111 Ionizing radiation effectively destroys Mycobacterium paratuberculosis in milk. Judith Stabel*[1], Charles Waldren[2], and Frank Garry[2], [1]*USDA-ARS, National Animal Disease Center, Ames, IA,* [2]*Colorado State University, Fort Collins, CO.*

Crohn's disease in humans has strong similarities to Johne's disease in cattle, but with a poorly defined etiology. Mycobacterium paratuberculosis, the causative agent of Johne's disease, has been linked as a causal agent of Crohn's disease in humans. However, evidence to date does not prove that Crohn's disease is caused by M. paratuberculosis and may, in fact, be a multifactorial disorder. Regardless, it has become a concern for the dairy industry as consumer confidence has been influenced by reports that pasteurization may not destroy the bacterium. Efforts to eliminate M. paratuberculosis from the food chain would require rigorous control and management programs for this disease on the farm. Although voluntary programs are in place in many states in the US, it will still be many years before the spread of this disease is attenuated. In the meantime, we must rely on methods that guarantee that potential contaminating agents such as M. paratuberculosis are destroyed in food products post-harvest. Studies to determine optimal methods of pasteurization by heat treatment are underway in many countries, including the US. An alternative method of destruction is the use of ionizing radiation. In the present study, we examined the effects of radiation on milk experimentally inoculated with 10^4 and 10^8 cfu M. paratuberculosis/ml of milk. Milk was exposed to either 0, 5 or 10 kGy of radiation (1.2 kGy/hr) and cultured for viable M. paratuberculosis. Results from this study demonstrate that M. paratuberculosis was effectively destroyed by gamma radiation at either dose. Thus, ionizing radiation may prove to be an alternative method for destruction of potential contaminants in dairy foods.

Key Words: Mycobacterium paratuberculosis, Milk, Radiation

112 Microbiological and Rheological Characteristics and Their Association with Shelf-life of Fresh Soft Goat Milk Cheese. Young W. Park*[1], Aref Kalantari[1], Diane L. Van Hekken[2], and M. H. Tunick[2], [1]*Agricultural Research Station, Fort Valley State University, Fort Valley, GA,* [2]*Eastern Regional Research Center, USDA/ARS, Wyndmoor, PA.*

Microbiological and rheological characteristics of commercial fresh soft goat milk cheeses were evaluated for their food safety and shelf-life for an extended refrigerated storage period. The soft goat cheeses were purchased from a farmstead grade A goat dairy, and stored at 4°C for 0, 7, 14, and 21 days. Microbiological assays for total aerobic, *E. coli* and coliform, yeast and mold, and *Staphylococcus aureus* counts were performed using 3M petrifilm plates techniques according to the manufacturer's recommended procedures. Rheological parameters were determined using a universal testing machine and a small strain oscillatory analyzer. Total aerobic counts of the soft goat cheeses were too numerous to count (TNTC) regardless of treatments and lengths of storage periods, probably due to the lactobacilli counts. The respective ranges of yeast and mold counts were 10^4 -10^6 cells/g and 10^3 -10^4 cells/g cheese, and both counts were increased with storage time. Coliforms, *E. coli* and *S. aureus* were not detectable, while non-coliform organisms such as *Pseudomonas* and many unidentified presumptive *Staphylococcus* species appeared to be present. Rheological properties revealed that cohesiveness of the soft goat cheeses significantly decreased, while viscoelastic properties remained unchanged as aging time progressed. An inverse correlation was observed between the yeast and mold counts and cohesiveness of the tested cheeses. The significant increase in deterioration of the cheese quality at 21 days storage may be attributed to the elevated *Pseudomonas*, yeast and mold counts, which might have accelerated the lipolytic and proteolytic processes in the stored products.

