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Geographic information system (GIS) and epidemiological associations among foodborne pathogens at the farm

Kimberly Denise Lamar

To the Graduate Council:

I am submitting herewith a dissertation written by Kimberly Denise Lamar entitled "Geographic information system (GIS) and epidemiological associations among foodborne pathogens at the farm." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Food Science and Technology.

Frances Ann Draughon, Major Professor

We have read this dissertation and recommend its acceptance:

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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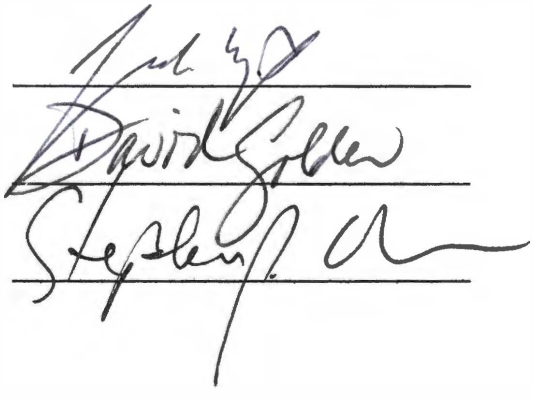

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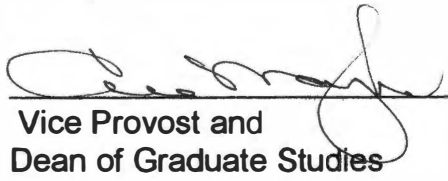

Vice Provost and
Dean of Graduate Studies

GEOGRAPHIC INFORMATION SYSTEM (GIS) AND EPIDEMIOLOGICAL ASSOCIATIONS AMONG FOODBORNE PATHOGENS AT THE FARM

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Kimberly Denise Lamar
August 2003

DEDICATION

This dissertation is dedicated to my mother

Samella B. Lamar

Whose hard work, sacrifice, and love has made my accomplishments possible

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I want to thank You, GOD, for all the blessings you have given me. It is only by your grace, and through your divine mercy, that I have made it this far.

Thank you, Mom, for being my rock and supporting me when times were hard. Thank you for believing in me and reminding me to "keep going, it will pay off in the end." I Love You!

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ABSTACT

Geographic Information System (GIS), a computer mapping and analysis technology, has emerged as an innovative epidemiological tool in a variety of disciplines. However, the application of GIS to food safety research has received little attention. This study utilized GIS and automated riboprinting technology to examine relationships that existed between animals and their environments, monitoring transmission of pathogens on the farm environment and to nearby surface water environments.

A comprehensive epidemiological survey was conducted at The University of Tennessee, Knoxville Experiment Station research dairy farm. More than 40,000 animal and environmental samples were analyzed for *Salmonella*, *Campylobacter jejuni* and *Escherichia coli* O157:H7. A survey of the Tennessee River, adjacent The University of Tennessee research dairy farm, was also conducted to determine the incidence of these pathogens in the river. Automated riboprinting was used to compare bacterial isolates from various species, locations, and sample types.

Salmonella (32%) was the most frequent pathogen isolated on the farm, followed by *C. jejuni* (21%) and *E. coli* O157:H7 (2%). Feed, bedding, water, insects and bird droppings were identified as significant vectors of transmission of pathogens to animals and farm environments. Results of this study indicate that controlling access to animal feed and water sources by insects and wild birds could reduce transmission of pathogens to dairy animals and farm environments.

Neither *C. jejuni* nor *E. coli* O157:H7 were recovered from the Tennessee River. However, *Salmonella* was isolated from sampling sites upstream and downstream from the dairy farm. *Salmonella* was recovered at increased frequency in the Tennessee River at the dairy farm and sites upstream from the farm. *Salmonella* ser. Senftenberg, Typhimurium, Havana and Newport were the most frequently isolated Serotypes at the dairy farm and from the river. *Salmonella* ser. Havana, isolated from farm and river water samples, was the only detected serotype showing similar riboprint patterns. Based on pathogens isolated at the farm and not in the river, the variable pattern of *Salmonella* isolation in the river, and detection of few similar *Salmonella* serotypes, it was concluded that the dairy farm did not contribute significantly to contamination of the river.

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PART I

LITERATURE REVIEW

In the United States, foodborne disease contributes to an estimated 76 million cases and 500 deaths annually (CDC, 2002a). Current research indicates that a broad array of pathogens contaminate our food supply. Although most foodborne pathogens can be controlled in the kitchen by consumers, they are not; therefore, risk reductions are needed at every point from farm-to-table. *Salmonella*, *Campylobacter jejuni* and *Escherichia coli* O157:H7, frequently associated with foods of animal origin, are among the most common pathogens implicated in cases of foodborne disease (Potter, 1994). Knowledge of factors that affect shedding of these pathogens by food producing animals could help reduce transmission of pathogens at the farm, thus decreasing the risk of contamination throughout the rest of the food chain.

An estimated 2 million to 4 million cases of salmonellosis occur annually in the United States (FDA, 1992). In the United States, the annual cost of foodborne salmonellosis in humans has been estimated at $0.6 billion to $3.5 billion, making salmonellosis one of the most costly human foodborne diseases (Busby et al., 1996). Human outbreaks of salmonellosis in the United States are frequently associated with foods of animal origin. Eggs, poultry, meat and meat products are commonly identified vehicles for transmission of salmonellosis to humans (Jay, 2000). The occurrence of *Salmonella* in food products poses significant health risks to consumers. Non-typhoid and non-paratyphoid strains of *Salmonella* generally cause entero*coli*tis, characterized by self-limiting diarrhea, severe abdominal pain, nausea, vomiting, and fever. Symptoms of

Salmonella typhi and *Salmonella* paratyphi differ from non-typhoid *Salmonella*.

Enteric fever is the illness resulting from exposure to S. typhi and S. paratyphi, characterized by translocation from the intestinal tract deep into human tissue. The disease can lead to a chronic carrier state, causing asymptomatic carriers to intermittently shed *Salmonella* (D'Aoust, 2001).

Farm animals may frequently be intestinal carriers of *Salmonella*, and fecal shedding of the organism is the primary mode of on-farm contamination (Oosterom, 1991). *Salmonella* can be shed in milk and can cause illness in people consuming raw milk or milk contaminated after pasteurization (Werner et al., 1984). Additionally, milk may also become contaminated by fecal material during collection. In 1984, cheddar cheese made from both pasteurized and heat treated (non-pasteurized) bovine milk was incriminated as the source of 2700 cases of humans salmonellosis (D'Aoust et al., 1985). This was the largest recorded *Salmonella* outbreak in the United States. A cross-connection between raw and pasteurized milk lines was responsible for this outbreak (D'Aoust et al., 1985).

Salmonella may also contaminate carcasses at slaughter and cause illness in people consuming contaminated meat (Smith et al., 1994). Raw ground beef is a well-known vehicle for transmission of *Salmonella*. Cull dairy cattle, which are the source of much of the hamburger consumed in the United States, represent important potential reservoirs for human salmonellosis. The rate of *Salmonella* shedding by cull dairy cattle reportedly ranges from 0.46 to 18.1% (Gay et al., 1994; Murinda et al., 2002). Gay et al. (1994) surveyed 1289

cull dairy cattle in Washington State for fecal shedding of *Salmonella*. The rate of fecal shedding by cull dairy cattle in Washington state was estimated at 0.46 to 0.92%. In a survey of cull dairy cattle from 30 dairy farms in east Tennessee, *Salmonella* were isolated from 23.3% of the farms surveyed, with 2.17% of fecal samples testing positive for *Salmonella* (Murinda et al., 2002). Differences in isolation rates of *Salmonella* can be attributed to variations in isolation and confirmation methodologies used in the studies. Higher isolation rates for *Salmonella* were observed in healthy beef cattle. In 1998, a national study was conducted on health and management of cattle in feedlot environments (Fedorka-Cray et al., 1998). A total of 4,977 samples were collected from 100 feedlots in 13 states having the majority of feedlot cattle production in the United States. *Salmonella* were recovered from 38% of the feedlots surveyed, however results varied by season and geographic region (Fedorka-Cray et al., 1998).

Campylobacter jejuni is the most frequent cause of foodborne disease in humans in the United States, causing approximately 2.4 million illnesses annually, and causes and estimated 100 deaths per 10,000 cases (Tauxe, 1992). The high frequency of infection may be due to the low infectious dose of *C. jejuni*, which ranges from 100 to 500 organisms (Mead et al., 2000). Symptoms of *Campylobacter*iosis include diarrhea, abdominal cramps, abdominal pain, and fever. The pathogen is also known to contribute to the development of Guillain-Barré syndrome, an illness characterized by nerve damage and possible paralysis (CDC, 2002a). An estimated one case of Guillain-Barré syndrome

occurs per every 1000 cases of *Campylobacter*iosis. Up to 40% of patients with the syndrome have evidence of prior *Campylobacter* infection (Allos, 1997).

Campylobacter infections are usually sporadic, occurring during summer and early fall, and usually follow ingestion of improperly handled or improperly cooked foods (Tauxe, 1992). Although human infection from *Campylobacter* is commonly associated with consumption of contaminated poultry products, several sporadic cases have been linked to consumption of contaminated beef, pork, water, and raw milk (Nielson et al., 1997). Raw milk was implicated as the source of infection in 30 of 80 outbreaks of human *Campylobacter*iosis reported to CDC between 1973 and 1992 (Hopkins et al., 1984; Schmid et al., 1987). In a Seattle case control study conducted by the King County Department of Public Health (1984) consumption of raw milk was implicated in 17% of 218 illnesses related to *C. jejuni* infection. In Iowa, another milk related outbreak occurred in which 30% of 46 illnesses from *C. jejuni* were linked to consumption of raw milk (Schmid, 1987).

