

**IDENTIFICATION OF CRITICAL CONTROL POINTS AND VALIDATION
CRITICAL LEVELS OF FOOD-BORNE PATHOGENS IN THE HARVESTING
AND STORAGE OF RAW MILK (E11-0178-011)**

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Goals of this research project are to provide science-based information on critical control points and critical levels of food-borne pathogens during the harvesting of milk. Data from this project is central for developing management routines for minimizing contamination of milk. During last year, 32 microbiological and milking practice surveys were conducted on four selected dairy farms. Over 1800 samples for coliform counts and 288 samples for detection of mastitis pathogens were analyzed. Initial results indicated that milking parlor and milk liner contamination correlated with bulk tank milk contamination that increased as milking progressed. Results also demonstrated that 10 of 35 milking practice critical points scored showed direct correlation with bulk tank coliform contamination and increased somatic cell counts. These 10 critical points were related to general hygiene, pre- and post-milking techniques and milker performance. *Staphylococcus aureus*, *Streptococcus uberis* and *Streptococcus dysgalactiae* subsp. *dysgalactiae* were the mastitis pathogens isolated most frequently.

**PREVALENCE OF POTENTIAL ZOO NOTIC ENTERIC BACTERIAL PATHOGENS
IN DOGS AND CATS WITH DIARRHEA (E11-0178-021)**

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Many potentially zoonotic organisms are associated with cats and dogs, many of which can cause potentially life-threatening infections in immunosuppressed human beings. There are reports of transmission of zoonotic enteric bacteria from dogs and cats to immunocompromised human beings including those with HIV-infection, young children, elderly, and cancer patients undergoing chemotherapy and/or radiation therapy. There are no surveys on prevalence of zoonotic enteric bacteria in dogs and cats with diarrhea. The purpose of this proposal is to determine the prevalence of potentially zoonotic enteric bacteria in dogs and cats with acute and chronic diarrhea. We began this project approximately 2 months ago and are in the process of acquiring samples as well as epidemiologic information. We hope to complete the project by December 2003.

**CONTROL OF AMMONIA AND LITTER PATHOGEN LEVELS
IN BROILER PRODUCTION FACILITIES TREATED WITH
ALUM AS A LITTER AMENDMENT (E11-0178-031)**

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Use of dry alum (aluminum sulfate) as a broiler litter amendment has been shown to reduce ammonia emissions, but little information is available on application of liquid alum to poultry litter to reduce *Salmonella* and *Campylobacter*. Our objective was to test the survival of both pathogens and to enumerate them in four adjacent broiler facilities of the same design that were treated with the following rates of liquid alum: 0 L/m², 0.82 L/m² (45kg), 1.64 L/m² (91kg) and 2.46 L/m² (136kg). Each broiler house contained approximately 30,000 birds with a six-week grow-out period and approximately two weeks between harvest of birds and introduction of the next flock. Alum had an accumulative effect over six months in reducing *Salmonella*. For reduction of *Salmonella*, the 1.64 L/m² and 2.46 L/m² alum applications were more effective than the 0.82 L/m² alum application. There was a strong correlation between litter moisture content and *Salmonella* levels but not with *Salmonella* levels and pH of litter except when litter pH was reduced below 3.5. *Campylobacter* was detected for four months during the six-month study. The 1.64 L/m² alum application reduced campylobacter levels by log 2 CFU/ml and the 2.46 L/m² rate reduced campylobacter levels by log 3 CFU/ml. These findings suggest that use of alum can reduce *Salmonella* and *Campylobacter* in a poultry broiler production facility.

**EFFECTIVENESS OF DAIRY-BASED ANTIMICROBIALS AGAINST FOODBORNE
PATHOGENS AND THEIR APPLICATION IN FOODS (E11-0178-041)**

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Many food products contain naturally occurring compounds that have antimicrobial activity. In the natural state, these compounds may play a role in extending the shelf-life of a food product. Enzymatic hydrolysis has been shown to produce antimicrobial peptides from milk proteins that are potentially useful in the medical field. However, none of these compounds has been evaluated for use as food antimicrobials. The objective of this research is to evaluate the effectiveness of naturally occurring peptides isolated from milk. Casein was isolated from raw whole milk by HCl precipitation. The casein was hydrolyzed with crystalline chymosin and peptides precipitated with trichloroacetic acid. The peptides (isracidin) were concentrated by centrifugation and frozen in ampules. The antimicrobial effectiveness of the peptides is being evaluated against strains of *Salmonella* Typhimurium and *Listeria monocytogenes* using a modified broth microdilution assay. Individual fractions will be evaluated in raw and ultrahigh temperature pasteurized milk.

GEOGRAPHIC INFORMATION SYSTEM (GIS) AND EPIDEMIOLOGICAL ASSOCIATIONS AMONG PATHOGENS AT THE FARM (E11-0178-051)

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A comprehensive epidemiological survey was conducted to determine prevalence of foodborne pathogens in dairy farm animals and farm environments. Geographic Information system (GIS) was used to examine relationships between presence of *Salmonella* spp., *Campylobacter jejuni*, and *E. coli* O157:H7 isolated from animals and presence of these pathogens in their environment including a river adjacent to the dairy. Neither *C. jejuni* nor *E. coli* O157:H7 were recovered from river water samples. However, *Salmonella* (33%) was isolated from all sampling sites along the river. GIS analysis revealed higher frequencies of *Salmonella* isolated at sites upstream from the dairy farm, indicating that sources other than the dairy farm contributed to river contamination. *Salmonella* isolates (n=190) were ribotyped for confirmation and comparison of isolates from various species, locations, and sample types. *Salmonella* ser. Senftenberg (26), Typhimurium (25), Havana (8), and Newport (8) were the most frequently isolated serotypes from all sampling locations. Isolates recovered from Washington State and Tennessee were similar and common to dairy cattle and dairy farm environments.