Key Words: Goat, soft cheese, microbiology, rheology, shelf-life

113 Real-time Assessment Of The Microbial Quality Of Fluid Milk Using A Simple Noninstrumental Microrespirometer. Y-H.P. Hsieh*[1], Z. Ren[1], and Y.P. Hsieh[2], [1]*Auburn University, Auburn, AL, USA,* [2]*Florida A & M University, Tallahassee, FL, USA.*

An innovative approach using a recently developed non-instrumental microrespirometer for detection of spoilage bacteria in pasteurized fluid milk was studied. The microrespirometer, which sensitively measures the minute amounts of CO_2 (l/h) evolved by bacterial respiration, efficiently determined the total spoilage bacterial activity in real time (within one hour). The technique was validated using the official Petrifilm total aerobic count (TAC) method in conjunction with the sensory characteristics of the milk sample. In the low microbial growth range (10^2 - 10^3 CFU/ml), the rate of CO_2 evolution was more consistent and sensitive in assessing the microbial quality of the milk than the TAC. The cut-off point for milk suitable for human consumption was set at a CO_2 rate of 25 l/ml/h, corresponding to a viable count approaching 10^7 CFU/ml. This research demonstrated a novel and economic means of improving the speed of microbial detection and ensuring the high quality and safety of perishable foods such as milk.

Key Words: milk, real-time assessment, microbial quality

114 Comparisons of meat carcass surface bacterial collection efficiencies utilizing a novel wet-vacuum Microbial Sampler and the Sponge method. J. Bruce Bradley* and S. Filomena Saddler, *Rocky Mountain Resource Labs, Inc., Jerome, Idaho/USA.*

A novel wet-vacuum sampler, Microbial-Vac^{TM} (M-Vac), currently under development with NIH funding, was compared to the Sponge (SP) method for collection efficiency of surface bacteria off chilled beef carcasses. The M-Vac allows contained application of sterile, food safe Surface Rinse Solution (SRS) and subsequent retrieval of the liquid and suspended microbes off food surfaces. Microbes present in high numbers (APC) may be detected in recovered liquid aliquots (ca 50ml/100cm^2) of SRS. For improved recovery and detection of bacteria present in low numbers, the collected liquid may be filtered through the M-Vac's 0.45μm final filter for rapid or conventional detection and quantification directly on the removable filter. Twenty-four samples were separately collected off adjacent sites of brisket, flank and rump (100 cm^2) using the two methods. Sponge samples, pummeled in peptone and M-Vac SRS aliquots were plated on Petrifilm for APC, TCC and ECC. Following SRS filtration, M-Vac filters were removed and placed directly on EMB media for TCC. Results indicate there were no differences ($P \geq 0.05$) among sampling sites within methods but the M-Vac collected higher mean APC/s than SP ($\log_{10}$), 3.91 $\pm$ 0.51 vs 3.11 $\pm$ 0.57 (all sites) $P \leq 0.05$) in all cases. No coliforms were detected in diluted liquid samples from either method but low level (1-5 CFU/100 cm^2) E.coli was confirmed on the M-Vac's filter in 8 of 12 samples. These observations indicate the M-Vac may provide a more efficient, non-destructive sampling method for low-level pathogen collection and detection on meat carcasses, as well as food-prep and processing equipment surfaces.

Key Words: Microbial, Collection, Meat

115 Novel Biosensors for the Rapid Detection of Campylobacter in Various Food Matrices. Richard Obiso* and Jill White, *IGEN International, Inc., Gaithersburg, MD.*

Campylobacter is considered to be the leading cause of enteric illness in the United States. Symptoms of a Campylobacter infection include mild to severe diarrhea, including loose and watery stools followed by bloody diarrhea. *C. jejuni*, *C. lari*, and *C. coli* represent the three main species that lead to enteric disease in humans, however, procedures to detect these Campylobacter species may take as long as 5 days to complete. Therefore, more rapid methods are required to determine the presence of Campylobacter in food. In this study, the utility of an ORIGEN®-based Campylobacter test was evaluated in different food matrices. This technology is based on a technique called electrochemiluminescence, which permits the detection of Campylobacter within an extensive array of samples. The food matrices tested included, poultry products, fruits and vegetables, shellfish, cheese products, milk products, and water samples. These food matrices (100 samples) were tested by microaerophillic incubation in Bolton broth using both 24 h and 48 h enrichment methods, followed by testing using the PATH*IGEN*™ Campylobacter test or B.A.M. standard plating methods. Food samples representative from each matrix were also inoculated with known levels of *Campylobacter jejuni* and tested using the Campylobacter test. The Campylobacter test was able to detect the equivalent of less than 100 CFU/25 g of sample. Detection of Campylobacter was possible in 24 h or in 48 h. The PATH*IGEN* test performed equivalently when compared

to B.A.M. standard plating methods, however, detection was reduced to 24-48h. Each assay takes approximately 1.5 h after enrichment and can be run simultaneously with other PATH*IGEN* tests.