Campylobacter jejuni is a common organism of the intestinal tract of cattle. Feedlot cattle are more likely than grazing animals to carry *Campylobacter* species (Giacoboni et al., 1993). In a survey of 100 beef cattle at slaughter, *C. jejuni* was recovered from 50% of animals tested (Garcia et al., 1985). Also, a 1985 retail survey indicated that *C. jejuni* was detected in 2 to 5% of raw ground beef and beef flank samples (Stern et al., 1985). Results from these studies indicate that risk of human infection may be reduced by targeting controls in food animal production, processing, and handling of foods of animal origin.

Escherichia coli O157:H7 causes an estimated 62,000 illness and 52 deaths annually in the United States (Mead et al., 2000). *Escherichia coli* O157:H7 produces one or two potent cytotoxins, designated as Shiga toxins 1 and 2, or verotoxins 1 and 2. While other serotypes of shiga toxin producing *E. coli* may cause human illness, *E. coli* O157:H7 is the most commonly identified and most important member of the shiga toxin-producing group of pathogens (Tarr, 1994). *Escherichia coli* O157:H7 is a major public health threat because of its ability to cause serious and potentially life-threatening illnesses. Illness resulting from infection with *E. coli* O157:H7 can range from self-limiting, watery diarrhea to development of hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (Padhye and Doyle, 1992).

A number of reservoirs of *E. coli* O157:H7 have been identified, the most common being cattle, sheep, deer and water (Doyle et al., 1997; Keene et al., 1997; Kudva et al., 1996). Animals shed the organism in their feces, resulting in the possibility of cross-contamination of a wide variety of foods and subsequent foodborne transmission to humans. In 1992, two major outbreaks of *E. coli* O157:H7 occurred in Oregon and Michigan (Riley et al., 1983). The Oregon outbreak involved 26 cases with 19 hospitalizations. The Michigan outbreak involved 21 cases with 14 hospitalizations. The illnesses were epidemiologically linked to undercooked hamburgers from the same fast food restaurant chain. *Escherichia coli* O157:H7 was isolated from patients as well as from frozen ground beef patties. The same meat processor supplied the hamburger patties to restaurants in both states (Riley et al., 1983).

In a study by Martin et al. (1986), raw milk was recognized as a vehicle of transmission of *E. coli* O157:H7 in 1986. Two children from different families developed hemorrhagic *coli*tis and HUS after drinking raw milk from dairy farms. *Escherichia coli* O157:H7 was isolated from the stool of patients and the feces of healthy heifers on both farms (Martin et al., 1986). Duncan et al. (1987) isolated shiga toxin-producing *E. coli* from seven cows at a farm where a class of kindergarten children became ill after drinking raw milk. Three of the children developed HUS (Duncan et al., 1987).

Escherichia coli O157:H7 infection associated with undercooked ground beef and raw milk has led investigations to the role of cattle as the major reservoir of the pathogen. Despite the disease-causing potential for *E. coli* O157:H7 in humans, the organism does not appear to cause disease in cattle (Montenegro et al., 1990). Therefore, healthy cattle harboring the pathogen may enter the food chain. Estimates of the prevalence of *E. coli* O157:H7 in health cattle range from 3.5 to 40% depending on diagnostic methods and on the cattle population under study (Busato et al., 1998). However, according to Cullor (1995), there appears to be no difference in the prevalence of *E. coli* O157:H7 in animals raised on dairies, in beef feedlots, or on cow-calf operations.

Hancock et al. (1994) demonstrated that *E. coli* O157:H7 can be isolated from the feces of asymptomatic cattle. In a Washington State study of 60 dairy cattle and 25 beef cattle, *E. coli* O157:H7 was found in 0.28, 0.71, and 0.33% of fecal samples from dairy, pastured beef, and feedlot beef cattle, respectively (Hancock et al., 1994). As many as 16% of the beef cattle and 8.3% of dairy

cattle herds were infected with the pathogen. Cattle management practices, in this case, were shown to reduce human exposure to *E. coli* O157:H7. *E. coli* O157:H7 positive herds were smaller and tended to irrigate grazing land with fecal slurry. Also, positive herds showed a shorter interval between application of the fecal slurry and grazing of the land by cattle (Hancock et al., 1994). In a second study, Hancock et al. (1997) estimated the prevalence of *E. coli* O157:H7 in feedlot cattle in the United States. Fecal samples from cattle in 100 feedlots in 13 states were cultured bacteriologically for *E. coli* O157:H7. *Escherichia coli* O157:H7 was isolated from 1.8% of 11,881 fecal samples. One or more samples tested positive for the pathogen in 63 of the 100 feedlots tested. The prevalence of *E. coli* O157:H7 was highest in the pens with cattle shortest on feed. Also, animal clustering in several pens may have contributed to the high prevalence of *E. coli* O157:H7 at many of the feedlots. Results from these studies indicate that there are differences in prevalence of cattle shedding *E. coli* O157:H7 among herds and that associations exist between prevalence in cattle and herd management practices.

SURVEILLANCE OF FOODBORNE PATHOGENS

The word "surveillance," derived from the French word "surveiller," means to watch over, to supervise, or to control (Bryan et al., 1997). Surveillance implies the continuous observation of all aspects of the occurrence and spread of a disease that are pertinent to its ultimate control (Bryan, 1988). The reporting of foodborne and waterborne disease in the United States began more than 50 years ago. During this time, state and territorial health officers, concerned with

high morbidity and mortality associated with typhoid fever and infantile diarrhea, recommended that cases of "enteric fever" be investigated and reported. The objective of investigating and reporting these cases was to obtain information regarding the role of food, water and milk in outbreaks of intestinal illness. In 1923, the Public Health Service began publishing summaries of outbreaks of gastrointestinal illness attributed to milk. However, in 1938, they began publishing summaries of outbreaks attributed to all foods. In 1961, the Centers for Disease Control (CDC) assumed the responsibility for publishing summaries of foodborne disease. These early surveillance and reporting measures led to the enactment of public health measures that had a profound impact in decreasing the incidence of enteric disease. Since the 1960's the quality of foodborne disease reports has improved due to more active participation by state and federal agencies in the investigation of outbreaks (Bean et al., 1996).

Surveillance of foodborne pathogens is necessary for preventing the spread of foodborne disease. As foodborne pathogens are identified, they become the subject of epidemiological investigations and studies of their pathogenicity, ecology, and methods of detection. A major component of foodborne pathogens surveillance includes gathering data on prevalence of the etiological agents of disease, vehicles of spread of these agents, and their common reservoirs. Newly identified vehicles may indicate changes in practices that commonly lead to contamination, survival, and amplification of etiological agents or factors that influence these events. In addition, data on reservoirs of

important pathogens can suggest needs for modifications in agricultural, food processing, and sanitation practices (Guzewich et al., 1997).

Although there are several types of disease surveillance systems, two types are important to foodborne pathogens surveillance. These include laboratory isolation of foodborne pathogens from humans and animals and hazard surveillance (Guzewich et al., 1997). Isolations of foodborne pathogens from humans usually come from ill persons, from which physicians obtain clinical specimens. Isolations from animals are usually obtained from animals being treated by veterinarians or as a result of surveys of a foodborne pathogen of contemporary concern (Bryan et al., 1997).

The CDC Emerging Infections Program Foodborne Diseases Active Surveillance Network (FoodNet) collects data on 10 foodborne diseases in 9 states (California, Colorado, Connecticut, Georgia, New York, Maryland, Minnesota, Oregon, and Tennessee) to monitor foodborne illness (CDC, 2002a). FoodNet's goals include estimating the annual frequency and severity of foodborne diseases, and determining how much foodborne illness is due to consumption of specific foods such as meat, poultry, and eggs. The core of FoodNet is laboratory-based active surveillance at over 300 clinical laboratories. Information is collected on every laboratory-diagnosed case of bacterial pathogens including *Salmonella*, *Shigella, Campylobacter*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, Vibrio and parasitic organisms. For more precise classification, clinical isolates are sent from FoodNet sites to CDC for

further testing, including antibiotic resistance, phage typing, and molecular subtyping (CDC, 2002a).

PulseNet is a national network of public health laboratories that performs DNA fingerprinting on foodborne bacterial isolates from outbreak cases. PulseNet performs DNA fingerprinting by Pulsed Field Gel Electrophoresis (PFGE) on disease causing bacterial isolates from humans and from suspected foods. The network identifies and labels fingerprint patterns and compares patterns through an electronic database at CDC to identify related strains. Through PulseNet, scientists are able to rapidly determine whether an outbreak is occurring, even if affected persons are geographically separated. Outbreaks and their causes can be identified in a matter of hours rather than days or weeks (CDC, 2002b).

Hazard surveillance has been used primarily in the assessment of occupational diseases and environmental exposures. It is the surveillance of the occurrence of and distribution of biological, chemical or physical hazards. Foodborne hazards are those hazards that affect contamination, survival, or proliferation of pathogens (Bryan et al., 1997). Hazard surveillance is the main focus of the hazard analysis component of the Hazard Analysis Critical Control Point (HACCP) approach. Surveillance information regarding vehicles and contributing factors of foodborne disease is used to identify critical control points where monitoring is necessary to ensure elimination, prevention, or reduction of foodborne hazards (Bryan, 1988).

Several hazards, including certain chemical, physical, and microbiological hazards, originate on farms. As processors develop programs to control these hazards, they are likely to look to farmers to reduce the risk of these hazards in primary production. Development of on farm systems to control hazards on farms, such as application of the HACCP approach, has been advocated as a relatively simple approach that integrates with other systems developed for slaughter and processing (Noordhuizen and Welpelp, 1996).