GENETIC AND PHYSIOLOGICAL DIFFERENTIATION OF SALMONELLA AND HEMORRHAGIC E. COLI (E11-1078-061)

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Epidemiological data suggest that our most important foodborne hazards are *Salmonella*, *Campylobacter jejuni*, and *E. coli* O157:H7. *Salmonella*, *C. jejuni*, and *E. coli* O157:H7 all share the common characteristic of having an animal reservoir from which they spread to humans; therefore, risk reductions at every point from farm-to-table are necessary. This study compared ribotyping and traditional biochemical methodology to examine relationships that exist between animals and their environments. Samples (n=12,240) were collected monthly for 12 months from dairy cows, calves, and farm environments and analyzed for *Salmonella*, *C. jejuni*, and *E. coli* O157:H7. Surface water adjacent to the farm was evaluated also. *Salmonella* was isolated at greatest frequency during summer months (54%). *Campylobacter jejuni* was isolated with increased frequency during the winter (33-35%). The overall prevalence of *Salmonella* spp. recovered from the Tennessee River (33%) paralleled that of *Salmonella* recovered from dairy farm animal and environmental samples (33%). The most frequently isolated *Salmonella* serotypes were Senftenberg (46%), Havana (24%), and Typhimurium (20%). *Salmonella* ser. Havana was the only serotype, showing similar riboprint patterns, isolated from the farm animal, environment, and surface water samples. Feeds, insects, and bird droppings were key sources of *Salmonella* ser. Havana at the farm. Elimination of *Salmonella* from feed and control of insects and wild birds on the farm could reduce transmission of *Salmonella* in dairy cows and dairy cow environments.

MECHANISM OF INHIBITION OF SELECTED ESSENTIAL OIL COMPONENTS ON BACTERIAL PATHOGENS (E11-0178-071)

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Antimicrobial properties of many herbs have been investigated in vitro, but few studies have been done in vivo. Our objective was to create an herbal chicken marinade, which might inhibit the growth of spoilage and pathogenic microorganisms and to evaluate factors affecting the inhibition. Fresh chicken breasts were inoculated ($\sim \log 4$ CFU/g) with *Campylobacter jejuni*, *Salmonella* Typhimurium, *Listeria monocytogenes*, and *E. coli* O157:H7. The following marinade treatments (pH 4.4-4.5) were evaluated using fresh inoculated chicken breasts (20% marinade by weight): control (no herbs), grapefruit seed extract (GSE-0.3%), oregano essential oil (ORG-0.3%), thyme essential oil (THY-0.3%), combination of GSE, ORG, and THY (0.3% of each herb), and dried oregano leaves (1%). The GSE marinade reduced growth of APC and *C. jejuni* ($< 2 \log$ CFU/g) but did not significantly inhibit *S. Typhimurium*, *E. coli* O157:H7, or *L. monocytogenes* ($p > 0.05$). THY, ORG, and the combination treatment significantly reduced microbial counts ($p < 0.05$). The combination treatment was most effective in reducing APC and *E. coli* O157:H7, and was highly lethal ($\sim \log 4$ CFU/g reduction) to *S. Typhimurium*, *C. jejuni*, and *L. monocytogenes*. These data suggest that selected herbs in a poultry marinade may be useful to increase the shelf life and safety of poultry.

CLONING JERSEY COWS: A USEFUL TOOL & NOVEL STRATEGY TO EVALUATE GENETIC INFLUENCES ON SUSCEPTIBILITY OR RESISTANCE TO MASTITIS IN JERSEY COWS (E11-0178-081)

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Mastitis, an inflammation of the udder affecting a high proportion of dairy cows throughout the world, significantly decreases production, alters milk composition, reduces reproductive performance and costs an estimated \$2 billion annually. Some cows are significantly more resistant to mastitis than herdmates in the same environment. Reasons for this are unknown but are likely due to yet identified genetic factors. Cloning technology that is well established in our laboratory was used to produce genetically identical Jersey cows that were previously confirmed either resistant or susceptible to mastitis. This novel approach has resulted in a unique set of genetically identical Jersey cows for use in subsequent studies to determine factor(s)/genes responsible for mastitis disease resistance/susceptibility. Identification of such factors could lead to improved selection strategies and/or novel approaches for eradicating or reducing the incidence of mastitis. **Doing so would greatly decrease incidence of antibiotic use; thereby, reducing potential for increased antibiotic resistance in humans.** Furthermore, this exciting research strategy could result in a very useful model for studying and developing prevention and control strategies for other diseases of significance to the dairy industry.

DEVELOPMENT OF A POST-PROCESSING PASTEURIZATION TREATMENT FOR PACKAGED HOT DOGS IN INACTIVE *LISTERIA MONOCYTOGENES* (E11-0178-091)

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This proposed research project will focus on the development and evaluation of post-processing pasteurization of packaged hot dogs as a method of controlling surfact-contaminate *L. monocytogenes*. Hot dogs will be individually packaged, inoculated with *L. monocytogenes*, and vacuum packaged. Packaged hot dogs will be pasteurized in hot water and low pressure steam (85 to 90⁰C), and survival of *L. monocytogenes* will be evaluated. Results of this investigation will be useful to the USDA and the ready-to-eat meat industry by demonstrating the applicability of an antilisterial process that is relatively inexpensive and simple to incorporate into existing processing operations.

