments were 200 ppm hypochlorite solution, 5% acetic acid followed by 3% hydrogen peroxide, 5% acetic acid, and a commercial phosphoric acid based fruit wash. Apples (1.7×10^3 CFU *E. coli* O157:H7) were either immersed or sprayed with each treatment for two minutes. The immersion method was more effective than the spray method. *E. coli* O157:H7 survived on apples that received the hypochlorite and the 5% acetic acid treatments. The acetic acid/hydrogen peroxide and the phosphoric acid treatments eliminated *E. coli* O157:H7 from the apples. The results of this study will serve as the basis for guidelines for apple cider processors to implement to improve the safety of their product.

(P16) TOLERANCE OF ACID-ADAPTED AND NON-ADAPTED *E. COLI* O157:H7 TO REDUCED PH AS AFFECTED BY TYPE OF ACIDULANT

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Exposure of *E. coli* O157:H7 to acidic conditions has been reported to enhance acid tolerance to secondary stresses. However, studies describing survival and growth of cells adapted in broth containing glucose, upon subsequent exposure to reduced pH environments containing organic acids, have not been reported. The objective of this study was to determine if three exceptionally acid-tolerant strains of *E. coli* O157:H7 grown in tryptic soy broth (TSB), with and without 1% glucose, exhibited changes in tolerance when plated on acidified tryptic soy agar (TSA). Diluted 18-h cultures were plated on TSA (pH 3.9, 4.2, 4.5, 4.8, 5.1 and 5.4) acidified with acetic, citric or malic acids. All test strains grew well on TSA acidified with acetic acid at pH≥5.4 or malic acid at pH≥4.5; two strains grew on TSA acidified with citric acid at pH≥4.5, while the third strain grew at pH≥4.8. Growth curves of acid-adapted and non-adapted cells in TSB containing acetic acid (pH 5.4 and 5.7) and citric or malic acids (pH 4.2 and 4.5) were also determined. There was no difference in growth characteristics of acid-adapted and non-adapted cells of *E. coli* O157:H7 in TSB acidified to a given pH with a given acid. The ability of exceptionally acid tolerant *E. coli* O157:H7 cells to adapt to low pH when grown in TSB containing 1% glucose was confirmed. Tolerance of acid-adapted and non-adapted cells upon subsequent exposure to low pH is influenced by the type of acidulant. Survival and growth characteristics in foods at reduced pH and containing various types of organic acids should be studied.

(P17) ANTIBACTERIAL EFFECT OF LACTOPEROXIDASE SYSTEM AGAINST *L. MONOCYTOGENES* AND *E. COLI* O157:H7 IN RAW AND UHT MILK

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L. monocytogenes and *E. coli* O157:H7 have been reported to be a causative agent of food poisoning

outbreaks involving milk and milk products. In this work, the antibacterial activity of LP system on the growth of *L. monocytogenes* and *E. coli* O157:H7 in raw milk and UHT milk was determined at initial inoculation levels of 10^2 CFU/ml and 10^4 CFU/ml at storage temperatures of 5, 10, and 15°C and at LP concentrations of 10, 20, and 30 ppm of LP system added with 0.25mM of H_2O_2 and SCN, respectively. Under these conditions, the antibacterial effect of the LP system was highest in the initial inoculation level of 10^2 CFU/ml, and 10 ppm of LP system in both raw milk and UHT milk. During the first 4 hours of incubation, the number of both pathogens markedly decreased at each temperature, and did not increase over 24 hours at 5°C but, thereafter, increased rapidly at 10°C and 15°C. *E. coli* O157:H7 in UHT milk was more resistant than in raw milk, and *L. monocytogenes* was more resistant to the LP system.

(P18) SURVIVAL OF *E. COLI* O157:H7 IN APPLE CIDER CONTAINING DIMETHYL DICARBONATE, SULFUR DIOXIDE, AND SODIUM BENZOATE

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Survival of *E. coli* O157:H7 at 4, 10, and 25°C in apple cider containing no preservatives, 0.025% dimethyl dicarbonate (DMDC), 0.045% sodium benzoate (SB), 0.003% sulfur dioxide (SD), and a combination of 0.003% SD and 0.045% SB (SD/SB) was evaluated. A two-strain mixture of *E. coli* O157:H7 was inoculated (log 7 CFU/ml) into ciders, and populations were determined during storage (up to 18 days) by surface plating samples onto TSA and modified EMB agar. *E. coli* O157:H7 survived well in unpreserved cider and was detected for up to 18 days at 4, 10, and 25°C. When stored at 4°C, *E. coli* O157:H7 was not detected after 3, 15, 9, and 12 days in cider containing DMDC, SD, SB, and SD/SB, respectively. At 10°C, *E. coli* O157:H7 was not detectable in cider containing DMDC, SD, SB, and SD and SB after 9, 15, 12, and 9 days, respectively. *E. coli* O157:H7 populations were reduced to undetectable levels after 2, 2, 1, and 1 day at 25°C in ciders containing DMDC, SD, SB, and SD/SB, respectively. *E. coli* O157:H7 was more resistant to preservatives at 4°C than at 25°C ($P<0.05$). *E. coli* O157:H7 was sublethally injured in cider containing preservatives, and to a lesser extent, in unpreserved cider. Injury was more pronounced in cider containing DMDC, followed by SD/SB, SB, and SD ($P<0.05$). While currently not approved for use in cider, DMDC may serve as a preservative that is effective against *E. coli* O157:H7.

(P19) SUITABILITY OF SELECTIVE MEDIA FOR RECOVERY OF HEAT-STRESSED *E. COLI* O157:H7

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When microorganisms are subjected to environmental stresses, they often are unable to form colonies on selective agar media. Thus, when using selective media for enumeration of *E. coli* O157:H7 from foods in which the organism has been exposed to stress, provisions should be made to ensure maximal recovery of stressed organisms. The purpose of this investigation was to evaluate the performance of tryptone soya agar (TSA; control medium) and three selective media, BCM™ O157:H7(+) agar (BCM), modified eosin methylene blue agar (MEMB), and sorbitol MacConkey agar (SMAC), for recovery of heat-stressed *E. coli* O157:H7. Two test strains of *E. coli* O157:H7 were inoculated (independently; about log 9 CFU/ml) into tryptone soya broth and heated in a water bath at 56, 58, and 60°C for up to 60 min. At specified intervals, samples were removed, diluted, and surface-plated onto test media. At all heating temperatures, TSA and MEMB were equally effective at recovery of heat-stressed *E. coli* O157:H7 and superior to SMAC and BCM ($P>0.05$). At 56 and 53°C, BCM provided the poorest recovery of heat-stressed *E. coli* O157:H7. However, there was no significant difference in recovery of *E. coli* O157:H7 on BCM and SMAC after heating at 60°C ($P>0.05$). The salami strain was more sensitive to heat stress than the cider strain ($P<0.05$). This study demonstrated that MEMB can be used for selective enumeration of heat-stressed *E. coli* O157:H7 while providing recovery equal to that of non-selective TSA.

(P20) THERMAL RESISTANCE OF *SALMONELLA* SPP. IN CHICKEN BROTH AS DEFINED BY D- AND Z-VALUES

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The heat resistance of 27 *Salmonella* spp. isolated from different meat species was determined at 56 to 62°C in chicken broth (pH 6.3). Thermal death times were determined using a submerged-coil heating apparatus. The surviving cell population was determined by spiral plating heated

samples onto tryptic soy agar supplemented with 0.6% yeast extract and 1% sodium pyruvate. D-values at 56°C ranged from 8.27 min for *S. enteritidis* phage type 13a (poultry outbreak strain) to 3.6 min for *S. derby* 8453 (pork isolate); the D-values at 62°C were 0.39 and 0.13 min, respectively. The D-values of a mixture of *S. kentucky* (chicken isolate), *S. muenster* (turkey isolate), *S. enteritidis* phage type 13a, and *S. derby* (pork isolate) were 10.4, 3.16, 0.86, and 0.41 min at 56, 58, 60, and 62°C, respectively. The z-values of all strains were very similar, ranging from 4.17 to 4.93°C. No correlation between the heat resistance and the origin of the *Salmonella* spp. could be established due to significant variation in the heat resistance among strains. Understanding these variations in heat resistance should help to ensure and to design adequate thermal regimes to eliminate *Salmonella* in thermally processed foods.

(P21) EFFECT OF REFINED LIQUID SMOKE ON ATTACHMENT OF *SALMONELLA* SP. ON PORK SKIN

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Refined liquid smoke solution was tested to determine its effectiveness in reducing *Salmonella typhimurium* attachment to pork skin. Preliminary studies were conducted to determine the effect of different concentrations of refined liquid smoke on survival of *Salmonella typhimurium* in Butterfield's phosphate diluent (BPD). One ml of 5.5×10^8 CFU/ml of *Salmonella typhimurium* cells was placed in 9 ml volumes of 0.5, 1, 5 and 10% concentrations of refined liquid smoke solutions. Surviving populations were enumerated on selective brilliant green sulfa (BGS) agar with 200 ppm of nalidixic acid (Nal). Triplicates samples of 25 cm^2 pork skins were inoculated with 1 ml of 10^6 or 10^4 CFU/ml of nalidixic acid resistant, *Salmonella typhimurium* with 10 minute contact time. 1 ml of 100% liquid smoke was spread over the skin. After 10 minutes, the skin was stomached in 45 ml of BPD for 2 minutes and bacterial populations were recovered on BGS + 200 ppm Nal agar. The average means of recovered *Salmonella typhimurium* were 2.89×10^3 CFU/cm^2 and $< 3.59 \times 10^1$ CFU/cm^2 for the inoculation levels of 2.20×10^5 CFU/cm^2 and 2.20×10^3 CFU/cm^2, respectively. The % average reduction in the bacterial load was 98.67 and 98.4 for the inoculum of 10^6 and 10^4 CFU/ml, respectively. The most effective of the solutions was the 10% liquid smoke on 7 logs of *Salmonella typhimurium* cells with a 99.9% reduction. Preliminary results indicate that liquid smoke could be an effective rinse solution in controlling *Salmonella* spp. on pork skin.

(P22) REDUCTION OF *SALMONELLA* SPP. IN CUT CANTALOUPE

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Methods to reduce microbial populations during the processing of fresh-cut melons are limited and their efficacy not well studied. The upper surface of fresh aseptically prepared cantaloupe cylinders (1.7 cm diameter × 2.0 cm high) was inoculated (10^7 CFU) with a five-strain mixture of *Salmonella* spp. The inoculated cylinders were subjected to various 1-minute wash treatments (25 to 30 ml-volume) or exposed to pulsed ultraviolet (UV) light (248 nm). After treatment, cylinders were macerated with 0.1% peptone water using a stomacher blender before plating onto Bismuth Sulfite Agar (BSA) and Tryptic Soy Agar (TSA). None of the wash treatments resulted in a greater than 1 log cycle decrease in the population of *Salmonella*. The order of efficacy was 200 ppm chlorine>60°C water>unchlorinated agitated water>unchlorinated water. Despite the small reduction in surface population, the presence of chlorine (50 ppm lowest level tested) was sufficient to reduce the population of *Salmonella* in the wash water to below detectable limits. In contrast, 10^5 CFU/ml *Salmonella* could be recovered from the unchlorinated wash water. Significant reduction in *Salmonella* (7 log cycles) at lower energy doses (3.3 mJ/cm^2) could be achieved when the organism was inoculated on the surface of TSA plates. However, a maximum 2.5-log reduction was achieved on cut cantaloupe surfaces even at high energy doses (500 mJ/cm^2). Processors should work to minimize contamination throughout production and processing as it is difficult to reduce populations of *Salmonella* spp. once present on cut cantaloupe surfaces.

(P23) ENHANCED THERMAL DESTRUCTION OF *S. ENTERITIDIS* IN LIQUID EGG PRODUCTS USING LYSOZYME LACTOFERRICIN-SS AND EDTA

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The consumption of liquid egg (LE) products has increased greatly over the past 10 years. LE products offer an alternative to whole shell eggs

and are more convenient to use. Generally about 0.01% percent of eggs are contaminated with *Salmonella*, which is then passed on to LE products during cracking. *Salmonella enteritidis*, which is more heat resistant than other *Salmonella* strains, is the most common pathogen associated with eggs. Because LE products are heat sensitive, the current pasteurization temperatures of 55.6 to 66.3°C are limited. However, such temperatures may allow survival of *S. enteritidis* in LE products. Hence, in this study, antimicrobial agents like lysozyme, lactoferricin-ß, and EDTA were applied prior to heat to enhance the destruction of *S. enteritidis* in LE products. Three treatment combinations of control, lysozyme and lactoferricin-ß, and lysozyme, lactoferricin-ß and EDTA were tested in LE products at temperatures of 55°C and 58°C. At 55°C, a 2 and 3 log reduction in population was detected in liquid whole eggs (LWE) and liquid egg white (LEW) respectively, within 3 minutes when all three chemicals were added. However, the same treatments at 58°C resulted in a complete destruction of *S. enteritidis* in LEW compared to a 3 log reduction in the control (heat only). Thus, an intervention with antimicrobial agents appears to enhance the efficacy of heat pasteurization of LE products.

(P24) EFFECT OF INOCULUM CELL PHASE, HEAT SHOCK, AND OSMOLYTES ON THE LAG PHASE DURATION OF *L. MONOCYTOGENES* SCOTT A AT 6°C

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Heat shock (HS), inoculum cell phase, and naturally occurring osmolyte effects on the lag phase duration (LPD) of *L. monocytogenes* following a temperature downshift is not well characterized. Stationary or log cells grown at 37°C, were subjected to HS (48°C, 30 min) and osmolytes in Pine's defined medium. After a cold shock at 6°C, LPD's were calculated. Protein synthesis during LPD was measured using ^{35}S-labeled methionine and cysteine. LPD of untreated log phase cells was 70 h, while LPD of 1 mM betaine, 10 mM trehalose, and 10 mM L-carnitine were 45, 45, and 55 h, respectively. HS log cells prior to temperature downshift increased LPD by 25, 48, 51, and 34% for controls, betaine, trehalose, and L-carnitine, respectively. LPD of temperature down-shifted stationary cells were longer than log phase cells being 89 h for untreated cells, 74 h for glycine betaine, 75 h for trehalose, and 99 h for L-carnitine. After heat shocking LPD's were 130, 90, 155, and 110 h for the same samples, respectively. Protein synthesis decreased ca three-fold, six hours post downshift, and continued to decrease for 31 h in non-HS, log phase control and glycine betaine samples. Synthesis then increased 18-fold in betaine treated cells prior to the end of LPD and six-fold in untreated cells. Thus, inoculum cell phase, HS, and osmolytes can affect the LPD of *L. monocytogenes* cells at cold temperatures. This has implications for predictive microbiology and for the development of food preservation systems.

(P25) CONTROL OF *L. MONOCYTOGENES* ON GROUND TURKEY BY IRRADIATION AND MODIFIED ATMOSPHERE PACKAGING

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When sterile ground turkey meat was inoculated with *L. monocytogenes*, packaged under mixtures of nitrogen and carbon dioxide, and treated with gamma radiation doses of 0 to 3.0 kGy, there was less survival in the presence of 100% carbon dioxide than in 100% nitrogen. Radiation resistance was not significantly different in air or in modified atmosphere packaging (MAP) mixtures containing 20, 40, 60, and 80% CO_2. Multiplication of *L. monocytogenes* at 7°C was delayed by a MAP mixture consisting of 50% CO_2 and 50% N_2 compared to aerobic packaging and irradiated cells decreased in number. The effects of MAP mixtures containing 25, 50, and 75% CO_2 and nitrogen were compared to aerobic and vacuum-packed turkey inoculated with approximately 5×10^3 colony-forming units per gram. Samples were irradiated to doses of 0, 0.5, 1.0, 1.5, 2.0, and 2.5 kGy and stored at 7°C for up to 28 days. Irradiation treatments were significantly more lethal in the presence of air than in either vacuum or MAP, and in those samples that received > 1.0 kGy there was a concentration dependent inhibition by CO_2 of multiplication of *L. monocytogenes*.

(P26) FAT CONTENT, STORAGE TEMPERATURE AND BACKGROUND MICROFLORA INFLUENCE THE GROWTH OF *L. MONOCYTOGENES* IN VACUUM PACKAGED GROUND BEEF

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The ability of three strains of *Listeria monocytogenes* (HPB 65, HPB 642, and list 4) to grow in normal (5.6 to 5.7) pH vacuum-packaged ground beef was determined based on a two

factorial central composite experimental design in which fat content of the samples ranged from 11 to 39% and temperature ranged from 0 to 10°C. Ground beef samples (inoculated at 10^3 CFU of *Listeria*/g) were analyzed for *Listeria* and total counts, and pH. The experiment was repeated with meat from two sources, a commercial meat plant and a research abattoir. The change in Log CFU of *Listeria*/g after 10 days of storage was used as the experimental response. Results for commercial meat samples showed that the inter-action between fat content and temperature of storage was not significant, but temperature of storage and fat content were significant factors influencing *L. monocytogenes* growth. *L. monocytogenes* did not grow at 0 or 1.5°C, but at ≥5°C there were differences in *L. monocytogenes* growth in meat samples prepared from the two sources. *L. monocytogenes* strains were differentiated by Random Amplification of Polymorphic DNA technique and results showed that all three strains of *L. monocytogenes* grew in the meat during storage. The naturally occurring microflora of the meat from the research abattoir was dominated by bacteriocin-producing *Leuconostoc* sp. that inhibited *L. monocytogenes* growth. The results of these studies demonstrate that *L. monocytogenes* strains can grow in refrigerated ground beef but that the background microflora can have a major effect on their growth.

(P27) ACID ADAPTATION OF *L. MONOCYTOGENES* OFFERS CROSS PROTECTION AGAINST AN ACTIVATED LACTOPEROXIDASE SYSTEM

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Foodborne pathogens are capable of tolerating extreme environmental stress conditions and exposure to one stress often offers cross protection against another subsequent stress. The effect of acid adaptation on subsequent exposure to an activated lactoperoxidase system in *Listeria monocytogenes* strains V7, V37 and CA was examined. Adapted (pH 5.5) and non-adapted cells were exposed to an activated lactoperoxidase system in TSB w/o dextrose at pH 4.5. Enumerations and pH measurement were performed at 0, 1, 2, 3, 6, 10, 24 and 48 h after challenge at pH 4.5 in all cases. The protein expression patterns of adapted and non-adapted controls and treated cells were analyzed 1 h after challenge using two-dimensional gel electrophoresis. Acid adapted cells exposed to lactoperoxidase system survived better (at least 5-fold) than non-adapted cells at 24 and 48 h, with variation in the degree of survival among the strains. Two-dimensional gel electrophoretic analysis revealed a change in the protein expression patterns of treated and control cells. There was a change in the expression of 43, 32 and 42 proteins in case of strains V7, V37 and CA, respectively. Acid adaptation offered some cross protection towards an activated lactoperoxidase system.

(P28) THE EFFECTS OF VARYING THERMAL PROCESSING SCHEDULES ON *L. MONOCYTOGENES* AND INDICATIVE MICROORGANISMS IN BLUE CRAB (*CALLINECTES SAPIDUS*) MEAT

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In this study, blue crabs (*Callinectes sapidus*) were cooked at 250°F, 15 psi for 5,7 and 8 minutes and evaluated for microbial reduction and time temperature profiles. Samples from each cooking time were analyzed for aerobic, anaerobic, coliform, fecal coliform, and *E. coli* counts using current methods of the American Public Health Association. Surviving species were isolated and typed for identification. Time and temperature profiles were used to form a mathematical model for the decimal reduction of *Listeria monocytogenes*. Crabs from the 8 minute cook, the current industry practice, were profiled through cooling, overnight refrigerated storage, and storage on the picking table during the next day. Coliforms, fecal coliforms and *E. coli* were eliminated during the 7 and 8 minute cooks, while found at low levels after a 5 minute cook. Reductions in surviving aerobes, anaerobes and *L. monocytogenes* were found with each cooking time. A minimum of 5 cooking minutes provided a sufficient reduction of *L. monocytogenes* and other microorganisms to meet regulatory action level criteria.

(P29) SENSITIVITY OF STRAINS OF *L. MONOCYTOGENES* TO TEMPERATURE AND LYSOZYME IN LIQUID EGG PRODUCTS

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The presence of *Listeria monocytogenes* has been reported in various foods including poultry and egg products. This pathogen is heat resistant and can survive the liquid egg (LE) pasteurization temperature. Furthermore, *Listeria* can survive at refrigerator temperature, which causes a major concern for consumers and food producers. In

this study, growth/survival of six different strains of *L. monocytogenes* in LE products was compared at different temperatures. *L. monocytogenes* ATCC 1911, Scott A, Scott A 2045, 2284, V7, and ST.L were inoculated at levels of 10^4 to 10^5 CFUs/ml in liquid egg white (LEW) and liquid whole egg (LWE). At 20°C in LEW, *L. monocytogenes* strains, Scott A 2284, V7, and St. L were found to survive, whereas strains ATCC 19111, Scott A, and Scott A 2045 failed to survive. In LWE, all six strains survived with no change in population through 72 hours. At refrigerator temperature (4°C), all strains survived for 72 hours in LWE and LEW except ATCC 19111, which did not survive in LEW. As lysozyme is the natural antibacterial agent found in egg white, all six *L. monocytogenes* strains were tested for sensitivity. The results showed that *L. monocytogenes* Scott A 2045 was the most sensitive to lysozyme while *L. monocytogenes* 2284 was the least sensitive. The addition of natural antimicrobial agents may help enhance the safety margin of low temperature stored LE products.