The Food Safety Inspection Service (FSIS) of the U.S. Department of Agriculture, is responsible for ensuring the safety, wholesomeness, and accurate labeling of meat, poultry, and egg products. In 1996, FSIS issued the Pathogen Reduction; HACCP rule, which sets pathogen reduction performance standards for *Salmonella* in slaughter plants and plants producing raw ground meat products. FSIS collects and analyzes HACCP *Salmonella* samples in order to verify plant compliance with the Pathogen Reduction and HACCP rule (Food Safety and Inspection Service, 2002). Recent data released by the CDC indicate that foodborne illness is declining in the United States, and that the prevalence of *Salmonella* in meat and poultry has declined since the implementation of the Pathogen Reduction/HACCP Rule (CDC, 2002a).

RISK ASSESSMENT AND RISK MANAGEMENT

Outbreaks of foodborne illness and recalls of various meat and poultry products over the past few years have reinforced the need for more effective measures to control foodborne pathogens at the farm. In 1998 the United States Department of Agriculture recommended a shift from traditional plant-based

inspections to prevention-oriented systems based on risk assessment at the farm (Food Safety and Inspection Service, 1998).

Risk is the chance that an event or exposure will lead to some disease, condition, disability, or even death. Risk factors are those behaviors, events, or exposures associated with occurrence of disease, condition, disability or death. The more exposure to risk factors, the greater the probability of occurrence of a specific disease or other negative result (Timmreck, 1994). Risk assessment is defined as the qualitative and quantitative process used to evaluate hazardous conditions and characterize the resulting risk. Risk assessment uses the tools of science, engineering, and statistics to analyze risk-related information and to estimate and evaluate the magnitude of outcomes harmful to humans and the environment. The process of risk management integrates results of risk assessment with economic, social, political and legal concerns to develop a course of action to prevent a problem (Walker, 1997).

Components of risk assessment include hazard identification, exposure assessment, dose-response assessment, and risk characterization (Timmreck, 1994; Walker, 1997). When risk assessment of foodborne pathogens is applied at the farm, hazard identification is used to identify foodborne pathogens of interest in farm animals and farm environments. Exposure assessment is used to identify routes of exposure as well as the number of species and areas of exposure to the foodborne pathogens of interest. The amount or dose response assessment is used to determine the incidence of these pathogens in farm animals and farm environments and to determine the relationships between

similar pathogens isolated from different sites. Utilizing sound principles of risk assessment is vital to communicating risk, setting priorities, developing risk management programs, and evaluating control efforts (Walker, 1997).

GEOGRAPHICAL INFORMATION SYSTEMS (GIS)

Surveillance systems used to monitor foodborne pathogens at the farm require the application of epidemiological sciences to data concerning reservoirs, environmental factors and farm management practices that favor the existence and spread of pathogens throughout the animal population. The Geographic Information System, or GIS, has recently emerged as an innovative and important component of many projects in public health and epidemiology. GIS is a powerful computerized mapping and analysis technology that allows large quantities of information to be viewed and analyzed within a geographic context (Clarke et al., 1996). GIS offers a coordinated and integrated approach to manage, analyze, and present large amounts of spatial and non-spatial data. GIS links non-graphic data (e.g. levels of pathogens) with graphic map features to allow a wide range of information processing and display operations, as well as map production, analysis, and modeling (Vine et al., 1997). Epidemiologists have traditionally used maps when analyzing associations between location, environment, and disease. GIS technology is currently being applied to a variety of public health issues, including the study of variations in disease frequency and health status, and measurement of health care delivery and resource allocation (Tim, 1995). Public health researchers, for example, have used GIS to map and study cancer mortality. Other disciplines now utilizing GIS include forestry,

transportation planning, emergency services delivery, marketing, surveying, and criminal justice (Tim, 1995).

Environmental health professionals are asked frequently to address whether exposure to certain environmental contaminants have led to adverse health effects. However, they are usually limited in their ability to properly address the issues in a timely and cost-effective fashion. Modern computer technologies, such as GIS, provide cost-effective epidemiological tools for evaluating relationships that exist between the environment and factors potentially affecting health outcomes.

Advances in GIS may prove to be a valuable tool in food safety research and surveillance of foodborne diseases. Utilization of GIS in food safety research will allows for improved monitoring and instantaneous visualization of the transport of foodborne pathogens in the environment. The use of this technology may reduce the time required to analyze numerical output and enable users to identify critical areas of non-point source pollution and to perform various "what if" scenarios to support the decision making process. GIS will allow researchers to visualize and analyze information in new ways, and reveal previously hidden relationships, patterns, and trends.

PURPOSE OF CURRENT RESEARCH

Epidemiological data suggest that our most important foodborne hazards are *Salmonella*, C. *jejuni*, and *E. coli* O157:H7, and foods of animal origin are most often associated with infectious foodborne disease than other foods (Potter, 1994). *Salmonella*, C. *jejuni*, and *E. coli* O157:H7 all share the common

characteristic of having an animal reservoir from which they can spread to humans; therefore, risk reductions at every point from farm-to-table are necessary. Development of efficient, on-farm strategies to control foodborne pathogens requires knowledge of basic epidemiology such as the prevalence and distribution of pathogens on animals and in the environment. The purpose of this research was to provide a detailed and comprehensive epidemiological characterization of *Salmonella*, C. *jejuni*, and *E. coli* O157:H7 in dairy cattle animals, farm environments, and nearby surface water. GIS and molecular typing methodology were used as epidemiological tools to examine relationships that exist between animals and their environments. Risk assessment and risk analysis were used to evaluate how various farm management practices influence factors such as contamination, proliferation, and transmission of *Salmonella*, C. *jejuni*, and *E. coli* O157:H7 on the farm. The overall goal of this research was to generate a descriptive risk assessment model to which future data could be applied to develop farm management strategies to reduce contamination with foodborne pathogens in production environments.

LITERATURE CITED

Allos, B.M. 1997. Association between *Campylobacter* infection and Guillain-Barré syndrome. J. Infect. Dis. 176:125-128.

Bean, N.H., J.S. Goulding, M.T. Daniels, F.J. Angulo. 1997. Surveillance for foodborne disease outbreaks-United States, 1988-1992. J. Food. Prot. 60(10): 1265-1286.

Bryan, F.L. 1988. Risks associated with practices, procedures, and processes that lead to outbreaks of foodborne diseases. J food Prot 51: 663-673.

Bryan, F.L., E.C.D. Todd, and J.J. Guzewich . 1997. Surveillance of foodborne diseases. Part II. Summary and presentation of descriptive data and epidemiologic patterns; their value and limitations. J food Prot 60:567-578.

Busato, A., D. Hofer, T. Lentze, C. Gaullard, A. Burnens. 1998. Prevalence and infection risk of zoonotic enteropathogenic bacteria in Swiss cow-calf farms. Vet. Microbiol. 69(4): 251-263.

Busby, J.C., C.T. Jordan Lin, and J.M. MacDonald. 1996. Bacterial foodborne disease: medical costs and productivity losses. USDA. Agricultural Economic Report No. 741, Washington, DC, USA.

CDC. 2002a. Preliminary FoodNet data on the incidence of foodborne illnesses: Selected sites, United States, 2001. MMWR 51(2): 325-329.

CDC. 2002b. National molecular subtyping network for foodborne disease surveillance (PulseNet). Location: http://www.cdc.gov/pulsenet. Accessed: 2 October, 2002.

Clarke, C. K., Mc Lafferty. S. L. and Tempalski. B. J. (1996). On Epidemiology and Geographical Information Systems: A review and discussion on future directions. *Emerging Infectious Diseases,* 12(2), 85-92.

D'Aoust, J.Y., et al. 1985. *Salmonella* Typhimurium phage-type 10 from cheddar cheese implicated in a major Canadian Foodborne outbreak. J. Food Prot., 48(12): 1062-1066.

D'Aoust, J.Y. 2001. Foodborne salmonellosis: Current international concerns. Food Safety Magazine, 7(2): 10-16.

Doyle, M.P., T. Zhao, J. Meng, and S. Zhao. 1997. *Escherichia coli* O157:H7. In Food Microbiology: Fundamentals and Frontiers, ed. M.P. Doyle, L.R. Beuchat, and T.J. Montville. ASM Press, Washington, D.C.

Duncan, L., V. Mai, A. Carter, A.K. Carlson, A. Borczyk, and M.A. Karmali. 1987. Outbreak of gastrointestinal disease-Ontario. Can. Dis. Weekly Rep. 13: 5-8.

Fedorka-Cray, P.J., D.A. Dargatz, L.A. Thomas, and J.T. Gray. 1998. Survey of *Salmonella* serotypes in feedlot cattle. J. Food Prot., 61(5): 525-530.

Food Safety and Inspection Service. 1998. Moving from a plant-based inspection workforce to a farm to table consumer safety workforce. Food Safety Inspection Service, U. S. Department of Agriculture, Washington, D. C.

Garcia, M.M., H. Lior, R.B. Stewart, G.M. Ruckerbaur, J.R.R. Trudel, and A. Skljarevski. 1985. Isolation, characterization and serotyping of *Campylobacter jejuni* and *Campylobacter coli*, from slaughter cattle. Appl. Environ. Microbiol. 49(3): 667-672.

Gay, J.M., D.H. Rice, and J.H. Steiger. 1994. Prevalence of fecal *Salmonella* shedding by cull dairy cattle marketed in Washington State. J. Food Prot. 57(3): 195-197.

Giacoboni, G.I., K. Itoh, K. Hirayama, E. Takahashi, and T. Mitsuoka. 1993. Comparison of fecal *Campylobacter* in calves and cattle of different ages and areas in Japan. J. Vet. Med. Sci. 55: 555-559.

Guzewich, J.J., F.L. Bryan, and E.C.D. Todd. 1997. Surveillance of foodborne disease I. Purposes and types of surveillance systems and networks. J food Prot 60(5): 555-566.