CHARACTERIZING SOURCES AND RESERVOIRS OF GENETIC ANTIBIOTIC RESISTANCE ELEMENTS (E11-0178-101)

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To characterize sources, reservoirs, and transfer of genetic resistance factors among bacteria associated with livestock, bacterial isolates will be subjected to molecular determinations using DNA probes and PCR, for detection of integrons and associated resistance genes. Bacterial isolates which have been previously identified to species or subgroup, and antibiotic resistance patterns (including susceptible isolates), will be selected from environmental sources and animals subjected to defined treatments and management conditions. Sources of test isolates will include bacteria from: 1) an on-going study of chronological occurrences of foodborne pathogens and antibiotic resistance from a newly-established swine facility, 2) swine produced with and without exposure to antibiotics, and 3) two generations of swine, subjected to various combinations of exposure to, or exclusion of antibiotics. The prevalence of genetic resistance elements will be correlated with management practices and antibiotic use to establish Hazard Analysis Critical Control Point (HACCP) strategies for control of antibiotic resistance in livestock systems.

EFFECTS OF IRRADIATION ON ANTIBIOTIC RESISTANCE IN BACTERIA IN MEAT PRODUCTS IRRADIATED WITH ELECTRON BEAM IRRADIATION (E11-0178-111)

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Ionizing radiation can eliminate pathogenic microorganisms from meats, fruits and vegetables. However, it is possible, that a small number of pathogens could survive the irradiation process. The objective of this study was to determine the lethality of ionizing irradiation to *Salmonella* and *Listeria* on bologna and to determine if changes in antibiotic resistance patterns occurred due to the irradiation process. *Salmonella* was not destroyed at 1 kGy since 9 out of 9 samples spiked with log 6 CFU/g *Salmonella typhimurium* grew typical salmonellae colonies. Controls (spiked but not irradiated) also provided 9 out of 9 positive salmonellae isolates. Only 1 out of 9 spiked samples of meat treated with 3 kGy electron beam irradiation showed survival of *S. typhimurium*. *Listeria monocytogenes* was more resistant to irradiation than *Salmonella* since *L. monocytogenes* was recovered from 6 out of 9 spiked samples treated with 3 kGy. Preliminary evaluation of isolates surviving irradiation showed that resistance to tetracycline, gentamycin and ampicillin remained unchanged. Studies are currently underway to determine the effect of irradiation on loss of plasmids and on resistance to other antibiotics.

CHARACTERIZING ANTIBIOTIC RESISTANCE IN BACTERIA ASSOCIATED WITH DAIRIES (E11-0178-121)

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To determine prevalence and source of antibiotic resistant bacteria on dairy farms, a total of 15 farms will be selected and categorized with respect to type and level of management. Ten lactating dairy cows from each farm will be randomly selected and bacterial samples will be collected from the mouth, anus, and teats. Samples will also be obtained from the bulk milk tank, manure, bedding, and other prominent environments. *Escherichia coli* and *Streptococcus uberis* will be isolated to act as gram negative and gram positive sentinel organisms, respectively, for resistance testing against a panel of 18 antibiotics, as currently used by the National Antimicrobial Resistance Monitoring System. Foodborne pathogens will also be isolated from samples and subjected to similar resistance analysis. Prevalence of single and multiple resistance patterns will be determined, sources of resistant isolates will be identified, and resistance patterns will be correlated with management practices and antibiotic use, to aid in the formulation strategies to control antibiotic resistance within the dairy environment.

INTERNET-BASED FOOD SAFETY EDUCATION FOR FOOD HANDLERS SERVING AT-RISK POPULATIONS (E11-0178-131)

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A section of a food safety education course will be developed for delivery via the Internet for those food handlers who serve at-risk populations such as infants and children, elderly, chronically ill and pregnant women. The course will contain science-based information on causes and controls of foodborne illnesses that are most dangerous for each at-risk group. The course will initially be available on CD-ROM for evaluation before placing it on the Internet. The effectiveness of the courses will be determined by analysis of pre-and post-examination, follow-up site visits and interviews with participants. The major contributing factor for foodborne disease outbreaks in the U.S. is improper food holding temperatures. Research will be conducted to validate information from the US Food Code on hot and cold holding as to adequacy for intended purposes and to use as illustrations for the food safety education course.

MULTIPLEX POLYMERASE CHAIN REACTION FOR DETECTION & CONFIRMATION OF SHIGA-TOXIN PRODUCING *ESCHERICHIA COLI* (E11-0178-141)

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The objective of this study was to determine prevalence of virulence determinants associated with Shiga toxin-producing *E. coli* (STEC). Multiplex polymerase chain reaction (PCR) was used to detect presence of genes encoding Shiga toxin 1 and 2 (*stx*₁ and *stx*₂), enterohemolysin (*hly*₉₃₃), intimin (*eaeA*) and flagella H7 (*flicC*_{H7}) in *E. coli* isolates (n=402) from diverse sources. *stx*⁺ isolates were tested for production of Shiga toxins (Stx1 and Stx2), enterohemolysin and agglutination with H7-specific flagella antiserum. Of *E. coli* O157:H7/H- strains, 91.5% (150 of 164) were *stx*⁺. Most O157 STEC (98%) had sequences for genes encoding intimin and enterohemolysin. Five of 20 *E. coli* O111, 4 of 14 O128 and 4 of 10 O26 were *stx*⁺. Five of 6 *stx*⁺ O26 and O111 produced Stx1, however, *stx*⁺ O128 were Stx-negative. Multiplex PCR was a powerful tool for typing and subtyping *E. coli* strains for pathogenic profiles associated with STEC.