Key Words: Campylobacter, Diagnostic, Detection

116 Novel Biosensors for the Rapid Detection of Salmonella Species in Various Food Matrices. Eddie Jefferies*, Shelia Rowe, and Jill White, *IGEN International, Inc., Gaithersburg, MD*.

Salmonella are implicated in causing 2 to 4 million cases of salmonelosis annually in the United States. Symptoms of a Salmonella infection include nausea, vomiting, abdominal cramps, diarrhea, fever and headache. *S. typhimurium* and *S. enteritidis* represent the two main species that lead to enteric disease in humans, however, procedures to detect these Salmonella species may take as long as 4 days to complete. Therefore, more rapid methods are required to determine the presence of Salmonella in food. In this study, the utility of an ORIGEN®-based Salmonella test was evaluated on different food matrices. This technology is based on a technique called electrochemiluminescence, which permits the detection of Salmonella species within an extensive array of samples. Food matrices included, poultry products, fruits and vegetables, shellfish, cheese products, milk products, chocolate, and water samples. 100 samples were tested by pre-enrichment in buffered peptone water followed by selective enrichment in Rappaport Vassiliadis soya peptone broth and testing using the PATH*IGEN*™ Salmonella test or B.A.M. standard plating methods. Food samples representative of each matrix were also inoculated with known levels of *Salmonella typhimirium* and tested using the Salmonella test. The Salmonella test was able to detect the equivalent of less than 1 CFU/25 g of sample. The sensitivity of the PATH*IGEN* test was equivalent to the B.A.M. standard plating methods, however, detection time was reduced to 24-30 h. Each assay takes approximately 1.5 h after enrichment and can be run simultaneously with other PATH*IGEN* tests.

Key Words: Salmonella, Diagnostic, Detection

117 Comparison of cultivation to PCR-hybridization for detection of *Salmonella* in porcine fecal and water samples. Ingrid Feder[1], Jerome C. Nietfeld[2], John Galland[3], Teresa Yeary[2], Jan M. Sargeant[3], Richard Oberst[3], Mark L. Tamplin[1], and John B. Luchansky[1], [1]*U. S. Department of Agriculture, Wyndmoor, PA/U.S.A.*, [2]*College of Veterinary Medicine, KSU, Manhattan, KS/U.S.A.*, [3]*Food Animal Health and Management Center, KSU, Manhattan, KS/U.S.A.*.

Salmonella in swine is an economic concern to swine producers and a human health risk. A reliable and rapid technique for *Salmonella* detection in swine feces and water exposed to swine feces would be useful. The purpose of this research was to compare cultivation methods and PCR-based methods for the detection of Salmonella in the feces of healthy pigs and in water. In the present study, three cultivation techniques were compared to a polymerase chain reaction (PCR)-hybridization technique for the detection of *Salmonella*. A total of 150 fecal and water samples were tested for the presence of *Salmonella*: 1) 92 fecal samples were pre-enriched overnight in tryptic soy broth (TSB) followed by overnight enrichment in Rappaport-Vassiliadis R10 (RV10) broth; 2) 34 fecal samples were enriched overnight in RV10 broth with no additional enrichment; and, 3) 24 water samples were pre-enriched overnight in 3MC broth followed by overnight enrichment in RV10 broth. For the PCR detection of *Salmonella*, samples were tested after the first overnight enrichment. The DNA was extracted via boiling and concentrated using a Sepharose CL-6B spin column. A total of 65 samples tested positive by both cultivation and the PCR or either method alone. *Salmonella* was detected by both methods in 68.8% of the positive samples pre-enriched in TSB, in 73.3% of the positive samples pre-enriched in 3MC, and in 24.0% of the positive samples enriched in RV10. Using the kappa statistic, agreement was 76% between cultivation with pre-enrichment and the PCR for *Salmonella* detection but was 5.7% when using cultivation without pre-enrichment compared to the PCR. These data provide evidence that the PCR could be used in combination with cultivation to improve *Salmonella* detection as the PCR worked as well or better than culture for delineating positive samples. However, the PCR detected only 72% of those samples which culture identified as positive, indicating that additional improvements are warranted before the PCR replaces cultivation as the gold standard for detection of *Salmonella* from swine.