Hancock, D.D., T.E. Besser, M.L. Kinsel, P.I., Tarr, D.H. Rice, and P.C. Paros. 1994. The prevalence of *Escherichia coli* O157:H7 in dairy and beef cattle in Washington State. Epidemiol. Infect. 113: 199-207.

Hancock, D.D., D.H. Rice, L.A. Thomas, D.A. Dargatz, and T.E. Besser. 1997. Epidemiology of *Escherichia coli* O157 in feedlot cattle. J. Food Prot. 60(5): 462-465.

Hopkins, R.S., R. Olmsted, and G.R. Istre. 1984. Endemic *Campylobacter jejuni* infection in Colorado: identified risk factors. Am. J. Public Health 74:249-250.

Jay, J.M. 2000. Modern food microbiology, 6th ed. Aspen Publishers, Gaithersburg, MD.

Keene, W.E., E. Sazie, J. Kok, D.H. Rice, D.D. Hancock, V.K. Balan, T. Zhao, and M.P. Doyle. 1997. Outbreak of *Escherichia coli* O157:H7 infections traced to jerky made from deer meat. J. Am. Med. Assn. 277: 1229-1231.

Kudva, I.T., P.G. Hatfield, and C.J. Hovde. 1996. *Escherichia coli*O157:H7 in microbial flora of sheep. Appl. Environ. Microbiol. 34: 431-433.

Mead, P.S., L Slutsker, V. Dietz, L.F. McCaig, J.S. Bresee, C. Shapiro, P.M. Griffin, and R.V. Tauxe. 1999. Food-related illness and death in the United States, Emerging Infect. Dis. 5: 607-625.

Montenegro, M.A., M. Bulte, and T. Trumpf. 1990. Detection and characterization of fecal verotoxin-producing *Escherichia coli* from healthy cattle. J. Clin. Microbiol. 28: 1417-1421.

Murinda, S.E., L.T. Nguyen, S.J. Ivey, B.E. Gillespie, R.A. Almeida, F.A. Draughon, and S.P. Oliver. 2002. Prevalence and molecular characterization of *Escherichia coli* O157:H7 in bulk tank milk and fecal samples from cull cows: A 12-month survey of dairy farms in east Tennessee. J. Food Prot. 65(5): 752-759.

Nielson, E.M., J. Engberg, and M. Madsen. 1997. Distribution of serotypes of *Campylobacter jejuni* and C. *coli* from Danish patients, poultry, cattle, and swine. FEMS Immunol Med. Microbiol. 19:47-56.

Noordhuizen, J. and H.J. Welpelo. 1996. Sustainable improvement of animal health care by systematic quality risk management according to the HACCP concept. Veterinary Quarterly 18(4): 121-126.

Oosterom, J. 1991. Epidemiological studies and proposed preventive measures in the fight against human salmonellosis. Int. J. Food Microbiology, 12: 41-52.

Padhye, N.V. and M.P. Doyle. 1992. *Escherichia coli* O157: H7: Epidemiology, pathogenesis, and methods for detection in food. J. Food Prot., 55(7): 555-565.

Potter, M.E. 1994. The role of Epidemiology in risk assessment: a CDC perspective. Dairy, Food, and Env. Sanitation, 14(12): 738-741.

Riley, L.W., R.S. Remis, S.D. Helgerson, H.B. McGee, J.G Wells, B.R. Davis, R.J. Herbert, et al. 1983. Hemorrhagic *coli*tis associated with a rare *Escherichia coli* serotype. N. Engl. J. Med. 308: 681-685.

Schmid, G.P., R.E. Schaefer, B.D. Plikaytis, J.R. Schaefer, J.H. Bryner, and L.A. Wintermeyer. 1987. A one-year study if endemic *Campylobacter*iosis in a Midwestern city: association with consumption of raw milk. J. Infect. Dis. 156:218-222.

Smith, B.P., L.D. Roden, M.C. Thurmond, G.W. Dilling, H. Konrad, J.A. Pelton, J.A. Picanso. 1994. Prevalence of *Salmonella* in cattle and in the environment on California dairies. J. Am. Vet. Med. Assoc. 205, 467-471.

Stern, N.J. and J.E. Line. 1992. Comparison of three methods for recovery of *Campylobacter* spp. from broiler carcasses. J. Food Prot. 55:663-666.

Tarr, P.I. 1994. *Escherichia coli* O157:H7: Overview of clinical and epidemiological issues. J. Food Prot. 57(7): 632-636.

Tim, U.S. 1995. The application of GIS in environmental health sciences: Opportunities and limitations. Env. Res. 71: 75-88.
Timmreck, T.C. 1994. An introduction of Epidemiology. Jones and Bartlett Publishers, Inc. Boston, MA.

Timmreck, T.C. 1994. An introduction to Epidemiology. Joans and Bartlett Publishers: Boston.

Vine, M.F., D. Degnan, and C. Hanchette. 1997. Geographic Information Systems: their use in environmental research. Env. Hlth. Persp. 101(6): 105-111.

Walker, B. 1997. Environmental planning. Environmental Health, 2nd Edition. Morgan et al. Eds. Morton Publishing, Engelwood, Colorado.

Wells, S.J., P.J. Fedorka-Cray. D.A. Dargatz, K. Ferris, and A. Green. 2001. Fecal shedding of *Salmonella* spp. by dairy cows on farm and at cull cow markets. J. food Prot. 64: 3-11.

PART II

ISOLATION OF FOODBORNE PATHOGENS FROM DAIRY COWS, CALVES AND FARM ENVIRONMENTS

ABSTRACT

A comprehensive epidemiological survey was conducted to determine the prevalence of *Salmonella, Campylobacter jejuni*, and *Escherichia coli* O157:H7 in dairy animals and dairy farm environments. The experimental design included 321 dairy cows (lactating and non-lactating) and calves from The University of Tennessee, Knoxville Experiment Station research dairy farm. Samples were collected monthly for 12 months from dairy cows, calves, feed and the farm environment, which included pastures, barns, bedding, soil, bulk tank milk, milking equipment, air, insects, and wild birds. A total of 45,732 samples were analyzed for *Salmonella*, *C. jejuni*, and *E. coli* O157:H7 using modifications of the Food and Drug Administration, Bacteriological Analytical Manual enrichment, isolation, and confirmation protocols, previously validated for each sample type. A Geographic Information System (GIS) was used to examine relationships that exist between animals and their environments.

A strong seasonal influence occurred with the isolation of *Salmonella* and *C. jejuni* at the farm. The prevalence of *Salmonella* isolated from dairy animals, feed, and dairy farm environments was 32%. *Salmonella* isolation was highest in the summer (48%) in both cows and calves. *Salmonella* isolation during winter, spring, and summer months was between 24% and 26%. Feed (59%), bedding materials (47%), water (40%), bird droppings (34%) and insects (39%) were identified as significant sources of *Salmonella*. The prevalence of *Campylobacter jejuni* isolated at the farm was 21%. Isolation of *C. jejuni* was significantly higher during winter (28%) and fall (29%). Bird droppings (33%)

were strongly correlated ($P \leq 0.05$) with contamination of feed (40%) and water (60%) sources. The prevalence of *E. coli* O157:H7 at the farm was only 2 %. Isolation was very infrequent throughout the entire study period. No statistical differences ($P \geq 0.05$) were observed among sample type. Data generated from the current research were used to devise strategies to reduce pathogen contamination at the farm through environmental management and risk assessment.

INTRODUCTION

Environmental sources of contamination are recognized as important links in the transmission chain of foodborne pathogens. Foods of animal origin are more often associated with foodborne disease than other foods (Potter, 1994), however, very little information is available on the significance of the farm environment's role in the survival and transmission of foodborne pathogens.

Although carriage of *Salmonella* is common in all types of cattle, specific reports on the disease and recovery of *Salmonella* from cattle tend to focus on dairy cattle (Smith et al., 1994; Kabgambe et al., 2000). Smith et al. (1994) evaluated the prevalence of *Salmonella* in cattle and in the environments of 60 California dairy farms. Samples for bacteriologic analysis were collected from pooled feces from calves, swabs of wet areas and feces from calf pens and hospital pens, drag samples from wastewater lagoons, and animal feed. Overall, 45 of 60 (75%) California dairies tested positive for *Salmonella*. This high percentage of California dairies testing positive for *Salmonella* was not necessarily surprising, in view of the poor farm management practices used on

many of the farms. The overall sanitation at many of the farms was poor. Farm management practices identified as contributing to the *Salmonella* contamination included the following: Feed was not routinely tested for contamination with pathogens; new animals added to the herd were not tested for pathogens; sick animals or known *Salmonella* carriers were not culled; control of rodents and wild birds was poor; and lagoon wastewater was recycled and used as flush water (Smith et al., 1994).

In 1996, Kabagambe et al. (2000) examined farm management practices associated with fecal shedding of *Salmonella* by dairy cows. Fecal samples from 4299 dairy cows in 91 herds were tested for fecal shedding of *Salmonella*. *Salmonella* fecal shedding was detected in 27.5% of the dairy herds surveyed. The most important risk factors identified for fecal shedding of *Salmonella* included use of flush water systems, feed, herd size, and region. However, the precise roles of each of these factors in the shedding of *Salmonella* were not identified (Kabagambe et al., 2000). The results from this study identified factors associated with increased risk of shedding of *Salmonella* in dairy cattle. Factors that have been postulated to increase the risk of fecal shedding of *Salmonella* include season of the year, feeding contaminated feeds to cattle, and improper manure management (Wray and Davies, 1996; Anderson et al., 1997). Also, new cattle introduced into herds, feed, rodents and birds with access to cattle feed sources may be sources of *Salmonella* at the farm (Evans, 1996). An integrated approach to control these multiple sources of contamination at the

farm is necessary for reducing the risk of *Salmonella* shedding among dairy cattle (Kabgambe et al., 2000).