(P30) EVALUTION OF ISSC INTERIM CONTROL PLAN FOR LIMITING *VIBRIO VULNIFICUS* GROWTH IN OYSTERS

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Vibrio vulnificus can cause severe illness in immunocompromised persons who consume raw oysters. Maintaining low numbers of *V. vulnificus* in oyster shellstock may be an effective means to reduce risk of disease. The recently implemented Interstate Shellfish Sanitation Conference (ISSC) Interim Control Plan (ICP) reduces the time between oyster harvest and refrigeration, and was proposed to prevent multiplication of *V. vulnificus*. This report compares the effect of the ICP protocol to the previous harvest method, on *V. vulnificus* concentration in oyster meats. The concentration of *V. vulnificus* in oysters, and storage temperature, were measured at regular time intervals. During summer months (ICP level IV), ICP-maintained oysters had 4.9-fold less *V. vulnificus* than those maintained under the traditional system. The greatest effect of the ICP protocol was found in winter months (ICP level I) where ICP-maintained oysters had 82.5-fold fewer *V. vulnificus*/g oyster meat. The least effect was observed in May and October (ICP level III) where only a 1.3-fold difference was observed. Furthermore, *V. vulnificus* levels were stable in ICP-maintained oysters stored at a retail market for an additional 13 days. We conclude that the ICP plan provides better safeguards against *V. vulnificus* disease than the previous oyster harvest handling system.

(P31) INHIBITION OF *C. BOTULINUM* BY PHOSPHATE-BASED SALTS IN MEDIA AND PROCESS CHEESE SPREAD

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Phosphate-based salts were evaluated for their ability to inhibit *Clostridium botulinum* in media and in a process cheese spread. Disodium phosphate (DSP), Bekaplus® FS, Joha® K, Joha HBS, T_{new}, S9, $C_{special}$, and C_{new} were added (0.1, 0.5, 1, and 2%; wt/vol) to Trypticase-peptone-glucose-yeast extract (TPGY) liquid medium and the pH adjusted to 5.7 or 6.5. Media was inoculated with 10^3 spores/ml nonproteolytic or proteolytic *C. botulinum* spores and incubated at 12.8°C for 20 days or 30°C for 4 days, respectively. The presence of toxin was determined by mouse bioassay. For tubes incubated at 30°C, botulinal toxin production was delayed in media supplemented with 2.0% BekaplusFS, HBS, and T_{new} compared with the TPGY control and with media supplemented with the other salts tested. At 12.8°C, 1 and 2% BekaplusFS, K, HBS, S9, $C_{special}$, and C_{new} consistently delayed toxin production by nonproteolytic *C. botulinum* compared with DSP and the TPGY controls. Subsequently, the antibotulinal activity of several combinations of 2% HBS+S9 was compared with 2% DSP in an inoculated process cheese spread (56% moisture, pH 5.75) stored at 30°C for 7 days. Results revealed that 2% HBS+S9 exhibited equivalent or slightly greater antibotulinal activity than DSP. These data suggest that HBS+S9 may be considered as a replacement for DSP in process cheese products.

(P32) PREVALENCE OF *BACILLUS* DIARRHEAL ENTEROTOXIN-PRODUCING ORGANISMS IN DAIRY PRODUCTS

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B. cereus is a common contaminant of processed foods due to the ability of the spores to survive heat-treatment. A number of strains has the ability to produce a diarrheal enterotoxin that can result in illness 10 to 16 hours after ingestion. However, the proportion of *B. cereus* isolates from dairy products that have the ability to produce this toxin had not previously been investigated.

In this study, 101 dairy products were tested to determine the level of *B. cereus* contamination (mpn) according to the Australian Standard Method. Each food was then enriched overnight in Brain Heart Infusion broth with glucose and examined for *B. cereus* contamination as well as for diarrhoeal enterotoxin production using the Tecra BDE VIA. The foods tested included cheese, milk powder, pasteurized milk and cream, butter, cultured products, custard, dairy desserts, ice cream and raw milk.

The results showed that of the 101 samples tested, 50 contained strains of *B. cereus* capable of producing diarrheal enterotoxin. An additional two organisms identified as spore-forming rods were also found to be enterotoxigenic. There was no observed correlation between the mpn and the level of enterotoxin that was detected following enrichment.

Analysis of some of these enterotoxigenic isolates by SDS-PAGE and Western blot showed that the BDE VIA detects a protein of molecular weight approximately 45kDa and that the level of production varies depending on the isolate.

The results of this study show that even with low mpn values for *B. cereus*, there is a high probability that these organisms can produce diarrheal enterotoxin if allowed to multiply. This could present a significant health risk if time-temperature abuse of the food occurred following manufacture and release. The use of the BDE VIA to screen for enterotoxigenic Bacilli following a simple overnight enrichment is therefore an extremely useful tool for the food microbiologist when assessing product quality.

(P33) DEVELOPMENT OF HYBRIDOMA CELL LINE FOR THE PRODUCTION OF MONOCLONAL ANTIBODY TO RESIDUAL HERBICIDE ATRAZINE

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To produce atrazine specific monoclonal antibodies(mAb), hybridoma cell lines were developed by the fusion of mouse myeloma cells(P3X63Ag8.V653) and spleen cells form mice immunized with atrazine coupled to keyhole limpet hemocyanin (KLH). After screening with an indirect competitive enzyme-linked immunosorbent assay (ELISA), a monoclonal antibody with a high binding affinity for atrazine was selected and used to develop a sensitive ELISA for detecting and quantifying atrazine in a microtiter plate. The standard curve based on a homologous system using the above atrazine hapten conjugated to bovine serum albumin displayed a detection range from 0 to 5ng/ml for atrazine. The mAb cross-reacted predominantly with atrazine and to a lower extent with various related fungicides. We tried to make experimental immunoaffinity column(IC) coupled with mAb and affi-gel for furification of atrazine in samples, and also to develop the IC-ELISA system. The detection system appears to be a versatile device for the environmental monitoring of atrazine.

(P34) PARTIAL CHARACTERIZATION OF AFLATOXIN B_1 REMOVAL BY CRUDE EXTRACT FROM *FLAVOBACTERIUM AURANTIACUM*

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F. aurantiacum has been characterized as having the ability to degrade Aflatoxin B_1 (AB_1). However, research is needed to characterize the protein(s) thought to be responsible for the degradation of AB_1. The crude extract of *F. aurantiacum* was tested for the ability to degrade AB_1 at selected pH values and after treatment with Proteinase K and DNase I. A slightly alkaline pH (7.4 to 8.0) demonstrated the largest decrease (50%) in AB_1 levels. Proteinase K decreased the amount of AB_1 degraded by the crude extract. Treatment with DNase I did not affect the removal of AB_1 from solution. When ammonium sulfate precipitation (ASP) was tested as an initial purification step, 55% removal of AB_1 was observed in the 60% saturation fraction with only slight removal of AB_1 occurring in the 20% and 40% saturation fractions. Fractionation of the crude extract by ultra-filtration (UF) demonstrated that a protein fraction between 20,000 to 50,000 MW was responsible for removing up to 60% AB_1 from solution. Fractionation of the crude extract by gel-filtration (GF) yielded two broad peaks, each responsible for the removal of approximately 25% AB_1 from solution. Fractionation of the crude extract by anion exchange chromatography (AEC) yielded two fractions each capable of removing approximately 45% AB_1 from solution. This work has shown that removal of AB_1 from solution is due to protein(s) located in the cytosol of *F. aurantiacum* and that these proteins

may be partially fractionated by ASP, UF, GF and AEC.

(P35) SCREENING OF T-2 TOXIN PRODUCING FUNGI FROM AGRICULTURAL COMMODITIES IN KOREA BY ELISA METHOD

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To screen T-2 toxin-producing fungi from agricultural commodities in Korea, specific and high affinity monoclonal antibodies against T-2 toxin were produced. By using these antibodies, an ELISA method was established. *Fusarim* species were also isolated from agricultural commodities in Kyoungnam province, Korea, and T-2 toxin producing fungi were screened. T-2 hemiglutarate (T-2-HG) was conjugated with BSA. One mole of BSA was reacted with approximately 14 moles of T-2-HG conjugate. Three hybridoma cell lines secreting monoclonal antibodies against T-2 toxin were obtained by fusion and cloning. The subclasses of monoclonal antibodies produced by 3 hybridoma cells were identified as IgG1 and their light chains were identified as *k*-chain.

Among them, the monoclonal antibody produced by No. T-9 hybridoma cell had the best titer and showed 23% cross-reactivity with HT-2 toxin and 15% with T-2 tetraol. However, it did not react with deoxynivalenol, diacetoxyscirpenol, nivalenol or zearalenone. The detection limit of T-2 toxin by ELISA method using the monoclonal antibody was 0.1 ppb. From 185 samples of rice, fruit, soil and corn in Kyoungnam province, Korea, 127 strains of *Fusarium* species were isolated and only 4 isolates were confirmed as T-2 producing strains by ELISA. The FS-10-2 isolate produced 50.3 μg/ml of T-2 toxin.

In conclusion, the ELISA method developed in this study was suitable for a rapid and simple analysis of T-2 toxin in agricultural commodities.

(P36) MONTE CARLO SIMULATION OF MILK SPOILAGE AS INFLUENCED BY TEMPERATURE AND INITIAL POPULATION

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The objective of this study was to develop a computer simulation of milk shelf life to be used in troubleshooting a recent increase in milk spoilage complaints. Cartons of milk were collected from local supermarkets and analyzed for total microbial population at the beginning and end of their 14-day shelf life. The temperatures of a representative number of milk cartons from local supermarkets were also determined. Several different simulation scenarios were evaluated. In the first round of simulations, the effect of dropping the average in-store storage temperature from 44°F to 42 and 40°F was evaluated. As temperature was reduced, the percentage of simulated samples which showed less than the target 14-day shelf life decreased significantly (52, 26 and 11%, respectively). The cumulative effect of reducing storage temperature to 40°F and reducing the variability of the initial population was simulated. Making these two changes produced a dramatic change in product shelf life, with 1% of the simulated samples having a shelf life equal to 14 days, and no samples having a shelf life less than 14 days. This simulation represents a useful starting point for further actions to reduce the number of consumer complaints due to spoiled milk.

(P37) PREDICTIVE MODEL TO DETERMINE THE EFFECTS OF MILKFAT, PH, AND TEMPERATURE ON THE THERMAL INACTIVATION OF *L. MONOCYTOGENES*

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Listeria monocytogenes is important to the food industry because of its heat resistance, zero tolerance in ready-to-eat foods, and growth at refrigeration temperatures. A study was done to model the effects of milkfat, pH, and processing temperature on heat resistance of *L. monocytogenes* in a formulated and homogenized milk system. D-values were determined at all combinations of milkfat (0%, 2.5%, 5%), pH (5.0, 6.0, 7.0), and temperature (55°C, 60°C, 65°C). D-values (min) ranged from 25 to 145, 2.2 to 5.5, and 0.22 to 1.05 at 55°C, 60°C, and 65°C, respectively. Data was fit to a modified Gompertz equation where parameter estimates characterized three regions of a survival curve, the shoulder, maximum slope and tail. A by-cell statistical analysis was done at each treatment condition to determine effects on parameter estimates. The shoulder increased with increasing pH and decreasing temperature. The maximum death rate was at 65°C and pH of 5.0 regardless of temperature. Generally, the tail increased with increasing pH and decreasing temperature. A full model was generated to determine single and interactive effects ($P<0.05$) of milkfat, pH and

temperature on the logarithmic surviving fraction (LSF) of *L. monocytogenes*. This study provides a better understanding of how extrinsic food properties affect microbial inactivation kinetics.

(P38) MICROBIOLOGICAL QUALITY AND SAFETY OF READY-TO-EAT STREET FOODS IN JOHANNESBURG CITY

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The microbiological quality and safety of 49 ready-to-eat street food samples from 6 typical vendors in Johannesburg City were determined. Meat and salad samples were collected from each vendor over 3 independent surveys and food temperatures measured. Standard methods were used to determine aerobic plate counts (APC), *Enterobacteriaceae* counts (EC), spore counts (SC) as well as the presence of common foodborne bacterial pathogens. Coliform counts (PC) were also determined using Petrifilm™ *E. coli* plates. Temperatures of the cooked foods ranged from 36°C to 94°C and those of uncooked salads from 25°C to 30°C. Mean APC's of 3.1 and 3.2 log CFU/g, mean EC's of 2.0 and 1.8 log CFU/g, mean PC's of 1.5 and 1.7 log CFU/g and mean SC's of 1.6 and 1.7 log CFU/g were determined for meat and salad samples, respectively. There were no significant differences ($P>0.05$) in any of the above count types between the 3 surveys. Out of the 49 food samples, *Bacillus cereus* was found in 22% (7 meat samples and 4 salad samples), *Clostridium perfringens* in 18% (9 meat samples), *Salmonella* in 2% (1 meat sample) and *E. coli* (an O157- negative, H- positive strain) in 2% (1 meat sample) of the samples. Based on these results and published data, the safety and quality of the street foods in Johannesburg, South Africa were judged to be acceptable.

(P39) EFFICACY OF LACTATE-BASED COMPOUNDS AS BREAD PRESERVATIVES

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Rope and mold spoilage result in substantial economic losses to bread manufacturers, often necessitating the use of chemical preservatives.

Nine test bakes using a standard brown bread recipe were carried out to determine the efficacy of lactic acid, calcium lactate and a commercial lactate-containing cocktail, singly and in combination with calcium propionate. Baked loaves were stored at 30°C and analyzed up to fourteen days post-baking by plate counting, viable staining and mold-free shelf-life determination. Efficacies of treatments were ranked statistically. Lactic acid (0.25%), calcium lactate (0.75%) and the commercial lactate-containing cocktail (1.0%) did not achieve significant ($P>0.05$) increases in rope or mold-free shelf-life.

Combinations at half the above concentration of each compound with 0.1% calcium propionate, however, showed significant ($P<0.05$) increases in rope-free shelf-life. Calcium propionate at 0.2% achieved the longest rope and mold-free shelf-life. The lowest reduction in yeast activity was exhibited by the commercial lactate-containing cocktail. Predominant bacterial colonies isolated from plates of the highest dilution showing growth were identified as *Bacillus* with high proportions of *Bacillus subtilis*.

(P40) EFFECT OF CHEMICAL SANITIZERS ON BACTERIAL CELL MORPHOLOGY

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The effect of three chemical sanitizers (iodophor, peracetic acid/hydrogen peroxide, chlorhexidine gluconate) on the morphology of *Pseudomonas fluorescens* and *Bacillus subtilis* cells was studied by scanning electron microscopy (SEM). In a parallel study, sanitizer efficacies based on plate counts were determined. Compared to untreated controls planktonic and attached cells of both bacteria exhibited surface roughness, indentations and shape and size distortions after treatment with all three sanitizers. None of these effects were confined to one specific sanitizer. The effects were, however, more pronounced for *Pseudomonas fluorescens* cells, although no clear-cut trend of increasing morphological change with increasing exposure time could be determined. Similarly, morphological changes were not always consistent with percentage kills determined in the parallel study. This highlighted the limited scope of SEM in quantitative studies, but confirmed its usefulness as a qualitative tool to asses cell morphology changes in studies of this nature.

(P41) BACTERIOCIDAL ACTIVITY OF SANITIZERS AGAINST *ENTEROCOCCUS FAECIUM* ATTACHED TO STAINLESS STEEL AS DETERMINED BY PLATE COUNT AND IMPEDANCE METHODS

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Enterococcus faecium attached (sessile) to stainless steel chips (100 mm^2) were treated with the following sanitizers: sodium hypochlorite (SH),

peracetic acid (PA), peracetic acid plus acid organic (PAS), quaternary ammonium (QA), organic acid (OA) and anionic acid (AA). The effectiveness of sanitizer solutions on planktonic cells (not attached) was evaluated by the AOAC suspension test. The number of attached cells was determined by impedance measurement and plate count after vortexing. The decimal reduction (DR) in numbers of *E. faecium* population was determined for the three methods and analyzed by analysis of variance ($P<0.05$) using Statview software. The adhered cells were more resistant ($P<0.05$) than non-adherent cells. The DR averages for all of the sanitizers for 30 s of exposure were 6.4, 2.2 and 2.5 for AOAC suspension test, plate count after vortexing and impedance measurement, respectively. Plate count and impedance showed a difference ($P<0.05$) after 30 s of sanitizer exposure but not after 2 min. The impedance measurement method was the best method to measure adherent cells. Using impedance measurement a quadratic regression (Log CFU/chip = $0.3285T^2 - 0.96T + 9.35$, $r^2 = 0.92$, $P<0.05$, T = impedance detection time in h) was obtained from 82 samples. This method showed that the sanitizers PAS and PA were more effective against *E. faecium* than the other sanitizers. At 30 s, the impedance method recovered about 25 times more cells than the plate count method after vortexing. These data suggest that impedance measurement is the method of choice when evaluating number of bacterial cells adhered to a surface.

(P42) RELATIVE HYDROPHOBICITY AND CHARGE OF PLANKTONIC AND ADHERED CELLS OF *ENTEROCOCCUS FAECIUM*

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Relative Surface Hydrophobicity (RSH) and Charge (RSC) are physicochemical factors that have an important role in the process of cell adherence to a solid surface. RSH and RSC of adhered cells of *Enterococcus faecium* and planktonic cells of *E. faecium* were determined by Hydrophobic Interaction (HIA) and Electrostatic Interaction (ESIA) Assays. Samples of adhered (chips) and planktonic cells were taken after 0,2,4,6,8,10 and 12 h of incubation in 0.275% MRS broth at 30°C. The number of cells in the control, adsorbed to phenylsepharose (HIA) and to anionic exchange resin (ESIA) were determined by Plate Count Method (PCM) and Impedance Measurement (IM). In addition, before IM, some samples were maintained on ice (0°C). Sessile or planktonic cells of *E. faecium* exhibited relatively high RSH and RSC regardless of the experimental conditions used. With both, HIA and ESIA, results showed a variation in the percentage of sessile cells at the different sampling times. Differences were not detected ($P<0.05$) in the RSH and RSC for planktonic cells and adhered cells as measured by IM and PCM. Also, differences were not detected ($P<0.05$) when the samples were stored at 0°C before IM. Impedance measurement was shown to be a viable alternative to the PCM to determine the number of cells on phenylsepharose (RSH) and on anionic exchanger resin (RSC).

(P43) CHARACTERIZATION OF TWO BACTERIOCINS PRODUCED BY ATYPICAL *ENTEROCOCCUS* SPECIES

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Two apparently new bacteriocin-producing bacterial strains were isolated from corn silk and yucca root by the agar overlay method. The bacteria were identified as atypical *Enterococcus* sp. by fermentation patterns, fatty acid profiles and serological tests and the bacteriocins were assigned the names enteriocin YU4 (yucca isolate) and enteriocin CS1(corn silk isolate). Physiochemical properties and antimicrobial spectra of the bacteriocins were determined by the spot-on-lawn method. The bacteriocins were inactivated by protease-IV, proteinase-A and -K, trypsin and a-chymotrypsin but not by pepsin, papain, ribonuclease A, lysozyme, dextranase, lipase, catalase or α-amylase. Enteriocin CS1 and enteriocin YU4 had relatively broad antimicrobiol spectra, inhibiting *Bacillus, Clostridium, Listeria, Enterococcus, Leuconostoc, Pediococcus*, and *Staphylococcus* species. These bacteriocins remained active when heated at 90°C for 15 min or 120°C for 20 min, over a pH range of 2.0 to 9.0 and in various organic solvents. The molecular weight values were 3.9 kDa for enteriocin YU4 and 4.0 kDa for enteriocin CS1. These bacteriocins differ from other reported enteriocins in being resistant to pepsin and/or papain.

(P44) WITHDRAWN

(P45) COMPARISON OF DILUENTS AND MEDIA FOR RECOVERING *ZYGOSACCHAROMYCES ROUXII* IN HIGH-SUGAR FOODS

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Zygosaccharomyces rouxii causes spoilage of intermediate-moisture high-sugar foods. This study was conducted to determine the performance of three diluents (0.1% peptone, 40% glucose, and 30% glycerol) in combination with three enumeration media (tryptone yeast extract 10% glucose [TY10G] agar, malt extract yeast extract 50% glucose [MY50G] agar, and dichloran 18% glycerol [DG18] agar) in recovering three strains of *Z. rouxii*. Boysenberry syrup, corn syrup, two types of chocolate syrup, condensed milk, caramel topping, marzipan, raspberry spread, and poppy seed filling with a_w ranging from 0.76 to 0.84 were inoculated with *Z. rouxii* and incubated for 10 to 34 days at 30°C before analysis. Regardless of the enumeration medium, the use of 0.1% peptone diluent resulted in detection of significantly ($P \leq 0.05$) lower numbers of viable cells in all foods except condensed milk. Recovery of *Z. rouxii* using 40% glucose diluent was occasionally higher than recovery using 30% glycerol diluent for some foods, and TY10G and MY50G agars recovered higher numbers than DG18 agar. Overall, however, populations were not significantly different using 40% glucose diluent or 30% glycerol diluent in combination with a particular enumeration medium. Considering the number of *Z. rouxii* recovered, practical and economical aspects of preparation of media, and ease of counting colonies, the use of 40% glucose diluent in combination with TY10G agar is recommended for enumerating *Z. rouxii* in high-sugar foods at a_w 0.76 to 0.84.

(P46) EFFECT OF THE EXTRACT OF *ANGELICA ACUTILOBA* AND *GLYCYRRHIZA URALENSIS* ON THE GROWTH OF INTESTINAL MICROORGANISMS

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Chinese herbs have been used for medical treatments. However, there were limited studies on its antimicrobial effect. Intestinal microorganisms such as *Bifidobacterium longum*, *Bifidobacterium adolescentis, Clostridium butyricum*, *Escherichia coli* and *Eubacterium limosum* were cultured in the modified Peptone-Yeast-Fildes broth (PYF), supplemented with different carbon sources such as 0.5% (w/w) glucose, or the extracts of *Angelica acutiloba* or *Glycyrrhiza uralensis*. As a control, the same microorganisms were cultured in modified PYF broth treated with no carbon additives. Treatments were incubated anaerobically at 37°C for 24 h and the optical density at 650 nm was measured during the incubation. The addition of the extract of *Angelica acutiloba* or glucose to the modified PYF broth increased the growth of all microorganisms tested. However, the addition of the *Glycyrrhiza uralensis* extract significantly inhibited the growth of *Eubacterium limosum*, but it had no effect on the growth of the other microorganisms which were tested in this study.