MOLECULAR SUBTYPING OF *SALMONELLA* SPECIES USING A POLYMERASE CHAIN REACTION-ENZYMELINKED IMMUNOSORBENT ASSAY (E11-0178-151)

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The objective was to continue development and evaluation of a PCR-ELISA-based detection system for *Salmonella* serovars and serogroups prominent in disease. A PCR-ELISA was developed to identify *Salmonella* somatic groups B, C1, C2, D and E1. Primers were selected from the *rfb* gene cluster, which is responsible for biosynthesis of O antigens of *Salmonella* lipopolysaccharide. Previously serogrouped *Salmonella* isolates (n=169) were tested using the PCR-ELISA procedure to determine sensitivity and specificity. DNA was amplified using the PCR procedure for selected somatic groups and amplified products were visualized on agarose gels, and subjected to the ELISA procedure. The sensitivity of this procedure to correctly identify *Salmonella* somatic groups was 96% and the specificity was 98%. Ninety-one percent of somatic group D, 92% of somatic group B, 97% of somatic group C1, 97% of somatic group C2 and 87% of somatic group E1 were identified correctly. Results indicate that the PCR-ELISA procedure is a rapid and accurate method for serogrouping *Salmonella* isolates.

THE INTERACTION OF INTERLEUKIN-8 RECEPTOR EXPRESSION WITH NEUTROPHIL FUNCTION AND DISEASE RESISTANCE (E110178-161)

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Our objective evaluates the frequency of interleukin-8 receptor (IL-8R) single nucleotide polymorphisms (SNPs) and their association with mastitis and neutrophil function. SNPs were identified by single-strand conformation polymorphisms (SSCP) in 53 Jerseys from the Dairy Experiment Station, Lewisburg, TN. Eight SSCP patterns have been identified. Four patterns represent ~77% of these cows. Cows with patterns 1 and 2 had significantly ($p < 0.05$) fewer quarters infected (6.7% and 4.2%) than those with pattern 5 (32.3%). Patterns 1 and 2 also tended to have lower proportions of cows with positive quarters when compared to 5 (61%, 50%, and 100%, respectively). These results suggest that IL-8R SNPs are present at defined frequencies, may be linked with mastitis susceptibility, and has the potential to allow for selection of healthier animals for production and research. The resulting knowledge from better-defined populations also would provide greater insight into the development of new and/or improved mastitis therapies and control measures.

DEVELOPMENT OF A NOVEL THERMOSONICATION PROCESS TO INACTIVATE MICROORGANISMS (E11-0178-171)

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The research involved two distinct parts. A laboratory-scale ultrasonic reactor with high-pressure, high-temperature capabilities suitable for food processing/inactivation of microorganisms was designed, constructed and tested. The reactor consists of a double-glass mantle, made from borsilicate able to withstand pressures >30bars with a minimal/maximal fill volume of 250/400ml. Operational conditions were determined to adhere to their design specifics: temperature control between 4 and 148°C, pressurization up to 8 bars with a 30% over-pressurization maximum, batch operational mode for semisolid systems and concentrated suspensions (<32v/v%), continuous mode for viscous systems up to viscosities of 1Pas using and external volumetric pump and integrated stirrer. In part 2, a study was conducted to determine the effect of high-intensity ultrasound and NaCl concentration (0, 0.5, 1.0, 2.0, and 5.0%) on inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes*. NaCl concentration or treatment time at 18 and 48 Watts had little effect on survival of *E. coli* O157:H7 (< 1 log reduction). At 48 and 111 Watts, NaCl concentrations > 0.5% adversely affected survival (~2 log reduction). For *L. monocytogenes*, reductions of < 1.2 log were observed after 10 minutes of treatment at 111 Watts. While increased solute concentration typically enhances microbial thermotolerance, this study demonstrates that this may not be the case with ultrasonication. Our results provide new and exciting insights into the fundamental theoretical nature of microbial deactivation by ultrasound. Results will be presented in two papers at meeting of professional societies and two publications are currently in preparation. Based on the seed grant funding, a USDA IREESG (~\$375,000) was awarded in 2002.

MAXIMIZING RECOVERY AND PLASMID STABILITY IN *YERSINIA ENTEROCOLITICA* (E11-0178-271)

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Virulent serotypes of *Yersinia enterocolitica* may carry a plasmid (pYV) encoding a family of proteins that are released into the medium and whose expression is highly dependent on cultural conditions. The plasmid is easily lost from cells during their growth in the laboratory. Since both virulent (plasmid-carrying) and avirulent strains (non-plasmid) of *Y. enterocolitica* are isolated from food, environmental and animal samples, maintaining stability of the plasmid is essential for appropriate recovery of virulent *Y. enterocolitica*. Avirulent strains of *Y. enterocolitica* have no public health significance, whereas, virulent strains are highly pathogenic and are also associated with numerous chronic health problems following foodborne infections (ie. Arthritis, endocarditis, meningitis, etc.). The objective of this study is to optimize stability of the plasmid in virulent serotypes of *Y. enterocolitica* by manipulation of calcium concentration, temperature and pH during isolation from environmental (including animal and plant) samples.