Healthy cattle may be reservoirs for a variety of *Campylobacter* species. *Campylobacter jejuni* and *C. coli* have been isolated frequently from healthy livestock and are assumed to be part of the normal intestinal flora of bovines (Busato et al., 1998). The prevalence of *C. jejuni* in dairy cattle ranges from 5 to 53%, depending on method of isolation, age of the animal, season the year, and type of samples analyzed (Wesley et al., 2000). Younger animals are more often colonized with *Campylobacter* than older animals (Giacoboni et al., 1993), and recovery rates are highest during the cold season (Carter et al., 1987).

Wesley et al. (2000) examined the prevalence of *C. jejuni* in healthy US dairy herds. Over 2000 fecal samples were collected from a combination of 31 milk cow dairy operations, 13 farms on which lactating cows were culled, and 36 market operations in 23 states. *Campylobacter jejuni* was detected in 37.7% of dairy cattle fecal samples. *Campylobacter jejuni* was recovered more frequently from fecal samples obtained from large versus small herds. Also, commingling of culled cattle with healthy cattle facilitated the transmission of *C. jejuni* throughout the herds and farm environments (Wesley et al., 2000).

In a survey of 13 dairy farms, *C. jejuni* was recovered from 5% of 904 milk samples and 22% of 904 cow fecal samples (Beumer et al., 1988). A second study, conducted by Humphrey and Beckett (1987), surveyed 12 English dairy farms for contamination of cattle with *C. jejuni*. *Campylobacter jejuni* was isolated from 24% of 668 cattle rectal swab samples. Dairy farms in which

Campylobacter was not isolated used chlorinated drinking water for animals. Dairies testing positive for *Campylobacter* used river water as an animal drinking water source. Several studies have identified unchlorinated drinking as a vehicle of transmission of *Campylobacter* to the dairy farm environment (Humphrey and Beckett, 1987). Several potential measures to control *C. jejuni* at the farm included sanitation, water treatment, and vector control.

Dairy cattle have been implicated as principle reservoirs of *Escherichia coli* O157:H7, with undercooked ground beef and raw milk being the major vehicles of foodborne outbreaks (Zhao et al., 1995). The involvement of cattle in the spread of the pathogen has focused research on the farm and farming practices that may contribute to the presence of the pathogen in herds.

Escherichia coli O157 is believed to be widespread on US dairy farms, but at very low prevalence (Hancock et al., 1994; Zhao et al., 1995). Estimates of the prevalence of *E. coli* O157:H7 in health cattle range from 3.5 to 40% depending on diagnostic methods and on the cattle population under study (Busato et al., 1998). The herd prevalence for *E. coli* O157:H7 can range from up to 70% in some dairy herds and 63% in US feedlots (Hancock et al., 1997). The prevalence for individual cattle within herds is relatively low and ranges from 0 to 5.5%. Surveys suggest that fecal shedding is intermittent and variable in cattle (Zhao et al., 1995).

The purpose of the present research was to determine the prevalence of *Salmonella, Campylobacter jejuni* and *E. coli* O157:H7 recovered from dairy farm animals and farm environments. A Geographic Information System (GIS) was

used to identify critical sources of contamination at the farm and identify the vehicles by which *Salmonella, Campylobacter jejuni*, and *E. coli* O157:H7 were spread among dairy cattle. Results of the study were used to devise farm management strategies to reduce the risk of transmission of foodborne pathogens at the farm.

MATERIALS AND METHODS

Experimental Design

The University of Tennessee, Knoxville Experiment Station Dairy Research Herd consisted of 251 cows and 170 calves (80% Holsteins and 20% Jerseys). The herd annually averages 10,896 kg of milk per cow for Holsteins and 7,718 kg of milk per cow for Jerseys. Cows in the research herd were milked twice daily in a 12-stall trigon parlor equipped with a DeLaval milking machine system (De Laval, Kansas City, MO). Pre-milking and post-milking teat disinfections were practiced regularly. Lactating cows were housed in free stalls bedded with sawdust and were fed a total mixed ration daily. All cows were dried off approximately eight weeks before expected calving and all quarters of cows were infused with antibiotic preparations approved for use in non-lactating cows following the last milking of lactation. Calves were housed in individual calf hutches and were fed discarded milk or milk replacer until weaning. After weaning, calves were moved to group pens bedded with sawdust.

All samples were collected and processed by the Food Safety Center Research Team at The University of Tennessee, which included Willie Taylor,

Philipus Pangloli, Michelle Saul, Andy Doan, and myself. Samples were collected monthly for a period of 12 months.

Animal Sampling

Oral, rectal and hair samples were collected from both cows and calves. All samples were collected in duplicate. Oral samples were taken from the inner surface of the oral cavity using sterile cotton swabs. Hair samples were obtained by swabbing the back and neck areas using swabs moistened with buffered peptone broth. The peri-anal area was cleaned with sterile gauze soaked in sterile saline and fecal samples were obtained from the rectum using occluded sterile swabs (Accu-Med Corporation, Milford, OH). After sample collection, swabs were placed into sterile tubes containing 5 ml of lactose broth (Becton, Dickinson and Company, Franklin Lakes, NJ) and kept on ice until processing. Foremilk samples were obtained from lactating cows by drawing 2 ml of milk into sterile tubes, without discarding the first four streams. Bulk tank milk samples were obtained by immersing a sterile container into the bulk tank. Milk samples were kept on ice until processed.

Environmental Sampling

Environmental samples included bedding, feed, water, air, manure slurry, and from milking equipment. Samples of bedding were collected from stalls, maternity pens, dry cow facilities, and calf facilities. Samples were collected from two locations in the center of the stall and from points approximately one-third the distance from each external wall to the center of the box stall or housing area.

Bedding samples (approximately 1 kg) were placed into sterile plastic bags, mixed thoroughly and kept at room temperature until processed.

Approximately 200 g of the total mixed ration at the time of feeding and 200 g of feed remaining in the feed bunk 4 h after feeding were collected from the feed bunk using sterile feed scoops. Samples were placed into sterile plastic bags and kept at room temperature until processed. Cattle drinking water samples were collected directly from water troughs. Water samples were obtained in duplicate.

Samples were collected from milking machine liners by swabbing areas of the liners that directly contacted teat skin with sterile cotton swabs moistened with sterile water. Swabs were placed into sterile tubes containing 5 ml LB and kept on ice until processed.

Air sampling was performed as described by Rahkio and Korkeala (1997) using an Andersen two-stage viable particle sizing sampler (Andersen Sampler Inc., Atlanta, GA) and differential plating media.

Insects and other wildlife samples including arthropods and flies were trapped using vinyl tube traps and fly strips as described by Gregory et al. (1997). Traps were placed 24 h prior to site visits, and specimens were collected the following day. Traps and fly strips were placed at representative locations within the cow and calf facility, with a minimum of 5 locations per facility. Droppings from wild birds were obtained from several farm environmental sites including fencing along the farm and gates enclosing animal housing areas.

Isolation and Identification of Salmonella

Culture media, reagents (FDA, 1998), equipment and materials used for isolation and confirmation of *Salmonella* are described in the BAM protocol. Sample preparation varied depending upon sample type. Positive control cultures of typical *Salmonella*, and atypical *Salmonella* (S. arizonae, American Type Culture Collection 12325, lactose +, sulfide +; S. abortus equi, American Type Culture Collection 9842, lactose -, sulfide -) were prepared and used as a reference for each analysis. Samples were direct plated onto bismuth sulfite agar (Becton, Dickinson and Company, Franklin Lakes, NJ) and XLT agar (Becton, Dickinson and Company, Franklin Lakes, NJ) for possible enumeration and also placed into lactose enrichment broth followed by enrichment in tetrathionate broth at 42º C and Rappaport Vassiliadis Broth (Becton, Dickinson and Company, Franklin Lakes, NJ) at 35º C. Differential plating was conducted on bismuth sulfite agar, XLD agar (Becton, Dickinson and Company, Franklin Lakes, NJ), hektoen enteric agar (Becton, Dickinson and Company, Franklin Lakes, NJ) and Brilliant Green Agar (Becton, Dickinson and Company, Franklin Lakes, NJ). Colonies typical of *Salmonella* were selected from differential plates (three from each plate) and inoculated onto triple sugar iron (TSI) medium (Becton, Dickinson and Company, Franklin Lakes, NJ). Colony morphology, biochemical testing, and serological testing were performed as described in BAM (Andrews et al., 1995; FDA, 1998). Procedure for the latex agglutination test involved adding a drop of latex solution to a sample card, mixing a loopful of sample with the latex solution (10 – 12 sec.) and observing for an agglutination

reaction. Agglutination positive reactions resulted in a thick, stringy appearance to the sample mixture.

Isolation and Identification of Campylobacter jejuni

Samples were pre-enriched for 5 h in *Campylobacter* enrichment broth (CEB) (Oxoid Inc., New York, USA) and Bolton Broth (BB) (Oxoid Inc., New York, USA). Samples were placed in a 30º C incubator for 3 h, then in a 37º C incubator for 2 h. After enrichment, all samples were streaked onto *Campylobacter* blood-free selective agar plates, with supplements (Oxoid Inc., New York, USA). The plates were incubated under microaerophilic conditions for 24 h at 42° C. Presumptive positive colonies were confirmed biochemically as *C. jejuni* with glucose fermentation, hippurate hydrolysis and oxidase reactions. Serological confirmation with polyvalent somatic O antigens latex agglutination test (Oxoid Inc., New York, USA). Procedure for the latex agglutination test involved adding a drop of latex solution to a sample card, mixing a loopful of sample with the latex solution (10 – 12 sec.) and observing for an agglutination reaction. Agglutination positive reactions resulted in a thick, stringy appearance to the sample mixture.