(P47) EFFECT ON SELECTED PATHOGENS OF EXPOSURE TO NATURALLY OCCURRING VOLATILE COMPOUNDS

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In vitro antimicrobial activity of nine naturally occurring volatile compounds (aldehydes, ketones and alcohols) released by green plants and produced through the lipoxygenase enzymatic pathway were evaluated over time against *E. coli*, 2 *E. coli* O157 strains, *Listeria monocytogenes*, *Salmonella enteritidis*, *Salmonella typhimurium*, and *Shigella flexneri*. One ml of culture (10^5/ml) was placed on an absorbent cellulose pad in a petri dish next to a vial containing 10, 25, or 50 µl of test compound or distilled water. The petri dish was wrapped with parafilm, placed in a hood at room temperature and the populations of the controls and treatments determined after 2, 4, and 6 h. The absorbent pad was placed in 10 ml of trypticase soy broth, vortexed for 30 sec and enumerated using the spiral plate method. Antimicrobial activity was determined by the difference in populations between the controls and treatments. The antimicrobial activity of the compounds in decreasing order of activity was Z-3-hexenal, E-2-hexenal, hexanal, 2-hexanone, Z-6-nonenal, E-2-nonenal, 2,4-hexadienal, hexyl alcohol and benzaldehyde. The populations of all species were reduced more than 3 logs after exposure for 4 h to 10 µl Z-3-hexenal. None of the other compounds showed similar activity against all species, even at the highest volume and exposure time. *Listeria monocytogenes* was the most resistant species studied.

(P48) GROWTH OF *ALICYCLOBACILLUS ACIDOTERRESTRIS* IN ACID PRODUCTS

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Alicyclobacillus acidoterrestris are sporeforming microorganisms which can survive a pasteurization process, germinate, grow and cause spoilage in acid products. The objective of this study was to investigate growth and subsequent spoilage of a variety of fruit juices and salsa. Commercially sterile apple juice, white grape juice, apple cranberry juice, orange juice, pineapple juice, a 10% juice fruit punch and salsa were inoculated *with Alicyclobacillus acidoterrestris* spores (10^1 and 10^4 spores per ml) and incubated at 20°C and 35°C. At each sampling time, juice was analyzed for appearance, odor, pH, and microscopic appearance, and a plate count was made on K medium. *A. acidoterrestris* spores grew in apple juice and orange juice held at 35°C. Juice spoiled within one to two weeks of inoculation. Spoiled juice had a medicinal off odor and a light sediment but no significant change in pH was observed. *A. acidoterrestris* did not grow in the other juices nor in salsa under the test conditions.

(P49) EFFECT OF SUGAR AND CITRIC ACID ON THE QUALITY OF CANNED LYCHEE

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Lychee is a tropical or subtropical fruit; its main production area is Taiwan, Mainland China, and Thailand. Except for fresh, canned lychee is a popular product of this region. The quality of canned lychee becomes poor and pink discoloration occurs. The texture of the fruit is also the indicator for the quality of the product. The purpose of this research is to discuss the effect of sugars and citric acid on the pink discoloration and texture of canned lychee.

The material of canned lychee includes five different varieties as Hau-yeh, Kwai-wei, No-mitzu, Sakan and Yu-ho-bow. Analyzing the soluble solid and the constitutes of sugar in fruit flesh from the above 5 varieties, it has fructose, glucose and little amount of sucrose, these are necessary for canning lychee. Meanwhile, the amount of citric acid to be added is 0.2, 0.4, or 0.6 to lower the pH. A supply of canned lychee stored at room temperature for one year has been measured and the panel test for the texture has also been done.

The results show that pink discoloration occurred while over-adding citric acid, and the texture of lychee was poor as well. The filled solution with 0.2% citric acid and using the same amounts of fructose, glucose, and sucrose as the sugar constitutions of the lychee flesh, restrained the onset of pink discoloration, and also got the best grade for texture by the panel test.

The sugar in filled solution should be adjusted close to fruit flesh amount and do not add too much citric acid when canning lychee, to prevent the pink discoloration and maintain the best texture of the fruit flesh.

(P50) MODEL FOR THE IMPLEMENTATION OF HACCP IN THE FOOD INDUSTRY OF DEVELOPING COUNTRIES

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Many food processors from our country have still not been implementing HACCP, and they probably will not implement it unless it becomes mandatory. However, even if HACCP is not included in the food law, regulatory agencies need to encourage the use of HACCP in the food industry. This study presents a model of cooperation between public health officials, food industry technologists, and academic experts for facilitating the use of HACCP in small and medium size food processors. The process starts with workshops where preliminary HACCP models for high risk or high consumption foods are given. Models are subsequently tested in selected industries, reviewed, and adjusted. Then generic HACCP models are prepared for teaching similar processors.

This collaborative approach is being applied in two areas of Colombia, and is beginning to show benefits. First, experts from industry, regulatory and academia are developing a new relationship; second, food safety and HACCP concepts are now being widely discussed and incorporated; third, foods are becoming safer. The models being prepared are basic. Further improvement of these models will require more technical expertise to be provided by international food safety experts, HACCP authorities, and HACCP organizations.

(P51) DEVELOPING HACCP TRAINING MATERIALS FOR FOOD SERVICE EMPLOYEES

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Academicians and regulatory officials have collaborated in developing HACCP training materials that are suitable for the front-line food service workers. The research team modified 7 HACCP principles into a 2-step procedure for easy adaptation in everyday practice. A training packet including a 55-min video, a workbook with recipe exercises and several job aids was produced.

The efficacy of these materials was evaluated by pre- and post-HACCP behavioral observations for a sample of food service workers using an instrument monitoring the trainees' food handling procedures. The result showed a significant reduction ($P<.001$) in HACCP violations 8 weeks after the training session. Motivating and retaining the employees seemed to be major obstacles for the success of the sanitation management. In this study, out of 60 workers invited, 37 participated in the training and only 28 workers were able to take a part in post-training observation after 8 weeks.

This study has produced effective HACCP training materials that are suitable for food handlers. Continuous training seems to be a main factor in successful HACCP programs in food service industry.

(P52) INDICATIVE MICROBIAL QUALITY OF GULF COAST SHUCKED OYSTERS PRIOR TO IMPLEMENTATION OF FDA SEAFOOD HACCP REGULATION

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Shucked oysters processed along the Gulf Coast states were analyzed for the quality and quantity of microbes. The nature and number of indicative bacteria was evaluated before implementation and advent of FDA seafood HACCP regulations. Five samples of shucked oysters were procured from each of eight oyster processors on the same processing day. Shucked oysters were analyzed for indicative microbiological (aerobic, psychrotrophic, *E. coli*, coliform, fecal, and *Enterobacteriaceae*) counts using appropriate 3M™ Plates. There was no significant difference ($P>0.05$) among the oyster processors in the aerobic (mean 5.2 ± 0.4 log CFU/g), psychrotrophic (4.6 ± 0.4 log CFU/g), coliform (58 CFU/g), fecal (3.0 CFU/g) and *Enterobacteriaceae* (2.6 ± 0.3 log CFU/g) counts for the shucked oyster products. *Enterobactericeae*, coliform and fecal coliform counts were present in all shucked oyster meats examined. *E. coli* was not detected in any of the shucked oyster meats. The indicative microbiological load in Gulf Coast shucked oyster meats was acceptable before the implementation of U.S. FDA seafood HACCP regulations.

(P53) BIOGENIC AMINE ANAYLSIS AND CHARACTERIZATION OF HISTAMINOGENIC BACTERIA FROM FROZEN ALBACORE

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Albacore specimens or extra quality were analyzed for biogenic amine contents after one, three, six and nine months of frozen storage either at -18°C and -25°C. An HPLC method involving a linear elusion gradient was optimized for the identification and determination of putrescine, cadaverine, histamine, spermidine and spermine in Albacore tuna. Putrescine showed the highest increase, reaching concentrations of 59.04 ppm (815% of the initial level) and 68.26 ppm (942% of the initial level) in white muscle of Albacore after nine months of frozen storage at -18°C and -25°C, respectively. Cadaverine, histamine and spermidine concentrations were below 3 ppm, 5 ppm and 11 ppm, respectively, after nine months of frozen storage, while spermidine significantly decreased at both storage temperatures. Microbiological analysis confirmed the absence of *Enterobacteriaceae* in 75% of the Albacore specimens after nine months of frozen storage, while coliforms were always below 3 CFU/g. The survival rate of the psychrotrophic microorganims after nine months of frozen storage at -25°C was 4.6%, while 38.9% and 92.1% of the aerobic mesophiles survived nine months of storage at -18°C and -25°C, respectively.

(P54) THE EFFECT OF REFRIGERATED STORAGE ON THE SAFETY AND QUALITY OF RAW OYSTERS (*CRASSOSTREA VIRGINICA*)

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Temperature abuse during raw oyster harvesting and storage may allow for the multiplication of natural spoilage microflora as well as pathogens (*Vibrio* sp., *Vibrio vulnificus* in particular); posing a health threat to susceptible consumers. On the other hand, spoilage of product may occur before there is significant growth of the pathogens. The objective of the study is to provide a scientific basis for determining whether or not different refrigeration temperatures for raw oysters will result in a spoiled product before it becomes unsafe. Raw shell stock oysters (*Crassostrea virginica*) purchased from a commercial VA processor were subjected to different temperature abuse conditions (storage at 7°C, 13°C, and 21°C) in the laboratory and sampled during a ten-day storage period. SPC, total *Vibrio* count, and *V. vulnificus* counts were estimated at each abuse condition. *V. vulnificus* isolates were confirmed by a specific ELISA. Olfactory analysis was performed in order to determine consumer acceptability of the oysters at each abuse stage. SPC increased while olfactory acceptance decreased over time. The length of storage had a greater effect on the bacterial counts and olfactory acceptance of the oysters over time than did the storage temperature. The results of the study will serve as the basis for meeting FDA HACCP regulations for fish and fishery products.

(P55) ANTIMICROBIAL SPRAY OF POULTRY CARCASSES: A PILOT PLANT STUDY

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Antimicrobial sprays of poultry carcasses in a poultry processing pilot plant were evaluated using an inside/outside birdwasher to reduce *Salmonella typhimurium* and total aerobic bacteria. Four chemicals including trisodium phosphate (TSP, 10%), lactic acid (LAC, 2%), cetylpyridinium chloride (CPC, 0.5%), and sodium bisulfate (SBS, 5%) were selected as antimicrobial agents. Each of pre-chilled chicken carcasses was inoculated by spraying its outside and inside with *S. typhimurium* at 10^5 CFU/carcass. All the chemical treatments reduced *Salmonella* on the chicken carcasses by approximately 2 log CFU/carcass. Total aerobes on the chicken carcasses carcass sprayed with 0.5% CPC, 5% SBS, 2% LAC or 10% TSP were reduced by 2.16, 1.66, 1.03 and 0.74 log CFU/carcass, respectively. The most effective antimicrobial spray treatment for reducing both *Salmonella* and total aerobes on pre-chilled chicken carcasses was 0.5% CPC. The changes in appearance and tenderness of chicken carcasses were dependent on the antimicrobial agents.

(P56) MICROBIOLOGICAL CHANGES DURING SWINE CARCASS DRESSING

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To develop a HACCP plan for food processing operations, critical control points (CCPs) must be determined. The swine slaughter and dressing operation was investigated to establish its CCPs. The microbiology of swine carcasses was monitored by surface swabbing the belly of carcasses at various steps during the process and quantitating total aerobic plate count (APC, Plate Count Agar) and total Gram negative bacteria (MacConkey agar). Starting with a dehaired carcass, the sequential steps monitored included pre- and post-singeing, post final polishing, hand shaving, evisceration, and pre- and post-chilling. Results indicate that singeing and chilling substantially reduced the level of APCs and total Gram negative bacteria, while evisceration did not alter their levels. Bacteria increased during polishing. While singeing and chilling did not yield a bacteria-free carcass, these steps can control the level of bacteria and contribute to lower numbers on carcasses.

(P57) FATE OF *ARCOBACTER* SPP. TO ENVIRONMENTAL STRESSES OF TEMPERATURE, PH AND NACL LEVELS

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Organisms belonging to the *Campylobacter*-related genus *Arcobacter* are considered to be emerging pathogens and have been linked to cases of gastritis in humans. In this study, six human isolates were used to determine the survival characteristics of *Arcobacter* spp. to various environmental stresses. Four strains of *A. butzleri*, believed to be the primary human pathogen of the genus, and two strains of *A. cryaerophilus*, (one from each of the two hybridization groups 1A and 1B) were exposed to pH levels of 3.5-7.5. All strains were able to grow over a pH range of 5.5-7.5, with optimal growth of *A. butzleri* strains at pH 6.0, and optimal growth of *A. cryaerophilus* strains at pH 7.0-7.5. When exposed to varying NaCl concentrations,

A. butzleri strains showed optimal growth at 0.5% NaCl, with a growth range between 0.09%-3.5% NaCl, while *A. cryaerophilus* strains showed optimal growth at 0.5% to 1.0% NaCl, with a growth range between 0.09%-3.0% NaCl. Thermal tolerance studies on one strain of *A. butzleri* determined the TDT (Thermal Death Time) for a culture of 1.0×10^6/ml density to be 2.5, 5, and 15 min at 60, 55, and 50°C, respectively.

(P58) ANTIBIOTIC RESISTANCE OF BACTERIA ISOLATED FROM SLAUGHTERED AND RETAIL CHICKENS IN SOUTH AFRICA

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Animal feed is increasingly being supplemented with antibiotics to decrease the risk of epidemics in animal husbandry. The aim of this study was to determine the level of antibiotic resistant bacteria present on 24 retail and 24 abattoir chicken samples. Staphylococci, *Enterobacteriaceae*, *Salmonella* and isolates from total aerobic plate counts were tested for resistance to vancomycin, streptomycin, methicillin, tetracycline, gentamicin and oxacillin. A large proportion of the bacterial strains displayed multiple antibiotic resistance (MAR). Results from the antibiotic code profiles indicated that a large proportion of both retail (39.4%) and abattoir (69.6%) of Staphylococci isolates were resistant to both tetracycline and oxacillin. In both retail (33.9%) and abattoir (59.6%) of *Enterobacteriaceae* isolates and 72.8% of *Salmonella* isolates were resistant to tetracycline and streptomycin. This was also evident in the total aerobic bacteria from both retail (15.2%) and abattoir (29.3%) samples. This resistance spectra of abattoir samples is a result of farmers adding low doses of antibiotics to their livestock's feed. The lower incidence of MAR pathogenic bacteria in the retail samples could be a result of the resistant flora being replaced by more sensitive flora during processing. The use of subtherapeutic levels of antibiotics for prophylaxis and as growth promoters remains a concern since the laws of evolution dictate that microbes will eventually develop resistance to practically any antibiotic. This study indicated that a large proportion of the bacterial flora on fresh chicken is resistant to a variety of antibiotics, and that resultant food related infections will be more difficult to treat.

(P59) COMPARISON OF TWO MOLECULAR TECHNIQUES FOR EPIDEMIOLOGICAL TRACING OF *C. JEJUNI*

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Two molecular typing methods were compared for ability to distinguish and characterize *Campylobacter jejuni* strains from chickens and humans. Chromosomal DNA fragments from 10 chicken and 4 human isolates were probed with pMO2005 and no patterns were revealed. *Campylobacter jejuni* isolats were analyzed by polymerase chain reaction (PCR) amplified flagellin gene (*flaA*), digested by *Dde*I enzyme, and separated by electrophoresis (restriction fragement length polymorphism [RFLP]). This second techinque provided various simple patterns, easy-to-read and consistent *flaA* gene types among 78 chicken and 39 human *C. jejuni* isolates analyzed. The PCR-*flaA*-RFLP analysis provided sufficient discrimination for tracing *C. jejuni* isolates through ecosystems; hybridization with the DNA probe was less discriminatory.

(P60) PHENOTYPING AND GENOTYPING OF FOODBORNE *CAMPYLOBACTER* FROM RECURRENT DISEASE

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Seven cases of *Campylobacter* infection, each of them involving two isolates, were analyzed. Study of their biochemical profiles and susceptibility patterns allowed the identification of *C. jejuni* and *C. coli* isolates and the effective typing of *C. jejuni* strains into biotypes. Genotyping was carried out by comparing chromosomal DNA-restriction patterns obtained by cleavage with *Bgl*II and *Eco*RV and by Southern hybridization experiments. These studies revealed clonal homogeneity between both isolates in five of the seven cases studied, this indicating that in these cases *Campylobacter* infection was caused by a single strain. Infection with two different strains was characterized in only two of the seven cases studied, two different species belonging to *C. coli* and *C. jejuni* subsp. *jejuni* biotype 1 being identified. Genetic analysis proved to be the most reliable technique to achieve precise identification of

strains and to elucidate clonal heterogeneity among *Campylobacter* isolates obtained from a single patient.

(P61) DETECTION AND DISTINCTION OF *C. JEJUNI* AND *A. BUTZLERI* IN CONTAMINATED FOOD PRODUCTS BY MULTIPLEX PCR

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Arcobacter is at present hard to distinguish by biochemical and microbiological methods from the closely related *Campylobacter* family. We have developed a mutliplex PCR that specifically detects both *Campylobacter jejuni* and *Arcobacter butzleri* in the same reaction tube. We demonstrate its use in seeded food samples that are ready to consume or might be subject to soil contamination or suboptimal temperatures for extended periods of time. These two pathogens were detected and distinguished using this multiplex PCR in strawberries, apple, kiwi, grapes, watermelon, pineapples, cantaloupe, broccoli, carrots, cauliflower, cheese, yogurt and a variety of deli-sliced meats. A PCR product of 159 bp was apparent for *C. jejuni*, while a 1223 bp product was seen on an agarose gel for *A. butzleri*. When both organisms were present, both bands could be detected on the gel. Each food and each organism has been repeated in triplicate and appropriate controls have been used. There is no cross reaction between specific primers and the other organism. All organisms detected by PCR were confirmed by standard microbiological methods. There was 100% agreement between the PCR and the standard methods.

(P62) REDUCTION IN MICROBIOLOGICAL COUNTS OF BEEF VARIETY MEATS EXPOSED TO VARIOUS DECONTAMINATION TREATMENTS

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The objective of this study was to evaluate interventions for decontamination of beef variety meats. Ten treatments, including hot (50°C) water (HW), and solutions of acetic acid (AA), lactic acid (LA) and trisodium phosphate (TS), applied by immersion or spraying; chlorine (CL) immersion; and, exposure to steam (ST) were tested to measure their effectiveness in reducing levels of bacterial contamination on cheek meat (CM), large intestine (LI), lips (LP), liver (LV), oxtail (OX) and tongue (TG) tissues. Five treated samples and five controls were analyzed for aerobic plate counts (APC) on tryptic soy agar, and for total coliform counts (TCC) and *E. coli* counts (ECC) on Petrifilm™. Reductions in log APC ($P<0.05$) ranged from 1.2 (TS/spraying) to 1.8 (AA/immersion) for LP, and from 1.4 (HW/immersion) to 2.7 (AA/immersion) for TG. When LI, LP, LV and OX were immersed in acetic acid, ECC were reduced by 1.4, 1.3, 1.8 and 1.2 logs, respectively. Immersing variety meats in a 2% acetic acid solution (50°C) was the treatment that reduced ($P<0.05$) bacterial counts on all products tested, while other treatments were not as widely effective. Results of this study documented that the decontamination treatments that have been efficacious for carcasses would also reduce bacterial counts on beef variety meats.

(P63) EFFECT OF ENVIRONMENTAL AND SUBSTRATE FACTORS ON SURVIVAL AND GROWTH OF *HELICOBACTER PYLORI*

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Helicobacter pylori is an etiologic agent of peptic ulcer disease and gastric cancer; to date epidemiologic studies have not fully elucidated reservoirs of the organism or vehicles of transmission. This study investigated the survival and growth characteristics of *H. pylori* in a nutrient-rich medium as influenced by environmental and substrate factors. The effect of temperature (4 to 42°C), NaCl concentration (0.5 to 7.5%), $NaNO_2$ concentration (0 to 400 µg/ml), water activity (a_w level of 0.6 to 0.995), pH (3.5 to 7.3) and urea (8 mM) on the survival and growth of *H. pylori* in a nutrient-rich laboratory culture medium was investigated. Under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2), the organism grew well in brain heart infusion broth supplemented with 7% horse serum and antibiotics in a temperature range of 30° to 37°C with agitation. The generation times for growth of *H. pylori* ranged from 2 to 4.6 hr. *H. pylori* (initial population of ca. 5×10^3 CFU/ml) survived for 14 days at 4°C, 2 days at 25°C, and less than 1 day at 40° and 42°C. The optimal NaCl concentration for growth of *H. pylori* was 0.5 to 1.0%; 2.0% NaCl inhibited growth. Up to 400 µg of $NaNO_2$ per ml did not prevent growth. The minimum a_w (adjusted with glycerol) and pH (acidified with HCl) for growth of *H. pylori* was 0.98 and 4.5, respectively. The addition of urea to broth

greatly enhanced the growth of *H. pylori* at both pH 4.5 and 5.5. Although *H. pylori* did not grow at pH 3.5, the presence of urea in broth enhanced its survival. Results indicate that *H. pylori* is unlikely to grow well, if at all, in most foods, but may survive for extended periods of time in low acid, high moisture environments under refrigerated storage.