**SURVIVAL AND MOVEMENT OF FOODBORNE PATHOGENS THROUGH SOIL
(E11-0178-281)**

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A study was conducted to determine the prevalence of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* in soil on a dairy farm. Sampling locations included water samples from up- and down-stream in a creek running through the farm, free stall bedding, silage, separator liquids, separator solids, pumped liquid from a holding pond, feed, and bulk tank milk. No *E. coli* O157:H7 was found in any of the samples. *L. monocytogenes* was present most often in the pumped holding pond liquid and the separator liquid, with each producing positives in 31% of the samples. Bedding and silage samples were negative for *L. monocytogenes*. A possible seasonal trend was identified in *L. monocytogenes*, with more positive samples collected during cooler months. *Salmonella* were ubiquitous in pumped liquid, with 34 (94%) of 36 samples testing positive; separator liquid and separator solid samples returned *Salmonella* positives in 72% and 81%, respectively. Downstream samples yielded 27% positives, whereas upstream samples identified 42% *Salmonella* positive indicating contamination from other sources upstream from the farm. After testing was completed, locations associated with waste, such as the manure solids separator and the holding pond, were identified as potential control points for *L. monocytogenes* and *Salmonella*.

**A NOVEL STRATEGY FOR EVALUATING GENETIC INFLUENCES ON
RESISTANCE TO MASTITIS IN JERSEY COWS (E110178-291)**

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Objective was to clone Jersey cows confirmed RESISTANT to mastitis and monitor immune status of clones derived from SUSCEPTIBLE cow UT3888 born last year. Data are being collected to assess immune status in cloned offspring. Efforts to clone resistant cows in Fall 2002 are depicted below. In total, 9 pregnancies are ongoing derived from resistant cows (2/18/03 date of last pregnancy check).

Cows	No. Clones	Embryo Transfer (%)	Pregnant 28 d (%)	*Pregnant 93-128 d
Resistant (UT4472)	44	5	3 (60.0)	1
Resistant (UT4585)	201	39	10 (25.6)	8
Susceptible (UT3888)	78	21	6 (28.5)	5

DETECTION & QUANTIFICATION OF PATHOGENS DIRECTLY FROM MILK BY REAL-TIME POLYMERASE CHAIN REACTION (E11-0178-301)

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Objectives were to develop a technique for bacterial DNA isolation directly from milk and to develop and evaluate Real-Time-PCR for detection and quantification of bacteria in milk. Primers were selected and evaluated for *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus uberis* and *Listeria monocytogenes*. Cross-reactivity studies have been conducted with no cross-reactivity detected. Initial work has focused on developing a multiplex Real-Time PCR reaction combining *S. aureus* and *S. agalactiae* specific primers, *S. aureus* and *S. uberis* specific primers and *S. agalactiae* and *L. monocytogenes* specific primers. Numerous techniques have been evaluated for isolation of bacterial DNA directly from milk; sensitivity varies considerably. Preliminary results using PrepMan Ultra reagent (Applied Biosystems) for isolation of bacterial DNA directly from milk demonstrated a sensitivity of ~500-600 colony forming units (CFU)/ml for *S. agalactiae*, ~3000 CFU/ml for *S. uberis* and ~40 CFU/ml for *L. monocytogenes* with Real-Time PCR using SYBR Green I dye as the fluorescent molecule.

EVALUATION OF CHEMICAL SANITIZERS, OZONE TREATMENT, AND STEAM PASTEURIZATION FOR DESTRUCTION OF *SALMONELLA* SPP. ON CANTALOUPE SURFACES (E11-0178-311)

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Pathogenic organisms have been isolated from a wide variety of fruits and vegetables, both imported and domestic. Cantaloupe, in particular, has been implicated as the vehicle of infection in several outbreaks of salmonellosis. From 1990 to 2001, more than 750 confirmed cases of *Salmonella* infection have been associated with consumption of contaminated cantaloupe. The increased frequency of foodborne disease outbreaks associated with consumption of contaminated raw fruits and vegetables has resulted in an increase in evaluations of chemical sanitizers and the development of new sanitizers, particularly targeted at application to fruits and attention on some form of chlorine (e.g. hypochlorite) and hydrogen peroxide. The purpose of the proposed study is to evaluate use of various chemical sanitizers, ozone, steam pasteurization, and combinations of the three treatments for their application as control measures for *Salmonella* on cantaloupes. Additionally, two standard methods and one new sampling technology will be compared for efficacy to recover and allow enumeration of *Salmonella* on cantaloupe surfaces.

CHARACTERIZING FOODBORNE RISKS ASSOCIATED WITH COMMERCIAL AQUACULTURE SYSTEMS IN THE SOUTHEASTERN US (E11-0178-321)

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To characterize potential foodborne risks associated with freshwater aquaculture, bacteria will be obtained from various components of culture ponds, including water, fish and/or prawns, naturally occurring aquatic invertebrates, and nearby livestock and manure, for isolation of foodborne pathogens and determination of antibiotic resistance. Bacterial isolates will be subjected to molecular determinations using PFGE analysis, DNA probes and random primer PCR, for characterization of resistance genes and to determine sources and relatedness between isolates found in aquaculture systems and nearby conventional livestock. The prevalence of genetic resistance elements will be correlated with management practices and antibiotic use in both aquaculture systems and adjacent livestock operations to establish Hazard Analysis Critical Control Point (HACCP) strategies for control and to determine if buffer zones should be utilized when aquaculture and livestock operations are in close proximity to each other.