Isolation and Identification of Escherichia coli O157:H7

Samples for *Escherichia coli* O157:H7 isolation were enriched in trypticase soy broth (Becton, Dickinson and Company, Franklin Lakes, NJ) and incubated in a shaking water bath at 37º C for 18– 24 h. Upon incubation, a loopful of enriched samples was streaked onto CT-SMAC (Becton, Dickinson and Company, Franklin Lakes, NJ) and EMB plates (Becton, Dickinson and

Company, Franklin Lakes, NJ) for isolation. Plates were incubated at 35º C for 18 – 24 h. Typical, non-sorbitol fermenting *E. coli* O157 colonies were picked up from each plate and inoculated on TSAYE slants (Becton, Dickinson and Company, Franklin Lakes, NJ). Slants were incubated at 35º C for 18– 24 h. The isolates on TSAYE were used for biochemical and serological testing. Isolated cultures were tested for indole and citrate biochemical reactions (FDA, 1998). Isolates were serologically confirmed with O157 antiserum and latex agglutination tests (Oxoid Inc., New York, USA). Procedure for the latex agglutination test involved adding a drop of latex solution to a sample card, mixing a loopful of sample with the latex solution (10 – 12 sec.) and observing for an agglutination reaction. Agglutination positive reactions resulted in a thick, stringy appearance to the sample mixture.

Geographic Information System (GIS) Analysis

An aerial photograph taken of the dairy farm was scanned into ArcView® GIS version 3.2 (Environmental Systems Research Institute, Redlands, CA) and used as the basemap onto which the spatial information was layered. Data generated from microbial analyses of animal and environmental samples (number of confirmed pathogens isolated at specific points on the farm) were imported into the ArcView® project. Data were sorted and grouped by quarter (1^{st}, 2^{nd}, 3^{rd}, and 4^{th}), analyzed to give percent of pathogens isolated at each point on the farm, and plotted onto the basemap. Cartographic software, Macromedia Freehand 9.0® (Macromedia Inc.,NY, USA), was used to visually

display data on the map. A series of colors and shapes were used for improved visualization of the data.

Data Analysis

Data were stored and coded as positive or negative for the presence or absence of *Salmonella* in animal and environmental samples. Categorical statistical data analyses were performed using SAS version 8.2 (SAS® Institute, Cary, NC, USA). Chi-Square (X^2) analysis was performed to determine correlations among sample types. ArcView® GIS version 3.2 was used to perform advanced statistical analyses of spatial data, including cluster analyses, and Poisson probability distributions.

RESULTS

The microbiological survey of The University of Tennessee research dairy farm represents over 40,000 samples from dairy cows, calves, and the farm environment. Dairy animals and environmental samples were analyzed for the presence of *Salmonella*, *Campylobacter jejuni*, and *Escherichia coli O157*:H7 (Table 1).

A total of 12,480 animal, feed, and environmental samples were analyzed for the presence of *Salmonella*. Of these samples 3994 (32%) tested positive for *Salmonella*. Bedding materials (47%), insects (39%), bird droppings (34%), dry cows (33%), and calves (33%) were significant sources of *Salmonella* on the dairy farm (Table 1). Isolation of *Salmonella* from dairy animals, feed, and farm environments varied significantly by quarter (Table 2). *Salmonella* isolation was highest in the summer (48%) in dry cows (42%), lactating cows (68%) and calves

Table 1. Prevalence of *Salmonella*, *Campylobacter jejuni* and *Escherichia coli* O157:H7 recovered from dairy cows, calves and farm environments.

Sample	Sample type	% Positive pathogen isolated (N = 37036)		
		Salmonella (n = 12480)	*C. jejuni* (n = 12546)	*E. coli* O157:H7 (n = 12010)
Lactating Cow	Animal[a]	14^{A}	34^{C}	4
Dry Cow	Animal[b]	33^{BC}	31^{C}	2
Calves	Animal[c]	33^{BC}	28^{BC}	1
Feedbunk	Feed	18^{A}	22^{B}	1
Silage	Feed	53^{DE}	12^{A}	ND
TMR	Feed	59^{E}	40^{D}	ND
Water Trough	Feed	46^{D}	60^{E}	6
Calf Sipper	Feed	24^{B}	11^{A}	ND
Bedding – Cow[d]	Environment	47^{D}	5^{A}	ND
Bedding –Calf	Environment	47^{D}	ND	1
Bird Droppings	Environment	34^{C}	33^{C}	ND
Soil – Lactating	Environment	36^{C}	20^{B}	9
Soil – Maternity	Environment	15^{A}	20^{B}	2
Soil – Calf	Environment	11^{A}	5^{A}	ND
Manure	Environment	16^{A}	7^{A}	ND
Air – Calf	Environment	15^{A}	14^{A}	ND
Insect – Calf	Environment	9^{CD}	17^{A}	2
TOTAL (average %)		32	21	2

[a]Lactating cow animal samples include oral, rectal, hair, teat and foremilk
[b]Dry cow samples included oral, rectal, and hair.
[c]Calf samples included oral, rectal, and hair.
[d]Bedding materials included sand, sawdust, and grass.
Means followed by different superscripts in the same column are significantly different at $P \leq 0.05$.
ND = not detected

Table 2. Seasonal incidence of *Salmonella* isolated from dairy farm cows, calves and farm environments.

Sample (N = 12480)	Sample type	% Positive *Salmonella* isolated season (quarter[a])			
		Winter (1st)	Spring (2nd)	Summer (3rd)	Fall (4th)
Lactating Cow	Animal[b]	39 ^{B}	25 ^{A}	68 ^{C}	33 ^{B}
Dry Cow	Animal[c]	45 ^{B}	24 ^{A}	42 ^{B}	23 ^{A}
Calves	Animal[d]	20 ^{A}	31 ^{A}	52 ^{B}	28 ^{A}
Feedbunk	Feed	12 ^{A}	27 ^{A}	23 ^{A}	9 ^{A}
Silage	Feed	22 ^{A}	62 ^{BC}	84 ^{C}	42 ^{B}
TMR	Feed	36 ^{A}	38 ^{A}	92 ^{B}	71 ^{C}
Water Trough	Feed	33 ^{A}	81 ^{C}	28 ^{A}	43 ^{B}
Calf Sipper	Feed	ND	11 ^{A}	63 ^{B}	21 ^{A}
Bedding – Cow[e]	Environment	56 ^{C}	19 ^{A}	66 ^{C}	46 ^{BC}
Bedding –Calf	Environment	ND	ND	19	ND
Bird Droppings	Environment	50 ^{BC}	13 ^{A}	72 ^{C}	ND
Soil – Lactating	Environment	23 ^{A}	17 ^{A}	68 ^{C}	37 ^{B}
Soil – Maternity	Environment	12 ^{A}	ND	26 ^{A}	20 ^{A}
Soil – Calf	Environment	26 ^{A}	10 ^{A}	8 ^{A}	ND
Manure	Environment	12 ^{A}	25 ^{A}	8 ^{A}	19 ^{A}
Air – Calf	Environment	22 ^{A}	19 ^{A}	17 ^{A}	ND
Insect – Calf	Environment	41 ^{B}	22 ^{A}	73 ^{C}	19 ^{A}
TOTAL (average %)		**26^{A}**	**25^{A}**	**48^{B}**	**24^{A}**

[a]Quarters during the 12-month sampling period: 1st (January – March); 2nd (April – June); 3rd (July-September); 4th (October – December).
[b]Lactating cow samples include oral, rectal, hair, teat and foremilk.
[c]Dry cow samples include oral, rectal and hair.
[d]Calf samples include oral, rectal and hair.
[e]Cow bedding materials sand, sawdust, and grass.
Means followed by different superscripts (A - C) in the same row are significantly different at $P < 0.05$.
ND, not detected.

(52%). Feed (84-92%), water (63%), bedding (66%), bird droppings (72%), and insects (73%) were significant sources of *Salmonella* during the summer. There was no significant difference ($P \leq 0.05$) observed with isolation of *Salmonella* during the winter, spring or fall. Feed and bedding samples were consistently positive for *Salmonella* throughout the entire sampling period.

Salmonella isolated from sippers (water; P = 0.029)) and bedding (grass, P = 0.053) were strongly correlated with *Salmonella* isolated from calves. *Salmonella* isolated from cow bedding (sand and sawdust) samples was correlated with *Salmonella* isolated from water troughs (P = 0.014) and from feed (TMR; P = 0.046) samples.

A total of 12,546 samples were analyzed for the presence of *Campylobacter jejuni*. Of these samples, 2,635 (21%) tested positive for the presence of *C. jejuni* (Table 1). Feed (60%), water (60%) and bird droppings (33%) were significant sources of contamination of lactating cows (34%), dry cows (31%) and calves (28%). Isolation of *C. jejuni* from lactating cows was strongly correlated with isolation of *C. jejuni* from water troughs (P = 0.015), TMR (P = 0.054), and bedding (P = 0.043).

Isolation of *C. jejuni* varied significantly by season (Table 3). Isolation of *C. jejuni* was highest during the winter (28%) and fall (29%). During the winter, lactating cows (30%), dry cows (40%), feed (50%), water (58%), bird droppings (70%) and insects (41%) were significant sources of *C. jejuni*. During the fall, *C. jejuni* was prevalent in both cows (45-61%) and calves (56%), as well as in Feed (28-83%), soil (42%) and bird droppings (34%).

Table 3. Seasonal incidence of *Campylobacter jejuni* isolated from dairy cows, calves and farm environments.