(P64) RESAZURIN AS AN INDICATOR OF GROWTH IN A NEW MEDIUM FOR MEASURING THE AEROBIC PLATE COUNT IN FOOD

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SimPlate™ for TPC (IDEXX Laboratories, Inc.) is a test which detects and enumerates aerobic bacteria in food by detecting specific bacterial enzymes. Some of these enzymes occur naturally in certain types of foods (i.e. liver, nuts, and flour). Therefore, testing these foods has proven troublesome. In this study, we describe a new medium which uses resazurin, a redox dye, to detect foodborne bacteria. With this test, the prepared food sample is added to a SimPlate device followed by media which is then distributed into the 84 separate wells of the SimPlate device. Viable bacteria are detected in each well by the biochemical reduction of the blue resazurin to the pink resorufin or the clear dihydroresorufin indicators. Enumeration is achieved by counting the number of pink or clear wells in the SimPlate device and referring to a table to determine the MPN of bacteria present. Validation of the method included side by side comparisons against standard plate count agar (SPC). Over 100 food samples including raw vegetables, raw meats, flour, liver, and nuts have been tested with this new medium and compared to the traditional SPC method. Simple regression analysis of the preliminary data show excellent correlation to SPC ($r>0.90$) and similar sensitivity (slope=1). We conclude that this new medium is a suitable alternative to TPC for determining the aerobic plate count of food.

(P65) RAPID DETECTION (1 TO 4 H) OF TOTAL AND THERMOTOLERANT COLIFORMS ON CARCASSES

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An effective HACCP program is dependent upon access to rapid and easy-to-use sanitation screening tests, especially at early stages in the production process. A novel method has been developed to assess the presence of total and thermotolerant coliforms on surfaces in which the swab is immersed in a selective liquid medium. A fluorogenic substrate, 4-methylumbelliferyl-ß-D-galactoside, is used to detect the target organisms. A predetermined pass level and incubation time of 30 min to 4 hours are established. Readings at initiation and the conclusion of the test provide a net fluorescence value correlated with the surficial contamination level. Total coliforms are determined at 35 to 37°C and thermotolerant coliforms at 42 to 44.5°C. Incubation temperatures are contingent upon prevailing regulations. In the study, coliform contamination was modelled by spiking 16 beef carcass surfaces (100 cm^2) with ambient water known to contain fecal material. The inoculum was varied to achieve approximately 5 to 500 CFU/cm^2. The 10 cm^2 area was sampled by a cotton swab which was then immersed in the assay medium and incubated at 44.5°C. The inoculum target organism concentration was assessed using membrane filtration and the m-FC method. The lowest level tested, about 5 CFU/cm^2 was detected at 3 hours, the highest level, about 500 CFU/cm^2 in 45 min.

(P66) DEVELOPMENT OF AN IMPEDANCE SELECTIVE METHOD FOR THE ENUMERATION OF LACTIC ACID BACTERIA

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Although Lactic Acid Bacteria (LAB) have long been used in the fermentation processes of many foods, specific species are responsible for spoilage or flavor defect. In this study, a rapid enumeration method using impedance has been developed to determine the specific level of LAB present in meat products. A selective medium incorporating the antibiotic, polymyxin B, was used to detect 10 to 10E5 CFU/ml of LAB in less than 24 hours under facultative anaerobic conditions. The LAB of 162 samples of four different meats (ground beef, steak, sausage and sirloin) were monitored at 30°C over a four week period. A correlation of $r^2=0.90$ was obtained between impedance detection times and lactic plate count for these types of meat. Identification of bacteria isolated from the meat was done using API 50 CHL (*Lactobacillus*) and sequencing 16S ribosomal RNA. LAB such as *Lactobacillus casei*,

Lactobacillus sake, *Leuconostoc mesenteroides*, and *Lactococcus cremoris* were the primary bacteria isolated. This impedance selective medium offers a rapid method for the enumeration of LAB in meat products.

(P67) A RAPID METHOD FOR THE DETECTION OF GRAM NEGATIVE ORGANISMS IN PASTEURIZED DAIRY PRODUCTS

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Gram negative organisms are the major cause of post-pasteurization contamination in dairy products. A U.S. dairy has evaluated a rapid screen for Gram negative contamination in pasteurized milk products based on ATP-bioluminescence technology. The study compared the results from the selective 24-hour ATP-bioluminescence screen for Gram negative organisms with those from traditional growth-based counts.

The results from 112 naturally contaminated (23 positive and 89-negative) samples showed that the ATP-bioluminescence method detected both coliform and non-coliform Gram negative organisms after 24 hours. All gram-negative contamination was confirmed by plating to Crystal Violet Tetrazolium agar. Data were analyzed using the Mcnemar's Test and there was a significant difference between methods (x^2 result of 2.30). The ATP-bioluminescence method therefore represented a more complete screen for post-pasteurization contamination than the traditional growth-based count alone.

(P68) PRACTICAL APPLICATION OF ATP-BIOLUMINESCENCE FOR THE ESTIMATION OF MICROBIAL POPULATIONS IN PORK

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Many microorganisms are responsible for final pork quality. A practical rapid application of ATP-bioluminescence, developed to monitor meat microbial levels throughout the slaughter process, has been investigated.

The ATP-bioluminescence assay took from 5 to 45 minutes from sampling to result and was found to give a reliable estimation of total microbial populations.

In a field trial conducted by Swift & Company in Marshalltown, IA, 91 pork samples were tested. The correlation of repeat ATP-bioluminescence results (in Relative Light Units, RLU) was 0.97. The correlation of the RLU result with traditional agar plating results was 0.86.

The speed of the test (< 1 hour vs. 2 days) makes it useful as a practical rapid method for the rapid estimation of microbial populations in pork.

(P69) EVALUATION OF METHODS USED FOR ENUMERATION OF THERMOPHILIC AND MESOPHILIC *BACILLUS* SPORES IN MILK POWDER

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Milk powder is a major constituent in many processed foods and thus microbial quality and safety must be maintained by establishing a standard method for detecting endospore-forming bacteria. The American Public Health Association (APHA) has established standards for enumerating endospore-forming bacteria from dairy products. However, dairy testing facilities are using a variety of methods, because each milk powder buyer demands results from different tests. The objective of this study was to test the various endospore assays for their ability to detect and enumerate both thermophilic and mesophilic *Bacillus* spores in milk powder. The assays were performed on sterilized reconstituted milk powder to which a known number of spores were added. The spores, from six different *Bacillus* species, were isolated from vegetative cells and quantified by direct microscopic count. Spore assay methods varied in heat-activation temperature and time, diluents for plating, plating media, as well as the time and temperature of incubation. All assay methods gave viable spore counts at least one order of magnitude less than the direct count. Those methods using diluents with higher mineral content, showed a lower thermophilic spore count than methods that used diluents with a lower mineral content, however this was not true for the mesophilic methods. Of the thermophilic methods, the APHA method appeared to detect more of the spores present in the samples. There was no particular method that was most accurate for enumerating endospore-forming mesophiles.

(P70) RAPID DETECTION OF *S. AUREUS* USING A MEMBRANE BIOSENSOR

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A simple, sensitive and rapid method for *S. aureus* detection in food is important for both laboratory and field testing. A membrane chemiluminescent biosensor was developed for this purpose. This is a one-step liquid phase immunorecognition by incubating the *S. aureus* cells with peroxidase (POD) labeled monoclonal antibody, followed by isolation of the cells on a membrane, and measurement of the POD catalyzed chemiluminescent signal with a fiber optic probe. The lowest background chemiluminescent signal due to antibody-POD conjugate binding to the membrane was obtained with polyvinylidene fluoride(PVDF) and polysulfone. The PVDF was selected to develop the sensor. Optimum pH, antibody binding temperature and time with *S. aureus* cell, and the sensitivity of the assay were determined. The optimum pH for the chemiluminescent reaction was at 8.5. No significant difference was found between ambient temperature (22°C-25°C) and 37°. One hour incubation gave a higher signal than 10 or 20 min; but no significant difference was observed between 10 and 20 min. To achieve a rapid and simple test, 10 min incubation at ambient temperature was chosen as the optimal binding conditions. The sensitivity of the biosensor was approximately 10^4 CFU/ml of *S. aureus*, making it sensitive enough to detect the organism at concentrations lower than the level that could result in food poisoning. The total assay time was much shorter, 15 to 20 min, when compared with most ELISA assays of 2 to 4 h.

(P71) DETECTION OF *C. BOTULINUM* TYPE A TOXIN IN CULTURE MEDIA AND FOOD SYSTEMS BY AN IMPROVED COLONY IMMUNOBLOT PROCEDURE

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In search for an alternative method for enumeration of *Clostridium botulinum* and confirmation of botulinum toxin which is more time efficient and less expensive, an immunoblotting procedure involving the transfer of the toxin from an agar medium to a membrane to be probed by type-specific antitoxins was studied. To determine the degree of the method's sensitivity, growth media, transfer membrane supports, antisera origins, and antisera concentrations were varied. By using type-specific primary antisera and type-specific anti-goat biotinylated secondary antisera conjugated with alkaline phosphatase, type A toxin production in both inoculated cultures and inoculated food systems was detected following only 72 h of anaerobic incubation at 32 to 35°C and approx. 6 h of preparation. By using working dilutions (1/1000) of primary antiserum and decreasing concentrations (1/500 to 1/4000) of the secondary antiserum-enzyme conjugate, color development, indicative of toxin production, could be detected. While the growth media types used (i.e., TPGY vs. liver veal) displayed no noticable increases in sensitivity, the polyvinylidine fluoride (PVDF) transfer membranes enhanced the detection by decreasing background color, as compared to the nitrocellulose (NC) transfer membranes. With these increases in sensitivity, this procedure can confirm toxigenic samples with greater efficiency at less cost.

(P72) RAPID DETECTION OF CYTOLETHAL DISTENDING TOXIN GENES IN *CAMPYLOBACTER* ISOLATES BY POLYMERASE CHAIN REACTION

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Campylobacteriosis is recognized as the most common bacterial cause of human gastroenteritis in the U.S. *C. jejuni* and *C. coli* probably account for at least 90% of these infections. We have been studying a novel potential virulence factor of diarrheagenic *Campylobacter*, cytolethal distending toxin (CDT), which was recently reported to cause diarrhea in a suckling mouse model. Since it is not known how widespread the presence of *cdt* genes are in *C. jejuni* and *C. coli* isolates, polymerase chain reaction (PCR) tests were developed based on *cdt* sequences as a means of rapidly screening *Campylobacter* isolates for the presence of *cdt* genes.

Eight different PCR primer pairs were tested; four of them, collectively, successfully detected *cdt* genes in all but one of the 124 *C. jejuni* and *C. coli* isolates tested. 120 isolates were shown to possess *cdt* genes with primer set 1; primer sets 2 and 3 detected *cdt* genes in 1, and primer set 4 detected *cdt* genes in 3, of the 4 remaining isolates. Restric-

tion analyses of the PCR products amplified by primer sets 1, 2 and 3 showed that all *C. jejuni*, but none of the *C. coli*, PCR products were cut once with *EcoR*I. In summary, PCR tests were successfully used to detect *cdt* genes in all but one of the *Campylobacter* isolates tested. In addition to showing the successful use of these PCR tests for detection of *cdt* genes, these results clearly indicate that *cdt* genes are widely distributed in both human and animal isolates. Furthermore, restriction analysis results of PCR products showed that we can distinguish between *C. jejuni* and *C. coli* simply by *EcoR*I digestion of the *cdt* PCR products.

(P73) IMPROVED ENRICHMENT PROTOCOL FOR RAPID DETECTION OF LOW LEVELS OF *SALMONELLA* IN FOODS

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Foods harboring low and injured levels of *Salmonella* represent a food safety hazard. The conventional protocol for the multiplication and isolation of *Salmonella* colonies from contaminated food is a 5-step protocol that requires 5 to 6 days before a confirmation is obtained. Therefore new approaches in food safety are needed for a rapid repair, multiplication and reliable detection of such low infectious levels. This study evaluates an improved 2-step enrichment protocol for the rapid repair, growth and detection of *Salmonella* spp. found in foods. The first step involves a combined pre-enrichment and selective enrichment using lactose broth supplemented with sodium pyruvate and yeast extract for the repair of injured cells and brilliant green for the repression of the competing flora. The first step is performed for 7 h at 40°C. The second step involves the isolation of *Salmonella* colonies onto selective and differential medium (xylose lysine deoxycholate, XLD) for 24 h at 35°C and the parallel serological confirmation using a rapid qualitative method (1-2 Test). The 2-step protocol requires 31 h for the repair, selective growth, isolation and confirmation. The protocol was successfully used in detecting as low as 2.0×10^0 CFU/ml in a 250 final enrichment volume of healthy *S. enteritidis* cells. This initial inoculum increased 4 logs and was confirmed by the 1-2 Test. Low levels of freeze-injured *S. newport* and *S. enteritidis* were as well repaired, detected and confirmed from artificially and naturally contaminated ice cream samples, respectively.

(P74) AUTOMATED, ONE-DAY, TWO-STEP DETECTION OF *S. ENTERITIDIS* IN EGGS

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We previously described an accelerated method to detect salmonellae in foods. It consisted of a short pre-enrichment step followed by immunomagnetic separation (IMS) and automated detection based on positive biochemical characteristics during overnight incubation. This method was refined and simplified, and the IMS step eliminated. Described here is its application to detect *Salmonella* in liquid eggs. Samples were inoculated (<10 CFU/25g) with *Salmonella enteritidis* or non-*Salmonella* organisms (*Proteus*, *Enterobacter* or *Citrobacter*). Following pre-enrichment in universal pre-enrichment broth at 35°C for 6 h, samples were transferred to vials containing sterile selective liquid media and incubated at 42°C in the BioSys instrument (MicroSys, Ann Arbor, MI). *Salmonella* positive samples were identified by black coloration of the media within 18 h, resulting from formation of hydrogen sulfide and the reaction with iron ions. The color changes were indicated by sharp declines of light transmittance recorded by the instrument. Results agreed with data from the conventional procedure for *Salmonella* isolation. A total of 24 h was required to detect ≤10 CFU of the pathogen in 25 g of eggs, compared to 72 h in the conventional methods. The procedure was confirmed in pure cultures of 24 *Salmonella* spp. and 37 non-*Salmonella* *Enterobacteriaceae*.

(P75) EVALUATION OF A NOVEL 24-HOUR TIMED RELEASE ENRICHMENT SYSTEM FOR THE RAPID ISOLATION OF *SALMONELLA* FROM FOODS

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Based on a greater understanding of traditional enrichment methodology and utilizing an innovative timed-release capsule delivery system a new method for the rapid isolation of *Salmonella* has been developed. Performance of the S•P•R•I•N•T *Salmonella* Kit from Oxoid was tested in two independent studies carried out in the U.K. Over 400 naturally contaminated food samples including beef, pork, lamb, chicken, sausages, raw egg and animal feed, and 70 processed food samples spiked

with very low levels of heat and acid-stressed *Salmonella* were tested. The new method was found to be at least equivalent in performance to the ISO reference method for both raw and processed foods with results obtained 24 h sooner. In addition, growth of 50 different *Salmonella* serotypes at low inoculum levels was at least equivalent to the traditional method and 25 common competitor microorganisms were similarly inhibited at relatively high inoculum levels.

(P76) DEVELOPMENT OF AN IMMUNOASSAY FOR DETECTING *SALMONELLA* ON CHICKEN CARCASSES

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An enzyme-linked immunosorbent assay (ELISA) employing goat anti-*Salmonella* antibody as the capture reagent and a peroxidase-conjugated monoclonal antibody (M181) to a conserved epitope in the core region of LPS of all *Salmonella* except Group O serovars as the detector reagent was developed and evaluated. The limit of detection of *Salmonella* in pure culture in the ELISA was 10^5 to 10^6 CFU/ml. Cultures of 51 fresh chicken carcass rinses were used to evaluate the ELISA. When boiled Muller Kauffmann Broth cultures of pre-enriched chicken carcass rinses were tested in the ELISA, the assay had a specificity of 100% and sensitivity of 96.7% compared with the Health Canada, Health Protection Branch standard cultural method for detecting *Salmonella*. The immunoassay and the standard method each misidentified one of the 30 true positive samples. We conclude that the ELISA shows promise as a rapid test for *Salmonella* on chicken carcasses.

(P77) A POLYMERASE REACTION PROCEDURE FOR THE DETECTION OF *S. ENTERITIDIS*

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Salmonella enteritidis is one of the most important foodborne pathogens worldwide. Conventional methods for the detection of *Salmonella* in foodstuffs are generally cumbersome and time consuming. Only a few techniques have been developed for the detection of *Salmonella enteritidis* in poultry, such as immunoblotting and DNA probing. Polymerase chain reaction (PCR) is a highly sensitive and rapid technique for the detection of *Salmonella* in food products. Common to many *Salmonella* is the presence of a large plasmid, 55 to 90kb, which contains the virulence (spv) genes. In this study, PCR primers were designed to amplify a 432bp product of the spvR region located on plasmid pNL2001 found in *Salmonella enteritidis*, *S. typhi*, *S. dublin* and *S. typhimurium*. The amplified product contained three *DraI* restriction enzyme recognition sites resulting in, 222bp, 179bp and 31 bp DNA fragments, of which the last two were unique to *Salmonella enteritidis*. Only two fragments, 222bp and 210bp, were generated when digesting the amplified PCR product of the other serotypes. This method proved to be an excellent tool for the rapid and sensitive detection of *Salmonella enteritidis* from poultry samples.

(P78) ISOLATION AND SIMULTANEOUS PCR DETECTION OF *E. COLI* O157:H7 AND *SALMONELLA* SPP. FROM ENRICHMENT CULTURES OF FOODS AND OTHER SAMPLES

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A method was developed for isolation and simultaneous detection of *Escherichia coli* O157:H7 and *Salmonella* spp. in various types of samples. Apple cider, beef carcass wash water, ground beef, and bovine feces were inoculated with both *E. coli* O157:H7 and *S. typhimurium*. Following enrichment culturing for 20 to 24 h at 37°C, subcultures from the enrichments were made onto Rainbow agar O157™ and CT-SMAC agars and onto XLT4 and BG Sulfa agars for isolation of *E. coli* O157:H7 and *Salmonella* spp., respectively. The samples were also subjected to a DNA extraction technique and to immunomagnetic separation then tested by a multiplex polymerase chain reaction (PCR) assay designed to allow simultaneous detection of both *E. coli* O157:H7 and *Salmonella* spp. Four pairs of primers were employed for amplification of *E. coli* O157:H7 *eaeA*, *stx* and plasmid sequences and for amplification of a portion of the *Salmonella invA* gene. Four fragments of the expected sizes were amplified in a single reaction and visualized following agarose gel electrophoresis in all of the samples inoculated with ≤ CFU/g or ml. Results can

be obtained in approximately 30 h. The method is rapid and sensitive and can be employed for testing foods, animal feces, and other types of samples for the presence of both organisms simultaneously.

(P79) DETECTION OF *SALMONELLA* IN DAIRY SAMPLES USING BIND®

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Salmonella is one of the primary bacterial agents causing food poisoning in the U.S. and many large scale outbreaks have been attributed to the contamination of dairy products. Two notorious examples are the pasteurized milk case in 1985 in the Midwest U.S. where over 16,000 people were infected, and the national scale outbreak associated with ice cream in the early 1990s.

In order to bring *Salmonella* testing of dairy samples to the hands of those doing the processing, BIND® (Bacterial Ice Nucleation Detection), was developed a simplified *Salmonella* screening system which requires a single overnight pre-enrichment step. BIND employs a cocktail of genetically-modified bacteriophages which are specific for the genus *Salmonella*. The test detects one live *Salmonella* cell in a 25-gram sample. Samples containing *Salmonella* express the recombinant ice-nucleation protein when cooled to -9.3°C and are identifiable by a visually-read assay. Validation data includes direct detection of *S. cubana*, *S. tennessee*, *S. seftenberg*, and *S. lille* at concentrations below 500 CFU/ml, along with results from low-level spiked samples of milk, ice cream, cheese, and yogurt.

(P80) WITHDRAWN

(P81) AUTOMATED ONE-DAY SCREENING METHOD TO DETECT LOW LEVELS OF *L. MONOCYTOGENES* IN MILK

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Outbreaks of foodborne listeriosis have raised the need for rapid screening methods, particularly for on-line testing of ready-to-eat foods. An automated screening method was developed for he detection of *Listeria monocytogenes* in milk. It consists of a short pre-enrichment step (6 h), followed by overnight incubation in selective broth at 35°C using the BioSys instrument (MicroSys, Ann Arbor, MI). Esculin hydrolysis by *L. monocytogenes* in the presence of ferric ions results in formation of black coloration. This causes a sharp drop in light transmittance, whereas no change is seen in negative samples. Changes in transmittance are registered continuously by an optical sensor and recorded in the computer during incubation. Although all *Listeria* spp. hydrolyze esculin, the hydrolysis rate for *L. monocytogenes* strains appears to be faster than for other *Listeria* spp. and non-*Listeria* organisms that hydrolyze esculin. The method can detect ≤10 cells of *Listeria* in 25 ml of milk in a total of 24 h, and is applicable to other foods, including eggs, raw and processed chicken and beef products. It is highly reproducible and labor efficient. Conventional or rapid confirmation tests can be applied directly to positive samples.

(P82) VIDAS® LISTERIA ASSAY: ENVIRONMENTAL SURFACE CHALLENGE STUDY

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This study, divided into three distinct phases, evaluated the efficacy of recovery of *Listeria* spp. from environmental surfaces using the VIDAS LIS Listeria Assay (bioMérieux Vitek, Inc., St. Louis, MO) and the USDA-FSIS method for recovery of *Listeria*. In the first segment of the study, three strains of *Listeria monocytogenes* (strains 1/2a, 1/2b, and 4b) were mixed with varying concentrations of *E. coli* and inoculated onto nine environmental surfaces. The surfaces were then swabbed to determine recovery of the *L. monocytogenes* species. In the second segment, inclusivity of the test was determined by inoculation of one environmental surface with the other six species of *Listeria* mixed with varying concentrations of *E. coli*. Exclusivity or specificity of the test was tested in the third segment of the study by inoculation of an environmental surface with 11 non-*Listeria* environmental species. The VIDAS method detected a total of 239 true positives and the USDA-FSIS method detected a total of 227 true positives. Using a paired *t*-test, no significant differences were observed between the number of true positive samples detected by the VIDAS *Listeria* assay and by the USDA-FSIS method for all environmental surfaces tested.