RISKS ASSOCIATED WITH ENROFLOXACIN USE IN CATTLE WITH CLOSE PROXIMITY TO VEGETABLE AND ORCHARD PLOTS (E11-0178-331)

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To determine risks posed by enrofloxacin use in livestock, beef cow-calf operations, previously identified as being adjacent to fruit and vegetable plots will be used in a survey analysis. Calves will be treated with enrofloxacin as per label directions and samples will be obtained over strategic time periods from animals, pastures, insects and nearby crops at defined distances. Bacteria, including foodborne pathogens and naturally occurring non-pathogenic *E. coli* will be isolated and typed via DNA fingerprinting to determine if isolates on produce originate from adjacent livestock. Antibiotic resistance patterns will be determined using the National Antimicrobial Resistance Monitoring System, and a minimum inhibitory concentration (MIC) analysis specific for enrofloxacin. From this work, recommendations will be established to provide guidance for commercial and private growers with regard to safe distances between gardens/orchard and livestock to reduce the risks posed by antibiotic-resistant bacteria.

**SIMULTANEOUS DIRECT DETECTION AND QUANTIFICATION METHODS FOR
FOODBORNE PATHOGENS USING REAL-TIME MULTIPLEX POLYMERASE
CHAIN REACTION (E11-0178-341)**

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Objectives of this study were to develop and apply simultaneous multiplex PCR protocols for real-time direct detection and quantification of *Salmonella*, *Escherichia coli* O157:H7 and *Campylobacter jejuni* in environmental and bulk tank milk samples from dairy farms. The study commenced with confirmation of presence of marker genes of interest in quality control target bacterial species. *Salmonella* were evaluated for presence of *invA* and *hima* sequences and *invA* primers were found to be more specific. *Campylobacter* spp. was evaluated for presence of hippuricase and thermophilic 23S rRNA gene sequences. Surprisingly, some of the quality control *Campylobacter* strains lacked 23S rRNA sequences. Additional quality control strains will be acquired to test sensitivity and specificity of this assay. *Escherichia coli* O157:H7 were evaluated for presence of *rfbE* sequences. The primers selected cross-reacted with other closely related test bacteria, i.e., *E. coli* O26 strains. Studies are being conducted to select more specific primer sequences.

**ISOLATION & CHARACTERIZATION OF A NOVEL BACTERIAL PROTEIN
INVOLVED IN ADHERENCE & INTERNALIZATION (E11-0178-351)**

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Virulence factors that favor adherence and internalization to host cells likely play a crucial role in the establishment, spread, and persistence of infection. Our research has focused extensively on host-pathogen interactions, and on identification and characterization of virulence factors associated with the pathogenesis of mastitis. We hypothesize that adherence to and subsequent internalization of mastitis pathogens into mammary epithelial cells is an important early event in the establishment of mastitis in dairy cows. We recently discovered a novel bacterial protein involved in adherence to and invasion of bovine mammary epithelial cells. We have isolated and purified this protein, generated antibodies to the purified protein, and have determined the N-terminal amino acid and predicted DNA sequence of this novel bacterial protein. Our research on identification and characterization of this novel bacterial protein resulted in the submission of an intellectual property disclosure to The University of Tennessee Research Corporation and filing of a U. S. Provisional Patent

IDENTIFYING GENES DIFFERENTIALLY EXPRESSED BY MASTITIS RESISTANT AND SUSCEPTIBLE DAIRY COWS (E11-1078-361)

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Our objective will test the hypothesis that neutrophils from mastitis susceptible and resistant dairy cows differentially express specific genes, thereby contributing to mastitis development. We are currently screening neutrophil adhesion molecule expression, chemotaxis, reactive oxygen species generation, and bactericidal killing of 20 mastitis susceptible and resistant cows. Six cows with the greatest and weakest neutrophil activity will be evaluated for differential gene expression by collecting RNA following in vitro stimulation. 720 genes specific to bovine leukocytes will be evaluated using a cDNA microarray developed at Michigan State University. We expect at least 10 genes to vary in relation to mastitis resistance. Identification of genes expressed at different levels by cows either resistant or susceptible to mastitis will provide valuable insight into mechanisms critical for neutrophil function. Based upon this information we can generate novel therapeutic and/or preventive strategies that would improve disease resistance and reduce antibiotic use, providing a safer milk supply.

DEVELOPMENT AND EVALUATION OF RAPID AND ACCURATE METHODS FOR THE DETECTION OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS (E11-0178-371)

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Amino acid sequences of Ag85 of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) (NCBI accession #AF280067) were compared to all other known sequences using the NCBI/GenBank Blast program. Unique amino acid sequences of MAP Ag85a were selected as prospective epitopes for antibody binding according to their antigenicity and the probability of being exposed on the surface of the tertiary protein molecule using the DNASTAR Inc. Protean Software. Polyclonal antibodies were generated in rabbits against two peptide sequences: GVRRRTRPGPATTRRC and GLAMGTPVATRPPTC (Genemed Synthesis Inc.) The two polyclonal antibodies directed against the above MAP sequences are currently being tested for binding in Western blots containing whole protein extracts from MAP, *Mycobacterium avium* subsp. *avium*, *Mycobacterium chelonae*, *M. fortitum*, *M. intracellulare*, *M. Kansasii*, *M. smegmatis* and *M. scrofulaceum*. If the polyclonal antibodies are capable of detecting MAP-specific proteins in the sera of infected animals, then mice will be immunized with the above amino acid sequences unique to MAP for the purpose of generating MAP-specific monoclonal antibodies.