Key Words: *Salmonella*, PCR-hybridization, Swine

ASAS/ADSA Growth and Development: Muscle Growth and Development

118 Cyclic stretch influences p21^{WAF1} promoter activity in myoblasts and myotubes. M.K. Webster*[1] and J.M. Reecy[1], [1]*Iowa State University, Ames, IA*.

The ability of skeletal muscle to adapt to an imposed workload has been well characterized, while the molecular events underlying this process are less defined. Cyclin dependent kinase inhibitors, such as p21WAF1, play an important role in cell cycle progression. These cell cycle regulators, in conjunction with members of the MyoD family, have been shown to induce the terminal differentiation of myoblasts. In previous studies, p21 expression was dramatically increased after as little as 12 hours of overload, as well as during terminal differentiation. These results suggest that either myoblasts differentiate, thereby increasing p21 expression, or p21 expression in the existing myofibers increases in response to work overload. We hypothesized that there was a stretch-responsive element within the p21 promoter. C2C12 myoblasts, plated at 1250 cells/cm2, were transiently transfected with a p21-Luciferase construct. The construct was made by inserting 2.1 kb of 5' p21 promoter upstream from the firefly luciferase gene. Cells were cyclically stretched for 24 or 48 hours with a Flexercell-3000 machine, or no stretch as a control. The stretch protocol consisted of a 20% sine stretch for 3 seconds, followed by ten seconds of rest. This cycle was repeated three times, after which the cells were allowed to rest for 30 minutes. This series was repeated for the duration of the experiments. The promoter activity in myoblasts appears to decrease in response to cyclic stretch. Additionally, C2C12 myoblasts were plated at 20,000 cells/cm2, transiently transfected, and induced to differentiate with low serum media for 15 hours. The differentiating myoblasts were then cyclically stretched for up to 48 hours. There was a 6.5-fold increase in promoter activity during differentiation. In addition, p21 promoter activity appeared to increase in response to stretch. We are currently examining the mRNA abundance of p21 under these same conditions. Based on these results, it would appear that the p21 promoter responds differentially in myoblasts and myotubes to cyclic stretch.

Key Words: p21^{WAF1}, cyclic stretch, skeletal muscle

119 Effect of intramuscular plasmid delivery and electroporation on circulating concentration of the plasmid-encoded reporter gene in the pig. A.G. Van Kessel*[1], B.G. Goldade[1], B.R. Krishnan[2], M.A. Morsey[2], L.D. Nelson[2], and P.J. Gaynor[3], [1]*University of Saskatchewan, Saskatoon, SK, Canada*, [2]*Pfizer Global Research and Development, Groton, CT* , [3]*Pfizer Global Research and Development, Terre Haute, IN*.

The effect of electroporation of skeletal muscle following plasmid injection on expression of a reporter gene was examined using 24 pigs (103.6 ± 0.79 kg BW). Pigs were assigned to one of four treatment groups including a vehicle control (CON) group, a group administered 2 mg plasmid in each of 2 sites (2P), a group administered 2 mg plasmid in each of 2 sites followed by electroporation (2PEP) and a group administered 1 mg plasmid in each of 2 sites followed by electroporation (1PEP). Prior to treatment, all pigs were sedated. The plasmid encoded secreted alkaline phosphatase (SEAP) driven by the CMV promotor and was injected in the right *biceps femorus* muscle suspended in 1 mL Na_2HPO_4 (150 mM, pH 7.2). Twelve electrical pulses (200 volts for 20 msec) were delivered across (1 cm gap) the injection site such that 6 pulses were delivered parallel with muscle fibres and 6 pulses were delivered perpendicular to muscle fibres. Polarity was reversed for 3 pulses delivered in each orientation. Electroporation had no adverse effects on the pigs. SEAP was detected in blood by day 2 following plasmid injection in electroporated pigs with maximal circulating SEAP activity observed