(P83) EVALUATION OF AN AUTOMATED IMMUNOASSAY FOR THE DETECTION OF *LISTERIA* IN FOODS

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Inoculated food studies were conducted to evaluate the performance of the VIDAS Listeria assay (LIS) for the detection of *Listeria* spp. in food. The LIS method was compared to USDA/FSIS and FDA standard methods. Inoculated and uninoculated replicate samples of 12 food types (ice cream, cheese, milk, shrimp, chocolate milk, lettuce, cauliflower, sausage, hot dogs, ground turkey, cubed beef, and pork sausage) were tested. Overall, 450 samples were tested with each method. *Listeria* was detected in 362 samples by the LIS assay, 360 of which were confirmed by culture. *Listeria* was detected in 337 samples by standard culture methods. Performance of the LIS assay was found to be equivalent to or better than standard methods.

(P84) EVALUATION OF DIFFERENT SELECTIVE AND DIFFERENTIAL MEDIA FOR DIRECT QUANTITATION OF *E. COLI* O157:H7 FROM IRRADIATED HAMBURGER MEAT

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E. coli O157:H7 infects more than 20,000 people in the U.S. and causes over 200 deaths annually. More than 70% of *E. coli* O157:H7 outbreaks are associated with bovine sources. Heat and irradiation have been recently approved for ground beef.

In challenge studies to evaluate the efficacy of irradiation on survival and recovery of *E. coli* O157:H7 in hamburger beef, appropriate selective and differential media are needed. Hamburger beef was inoculated with *E. coli* O157:H7 and irradiated at 0 to 1.5 KGy, stored in refrigerator and plated on a number of commercial media like: RBA (Rainbow Agar™ O157:H7, Biolog, Hayward California), BCM O157:H7⁺ (Biosynth), TBX (Oxoid), PRSA (Difco/Oxoid), TSA (Difco/Oxoid) and SMAC (Difco/Oxoid). The media were compared for the ability to recover sublethal injured cells and their selective and differential potential.

Among the media tested, BCM and RBA allow better recovery of *E. coli* O157:H7 from irradiated hamburger beef samples. Post irradiation recovery of *E. coli* O157:H7 on RBA seemed slightly higher than BCM. Compared to RBA, the *E. coli* O157:H7 colonies on BCM were slightly larger and intense in color. However, in both media, difficulties were encountered in accurate and consistent differentiation of *E. coli* O157:H7 from the background microflora that mimic *E. coli* O157:H7 in hamburger beef.

(P85) DEVELOPMENT OF A HOMOGENEOUS PCR ASSAY FOR THE DETECTION OF *E. COLI* O157:H7 IN FOOD SAMPLES

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BAX™ for Screening/*E. coli* O157:H7 is a commercially available PCR-based kit that detects *E. coli* O157:H7 in food and environmental samples. The assay detects *E. coli* O157:H7-specific PCR products using gel electrophoresis and ethidium bromide staining. In order to increase the ease of PCR product detection, a homogeneous detection method has been developed. The principle of the Temperature-Dependent Fluorescence (TDF)-PCR detection is based on a fluorescent intercalating dye, SYBR green I, that binds to the double-stranded PCR product. The TDF-PCR assay is an in-situ process, simultaneously amplifying the target DNA and directly detecting the increasing fluorescence signal during the annealing/elongation phase of PCR. At the completion of the current commercial PCR cycling program, one additional thermal cycle is run consisting of a denaturation step and a product annealing step. Fluorescence is monitored in the PCR tubes during this additional cycle. The rate of increase in fluorescence over time during the product annealing step yields a characteristic pattern for positive samples that can be differentiated from negative samples. This homogeneous assay has been evaluated using purified DNA, bacterial lysate DNA from pure cultures and pre-enriched food samples that have been spiked with target organisms. The commercial assay run in combination with homogeneous detection eliminates the need for gel electrophoresis and simplifies PCR product detection. These data indicate that the homogeneous assay offers all of the benefits of the commercial *E. coli* O157:H7 in an alternative user-friendly format.

(P86) INHIBITORY EFFECT OF GAMMA IRRADIATION AND EFFICACY OF PLATING MEDIA FOR RECOVERING IRRADIATED *E. COLI* O157:H7

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The lethal effect of gamma irradiation using dose levels from 0 kGy to 3.0 kGy at 50° to 60°C and -20°C and the efficacy of plating media for recovering gamma irradiated *E. coli* O157:H7 strains was determined. Non-*E. coli* O157:H7 was significantly more sensitive than O157:H7 strains to gamma irradiation treatment at room temperature. *E. coli* O157:H7 strain 933 showed the greatest irradiation resistance followed by strains 0019 and 933. Strain 933 showed significant resistance to heat and freezing, but the strain was significantly inactivated by combining these treatments with gamma-irradiation treatment. Recovery of gamma irradiated *E. coli* O157:H7 from tryptic soy broth was better on TSA than on TSA supplemented with 4-methylumbelliferyl-ß-D-glucuronide(MSMA), followed by sorbitol MacConkey agar (SMA). Recovery from beef treatment with a combination of heat or freezing with gamma irradiation beef showed similar results.

(P87) EFFECT OF SURFACE FINISH ON THE CLEANABILITY OF STAINLESS STEEL

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The objective of this study was to determine the effect of surface polish on cleanability. Stainless steel type 304 with no polish (hot rolled and pickled), #4 and 2B mechanical polish and electropolished were tested. A total of nine different samples were obtained. Cleanability was assessed by soiling with cultured milk containing spores of *Bacillus stearothermophilus*. After the soil was dried the samples were cleaned by immersion in a turbulent bath of 1.28% NaOH at 66°C for 3 minutes. Plates were then rinsed and sanitized by immersion in 100 ppm hypochlorite for 20 seconds. Plates were covered with PM Indicator Agar, incubated for 25 h at 58°C, and colonies counted. The number of colonies is an indicator of residual soil. The same plates were subjected to an additional 10 cleaning/soiling cycles and the residual protein measured by using epifluorescent microscopy. Results indicate that both spore count and microscopic examination are required to determine relative cleanability, but the spore count test was a more precise measure of cleanability for the polished samples. Polish type was not a good predictor of cleanability. However, surface roughness, as determined using a surface roughness gage, produced a correlation of 0.82 with spore counts. Results indicate critical applications, stainless steel should be purchased based on surface roughness measures rather than polish type.

(P88) A COMPARATIVE EVALUATION OF SPONGING AND EXCISING AS SAMPLING PROCEDURES FOR MICROBIOLOGICAL ANALYSIS OF BEEF CARCASS TISSUE

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This study evaluated sponging and excising as sampling procedures for microbiological analysis of beef carcass tissue prior to or following spray-washing treatments. Brisket samples (10 × 10 cm) were inoculated with 2 ml of *E. coli* (3×10^8 CFU/ml) cell suspension. After 30 min, samples were excised (EX) or swabbed with a sterile sponge (SP) and diluted with either 0.1% peptone water or Butterfield's phosphate buffer. The samples were analyzed for aerobic plate counts (APC) on tryptic soy agar (TSA) and for total coliform counts (TCC) and *E. coli* counts (ECC) on Petrifilm™. Another set of samples was analyzed after spray-washing with water (6 sec, 35°C, 3.4 bar), acetic acid (2% 6 sec, 35°C, 2.1 bar), final wash (20 sec, 42°C, 20.7 bar) and acetic acid (2% 6 sec, 35°C, 2.1 bar). Additional samples were analyzed after storage of 7°C for 24 hr. Variables having significant ($P<0.05$) effects on bacterial counts recovered included procedure of sampling (EX vs. SP), time of sampling (0.5 hr vs. 24 hr) and their interactions. Counts recovered 30 min after inoculation or spray-washing were similar between the two sampling procedures (EX, SP). However, counts recovered after 24 hr of sample storage were significantly ($P<0.05$) lower for the SP compared to the EX procedure. The results indicated that as the carcass tissue was stored, recovery of bacteria by SP was less efficient compared to EX.

(P89) SHIPPING, STORAGE AND SAMPLING-SPONGE EFFECTS ON BACTERIAL NUMBERS DETECTED FROM PORK CARCASS SKIN AND FAT SURFACES

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This study compared microbiological counts (aerobic plate counts-APC; total coliform counts-TCC; and *E. coli* counts-ECC) determined from sampling fat and skin of pork carcasses with two buffers (Butterfield's Phosphate–BP and Peptone Water–PW) and two commercially available sampling sponges (A and B), after overnight shipping and an additional 24 hr sponge storage. Pork carcasses were sponged (n=80) according to the United States Food Safety and Inspection Service (FSIS) regulations prior to the final carcass washing. The sponges were analyzed after overnight shipment (24 hr) and after an additional 24 hr of storage at 4°C (48 hr). Average ECC were generally <0.60 log CFU/cm^2 and similar among treatments. Factors affecting ($P<0.001$) APC and TCC were sponge type, sponge storage time and type of carcass surface. Surface of dehaired carcasses (skin) had higher counts than fat surface of skinned carcasses, and 48 hr storage had higher counts than 24 hr storage. Average APC of samples obtained with sponge A at 24 and 48 hr were 3.62 and 3.69 log CFU/cm^2 and average APC of samples obtained with sponge B at 24 and 48 hr were 4.48 and 5.01 log CFU/cm^2. These results suggest that sponge type, method of hair removal and shipment/storage may have significant effects on bacterial counts detected on pork carcasses.

(P90) RECOVERY OF *E. COLI* PURE CULTURE SUSPENSIONS FROM SPONGES FOLLOWING SHAKING OR STOMACHING

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This study evaluated recovery of a pure *E. coli* culture inoculated (1 ml of a 10^6 CFU/ml suspension) in each of two brands of commercial sponges in plastic bags with 25 ml of each of two hydrating buffers (Butterfield's Phosphate, 0.1% Peptone Water). The contents of sampling bags with sponges were analyzed by plating on tryptic soy agar and Petrifilm, following stomaching (2 min) or manual shaking (30 sec) immediately after inoculation and after 24 hr at 4°C. A set of samples was analyzed without any stomaching or shaking. Each treatment was replicated six times. Method of sample agitation (stomaching/shaking), type of sponge and buffer had no influence ($P>0.05$) on bacterial counts recovered. Time of storage had a significant ($P<0.001$) effect on counts determined on Petrifilm™. Overall counts obtained on tryptic soy agar following sponge stomaching, shaking and no agitation at 0 hr were 3.29, 3.33 and 3.29 log CFU/ml; the corresponding counts after 24 hr at 4°C were 2.83, 3.00 and 3.08 log CFU/ml. Overall counts obtained on Petrifilm™ (following sponge stomaching, shaking and no agitation at 0 hr were 2.83, 2.88 and 2.94 log CFU/ml; the corresponding counts after 24 hr at 4°C were 1.83, 2.00 and 2.38 log CFU/ml. These results should be useful in selecting the optimal sampling procedure for analyses of samples required by the new meat and poultry inspection regulations.

(P91) SURVIVAL OF *SALMONELLA* DURING 4°C STORAGE IN SPONGE BAGS HYDRATED WITH DIFFERENT MEDIA

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Three diluents, Butterfield's phosphate diluent (BPD), buffered peptone water (BPW), and neutralizing buffer (NB) were evaluated for their ability to maintain *Salmonella* viability in Nasco(N) Speci-Sponge bags, International Bioproducts Hydrasponge (IB)bags, and control test tubes (TT) during 4°C storage for 24 h. A 10^4 CFU/ml inoculum cocktail of five *Salmonella* strains was inoculated into individual sponge bags and test tubes containing the test media, and viable *Salmonella* were enumerated at 0, 1, 6 and 24 h. There was no reduction ($P>0.05$) in *Salmonella* populations within containers, media, or container × media at 0 and 1 h of storage. No reductions ($P>0.05$) in *Salmonella* populations were observed with any container over 24 h storage in BPW. At 6 h there were population reductions ($P\leq0.05$) in IB, N and TT containing BPD media of 47.9, 50. 3 and 63.6% respectively. At 24 h *Salmonella* population reductions ($P\leq0.05$) in IB, N and TT were 39.9, 47.3, and 56.6% in BPD and 28.7, 27.0 and 30.3% in NB, respectively. *Salmonella* survival in sponge-sampling bags seems to be much better than

generic *E. coli* (studies presented simultaneously). Use of BPW should be the media of choice in *Salmonella* sponge sampling protocols.

TECHNICAL SESSIONS

(T1) DETERMINATION OF END-POINT TEMPERATURE IN COOKED GROUND BEEF PATTIES BY NEAR-INFRARED REFLECTRANCE SPECTROSCOPY

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Inadequate heat treatment of meat is commonly cited as a factor in foodborne disease outbreaks. Therefore, meat must be heat treated to safe endpoint temperatures, to ensure destruction of pathogenic microorganisms. Near infrared (NIR) reflectance spectroscopy was studied as a potential method for determination of endpoint temperature (EPT) in ground beef *M longissimus dorsi* patties cooked fresh, patties frozen 2 weeks and thawed at room temperature 2 h or 4 h or by microwave, and patties from bulk frozen thawed (room temperature or microwave). Meat juices (N=288) after cooking patties to an EPT of 57.2°, 65.5°, 71.1°, and 79.4°C were analyzed by NIR in both wet and dried states. Near infrared spectra were related to EPT by multivariate analysis. The NIR method was able to determine the EPT of cooked ground beef patties with a prediction error of 1.8°C. Near infrared spectra showed a significant decrease in protein absorbance with increased EPT. These results indicate that this 15 min test could have potential as EPT indicator for verifying FSIS/FDA requirement for fully cooked hamburger patties.

(T2) ACID PHOSPHATASE ACTIVITY AND MYOGLOBIN DENATURATION AS END-POINT TEMPERATURE INDICATORS IN COOKED GROUND BEEF PATTIES

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Verification of end-point temperature (EPT) is needed in cooked meat products due to recent outbreaks of *E. coli* O157:H7. USDA and FDA have issued temperature requirements and visual color guidelines to help consumers cook pathogen-safe beef patty products. Acid Phosphatase (ACP) activity and myoglobin (MYO) content was studied as potential methods for determination of EPT in ground beef patties cooked fresh-ground, patties frozen 2 weeks and thawed at room temperature 2 h or 4 h or by microwave, and patties made from bulk frozen ground beef thawed at room temperature or by microwave. Pressed-out meat juice was analyzed from patties (N=288) cooked to 57.2, 65.5, 71.1, and 79.4°C target EPT's. Log_{10} ACP had a significant linear (R^2=0.99) response to EPT. Myoglobin had a significant quadratic (R^2=0.99) response to EPT. Bulk freezing with slow refrigerator or room temperature thawing caused significant loss of MYO in expressed meat juice. Results show these 3 to 5 min tests could be used by FSIS inspectors or food processors in quality assurance/HACCP programs.

(T3) RECOVERY OF *SALMONELLA*, *C. JEJUNI* AND *C. PERFRINGENS* FROM A POULTRY BROILER HOUSE

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To assess the prevalence and movement of *Salmonella*, *Campylobacter* and *C. perfringens* through an integrated broiler operation, a 20,000 broiler flock was studied through grow-out and processing. Samples were collected at placement and at 2, 4 and 6 weeks post-placement (PP). For *Salmonella*, 102/135 samples were positive at placement with recovery predominately from paper pads, egg shells, boots, and flies. *Salmonella* positive samples declined to 16/76, 11/74 and 4/65 at 2, 4 and 6 weeks PP. *Campylobacter* was not recovered until 5 weeks PP when only 1 feces sample was positive. At 6 weeks PP, 20/65 samples were positive with recovery predominantly from feces. *C. perfringens* were recovered throughout grow-out with peak recovery at 4 weeks (16 of 75). After processing, more carcass were positive for *Campylobacter* (19/25) than *Salmonella* (5/25) or *C. perfringens* (0/25). These data showed that *Salmonella* and *Campylobacter* may be more prevalent throughout the poultry environment and may colonize chicks earlier than *Campylobacter*. However, after processing, more *Campylobacter* than *Salmonella* or *C. perfringens* were recovered.

(T4) NONTHERMAL INACTIVATION MODELS FOR *S. TYPHIMURIUM* IN POULTRY PROCESSING

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Predictive microbial models of *Salmonella* during poultry processing are needed for HACCP analysis to ensure food safety of poultry products. The objectives of this study were to determine the effects of antimicrobial spray including spraying chemicals, time, temperature, pressure, and setting time on destruction of *Salmonella* on the pre-chill chicken carcasses, and to incorporate this information into the formulation of predictive microbial models. The chicken skin was inoculated with *S. typhimurium* and sprayed with antimicrobial chemicals (0.5% cetylpyridinium chloride (CPC), 10% trisodium phosphate (TSP) and 2% lactic acid (LA) at various setting time, spraying time, temperature and pressure. Most probable number technique (MPN) was used to enumerate *Salmonella*. The survivor data were described mathematically by fitting data using linear and non-linear primary models. The first order models were capable of fitting survival/inactivation data of *S. typhimurium* as a function of setting time, spraying time, temperature and pressure. The D values for *S. typhimurium* were 60, 75 and 100 sec for 0.5% CPC, 10% TSP and 2% LA, respectively. The R^2 for the model-fitting were between 0.5 to 0.7. The nonthermal inactivation models is useful for better understanding microbial elimination and enhancing scientific basis for HACCP programs in poultry processing.

(T5) QUANTITATIVE RISK ASSESSMENT FOR *C. JEJUNI* IN FRESH CHICKEN

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Quantitative risk assessments for *Campylobacter jejuni* associated with fresh poultry were undertaken using Monte Carlo simulation techniques. A dose-response model was fit to published data from human feeding trials and the average probability of infection from one *C. jejuni* organism was estimated to be 0.0187, which supports the reported low infective dose for *C. jejuni*. In North America, the prevalence of *C. jejuni* on retail fresh chicken has been reported to range from 30% to 80%. However, data on the numbers of *C. jejuni* on fresh chicken are not readily available. Results of the 1994-95 USDA baseline survey, which quantitatively measured *C. jejuni/coli* on broiler carcasses immediately after processing, were used and changes in concentration likely to occur between processing and consumer purchase were simulated. Initial results indicate that when contaminated chicken is brought into the home, the most likely number of organisms on a whole chicken is between 2 and 3 logs. It was, however, also estimated that approximately 16% of contaminated fresh chicken would have between 5 and 6 logs *C. jejuni* present. The high infectivity, prevalence and numbers of *C. jejuni* on fresh chicken suggest a potentially unacceptable risk for the consumer. A process risk model (PRM) was developed as a result of this potential risk. The PRM simulates the production and processing of poultry to highlight the stages that have the greatest influence upon the risk. The points in the processing line at which cross-contamination, survival, and reductions in numbers can be expected were modeled using published data and expert opinion. The goal of the PRM is to develop a tool to compare the effect of various intervention strategies on the final comparative risk outcome. As is true for most risk assessments, another useful outcome of the model is the identification of areas in which data are lacking.

(T6) EXPERIMENTAL INFECTION OF BIRDS WITH *A. BUTZLERI*

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Arcobacter butzleri is a Campylobacter-like organism which causes human enteritis and is frequently recovered from poultry. The source of poultry contamination is unknown. The purpose of this study was to determine the relative pathogenicity of *A. butzleri* for live birds. In Trial I, 3-day old chicks (n=62) were divided into 3 groups and infected per oz with (i) *A. butzleri* ATCC 49616, (ii) a suspension of 7 field strains of *A. butzleri* isolated from retail purchased chicken, and (iii) *C. jejuni* (positive controls). *Arcobacter* was not detected in cloacal swabs or in caecal samples of chicks through day 5 post-infection. In contrast, *C. jejuni* was recovered from the cloacal and caecal samples of all positive control birds. In Trial 2, 3-day old turkey poults (n=85) were infected as described above with the addition of a group infected with a suspension of 4 field strains of *A. butzleri* recovered from turkey meat. *A. butzleri* was

isolated from either cloacal swabs or caecal contents of 6.5% (4 of 62) of turkeys; *C. jejuni* was recovered from 100% of the positive control birds (n=21). In Trial 3, 3-day old turkey poults of the highly inbred Beltsville strain (n=142) were infected. In contrast to earlier trials, *A. butzleri* was recovered from birds infected with: ATCC 49616 (44%), turkey field strains (58%), and isolates recovered from retail purchased chickens (100%). In this study no colonization of chicken was observed with *A. butzleri*; however, variable colonization of turkey was observed.

(T7) OCCURRENCE OF *E. COLI* O157:H7, *SALMONELLA*, AND OTHER SHIGA-LIKE TOXIN-PRODUCING *E. COLI* IN RETAIL FRESH GROUND BEEF

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More than 1,000 fresh ground beef samples were purchased from Seattle, Washington area retail outlets. Ten-gram samples were homogenized in 90 ml of mTSB and enrichment cultured overnight with agitation. The cultures were then screened using the BAX™ system PCR-based assays for *Salmonella* and *E. coli* O157:H7, and for Shiga-like toxin-producing *E. coli* (SLTEC) using a non-commercial multiplex PCR assay (for Shiga-like toxin genes, the EHEC *eaeA* gene, and the *E. coli* O157:H7 60-MDa plasmid). Cultures positive by PCR for *Salmonella* and *E. coli* O157:H7 were confirmed by culture methods, while each SLTEC-PCR-positive culture was confirmed by colony hybridization using the SLT-I and II genes. More than 10% of the samples were positive for SLTEC by PCR and all were confirmed by colony-hybridization. Less than 1% of the samples were PCR-positive for *E. coli* O157:H7 and approximately half of these were confirmed by culture. Four percent of the samples were positive by PCR for *Salmonella* and all were confirmed by culture. Strains of *E. coli* other than O157:H7 were found to be SLT/ *eaeA*-positive. More than half of all the samples were positive for the 60-MDa plasmid, making this marker of little value. The *E. coli* O157:H7 isolates were compared to a collection of clinical isolates from Washington, Oregon, Utah and Nevada using the Micro Restriction Fingerprinting (MRF) method.