MICROCANTILEVER BIOSENSORS FOR THE DETECTION OF FOODBORNE PATHOGENS AND ANTIBIOTICS (E11-0178-381)

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A widespread need exists for portable, real-time, low power, in-situ sensors for detection of foodborne pathogens. Microcantilevers can be readily fabricated on silicon wafers and other materials for simultaneous detection of bending and resonance frequency variation to separate specific adsorption from non-specific adsorption. The dimension of the cantilever determines the sensitivity while selectivity is achieved by applying chemically selective coatings of the cantilever surface. Consequently, microcantilevers are a universal platform to base electro-mechanical sensors for measuring a multitude of physical, chemical, and biochemical factors, depending upon the selection of the coating. Detection limits have yet to be fully explored, but parts-per-billion to parts-per-trillion have been demonstrated. We have successfully demonstrated sensitive and selective detection of chemical and biological analytes including detection of prostate-specific antigen, DNA SNP, racing, and glucose. Goals of this proposal are to adapt this technology to rapidly identify foodborne pathogens and pathogens important in veterinary and human medicine.

DEVELOPMENT OF 'RELEASE-ON-DEMAND' ANTIMICROBIAL DELIVERY SYSTEMS TO IMPROVE FOOD SAFETY (E110178-391)

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The stability and antimicrobial efficacy of nisin and lysozyme encapsulated in phospholipid liposomes was studied. 10mM phosphatidylcholine was dried under a stream of N₂, rehydrated and ultrasonicated with 0.1x phosphate buffered saline containing 1mM EGTA, 50mM calcein and 0.1-5 mg/ml nisin, lysozyme or 1:1-nisin-lysozyme mixture to produce liposomes. Vesicles were separated from un-encapsulated antimicrobials by size exclusion chromatography. Entrapping calcein in liposomes caused fluorescence quenching of >65%, while co-encapsulating nisin and calcein decreased entrapment to >45%. At the same molar lipid/antimicrobial ratio, lysozyme induced more calcein release compared to nisin, and the nisin/lysozyme mixture had the lowest release, *e.g.* at a lipid/antimicrobial ratio of 4, 55% nisin, 50% lysozyme and 80% nisin-lysozyme was retained indicating that destabilization of liposomes depended on type of antimicrobial encapsulated. Encapsulated antimicrobials in PC-liposomes strains of *Listeria* over a period of 48 hr. Growth inhibition was more pronounced for the nisin:lysozyme mixture which was explained in terms of absolute concentration of antimicrobials present. The promising results of this research demonstrate that phospholipid liposomes may provide a novel carrier system capable of delivering antimicrobials in complex food systems to inhibit growth of pathogens. Results will be presented in a paper at the IFT Annual Meeting in Chicago, 2003 and two publications are currently in preparation. Results also supported a USDA grant application and a NineSigma application that are both currently pending.

TOXICOLOGY ISSUES WITH PLASTIC MULCHES USED AS ROW COVERS FOR TOMATO PRODUCTION IN TENNESSEE (E11-0178-401)

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This research is aimed at exploring the persistence of pesticide residues and microorganisms with regards to plastic mulches used as row covers. With the increasing use of such plastics for tomato production in Tennessee, a huge waste management issue has arisen. The fate of remaining chemical residues and/or microorganisms may be affected by the disposal method for used plastic. If the remaining material is buried, is enough material remaining to have environmental impact? An analytical method has been developed so that five different pesticides can be measured using one screening utilizing High Performance Liquid Chromatography. During the 2003 growing season, plastic sampling and extraction procedures will be developed so the mass of chemical residues on the plastic can be measured quickly and efficiently. In addition, various bacterial organisms will be monitored throughout the growing season. At the end of the growing season, the persistence of common pesticides and microorganisms will be determined.

DESTRUCTION OF PATHOGENS ON PRODUCE WITH PLASMAS (E11-0178-411)

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The specific aims of this proposal are to assess the feasibility of using a One Atmosphere Uniform Glow Discharge Plasma (OAUGDP) as a novel processing technology for inactivating foodborne pathogens on food surfaces. Previous research demonstrated that this technology is effective at destruction of foodborne pathogens on the surface of agar media. Our newly developed OAUGDP configuration consists of a reactor with a remote exposure chamber. Limitations of our original reactor (parallel plate design) were prohibitive for application to items greater than a few millimeters in thickness. The newly designed remote exposure reactor will accommodate a wide variety of food types, thus enabling OAUGDP treatment of foods. Based upon preliminary work demonstrating that large populations of foodborne pathogens such as *E. coli* O157:H7 and *Salmonella* can be significantly reduced on food surfaces, there is good indication that the remote exposure reactor could serve as a novel, non-thermal processing technology to be used for reducing microbial populations on various food surfaces.

**ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS INCORPORATED IN
CHITOSAN FILMS AGAINST *Listeria monocytogenes* AND *Escherichia coli*
(E110178-421)**

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Microbial growth is predominant at the surface of many food products. The objective of our research was to evaluate the antimicrobial effects of chitosan films enriched with essential oils (EOs) that can be used as edible coatings or food packaging material. The films were prepared from solutions containing 1% chitosan, 1% acetic acid, 0.5% Tween20, and 1 to 4% EO of anise, basil, coriander, and oregano. The antibacterial efficiency of films was tested by disk diffusion method at 35°C. All EOs incorporated in chitosan films had higher inhibitory effect toward *L. monocytogenes* than toward *E. coli*. Inhibition, regardless on the bacteria species, was strongest with oregano, followed by basil and coriander, and the weakest with anise EO. Antimicrobial activity of chitosan-essential oil films was proportional to concentration of EO. Chitosan-essential oil films have potential in ensuring food safety due to local application of antimicrobials and reduced losses of active volatiles.