Sample (N = 12546)	Sample type	% Positive *Campylobacter jejuni* isolated season (quarter[a])			
		Winter (1st)	Spring (2nd)	Summer (3rd)	Fall (4th)
Lactating Cow	Animal[b]	30 ^{A}	21 ^{A}	20 ^{A}	61 ^{B}
Dry Cow	Animal[c]	40 ^{B}	18 ^{A}	22 ^{A}	45 ^{B}
Calves	Animal[d]	16 ^{A}	34 ^{B}	4 ^{A}	56 ^{C}
Feedbunk	Feed	31 ^{B}	18 ^{A}	3 ^{A}	37 ^{B}
Silage	Feed	20 ^{A}	ND	ND	28 ^{A}
TMR	Feed	50 ^{B}	17 ^{A}	8 ^{A}	83 ^{C}
Water Trough	Feed	58 ^{B}	83 ^{C}	81 ^{C}	17 ^{A}
Calf Sipper	Feed	7 ^{A}	21 ^{A}	ND	16 ^{A}
Bedding – Cow[e]	Environment	6 ^{A}	ND	ND	12 ^{A}
Bedding –Calf	Environment	ND	ND	ND	ND
Bird Droppings	Environment	70 ^{C}	26 ^{A}	ND	34 ^{A}
Soil – Lactating	Environment	28 ^{AB}	11 ^{A}	ND	42 ^{B}
Soil – Maternity	Environment	33 ^{B}	22 ^{A}	ND	26 ^{AB}
Soil – Calf	Environment	20 ^{A}	ND	ND	ND
Manure	Environment	9 ^{A}	ND	9 ^{A}	11 ^{A}
Air – Calf	Environment	14 ^{A}	31 ^{A}	11 ^{A}	ND
Insect – Calf	Environment	41 ^{B}	ND	ND	26 ^{A}
TOTAL (average %)		**28^{B}**	**18^{A}**	**9^{A}**	**29^{B}**

[a]Quarters during the 12-month sampling period: 1st (January – March); 2nd (April – June); 3rd (July-September); 4th (October – December).
[b]Lactating cow samples include oral, rectal, hair, teat and foremilk.
[c]Dry cow samples include oral, rectal and hair.
[d]Calf samples include oral, rectal and hair.
[e]Cow bedding materials sand, sawdust, and grass.
Means followed by different superscripts (A - C) in the same row are significantly different at $P < 0.05$.
ND, not detected.

A total of 12,010 samples were analyzed for the presence of *E. coli* O157:H7. Of these samples only 240 (2%) tested positive (Table 1). Although the isolation of *E. coli* O157:H7 was infrequent throughout the entire study period, a key source of contamination was water from watering troughs (6%).

Raw milk stored as bulk tank milk (BTM) on dairy farms can be a source of foodborne pathogens. Several studies have identified pathogens, such as *C. jejuni*, *E. coli* O157: H7 and *Salmonella* in farm BTM. However, the prevalence rates of these pathogens varied considerably among surveys. Jayarao and Henning (2001) examined bulk tank milk from 131 dairy farms in eastern South Dakota and western Minnesota for the presence of *C. jejuni*, *E. coli* O157:H7 and *Salmonella* Results showed that 27% of BTM samples contained one or more pathogens. This prevalence paralleled the rate of 25% from BTM in eastern Tennessee and southwestern Virginia, which also contained one or more pathogens (Rohrbach et al., 1992).

A total of 8,696 samples were collected from the milking parlor and BTM and analyzed for the presence of *Salmonella, C. jejuni*, and *E. coli* O157:H7 (Table 4). The prevalence of *Salmonella* was 26% from the milking parlor, milking equipment and BTM. Significant sources of potential transmission of *Salmonella* included air (66%), insects (56%), and milking equipment (28%). *Salmonella* was isolated from the milking parlor floor (17%) and bulk tank milk (11%). Isolation of *Salmonella* in manure ($P = 0.004$) and bedding ($P = 0.014$) samples were strongly correlated with isolation of *Salmonella* isolation from the milking parlor floor. *Salmonella* isolation from lactating cows ($P = 0.03$) and soil

Table 4. Prevalence of *Salmonella*, *Campylobacter jejuni* and *Escherichia coli* O157:H7 recovered from the milking parlor and bulk tank milk supply.

Sample	% Positive pathogen isolated (N = 8696)		
	Salmonella (n = 3360)	*C. jejuni* (n = 2900)	*E. coli* O157:H7 (n = 2436)
Parlor Walls	2^{a}	15^{a}	ND
Parlor Floor	17^{b}	ND	ND
Parlor Air	66^{d}	23^{b}	6
Parlor Insects	56^{d}	39^{c}	4
Equip – Liners	28^{bc}	26^{bc}	1
Equip – Cups	1^{a}	14^{a}	ND
Bulk Milk	11^{a}	15^{a}	ND
TOTAL (average %)	**26**	**19**	**2**

Means followed by different lower case superscripts (a - d) in the same column are significantly different at $P < 0.05$.
ND, not detected.

(P = 0.05) were correlated with *Salmonella* isolation from milking equipment and BTM.

Campylobacter jejuni was isolated at a prevalence of 19% from the milking parlor and BTM. *Campylobacter jejuni* was isolated from the milking parlor floor (15%) and BTM (15%). Air (23%), insects (39%) and milking equipment (26%) were significant sources of contamination of *C. jejuni.* Lactating cow animal (P = 0.05) and lactating cow soil (P = 0.026) were correlated with the prevalence of *C. jejuni* on milking equipment.

Escherichia coli O157:H7 was not isolated from the milking parlor or BTM samples. Results emphasize the importance of continued diligence in the application of hygiene programs within dairies. However, *E. coli* O157:H7 was isolated from air (6%) and insects (4%) collected in the parlor as well as from milking equipment liners (1%).

The spatial distribution of *Salmonella*, *C. jejuni*, and *E. coli* O157:H7 isolated at The University of Tennessee research dairy farm are displayed in Figure 1. The GIS map reveals a higher concentration of pathogens isolated at the center of the farm. Pathogen isolation was less frequent along the perimeter of the farm. Overall, *Salmonella* was the pathogen isolated most frequently at the dairy farm (Figure 2). Isolation of *Salmonella* was more prevalent near the center of the farm. Lactating cows, feed, bird droppings, and insects were common sources of *Salmonella.* Isolation of *C. jejuni* and *E. coli* O157:H7 was sporadic throughout the dairy farm. Water, lactating cows, dry cows, and calves were common sources of both *C. jejuni* and *E. coli* O157:H7.

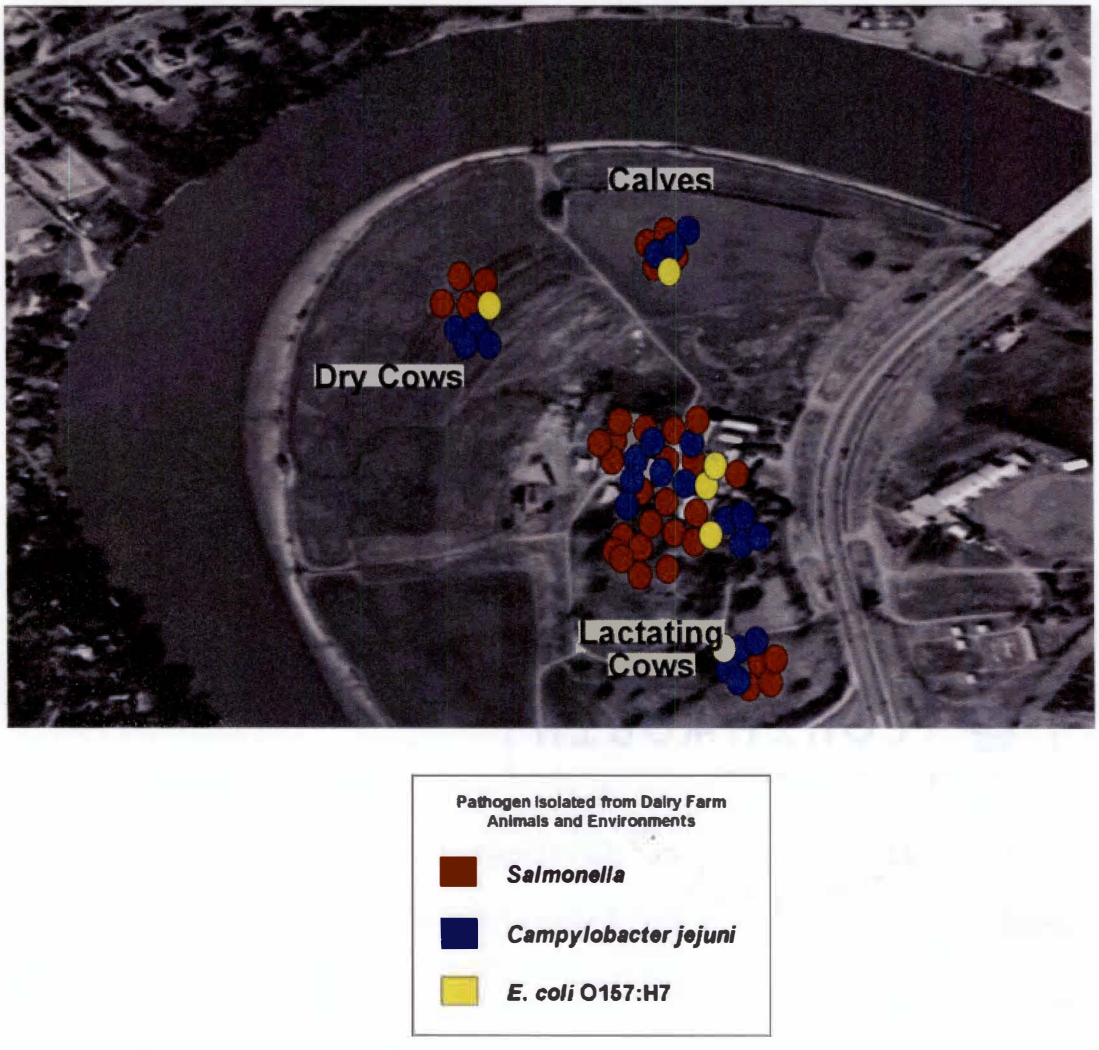
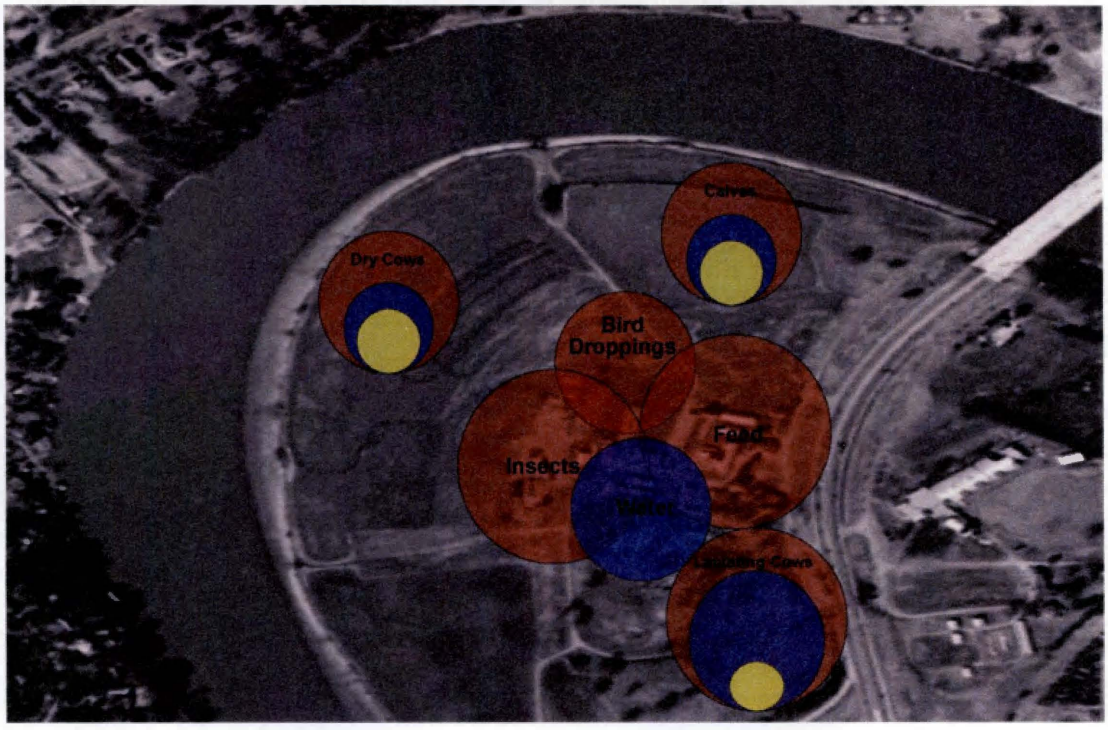