(T8) IMPROVED ISOLATION OF VEROTOXIN-PRODUCING *E. COLI* FROM GROUND BEEF

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Isolation of verotoxin (VT)-producing *Escherichia coli* (VTEC) from raw meat is time-consuming and often unreliable. We developed a VT-immunoblot method to isolate VTEC from enrichment cultures of ground beef. Dilutions of cultures positive for VTs by the verocell cytotoxicity assay (VCA) or a VT ELISA are each plated onto a filter membrane overlying a capture membrane coated with VT antibodies on a tryptic soy agar plate. After 12 to 18 hour incubation, the capture membranes are probed with monoclonal antibodies to VTs, peroxidase-labelled anti-mouse IgG and BCIP/NBT substrate. Stained dots on the membranes correspond to VT-producing colonies on the overlying filter which can be picked for typing. In tests of 200 samples of ground beef, we successfully isolated VTEC from more than 90% of the 70 samples positive for VTs by the VCA or VTELISA. Three of the isolates were *E. coli* O157:H7.

(T9) IMMUNOASSAYS FOR THE DETECTION OF SPINAL CORD IN PRODUCT FROM ADVANCED MEAT RECOVERY SYSTEMS

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Immunohistochemistry, histochemistry, and polarization microscopy was used to document the presence of spinal cord in a ground meat product produced by Advanced Meat Recovery Systems (AMRS) machines. Antibodies to neurofilament and glial fibrillary acidic proteins were quite useful for highlighting the presence of neural tissue in comminuted product. Immunostaining with synaptophysin, polariscopy, and trichrome staining were excellent methods for differentiating spinal cord from peripheral nerve. Spinal cord was found in two of 196 AMRS samples when only hematoxylin and eosin staining was used. In the immunohistochemical study, spinal cord was found in seven of 17 AMRS samples. Spinal cord tissue has also been found in three of 26 regulatory cases. AMRS product found to contain spinal cord cannot be sold as meat in the U.S. In countries where Bovine Spongiform Encephalopathy is present, spinal cord in AMRS derived product could be a source of contamination of the food supply.

(T10) TRICLOSAN-INCORPORATED PLASTIC FOR REDUCING BACTERIA ON MEAT SURFACES

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Triclosan (2,4,4´-trichloro-2´-hydroxydiphenyl ether) is a nonionic, broad spectrum, antimicrobial agent that has been incorporated into a variety of personal hygiene products, including hand soaps, deodorants, shower gels, mouthwashes, and toothpastes. Triclosan not only exhibits bacterial inhibition through direct contact but also imparts residual antimicrobial activity. In this study, plastic containing 1500 ppm of triclosan was evaluated in plate assays and meat experiments as a means of reducing populations of bacteria. Plate assays indicated that the triclosan-incorporated plastic (TIP) inhibited the following organisms: *Brochothrix thermosphacta* ATCC 11509, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 12598, *Bacillus subtilis* ATCC 6051, *Shigella flexneri* ATCC 12022, *Escherichia coli* ATCC 25922, and several strains of *E. coli* O157:H7 (ATCC, 35150, 43888, 43889, 43890, 43894, 43895). When beef surfaces were inoculated with *E. coli* O157:H7, *S. typhimurium*, *B. subtilis*, or *B. thermosphacta*, wrapped in TIP, vacuum packaged, and stored under refrigerated conditions, bacterial populations were reduced to a greater extent as compared to controls. Additional studies are underway to determine the effects of TIP against bacterial populations during long-term, refrigerated storage. This study demonstrates the potential use of an antimicrobial incorporated plastic for reducing bacterial populations on fresh meat surfaces.

(T11) STUDIES TO CHARACTERIZE AND OPTIMIZE THE *E. COLI* SPONGE SAMPLING METHOD FOR SLAUGHTER PROCESS CONTROL MONITORING

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Observations have indicated a low generic *E. coli* recovery using the USDA-FSIS defined sponge sampling method for monitoring slaughter process control. Studies were undertaken to quantify recovery and identify protocol components responsible for microbial reductions. **Study 1.** Sponge bags hydrated with Butterfield's Phosphate diluent (BPD) containing meat residues yielded a 70% *E. coli* reduction in 5 min and 93% in 24 h at 4°C. **Study 2.** *E. coli* were added to bags containing cellulose sponges that had been boiled and autoclaved. No reductions occurred during the first 12 h of storage in BPD, with a 45% reduction by 24 h. **Study 3.** The Microtox™ acute toxicity assay was used to determine inherent toxicity of commercial sampling sponges. BPD expelled from sponges indicated an EC_{50} of ca. 28%; indicating "moderate" toxicity. BPD without the sponge was not toxic (P=0.5) in the assay. **Study 4.** Chilled beef carcasses (28) were tested for generic *E. coli* at adjacent anatomical sites using FSIS-defined sponge (BPD diluent; 300 cm^2) and excision (60 cm^2) protocols. Sponge sampling provided a lower ($P \leq 0.05$) *E. coli* recovery frequency than excision; 0/28 and 6/28 positive (0.39 to 23.6 CFU/cm^2), respectively. **Study 5.** Pre-rigor beef was inoculated with ca. 100 *E. coli*/cm^2 and air chilled for 24 h. Sponge sampling recovered 13% of *E. coli* compared to excision sampling. Studies are underway to optimize *E. coli* recovery and survival in sponge bags to improve the effectiveness of the methods for slaughter process control monitoring.

(T12) ECOLOGY AND CONTROL STRATEGIES FOR SALMONELLAE IN BROILER CHICKENS

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Salmonellae is found to be present on about 20% of commercially processed broiler carcasses. From where does this salmonellae come and what can be done to control or eliminate it? Salmonellae can be found in hatchery, feed, environmental, animal, rodent, and numerous other samples. The relative importance of these different sources will be discussed in relationship to the degree of susceptibility of different age chickens. Without cooking or irradiation, the best way to reduce the presence of salmonellae on processed chickens is to reduce its presence on live birds leaving the farm and entering the processing plant. The use of competitive exclusion, biosecurity, vaccination, and other intervention strategies will be discussed with particular reference to different sources of salmonellae, level of stress and age of chicken.

(T13) CHARACTERIZATION OF THE ANTIBIOTIC RESISTANCE LOCUS IN *S. TYPHIMURIUM* DT 104

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Salmonella typhimurium DT 104 is a rapidly emerging new pathogen confronting the food industry. This strain is characterized by chromosomally encoded resistance to the antibiotics ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (R-type ACSSuT). As part of the 1995 National *Salmonella* Antimicrobial Resistance Study, the Centers for Disease Control and Prevention studied 976 *S. typhimurium* isolates and found 275 (28%) had R-type ACSSuT, compared to just 7% in 1990. In the United Kingdom DT 104 leaves 41% of its victims hospitalized and results in fatalities in 3% of victims, compared with only 0.1% fatality rates for all other *Salmonella* infections. We have constructed a lambda library of the DT 104 genome and cloned the antibiotic resistance genes into *E. coli* strain XLOLR. Using PCR we have demonstrated that antibiotic resistance is mediated by a class I integron and mobile gene cassettes. Currently we are working to discover the identity and reveal the sequence of the antibiotic resistance genes. We believe the arrangement and intergenic sequences of the antibiotic resistance locus will yield unique DNA sequences allowing rapid identification of this organism using PCR.

(T14) RESPONSE SURFACE MODELS FOR EFFECTS OF PREVIOUS PH, TEMPERATURE, AND PH ON LAG TIME AND GROWTH RATE OF *S. TYPHIMURIUM*

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Response surface models for effects of previous pH (pre-pH; 5.5 to 8.5), temperature (15 to 40°C) and pH (5 to 7) on lag time (LT) and growth rate (GR) of *Salmonella typhimurium* were developed in brain heart infusion broth. Growth curves were fit to a two-phase linear equation that directly estimated LT and GR. Response surface models for LT and GR as a function of pre-pH, temperature, and pH were obtained by regression analysis. Of the model variables, temperature had the largest effect on LT and GR followed by pH and then pre-pH, which had a small effect on LT and no effect on GR. None of the model variables interacted to affect growth of *S. typhimurium*. Models were tested against data not used in their development. Prediction error for LT and GR in broth culture were similar for data not used in model development and new data. In contrast, prediction error were higher for LT and GR on cooked chicken compared to broth culture. Thus, the models were better at predicting growth of *S. typhimurium* in broth culture than on cooked chicken.

(T15) USE OF AN AUTOBIOLUMINESCENT *S. HADAR* TO MONITOR THE EFFECT OF DECONTAMINATION METHODS

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In recent studies, application of lactic acid was shown to improve the quality and safety of fresh poultry. However, only some research has investigated the long-term effects of this treatment. In a novel approach to determine recovery of pathogenic bacteria, an investigation was conducted with a bioluminescent bacteria to examine and evaluate the efficacy and shelf life of lactic acid treatments. *Salmonella hadar* was isolated from poultry and was genetically engineered to become autobioluminescent by transformation with the a lux (CDABE) cassette. Viability was determined by measuring luminescence following lactic acid treatment on turkey breasts and storage at -12, 0, 5 and 10°C. The ability of the *S. hadar* (lux^+) to recover from the treatment was determined by incubating at 22°C for 10 h and monitoring light output. Both experimental trials were conducted as Randomized Complete Block Designs. Results showed that metabolic activity was significantly ($P<0.05$) affected by the lactic acid treatment and by storage temperatures of -12, 0 and 5°C. The lowest recovery rate was observed after treatment with lactic acid $P<0.05$) and storage at 5°C. The results demonstrated that an effective carcass decontamination method can lead to improved safety of poultry and that temperature plays an integral role with the recovery of *Salmonella hadar*.

(T16) EVALUATION OF A PCR-TAQMAN™ ASSAY FOR DETECTION OF *E. COLI* O157:H7 AND *SALMONELLA* FROM GROUND BEEF

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A PCR-based assay (TaqMan™) was evaluated for the ability to defect *E. coli* O157:H7 and *Salmonella*

from various enrichments and ground beef. Universal preenrichment broth (UPB), modified EC broth (MECB), and modified tryptic soy broth (MTSB) were used for detection and sensitivity characterization of the *E. coli* O157:H7 assay. Lactose broth (LB), buffered peptone water (BPW), and Butterfield's phosphate buffer (BPB) were used as enrichments for *S. typhimurium*. Ground beef (25 g) was added to 225 ml of enrichment broth, followed by incubation at 35°C for 6 h. Enrichment broths or ground beef samples were spiked with known concentrations of *E. coli* O157:H7 or *S. typhimurium*. All samples were stored at -20°C until DNA extraction using the Q1Aamp tissue extraction kit. The assay sensitivity for *E. coli* O157:H7 from all samples preenriched in mTSB was 10^4 CFU/ml (50 CFU/PCR reaction). The detection sensitivity for *Salmonella* from samples was 10^3 CFU/ml (5 CFU/PCR reaction). This PCR/TaqMan assay is a rapid and sensitive assay with high volume sample and potential in pathogen screening. Additionally, a previous large-scale field evaluation (>2,000 samples) of the PCR/Taqman assay to detect *E. coli* O157:H7 from cattle feces has indicated a high degree of specificity and repeatability compared to cultural procedures for pathogen detection from complicated matrices.

(T17) DEVELOPMENT OF A PCR ASSAY FOR THE DETECTION OF *LISTERIA* SPP. IN FOOD PRODUCTION ENVIRONMENTS

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A PCR-based method has been developed for the detection of *Listeria* spp. in food production environments. Specific primers were identified by a method that involved using Random Amplified Polymorphic DNA (RAPD) analysis of 45 strains of *Listeria* spp. The primer concentration was optimized to maximize the sensitivity of the assay and to minimize the potential of cross-reactivity with non-*Listeria* bacteria. Following the optimization, the primers were tableted along with deoxynucleotides and *Taq* DNA polymerase. After enrichment, the assay had a sensitivity of 10^4-10^5 CFU/ml for *Listeria* spp. and did not produce the specific 479-base pair PCR product with non-*Listeria* when tested at levels of 10^8 CFU/ml. An assay protocol was developed for PCR analysis of environmental samples following a 22 to 24 hour selective enrichment. The assay was used to analyze more than 200 samples from dairy, pork, and beef processing environments. Nine of 11 positive samples were confirmed as *Listeria* spp., while 48 of 49 samples inoculated with low levels of *Listeria* were positive by the PCR assay. This assay appears to be applicable to a wide range of environmental sample types, yielding accurate next-day results.

(T18) *CAMPYLOBACTER* RECOVERY AND ENUMERATION FROM BROILER CARCASSES

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Labor intensive MPN techniques were compared to a simple direct plating procedure for detection and enumeration of campylobacters from broiler carcass rinse samples. There was no significant difference between the MPN procedures used by FSIS and the direct plating method in recovery of campylobacters from freshly processed carcasses (*P*0.05). Similar comparisons were conducted on recovery of environmentally stressed campylobacters from carcasses stored at 4°C for 5 days. Combining direct plating with a back-up enrichment broth sample provided estimates comparable to the MPN techniques for enumeration of stressed campylobacters. Overall, the direct plating method provided a less expensive, more rapid alternative to traditional MPN procedures for estimating *Campylobacter* populations associated with broiler carcasses.

(T19) A RAPID AND SPECIFIC FLUOROGENIC PCR-BASED SYSTEM FOR THE DETECTION OF SHIGA TOXINS PRODUCING *E. COLI* FROM DIFFERENT FOOD SAMPLES

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More than one hundred different *Escherichia coli* serotypes other than O157:H7 can produce Shiga toxins, or verocytotoxins (VT), I and II. Patients may suffer from bloody diarrhea and possible life-threatening complications like hemorrhagic colitis or hemolytic uremic syndrome. A rapid and specific fluorogenic PCR-based assay has been developed for the detection of these

highly pathogenic *E. coli* strains. This novel molecular-based system enables the detection of specific target organisms by monitoring and automatically analyzing fluorescence signals that increase during the PCR amplification process. In assessing the specificity and sensitivity of our assays, we tested over 90 *E. coli* and 70 non-*E. coli* bacterial isolates representing 54 serogroups and 25 genera, respectively, for the presence of VTI and/or II. We found excellent agreement between our assay results and those obtained independently using serology or cytotoxicity tests. To assess the performance of these assays, naturally and artificially contaminated foods such as ground beef, milk, and alfalfa sprouts were tested and demonstrated very high correlation with culture results performed in parallel. These results illustrate that integration of this fluorogenic PCR-based assay with instrumentation and automated analysis provides a robust and powerful system for rapid evaluation, detection, and documentation of food samples that are contaminated with various strains of Shiga toxins producing *E. coli.*

(T20) *IN VITRO* PATHOGENICITY ASSAY OF *BACILLUS CEREUS* USING HYBRIDOMA CELLS

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Bacillus cereus is a major cause of foodborne gastroenteritis in humans. Development of a rapid, simple, and inexpensive method for detecting *B. cereus* cells and toxins is of great interest to the food industry. The purpose of this study was to determine the pathogenicity of *B. cereus* cells at various concentrations and to determine the cytotoxicity of culture filtrates containing *B. cereus* toxins on a tissue culture assay model developed in our laboratory. Ped-2E9 hybridoma cells (HC) were challenged with cells from ten *B. cereus* strains at concentrations of 10^6, 10^5, 10^4, and 10^3 CFU/ml and with cells from *Bacillus subtilis* at a concentration of 10^8 CFU/ml. The challenged HC were incubated for 8 h at 37°C in 7% CO_2 under humidified conditions. Samples were taken every 2 h and HC viability determined by Trypan blue exclusion method. All ten *B. cereus* strains at 10^6 CFU/ml caused >95% HC death in 2 h and in 8 h for 10^3 CFU/ml. *B. subtilis* caused no significant HC death in 8 h. Tests with 100, 50, 20, and 10 mg/ml of freeze dried supernatant from emetic strains of *B. cereus* produced >95% HC death at 4 h with 100 mg/ml and >75% HC death at 8 h with 50 mg/ml. Ped-2E9 HC can rapidly detect both diarrheal and emetic strains of *B. cereus* cells at concentrations required to cause human illness.

(T21) COMPARISON BETWEEN AUTOMATED RIBOTYPING AND RAPD ANALYSIS FOR 44 DIFFERENT *BACILLUS CEREUS* ISOLATES FROM THE DAIRY INDUSTRY

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Automated ribotyping using the RiboPrinter® Microbial Characterization System (Qualicon, Inc.) proved to be a useful, standardized and quick method to discriminate between *Bacillus cereus* strains. Discrimination by automated ribotyping and random amplified polymorphic DNA (RAPD) was compared for 40 different *B. cereus* isolates, four different *B. mycoides* isolates and six culture collection strains. RAPD was only slightly more discriminatory than automated ribotyping. On the basis of automatic ribotyping using the restriction enzymes *Eco*RI and *Pvu*II, the *B. cereus* and *B. mycoides* isolates/strains were separated into 36 different ribotypes. RAPD typing with two single primer reactions generated 38 different RAPD-profiles. Both analysis methods showed that 17 isolates were actually 8 unique strains. In all but one case, the isolates with the same pattern were isolated from the same dairy. The densitometric profile for automatic ribotyping seems to cluster the separate dairies more tightly than the RAPD-typing.

(T22) USE OF AUTOMATED RIBOTYPING TO TRACE SOURCES OF *PSEUDOMONAS* IN A READY-TO-EAT FOOD PRODUCT

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The aim of this study was to use genetic fingerprinting of bacteria in detecting routes of contamination. The process studied was manufacturing of ready-to-eat pea soup packed in a plastic film tube sealed with metallic clips. To test the tightness of the seals, a culture of easily

detectable bacteria was applied outside the metallic clips on 21 packages. When the contents were analyzed, these bacteria were found in every package tested. Accordingly, microorganisms may penetrate the packages during the chilling and transport process. An analysis pointed out several steps after packaging as critical to the microbial status of the product. Some of these steps totally lack preventative measures. A microbial analysis showed that the dominating spoilage organisms found in the product belonged to the genus *Pseudomonas*. The results also suggested that these bacteria entered the product after the packaging step. Therefore *Pseudomonas* were chosen as the indicator organisms in the process. To investigate routes and sources of contamination, genetic fingerprinting of 52 different strains of *Pseudomonas* found in products and plant environment was done using the RiboPrinter® Microbial Characterization System (Qualicon, Inc.). Thirty-nine ribotypes were defined showing a broad diversity and potentially many contamination sources of *Pseudomonas* in the plant. Isolates within the same ribotype were found in products processed on different dates and in different products manufactured on the same process line. Identical strains were also isolated from packed product and the air in the packaging room. These facts suggest that there are remaining hygiene problems with strains established in the plant and that strains in the plant environment may contaminate the product.

(T23) PASTEURIZATION PROCESS FOR DAIRY PRODUCTS

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A continuous process is provided for the pasteurization of dairy products including milk, cream, and yogurt mixes. A special feature of the process is the use of a holding tube that incorporates a static mixer. Thereby improved control is obtained over the residence time of the dairy product under pasteurization conditions. The static mixing device evens out the flow rates between the fastest moving particles of dairy product and the slowest moving particles. By providing improved control over the pasteurization process such a static mixer will assure the complete destruction of harmful microorganisms and at the same time reduce the development of off-flavors and other undesirable properties. This pasteurization process is described in greater detail in U.S. Patent No. 5,266,343.

(T24) DEVELOPMENT OF WHEY BEVERAGE USING LACTIC ACID BACTERIA

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A process was standardized to develop whey beverage without (100:00) and with skim (50 : 50) using strains of *Lactobacillus acidophilus* NCDC-15, *L. casei* NCDC-12 and *L. casei* RTS. *L. acidophilus* NCDC-15 with *L. casei* NCDC-12 produced highly acceptable whey beverage ($P<0.05$) by sensory panels with 5% sugar which had antagonistic properties against foodborne pathogenes such as *E. coli, Klebsiella phenumeniae, Salmonella typhi, Staphylococcus aureus* and showed maximum tolerance at pH 4.0 and in presence of 0.5 percent bile salt. Growth characteristics of selected strains of *Lactobacillus* alone and in combination exhibited highest lactic acid (0.80 to 1.86%) and rate of acid production (1.90 to 8.25 UM/sec), maximum viable counts (1.21 to 3.15×10^8 CFU/ml) and acetaldehyde production (0.34 to 1.31 ppm) during 16 h incubation at 37± 1°C.

(T25) EVALUATION OF THE FOOD SAFETY NETWORK AS AN EDUCATIONAL TOOL

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Anecdotal evidence suggests that the Food Safety Network (FSnet), established in 1995, is an effective educational tool, exposing students and others to current trends in microbial food safety. A preliminary analysis of four undergraduate courses involving some 200 students at the University of Guelph was undertaken to better quantify the role of FSnet and the Agriculture Network (AGnet) in modulating the perceptions of undergraduate students receiving the daily FSnet and AGnet postings. Results suggest that while course instructors value the availability of daily updates, individual students were overwhelmed by the breadth of information, responding more to the professor's instruction than to material posted as a daily electronic mail message. Future research will explore the value of providing a web-based repository of information, in conjunction with the timeliness of daily postings, to better meet the needs of time-constrained students.

(T26) AN EVALUATION OF FOOD SAFETY NETWORK (FSNET) AS A RISK ANALYSIS TOOL

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It has been previously demonstrated that the formation of public perception regarding microbial foodborne risks is strongly related to on-going media coverage. The Food Safety Network (FSnet) was established in 1995 to provide current, generalized food safety information to a wide audience. To better understand the formation of risk perceptions, two surveys of FSnet recipients were conducted, one in 1996 and one in 1997. Results demonstrate that FSnet is regarded as a useful tool to manage risks, providing recipients with a much broader notion of foodborne risk and helping in the formation of risk messages.