**SOCIOECONOMIC DETERMINANTS OF CONSUMER FOOD SAFETY
AWARENESS AND PERCEPTIONS:
The Case Of MicroOrganisms In Food (E11-0178-431)**

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This study investigated the factors determining consumer awareness and perception of food safety risk associated with micro-organisms such as *Salmonella*, *Campylobacter*, *Listeria*, *E. coli*, *Vibrio vulnificus*, and *Cyclospora*, and chemical contamination such as pesticide and antibiotic residues. National data collected by the FDA and USDA and innovative statistical procedures are developed and used in the investigation. Profile of consumers who are more likely to be aware of and concerned about these food safety issues are identified. A preliminary report on the chemical contamination has been accepted for presentation at the International Agricultural Economics Association Meeting in August 2003 (Durban, South Africa) and a revised manuscript is under consideration at *Risk Analysis*. A report based on the micro-organisms is under preparation and will be submitted for presentation at a professional meeting and to a refereed journal.

ASSESSMENT OF FOOD SAFETY EDUCATION MATERIALS AND TRAINING PROGRAMS IN DAY CARE CENTERS AND NURSING HOMES (E11-0178-441)

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The purpose of this project was to investigate the food safety education system utilized by the food service segment that prepares and handles food for day care centers and nursing homes. Goals of the research are to: (1) begin to develop a database of food safety educational materials available for food service workers and other segments of the population, (2) develop a survey instrument to determine what food safety educational materials and programs are being used by food service personnel, (3) validate the survey instrument with the assistance of day care center and nursing home administrators involved with food safety education, (4) analyze food safety education data gathered during a meeting of the day care center and nursing home administrators, and (5) revise the survey instrument in preparation for a statewide survey. The database will involve an Internet and literature search of materials to be gathered and reviewed for content, level, and method of delivery. The survey developed will assess types of food safety educational materials and programs used, the provider of the materials, actual individuals that use the materials, frequency of usage, etc. Once the draft survey is developed, day care center and nursing home administrators will be invited to a conference to assist in validating the survey. They will also receive a food safety education program. Approximately 20 food service directors or agency administrators for day care centers and nursing homes will be the participants in two conferences. The database information, survey and process will be used to assist in preparation of a grant proposal to the USDA to expand the project to a regional and, eventually, a national audience.

THE ROLE OF SYNANTHROPIC (FILTH) FLIES IN THE SPREAD AND MAINTENANCE OF SHIGA TOXIN *ECHERICHIA COLI*: O157 (STEC) AND ENHANCEMENT OF LABORATORY SKILLS FOR THE ISOLATION OF STEC AT UT (E110178-451)

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Pools of house and stable flies were collected biweekly from 1 April until 30 November, 2002 at the UT Dairy. Adult fly populations were monitored weekly by leg counts, sticky traps and fly speck cards. Thirty fresh manure samples were collected at the same time as the fly pools. All fly and fecal samples were shipped to the USDA Meat Animal Research Center, Clay Center, NE for determination of the presence of shiga toxin O157 *E. coli*. *E. coli* O157 was again present, but was much less prevalent in 2002 than in 2001 in both house flies and cattle. The low prevalence (and sometimes absence) of O157 in cattle was reflected in the reduced prevalence of O157 isolated from flies. The housefly continued to be the predominant species of fly harboring O157.

PREVENTION AND CONTROL OF FOODBORNE PATHOGENS IN FOOD EMULSIONS USING CHITOSAN (E11-0178-461)

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Studies were conducted to assess the antimicrobial efficacy of chitosan against foodborne pathogens in oil-in-water emulsions. Chitosan emulsions were prepared by adding acetic acid solutions of two chitosans (low molecular weight [LMW] with 150KDa and oligosaccharide lactate [OL]) to aseptic corn oil emulsions to give chitosan concentrations ranging from 0.1 to 0.7%. Emulsions were inoculated at $\sim 10^7$ CFU/mL with *Salmonella* Typhimurium DT104 2486 and 2576, *Listeria monocytogenes* Scott A and 310 and incubated at $24 \pm 1^\circ\text{C}$ and $10 \pm 1^\circ\text{C}$. Viable cells were enumerated at 0, 24, 48 and 96h. Addition of the lowest concentration (0.1%) of LMW chitosan was sufficient to reduce number of viable cells of both *Salmonella* strains by ~ 7 logs CFU/ml within 48h at $24 \pm 1^\circ\text{C}$. However, *Salmonella* 2586 and 2576 only decreased by ~ 4 logs at 10°C after 48h. Adding more chitosan improved inactivation. Similar trends were observed with both *Listeria* strains, however Scott A was slightly more resistant to chitosan than 310, OL's were dramatically less effective in reducing viable counts regardless of species and strain. Overall, our study shows that molecular weight greatly influenced antimicrobial efficacy of chitosan. Practical applications: Adding low molecular weight chitosan to food emulsions can significantly decrease the number of pathogens and increase the safety of such foods. Results will be presented in two papers at the IFT Annual Meeting in Chicago, 2003 and two papers are currently in preparation. Results also supported a USDA grant application that is currently pending.