(T27) THE PROVISION AND EVALUATION OF DAILY ELECTRONIC INFORMATION SUMMARIES TO IDENTIFY PUBLIC AND SCIENTIFIC ANIMAL AGRICULTURAL ISSUES WARRANTING RISK ANALYSIS ACTIVITIES: THE ANIMAL NETWORK

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The Animal Network was established in September 1997, based on previous experience with the Food Safety Network (FSnet), and subsequently analyzed to determine if there was a need for the daily provision of journalistic and scientific information regarding animal-related risks. A preliminary analysis based on quantitative assessment of the stories collected, anecdotal evidence, and a survey of AnimalNet recipients has found that such a service can be used as a tool to help manage animal-related risks. Case studies regarding avian influenza, *Pfisteria* and animal biotechnology will be discussed along with lessons for good risk communication and management.

(T28) FOOD SAFETY AND WATER SANITATION IN CAMBODIA AND CHINA

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The national systems in Cambodia and China for surveillance of diarrheal disease, conducting food safety, and supplying potable water were assessed on behalf of the WHO. In Cambodia, the Ministry of Health is being developed after a long period of political uncertainty. There are limited laboratory facilities for determining the burden of diarrheal disease and few agents are isolated except for cholera, dysentery and typhoid fever. Street vendors supply the cheapest foods but the preparation and serving of these is under poor sanitary conditions. Chlorination of drinking water is not generally available and bottled water is drunk by tourists and many residents. In China, water is also a major problem because of the high pollution in the rivers, and bottled water of different types and quality is widely distributed. In Beijing, good quality food is plentiful and street vendors are strictly controlled. Even though there is a good electronic reporting system for disease in the country, only the well-known diarrheal diseases and large foodborne outbreaks are recorded. Recommendations for both countries included a better knowledge of the agents causing foodborne and waterborne disease, an assessment of the quality of bottled water, the contamination rate of rodents for specific pathogens, improvement of laboratory facilities, the extent of antibiotic resistant pathogens and implementation of HACCP. In addition, an increasing awareness of the need for risk assessment was proposed. The disease status in developing countries is important for industrialized countries also, because of trade issues, tourism and the possible spread of infectious agents beyond borders.

(T29) COMPARISON OF CHEMICAL TREATMENTS TO ELIMINATE *E. COLI* O157:H7 ON ALFALFA SEEDS

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Consumption of alfalfa sprouts was associated with two outbreaks of *E. coli* O157:H7 infection in 1997. This study was conducted to determine the efficacy of several food-grade chemicals in reducing populations of *E. coli* O157:H7 on alfalfa seeds destined for sprouting. The following treatments were applied to alfalfa seeds inoculated with 2.79 to 3.35 log_{10} CFU of *E. coli* O157:H7/g: Acidified $NaClO_2$ (Alcide Corp. Redmond, WA) (500, 850, 1200 ppm) for 0.5 and 2 min; $Ca(Ocl)_2$ and NaOCl (200, 500, 1000, 2000 ppm), C_2H_3OH (30, 70%), and H_2O_2 (0.2, 1, 2, 5, 8%), all for 3 and 10 min. Significant ($P \leq 0.05$) reductions in the number of viable cells of *E. coli* O157:H7 were observed using

acidified $NaClO_2$ at ≥ 500 ppm for 0.5 and 2 min, $Ca(OCl)_2$ and NaOCl at ≥ 1000 ppm for 3 and 10 min, C_2H_3OH at ≥30% for 3 and 10 min, and H_2O_2 at ≥0.2% for 3 and 10 min. Despite reductions in population of *E. coli* O157:H7, no treatment eliminated the pathogen from alfalfa seeds. Germination percentage was significantly ($P \leq 0.05$) reduced by treatment with ≥30% C_2H_3OH for 3 and 10 min or by 2000 ppm $Ca(OCl)_2$ for 10 min. Acidified $NaClO_2$ and H_2O_2 show greatest potential as chemicals to reduce populations of *E. coli* O157:H7 on alfalfa seeds without detriment to germination.

(T30) SENSITIVITY OF *E. COLI* O157:H7 TO STORAGE IN FROZEN APPLE JUICE

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Short-term frozen storage of fresh juice is a common and accepted practice among small California apple juice processors. *E. coli* O157:H7 survives well in frozen meats but little is known about its survival in frozen acidic environments. Fresh apple juice (pH 3.6 or 3.9 to 4.1) was inoculated (10^7 CFU/ml) with *E. coli* O157:H7 ATCC 43890. In some cases the juice was neutralized to pH 7.0 with NaOH prior to inoculation. Samples (10 ml) were stored under frozen (-18°C) or refrigerated (+4°C) conditions for 14 days. Cell populations were determined at regular intervals by plating onto tryptic soy agar (TSA) or sorbitol MacConkey agar (SMA). When refrigerated, populations of *E. coli* O157:H7 in the juice remained unchanged (pH 3.9 to 4.1) or steadily decreased by 2 log cycles (pH 3.6) during the 14-day storage period. Colony counts on both TSA and SMA were similar indicating that minimal sublethal injury had occurred. During frozen storage, populations remained unchanged (pH 7.0), decreased by 1 log cycle (pH 3.9 to 4.1) or decreased by 4 log cycles (pH 3.6). The greatest population decrease was observed within 24 h of frozen storage. Significant cell injury also occurred in the acidic samples. Counts on SMA were < 1 log cycle (pH 7.0) or 1 to 2 log cycles (pH 3.6 or 3.9 to 4.1) lower than counts on TSA within the first 24 h of storage. These data suggest that short-term frozen storage may enhance the safety of some juices.

(T31) EFFECT OF BRIEF BLANCHING TREATMENTS ON THE MICROFLORA OF FRESH CUCUMBERS

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The objective of this research was to determine the effects of brief blanching treatments on microbial populations of whole, fresh, cucumbers, which have been shown to vary between 10^3 and 10^8 CFU/g. Cucumbers were blanched by submerging in water at various temperatures between 55 and 95°C. The cucumbers were then homogenized in 0.85% saline and microflora enumerated by spiral plating on agar media: PCA (total aerobes), VRBG (*Enterobacteriaceae*), YM (yeasts and molds), and modified MRS [containing 0.02% sodium azide, for lactic acid bacteria (LAB)]. Blanching for 15 seconds at 75°C was sufficient to reduce the total aerobe count from 10^6 to 10^3 CFU/g, and blanching at 95°C for 15 seconds did not significantly reduce the population further. Aerobic spore counts determined before and after blanching treatments remained unchanged at 10 to 20 CFU/g. These data suggest the surviving bacteria were predominantly vegetative cells, presumably protected from the heat treatment in the interior of the fruit. When fruit blanched at 80°C for 15 seconds were brined (2% NaCl) and refrigerated (5°C), LAB reached 10^7 CFU/g in 25 days, while unheated controls required only 15 days to reach this level. Optimization of blanching treatments in combination with addition of LAB as protective biocontrol cultures may prove useful in increasing shelf life and safety of minimally processed vegetable products.

(T32) ALLYL ISOTHIOCYANATE AS A PRESERVATIVE IN NON-ACIDIFIED, REFRIGERATED, PICKLED VEGETABLES

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The objective of this research was to explore the application of allyl isothiocyanate (AITC) as a natural preservative for non-acidified, refrigerated pickled vegetables. Fresh pickling cucumbers were brined (2% NaCl, pH 6.6), supplemented with 0 to 200 ppm AITC contained in mustard oil, and

refrigerated (4°C). The effects of AITC concentration and storage time on changes in the natural microbial population [total aerobes, *Enterobacteriaceae*, lactic acid bacteria (LAB)], pH and flavor acceptability were determined. Shelf life (days before visual turbidity due to microbial growth) of the product ranged from 11 days (no AITC) to 33 days (100 ppm AITC). Numbers of all groups of bacteria were reduced at 60 to 100 ppm AITC. However, *Enterobacteriaceae* counts were most affected, with rate of survival being reduced significantly at 30 ppm AITC and more dramatically at 60 and 100 ppm. LAB eventually increased in number in the presence or absence of AITC, and a reduction in pH resulted. Flavor threshold of AITC in the product was 2 to 8 ppm; and acceptability level among panelists varied widely, but averaged a maximum of 30 ppm. AITC may prove useful as a natural food preservative in products where its sensory acceptability and levels required an antimicrobial activity are compatible. AITC appears to be particularly effective as an antimicrobial to *Enterobacteriaceae.*

(T33) DEPOSITION OF SALMONELLAE FROM SOIL AND BLOSSOMS INTO INTERNAL TISSUE OF TOMATOES

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Experiments (exp) were conducted to determine if salmonellae inoculated onto blossoms or into soil would lead to contamination of the internal tissues of cherry tomato cultivars VFNT (exp 1) or Husky Red (exp 2). At onset of blossom formation, plants were divided into 4 treatment groups (4 plants/group) based on placement of *Salmonella* inoculum: 1. blossoms only, 2. soil only, 3. blossoms and soil, or 4. none (control). A composite suspension (10^7 CFU/ml) of *Salmonella enteritidis, S. typhimurium, S. mission, S. montevideo, S. poona, S. hartford, S. newport,* and *S. stanley* was used to spray blossoms daily (25 ml/plant) for 4 weeks, whereas diluted feces (10^2 to 10^4 CFU *Salmonella*/ml) from chickens shedding *S. enteritidis, S. typhimurium,* and *S. mission* was applied to soil (400 ml/plant) at 2 wk intervals for 6 wk. Two wks post-inoculation, 15 tomatoes from each group were collected, rinsed in 1:10 Clorox™, rinsed in ethanol, and flamed (exp 2 only). Internal contents (25 g) were aseptically collected and assayed for presence of *Salmonella* using enrichment-selective plating procedures. In exp 1, *Salmonella* was detected in tomatoes from all treatment groups, but not in fruit from the control plants. In exp 2, tomatoes from plants in which the blossoms only were inoculated yielded *Salmonella*, while all other samples were negative. Although extent of internal contamination varied between exps and appeared to be affected by cultivar and other variables, data suggest that environmental exposure to salmonellae can lead to internal contamination of tomatoes.

(T34) OUTGROWTH OF *BACILLUS COAGULANS* IN VARIOUS TOMATO PUREES AS AFFECTED BY PH AND ACIDITY

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The influence of pH and acidity on outgrowth of *Bacillus coagulans* spores in tomato puree was investigated. Tomato purees containing 5, 10 or 15% tomato soluble solids (TSS) were prepared from canned tomato paste and pH was adjusted to 4.0, 4.25, or 4.5 with 1 N citric acid or 1 N KOH; titratable acidity of each combination was determined. Fifty grams of each puree was weighed into sterile centrifuge tubes, preheated to 90.6°C, inoculated with 10^3, 10^1, or 10^0 CFU *B. coagulans* spores/g of puree and held for 3 minutes. Samples were immediately cooled in running water and incubated at 35° or 55°C for 0, 1, 2, 3, and 4 weeks. Uninoculated samples (control) were likewise prepared. Regardless of solids content, *B. coagulans* grew in purees (pH 4.5) inoculated with 10^3 and 10^1 CFU/g within 2 weeks storage at 35°C; growth also occurred in puree (5 and 10% TSS, pH 4.25) with 10^3 CFU/g within 3 weeks at 35°C. Tomato purees containing 5% TSS, pH 4.5 or 4.25 also supported growth at all inoculum levels when stored at 55°C for 3 weeks. *B. coagulans* was not isolated from any of the uninoculated samples. Growth did not occur in purees adjusted to pH 4.0 and/or titratable acidities >0.65% (as citric acid). Results suggest that acidification to pH 4.0 or titratable acidity greater than 0.65% (as citric acid) will confer shelf stability of tomato products.

SYMPOSIA

(S1) BASIC DAIRY FIELD WORKSHOP — PART I

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Basic Dairy Field Workshop Parts I and II are designed to provide information for new employees working as regulatory dairy farm inspectors or industry dairy field representatives. Topics covered will include an in-depth discussion of dairy farm sanitary regulations including use of the dairy farm inspection sheet, somatic cell issues, drug residues, industry quality control issues, waste management and hauling.

(S2) CHANGE—AND UNINTENDED MICROBIAL CONSEQUENCES — ALONG THE FARM TO FORK CONTINUUM

D. Zink, Nestlé, USA, Inc., 800 North Brand Blvd., M/S QM-15, Glendale, CA, 91203, U.S.A.; E. S. Garrett, National Marine Fisheries Service, NOAA, DOC, P.O. Drawer 1207, Pascagoula, MS, 39568-1207, U.S.A; J. Wilesmith, Central Veterinary Laboratory, Weybridge, New Haw, Addlestonse, Surrey KT15 3NB, United Kingdom; K. Sellner, National Oceanographic and Atmospheric Administration, SSMC3, Room 9752, 1315 East-West Highway, Silver Spring, MD, 20910, U.S.A.; and B. Herwaldt, U.S. Centers for Disease Control and Prevention, 4770 Buford Highway, MS F22, Atlanta, GA, 30341-3724, U.S.A.

Food safety has become a topic of considerable and increasing interest in many sectors including industry, government and public health. In the past several years, new diseases or newly emergent pathogens have appeared, presenting challenges for traditional approaches to disease control and regulatory frameworks. As more has been learned about these new pathogens, a recurring theme has begun to be recognized — namely, that changes anywhere along the farm to fork continuum may promote undesirable microbial consequences by creating unique opportunities for the emergence of old or new pathogens. The purpose of this symposium is to explore recent examples of this theme. A historical perspective on past foodborne microbial hazards will provide a backdrop against which to view current events. The globalization of the food supply now presents the means by which formerly "exotic" pathogens such as *Cyclospora* may be transmitted to non-immune populations far removed from the pathogen's geographic source. Alterations in rendering practices for preparation of animal feeds are a current focus of study on the circumstances surrounding the evolution and emergence of "mad cow disease" or Bovine Spongiform Encephalopathy (BSE). A relatively new industry, aquaculture, can be associated with shrouded public health hazards whose relationship to aquaculture has only recently been realized. The fish pathogen, *Pfiesteria*, is the latest newcomer to this roster of emerging pathogens and the current understanding of its role in disease and its ecological niche will be reviewed. A roundtable discussion will provide an opportunity for questions and discussing new observations.

(S3) SEAFOOD HACCP REFLECTIONS AFTER IMPLEMENTATION

D. DeVlieger, FDA, P.O. Box 3012, Bothell, WA 98041 U.S.A.; J. Johnson, Jr., Washington Crab Co., Inc., P.O. Box 2132, Washington, D.C. 27889, U.S.A.; R. Jones, Rich Seapak Corp., P.O. Box 20670, Airport Road, St. Simons Island, GA 31522, U.S.A.; R. Price, Food Science and Technology, University of California, Davis, CA 95616, U.S.A.; S. Otwell, University of Florida, Aquatics Products Program, P.O. Box 110370, Gainesville, FL 32611, U.S.A.; D. Ward, Dept. of Food Science, North Carolina State University, P.O. Box 7624, Raleigh, NC 27695, U.S.A.; J. Burkholder, Dept. of Biological Sciences, North Carolina State University, Raleigh, NC 27695, U.S.A.

This symposium is designed to reflect on the HACCP experience and observations of Federal Regulators, the small and large affected industry, and the university sector that assisted in its implementation and training. Speakers will address what impacts the federal mandate had on small family-owned seafood processors, was it worth their efforts to comply? Does HACCP ensure food safety? Are relationships between regulators, industry and consumers strengthened by this regulation? What follow-up training was needed by processors employing large numbers of workers? What changes needed to be made after the first formal inspection? What has been the experience of using the World Wide Web (WWW) to exchange information via the seafood@ucdavis.edu network? What role did the Seafood HACCP Alliance play in

training and what of future needs? These questions will be addressed during this symposium.

(S4) A SYMPOSIUM ON FRESH-CUT PRODUCE: FIELD SANITATION, PACKAGING, MICROBIOLOGY, CONTROL, PROGRAMS AND REGULATIONS — PART I

J. Farber, Health Canada, Microbiology Research Division, Postal Locator 2204A2, Barring Bldg., Tunney's Pasture, Ottawa, Ontario K1A 0L2, Canada; L. J. Harris, Dept. of Food Science and Technology, University of California, Davis, CA 95616-8598, U.S.A.; J. Ferrar, California Dept. of Health Services, Food and Drug Branch, P.O. Box 942732, MS-357, Sacramento, CA 94234-7320, U.S.A.; A. Hathcox, Cryovac GRACE Packaging, Cryovac North America, W. R. Grace and Co., 100 Rogers Bridge Road, Bldg. A., Duncan, SC 29334, U.S.A.; F. Pabrua, California Strawberry Commission, P.O. Box 269, Watsonville, CA 95077, U.S.A.

The fresh-cut produce industry has grown tremendously in the last five years, with more and more consumers being exposed to this type of product. In the past, the produce industry has not been highly regulated because traditionally fresh whole produce has been viewed as a safe product. Fresh-cut produce, however, which are usually cut, washed and then packaged, may present a possible safety risk. The microbiological safety of imported products has also been questioned. The microorganisms of concern not only include bacteria such as *Listeria monocytogenes*, non-proteolytic strains of *Clostridium botulinum*, and *Escherichia coli* O157:H7, but also viruses and protozoan parasites.

Control of the safety of fresh-cut produce starts at the field level and much more attention is now being paid to the development of safe and efficient field sanitation programs. One important critical control point is temperature control throughout the whole food chain. Inside the processing plant, strict attention to sanitation practices and an effective disinfectant wash water stage is essential. HACCP programs have emerged in both Canada and the United States. Novel combination type treatments are needed which can provide greater assurance of the safety of these products.

President Clinton announced in 1997 his plans to further ensure the safety of the U.S. food supply. As a part of this initiative, directives were given to issue guidance on good agricultural practices and good manufacturing practices for fruits and vegetables. As a result of this, produce safety guidelines have been drafted by several organizations and agencies.

This symposium will take a farm-to-fork approach and attempt to assimilate the latest information existing on fresh-cut produce in regards to field sanitation, microbiology, control, product packaging, produce industry safety programs, as well as industry and government produce guidelines. Panel discussions held at the end of each half-session will attempt to summarize current opinions and discuss issues of mutual concern.

(S5) BASIC DAIRY FIELD WORKSHOP — PART II

P. Dersam, Upstate Milk Coop, 27 Sullivan Road, Alden, NY 14004, U.S.A.; R. T. Burns, University of Tennessee, Knoxville, TN, 37996, U.S.A.; R. Nordtvedt, Land O'Lakes, Inc., 4001 N. Lexington Ave., Arden Hills, MN 55126-2998, U.S.A.; N. Linebaugh, Dairy Farmers of America, 8257 Dow Circle, Strongsville, OH 44136-9717, U.S.A.; and C. Price, FDA, 20 N. Michigan Ave., Room 510, Chicago, IL 60602, U.S.A.

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(S6) FARM TO TABLE: ECOLOGY OF PATHOGENS ASSOCIATED WITH POULTRY

M. E. Berrang, Russell Research Center, P.O. Box 5677, Athens, GA 30604-5677, U.S.A.; D. Conner, 245 Animal Sciences Bldg., Dept. of Poultry Science, Auburn University, Auburn, AL 36849-5416, U.S.A.; S. Craven, Russell Research Center, P.O. Box 5677 Athens, GA 30604-5677, U.S.A.; S. Russell, Livestock Poultry Bldg., Dept. of Poultry Science, University of Georgia, Athens, GA 30602, U.S.A.; S. Shane, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803, U.S.A.; and D. Swayne, Southeast Poultry Research Laboratory, 934 College Station Road, Athens, GA 30677, U.S.A.

With the 1998 implementation of HACCP by the USDA Food Safety Inspection Service and the recent outbreak of Hong Kong Flu in chickens, people are probably more aware and concerned about the safety of the foods they eat now than ever before. Poultry has been a food of concern because several human foodborne pathogens have

been associated with this raw food product. The purpose of this symposium is to discuss the Hong Kong flu virus as well as bacterial pathogens in poultry including: *Aeromonas, Campylobacter, Clostridium perfringens, E. coli, Listeria, Salmonella,* and *Yersinia*. The following aspects will be discussed for each pathogen: (1) How does the microorganism adversely affect the chicken? (2) How does the microorganism affect people when the product is mishandled? (3) Have the various sources of the microorganism into the production of the live animal or subsequent processing been identified? (4) Are there any intervention strategies either in place or proposed and how effective might they be? Additionally there will be a presentation on the effectiveness of indicator organisms with the various foodborne pathogens.

(S7) THE LEADING EDGE OF FOODBORNE DISEASE SURVEILLANCE

J. J. Guzewich, FDA, CFSAN, 200 C St. S.W., Washington, D.C. 20204, U.S.A.; T. Barrett, Foodborne and Diarrheal Disease Branch, Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA 30333, U.S.A.; D. Voetsch, Foodborne and Diarrheal Diseases Branch, Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA 30333, U.S.A.; P. Sockett, Division of Disease Surveillance, Bureau of Infectious Diseases, Laboratory Center for Disease Control, Health Protection Branch, Health Canada, 3rd Floor LCDC Building, Tunney's Pasture, Ottawa, Ontario, K1A 0L2 Canada

Surveillance has been the workhorse of foodborne disease control and prevention over many decades, but the passive systems in most countries are inadequate today to give the answers we need. In 1997, authors Guzewich, Bryan, and Todd prepared a series of four review papers on foodborne disease surveillance to improve the existing system at the state/province and national levels. Three countries are introducing programs to explore more useful ways of collecting data. In the U.K., a 3-year enteric study has thrown light on actual consumer practices that have led to disease. This is somewhat similar to the U.S. FoodNet program, which has been expanded to several specific studies since it began in 1995. In Canada, an Information Highway is in the initial stages where surveillance information can be transmitted in real time by electronic means; communications companies in collaboration with provincial and public health personnel are leading the project. All these areas will be reviewed and the latest findings presented at the symposium along with a panel for questions to the participants.

(S8) A SYMPOSIUM ON FRESH-CUT PRODUCE: FIELD SANITATION, PACKAGING, MICROBIOLOGY, CONTROL, PROGRAMS AND REGULATIONS — PART II

L. Bell, Fresh Express Farms, 607 Brunken Ave., Salinas, CA 93902, U.S.A.; J. Roberts, DFL Laboratories, 1548 Cummins Dr., Modesto, CA 95358-6412, U.S.A.; S. Zawel, United Fresh Fruit and Vegetable Association, 727 No. Washington St., Alexandria, VA 22314, U.S.A.; L. A. Jackson, FDA, 200 C St. S.W., Washington, D.C. 20204, U.S.A.; E. Garrett, International Fresh-Cut Produce Association, 1600 Duke St., Suite 440, Alexandria, VA 22314, U.S.A.; E. Forman, USDA, Agricultural Marketing Service, Fruit and Vegetable Division, Washington, D.C., U.S.A.

The fresh-cut produce industry has grown tremendously in the last five years, with more and more consumers being exposed to this type of product. In the past, the produce industry has not been highly regulated because traditionally fresh whole produce has been viewed as a safe product. Fresh-cut produce, however, which are usually cut, washed and then packaged, may present a possible safety risk. The microbiological safety of imported products has also been questioned. The microorganisms of concern not only include bacteria such as *Listeria monocytogenes*, non-proteolytic strains of *Clostridium botulinum, and Escherichia coli* O157:H7, but also viruses and protozoan parasites.

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information existing on fresh-cut produce in regards to field sanitation, microbiology, control, product packaging, produce industry safety programs, as well as industry and government produce guidelines. Panel discussions held at the end of each half-session will attempt to summarize current opinions and discuss issues of mutual concern.

(S9) CURRENT PERSPECTIVES ON THE USE OF ANTIBIOTICS IN ANIMAL PRODUCTION SYSTEMS

S. Thompson, Center for Veterinary Medicine, FDA, 7500 Standish Place, HFV-1, Rockville, MD 20855, U.S.A; P. Fedorka-Cray, Agricultural Research Service, USDA-ARS-RRC-PMSRU, 950 College Station Road, Athens, GA 30605, U.S.A.; F. Angulo, National Center for Infectious Diseases, Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA 30333, U.S.A.; J. Threlfall, Central Public Health Laboratory, 61 Colindale Avenue, London, NW9 5HT, United Kingdom; and R. Carnevale, Animal Health Institute, 501 Wythe St., Alexandria, VA 22314-1917, U.S.A.

The development of antibiotic resistant bacteria is of increasing concern to physicians and public health officials around the world. The recent development of the multiple antibiotic resistant *Salmonella typhimurium* DT 104 and the proliferation of this organism in different countries along with increasing evidence of resistance to fluoroquinolones has led to added pressures to reduce antimicrobial usage in animal production systems. These and related issues will be examined in depth by speakers from the U.S. FDA, the U.S. Centers for Disease Control and Prevention, the Public Health Laboratory Service in Great Britain, and the U.S. Department of Agriculture, and Animal Health Institute.

(S10) PEST MANAGEMENT AS WE APPROACH 2000

J. Sargent, Copesan Services, Inc., 3490 N. 127th St., Brookfield, WI 53005, U.S.A.; K. Kelley-Tunis, McCloud Pest Control, 1811 Executive Dr., Indianapolis, IN 46241, U.S.A.; T. Bruesch, Lipha Tech, Inc., 1721 Taylor Lane, West Bend, WI 53095, U.S.A., O. Dosland, Copesan Services, Inc. 3490 N. 127th St., Brookfield, WI 53005, U.S.A.; M. Gushwa, Nestlé USA, Inc., 800 North Brand Blvd., Glendale, CA 91203, U.S.A.

Pests have caused contamination, disease transmission and loss of product forever. However, the ways in which we prevent and control them has changed, and continues to change, as we approach the millennium.

Today's pest management industry has an ever expanding arsenal of weapons to use in our battle against pests. From ants to rodents to roaches to stored product pests, this symposium will discuss many of these changes and what to look for in the future. Dr. Jim Sargent, Copesan Services, Kim Kelley-Tunis, McCloud Pest Control and Ted Bruesch, Lipha Tech will each discuss new products and techniques in controlling pests.

One strong example of the changes taking place in the industry is the current consumer attitude toward chemicals, along with continuing restrictions, which have provided a challenge in controlling stored product insects in food plants without fumigants (i.e. methyl bromide). The use of heat is a feasible treatment in some applications. What are parameters that maximize control effectiveness of stored product insects? Ole Dosland, Copesan Services will discuss the findings of his research studies in the area.

The symposium will also discuss the client's perspective in choosing and working with a pest management company. What types of characteristics should you be looking for in a pest management company? Marty Gushwa from Nestlé USA will discuss several of the criteria that go into choosing and maintaining a partnership with a pest management service provider.

(S11) VIRAL AND PARASITIC FOODBORNE DISEASE ASSOCIATED WITH PRODUCE: EPIDEMIOLOGY, DETECTION, AND CONTROL

P. Orlandi, FDA, 200 C St. S.W., Washington, D.C. 20204, U.S.A; D. Friedman-Huffman, Dept. of Marine Science, University of South Florida, St. Petersburg, FL 33701, U.S.A.; Y. Hutin, Viral Hepatitis Branch, CDC, MS G37, 1600 Clifton Rd., Atlanta, GA 30333, U.S.A.; S. Sattar, Faculty of Medicine, Microbiology and Immunology, University of Ottawa, 451 Smyth Rd., Ottawa, Ontario, K1H 8M5, Canada; and M. Sobsey, Dept. of Environmental Sciences and Engineering, CB 7400, University of North Carolina, Chapel Hill, NC 27599-7400, U.S.A.

Human enteric viruses and protozoal parasites are significant causes of foodborne disease whose incidence and recognition is increasing. Recent outbreaks of *Cyclospora cayetanensis* and hepatitis A virus associated with the consumption of fresh and frozen produce have been reported and illustrate the importance of produce as a vehicle for the transmission of these agents. Historically, establishing causation in viral and parasitic

disease outbreaks has depended upon epidemiological investigation and detection of the agents in clinical, food and water specimens. However, both epidemiological investigation and traditional methods of detection are cumbersome, expensive, and frequently unavailable or unattempted for the important food and waterborne enteric viruses and protozoa. The recent introduction of regulatory testing mandates, alternative testing strategies, federal food safety initiatives, and increased epidemiological surveillance for food and waterborne disease should significantly improve the ability to detect and control these agents. In this symposium, speakers will address the significance of some of the more recent viral and parasitic foodborne disease outbreaks associated with the consumption of fresh and frozen produce. Others will discuss the importance of emerging technologies for the detection and control of viral and parasitic contamination of foods. Together, this symposium will provide timely information on the role of emerging technologies in the recognition, identification, and control of these important agents of human foodborne disease.

(S12) LIFE IN A FISH BOWL: ESSENTIALS FOR COMMUNICATIONS DURING A FOOD SAFETY CRISIS

S. Conley, USDA-FSIS, 1400 Independence Ave., S.W., Washington, D.C. 20250, U.S.A.; J. Scott, National Food Processors Association, 1401 New York Ave., N.W., Suite 400, Washington, D.C. 20005, U.S.A.; and D. Powell, Dept. of Food Science,University of Guelph, Guelph, Ontario, Canada N1G 2W1

The production of "safe" food using today's technology is not without risks from microbiological, chemical, and physical hazards. Yet we live in a country where consumers expect "safe" food to be totally risk free.

When a hazard is identified in a product, the communication of that risk to the public at large (consumers, Wall Street analysts, heath professionals, international Internet users, etc.) can mean the difference in a company's survival and keeping consumer confidence in a product name.

This symposium will address risk communication from the "real life" perspective of persons involved in food production, trade organizations, and consumer relations. If you ever find yourself communicating the risk of a contaminated product to the community at large, what you learn from this symposium may prove invaluable to yourself and your organization.

(S13) BRINGING SCIENCE TO THE RESTAURANT INSPECTION

G. Barnes, Multnomah County (Portland) Health Dept., 426 S.W. Stark, 3rd Floor, Portland, OR 97229, U.S.A.; O. P. Snyder, Hospitality Institute, 670 Transfer Rd., Suite 21A, St. Paul, MN 55114; D. Maxson, Clark County Health District, P.O. Box 3902, Las Vegas, NV 89127, U.S.A.; G. Warner, Multnomah County (Portland) Health Dept., 426 S.W. Stark, 3rd Floor, Portland, OR 97229, U.S.A.; and F. Bryan, 8233 Pleasant Hill Rd., Lithonia, GA 30058, U.S.A.

Current food service regulations present a web of safety net standards. This approach assumes that operators need not understand the science behind safe food handling. This symposium will present the science behind everyday activities in a food service operation. The closing panel will offer suggestions on how regulations could be modified to support the scientific basis of safe food handling.

(S14) COMPUTERIZED PROCESS CONTROL AND RECORD KEEPING IN THE DAIRY INDUSTRY

R. Coutlee, Dean Foods Technical Center, P.O. Box 7005, Rockford, IL 61125-7005, U.S.A.; W. Wilson, Anderson Instruments Co., 156 Auriesville Rd., Fultonville, NY 12072, U.S.A.; K. Anderson, Harold Wainess and Associates, 464 Central Ave., Northfield, IL 60093, U.S.A.; J. E. Schlesser, FDA, NCFST, 6502 South Archer Rd., Summit, IL 60501, U.S.A.; and S. T. Sims, Milk Safety Branch (HFS-626), FDA, 200 C St. S.W., Washington, D.C. 20204, U.S.A.

In 1988, the Milk Safety Branch of the U.S. FDA issued Memorandum M-I-88-11, which outlined the requirements for computer-based control and recording systems for the pasteurization of dairy products. Criteria for computerized process control were listed to determine compliance with Item 16p of the Pasteurized Milk Ordinance (PMO). The dairy industry and the regulatory agencies used these criteria to promote uniformity of equipment and promote compatibility of inter-related components. Circular or strip charts were still required to record and store the safety data for computer controllers. Electronic review of the safety data is potentially more accurate, more complete, and faster than visual review. However, little has been published on the accuracy and reliability of these monitoring systems, and therefore, they have not gained widespread acceptance by public health authorities.

At the 27th Meeting of the National Conference on Interstate Milk Shipments, provisions for alternative record keeping of the pasteurization process were considered. These provisions were accepted by the delegates at the conference and incorporated into the PMO, 1997 revision. One proposed alternative record keeping method is computerized record keeping. The information stored in this format must be shown to be equivalent to that currently monitored and recorded on the circular or strip charts. This symposium will review current techniques, and the research conducted in this area. Compliance of the computerized process control and record keeping with the PMO will be addressed. Topics covered will include: Plant Modernization with Computerized Process Control; Proper Maintenance and Calibration of Electronic Instruments; HACCP-based Monitoring in the Dairy Plant; Acquisition, Storage, and Review of Safety Data from a High Temperature, Short Time Pasteurization System; and Regulations for Computerized Process Control and Record Keeping.

(S15) FACTORS AFFECTING BACTERIAL ATTACHMENT TO MEAT SURFACES

J. S. Dickson, Iowa State University, Dept. of MIPM, 207 Science I, Ames, IA 50011, U.S.A.; T. McMeekin, School of Agricultural Science, University of Tasmania, GPO Box 252C, Hobart, Tasmania, 7001, Australia; J. W. Arnold, Russell Research Center, P.O. Box 5677, Athens, GA 30604-5677, U.S.A.; G. R. Siragusa, USDA-ARS, U.S. Meat Animal Research Center, Box 166, Clay Center, NE 68933, U.S.A.; J. Frank, University of Georgia, Dairy Science Building, Athens, GA 30602, U.S.A.; and W. J. Dorsa, John Morrell & Co., 805 E. Kemper Rd., Cincinnati, OH 45246-2515, U.S.A.

The recent focus on food safety issues and recalls of fresh meat products due to bacterial contamination underscores the need to review relevant issues associated with bacterial attachment of meat surfaces and to present strategies to alter, prevent, or evaluate attachment in order to reduce bacteria on meat surfaces. Bacterial attachment to meat surfaces may be mediated by one or a combination of factors including hydrophobicity, cell surface charge, bacterial appendages, surface composition, entrapment within the tissue or a superficial water film, reversible/irreversible binding, or interaction with connective tissue components. Following initial bacterial contamination, consolidation and colonization of meat surfaces occurs, giving rise to biofilms. Colonization requires matching the physiological characteristics of bacteria with properties of the meat surface and factors such as temperature, water activity and pH. These entities determine the composition and rate of development of the biofilm and thus, product shelf life and potential safety problems of meat products.

Food plant sanitation methods rely on physical and chemical treatments for decontamination of bacteria or biofilms from meat or food contact surfaces. Because of the nature of bacterial attachment and biofilm formation, not all interventions, cleaners, or sanitizers are effective. Therefore, the microbiological quality and safety of meat should be enhanced by improving upon existing interventions, implementing multi-hurdle approaches, or identifying novel compounds or technologies. Through the use of bioluminescent organisms and biophotonics, researchers can study interventions and physiology of bacteria on meat surfaces in real time without requiring exogenous sampling of tissue. Another technique, confocal scanning laser microscopy, provides a means to directly observe and differentiate the various interactions of bacteria on fully hydrated and relatively undisturbed meat surfaces. Finally, the implications of excision and sponge sampling techniques are relevant in recovering attached bacteria from meat surfaces.

(S16) ILSI NORTH AMERICA-SPONSORED RESEARCH UPDATE

C. Brown, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, U.S.A.; D. D. Hancock, Dept. of Veterinary Clinical Medicine and Surgery, Washington State University, Pullman, WA 99164, U.S.A.; C. Gyles, Dept. of Veterinary, University of Guelph, Guelph, Ontario, Canada N1G 3W4; S. Kathariou, Michigan State University, Plants and Soils Sciences Building, East Lansing, MI 48824, U.S.A.; H. Schraft, Dept. of Food Science, University of Guelph, Guelph, Ontario N1G 2W1, Canada; C. Haas, Drexel University, Building 29W, Philadelphia, PA 19104, U.S.A.; and M. Cassin, Decisionalysis Risk Consulting, Cambridge, United Kingdom

A primary objective of the ILSI North America Technical Committee on Food Microbiology is to promote improved understanding of microbial food safety hazards by sponsoring research. In this symposium, researchers will report the results to date of recent committee-funded projects in the following areas: factors associated with bovine acquisition and elimination of *E. coli* O157:H7,

differentiation of human pathogenic from non-pathogenic strains of *E. coli* O157:H7, *L. monocytogenes* virulence and pathogenicity markers; detection methodology for *Cryptosporidium* in foods; and quantitative microbial risk assessment for human infection with *L. monocytogenes* and *E. coli* O157:H7.

(S17) SYMPOSIUM OF SENSORY CHARACTERISTICS OF DAIRY FOODS

J. C. Bruhn, Dairy Research and Information Center, University of California, Davis, 101B Cruess Hall, One Shields Ave., Davis, CA 95616-8598, U.S.A.; E. Spear, EMS Associates, 4237 Biltmore Dr., Corpus Christi, TX 78413, U.S.A.; R. Bradley, Jr., University of Wisconsin, Babcock Hall, Room A203A, Madison, WI 53706, U.S.A.; M. Bates, Washington State University, WSU Creamery, 101 Food Quality Building, Pullman, WA 99164-6392, U.S.A.; R. Marshall, University of Missouri, Dept. of Food Science and Nutrition, 122 Eckles Hall, Columbia, MO 65211, U.S.A.; and C. M. Bruhn, Center for Consumer Research, University of California, Davis, CA 95616-8598, U.S.A.

The speakers in this unique symposium will discuss the sensory properties of several classes of dairy foods. After an introduction to the principles of sensory evaluations in a commercial setting, symposium speakers will discuss how one evaluates the sensory properties of fluid milks, cottage cheeses, hard and specialty cheeses and frozen dairy desserts, especially frozen yogurts. The concluding speaker will briefly present research highlighting the nutrient content of frozen desserts. The audience will then flavor dairy foods for a hands-on learning experience.

(S18) FOOD WORKER HAND HYGIENE: A FACTOR IN FOODBORNE ILLNESS

B. Bartleson, 2502 Division St. N.W., Olympia, WA 98502, U.S.A.; J. Damaré, Compounds Reg. Branch, FSIS, 300 12th St. S.W., Cotton Annex Building, Washington, D.C. 20250, U.S.A.; M. J. Dolan, GoJo Industries, Inc., 3783 State Rd., Cuyahoga Falls, OH 44223-2698, U.S.A.; N. Segal, Compliance Control Center, 8012 Fernham Lane, Forestville, MD 20747, U.S.A.; and D. L. Charbonneau, Procter and Gamble, Health Care Research Center, 8700 Mason-Montgomery Rd., Mason, OH 45040, U.S.A.

Poor personal hygiene is a factor in 25 to 30% of foodborne disease outbreaks. The goal of this symposium is to engender discussion on the importance of hand hygiene in the food processing and food service industries. The significance of personal hygiene will be introduced by a discussion of hands and the role they play in transmission of foodborne illness. The second discussion will examine the importance of handwashing in light of the Final Rule on Pathogen Reduction and the role of handwashing in a HACCP plan. The next presentation will concentrate on hand disinfectants themselves, what they are, how they are used, and current and propose regulations for these products. Foodworker compliance with proper handwashing guidelines is key to breaking the transmission sequence. Therefore, a presentation will focus on automated handwashing and handwash monitoring systems and their effects on compliance with proper handwash guidelines. A discussion on the usefulness of gloves to improve food safety will be held as they may provide an extra defense against the transmission of foodborne illness by food workers. The final portion of the symposium will be a round table discussion where the audience members will have an opportunity to question the speakers and other attendees on the issue of hand hygiene and foodborne illness.

(S19) MICROBIOLOGICAL ISSUES ASSOCIATED WITH PORK

R. Gamble, USDA-ARS, Building 1040, Room 2 BARC-East, Beltsville, MD 20705, U.S.A.; P. Fedorka-Cray, USDA-ARS-RRC, Russell Research Center, Poultry Microbiology Research Center, 950 College Station Road, Athens, GA 30604-2104, U.S.A.; I. Wesley, USDA-ARS, National Animal Disease Center, 2300 Dayton Road, Ames, IA 50010, U.S.A.; A. M. McNamara, USDA-FSIS-OPHIS, Suite 3714A Franklin Court, 1400 Independence Ave., S.W., Washington, D.C. 20250-3700, U.S.A.; J. Bender, Minnesota Dept. of Health, 717 Delaware St. S.E., P.O. Box 9441, Minneapolis, MN 55440-9441, U.S.A.; and A. Peterson, 11211 Sorrel Ridge Lane, Oakton, VA 22124, U.S.A.

Food safety is a continuum. Therefore, effectively addressing food safety issues requires a partnership among all of the participants in the food chain. This symposium is designed to provide insight into the microbiological concerns facing the pork industry and how they are being addressed from farm-to-table. Microbiological issues for pork range from parasites, which have remained a stigma associated with pork for

hundreds of years, to bacterial pathogens which are garnering more attention and publicity with the consumer and regulatory agencies. Though the prevalence of trichnia in U.S. swine is extremely low and the number of human cases is small, it continues to be a perception concern for U.S. pork. An additional focus has been placed on bacterial pathogens, such as *Salmonella* and *Campylobacter.* The increased prevalence of strains of *Salmonella* demonstrating a unique antimicrobial resistance such as *Salmonella typhimurium* DT 104, are of particular interest with regards to swine as the clinical disease is inapparent in the animal and asymptomatic carriers are more typical than in other animal species. Taxonomically, the RNA Superfamily VI includes the genera *Campylobacter, Helicobacter,* and *Arcobacter* which have all been implicated in cases of human enteritis and associated with hogs and/or hog carcasses.

An increase in the focus on foodborne pathogens has been a result from additional attention from governmental agencies such as USDA and CDC. In a move toward a more science-based inspection program, the results of microbiological testing programs for pork carcasses and more recently for ground pork have led to the development of microbiological performance standards and criteria. Outbreaks of yersiniosis, a pathogen primarily associated with pork products, have come to the attention of the CDC's FoodNet and have been targeted in food safety campaigns designed for specific consumer audiences.

(S20) RISK MANAGEMENT OF FOOD FROM FARM TO FORK

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There is no such thing as "zero risk" when a food is consumed by a person. All that can be done is to reduce/control the risk, in keeping with proper technology, under the surveillance of an effective management system, so that pathogenic substances are at a safe level when consumed. A problem arises that most of the time, a safe level of pathogenic microorganisms or size of physical object is not defined. "Safe" becomes a level/size that has not resulted in observed public health problems in the past. Rather than this approach, risk assessment provides a scientific way of evaluating the probability of disease or illness from a specific food in a specific supply system.

In the past, risk analysis has not normally looked at a whole supply system from farm to fork but at only one step, such as the probability distribution of the organism in the food in its raw state and probability distribution of the resistance of the person who will consume the food. All of the steps from farm to fork are not factored in. The risk management component that should reduce the risk to an acceptable level is often left out.

This seminar will look at the overall farm-to-fork risk analysis including risk management from the perspectives of the foodservice operator, food processor, European food operator and food company, lawyer, and consumer. Since we cannot get to zero risk, deciding on what is an acceptable and unacceptable risk is not easy. This seminar will discuss ways to determine when management can decide that there is no cost/benefit to further trying to reduce a particular risk in a food item being provided to the consumer.

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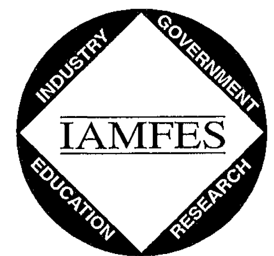

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