Figure 1. Spatial analysis of *Salmonella, Campylobacter jejuni* and *Escherichia coli* O157:H7 isolated from dairy farm environments.



Pathogen Isolated from Dairy Farm Animals and Environments

- *Salmonella*
- *Campylobacter jejuni*
- *E. coli* O157:H7

% Pathogen Isolated from Dairy Animals and Farm Environments

- 46 - 60
- 31 - 45
- 16 - 30
- 0 - 15

Feed samples includes feed bunk, silage, and total mixed rations
Water samples include watering troughs and calf sippers
Bird Droppings from fencing surrounding animal pasture and feeding areas
Insects from animal housing facilities

Figure 2. Geographic distribution of *Salmonella, Campylobacter jejuni* and *Escherichia coli* O157:H7 isolated from dairy cows, calves and farm environments.

DISCUSSION

The farm environment as a whole represents an array of possible reservoirs for *Salmonella*, *C. jejuni*, and *E. coli* O157:H7. Contamination is likely to occur from pathogens shed in the feces of farm animals. Once pathogens are present in the environment, they may be disseminated to other sites by rainwater, wind, removal and spreading of manure, as well as by the animals themselves. Results of this study demonstrate that feed, water and bedding were identified as common routes by which *Salmonella*, *C. jejuni*, and *E. coli* O157:H7 spread to cows and calves. Although sources of fecal contamination on farms are easy to identify, controlling transmission of pathogens throughout farm environments may be difficult to achieve. Based on survey results, several farm management strategies were suggested for control of foodborne pathogens.

Commons sources of contamination for all pathogens were feed and water. Access to water and feed sources by wild birds and insects was a major contributing factor to contamination of these sources. Replication in feed seemingly increased the potential for colonization in animals. Wild birds could readily contaminate cattle feed as well as the environment. According t a review by Davies and Wray (1997), contaminated bird droppings have been found in feedmill environments. Starlings, blackbirds, and pigeons are common pests on many farms. They feed directly from feedbunks or search for undigested food in livestock droppings. Feeding on feces is likely a means of ingesting large numbers of fecal microorganisms (Davies and Wray, 1997).

At The University of Tennessee research dairy farm, wild birds and insects were significant to the contamination of feed and water sources. Wild birds were a major reservoir of both *Salmonella* and *C. jejuni*. Investigators have reported an association between various bird species contaminating feed and the transmission of *Salmonella* to cattle (Coulson et al., 1983; Glickman et al., 1981; Johnson et al., 1979). Bird droppings have been shown to be associated with the presence of *Salmonella* in feeds on swine farms (Harris et al., 1997). *Campylobacter jejuni* has been recovered from gulls, waterfowl, cranes, geese, doves, and falcons (Whelan, 1988). Insects can also act as vectors for *C. jejuni*. In a study conducted by Rosef and Kapperud (1983), the carrier rate for *C. jejuni* in flies on a single poultry farm was 51% and 43% on a nearby swine farm.

Measures to reduce pathogens in feed and water should be implemented. Insects, birds, rodents and domestic animals should be excluded from farms and animal housing facilities. Animals can acquire a number of pathogens from water. Efforts should be made to limit contamination of water sources by grazing animals, farm effluent and human sewage. Pathogens present in the saliva and tonsils of calves and other animals contaminate drinking bowls and buckets. Good quality water should be used and regular cleaning of these sources is needed to limit the spread of infection.

Isolation of pathogens from bedding (cow and calf) at The University of Tennessee research dairy farm was correlated with isolation of pathogens from feed, water and animals samples. Bedding contributes to cow comfort, udder health and milk quality. Clean, dry bedding for animals promotes cleanliness and

inhibits microbial growth. Proper bedding management is critical for the effective control of pathogens in the farm environment. Bedding type and quality may impact udder health and the incidence of mastitis in animals (Smith and Hogan, 2000). Sand and sawdust were the two materials used as bedding for cows. Sawdust is beneficial in its ability to absorb moisture, however, this material will support growth of bacteria when mixed with manure and urine (Hogan et al., 1989). Bacterial counts of used sand bedding are often significantly lower than in organic bedding materials (Zehner et al., 1986). Accumulation of excessive amounts of manure, mud or urine may cause a rapid deterioration of bedding quality due to severe contamination of the bedding.

Hygienic conditions are the major factors to monitor when controlling pathogens at the farm. Heavy soiling of animals is caused by poor housing conditions where there is irregular removal of manure, inadequate bedding, and holding animals on muddy ground. The herd environment must be kept clean, dry and comfortable for animals. Also, farm management must minimize conditions that increase exposure to environmental pathogens, such as overcrowding, elevated temperatures and humidity in barns, poor ventilation, accumulation of manure, urine, and water in housing areas, and access to muddy lots. The design and construction of buildings in which animals are housed should allow effective cleaning and disinfection and removal of manure.

Data generated from this study permit science-based, risk management decisions to be made regarding the exposure and contamination of animals by pathogens and the significance of geographic and temporal factors on

management of pathogens on the farm. The conditions caused by environmental factors are complex. This made it difficult to clearly identify causal factors for contamination, as causation is usually from an array of sources and risk factors. The results of this study indicated that a wide array of animal and environmental factors contributed to the shedding of foodborne pathogens on the farm and subsequent environmental contamination. Significant sources of pathogen transmission on the farm were identified as feed, water, bedding, insects, and wild bird droppings. Therefore, it can be concluded that monitoring and controlling pathogens in these sources could reduce pathogen transmission to dairy cows calves and farm environments.

The development of computerized models using GIS provided robust analyses and depictions of how management practice at the farm influenced the occurrence and persistence of foodborne pathogens. Regardless of the high variation in shedding contamination from the farm survey, there is no doubt that on-farm food safety would benefit from programs that identify animal production practices and farm management practices that minimize pathogens at the farm.

LITERATURE CITED

Anderson, R.J., R.L. Walker, D.W. Hird, and P.C. Blanchard. 1997. Case-control study of an outbreak of clinical disease attributable to *Salmonella* menhaden infection in eight dairy herds. J. Am. Vet. Med. Assoc., 210: 528-530.

Andrews, W. H., V. R. Bruce, G. June, F. Satchell and P. Sherod. 1995. *Salmonella* in Bacteriological Analytical Manual (BAM). 8th ed. Food and Drug Administration, Washington, D.C.

Beumer, R.R., J.J.M. Cruysen, and I.R.K. Birtantie. 1988. The occurrence of *Campylobacter jejuni* in raw cows' milk. J. Appl. Bacteriol. 65:93-96.

Busato, A., D. Hofer, T. Lentze, C. Gaullard, A. Burnens. 1998. Prevalence and infection risk of zoonotic enteropathogenic bacteria in Swiss cow-calf farms. Vet. Microbiol. 69(4): 251-263.

Carter, A.M., R.E. Pacha, G.W. Clark, and E.A. Williams. 1987. Seasonal occurrence of *Campylobacter* spp. and their correlation with standard indicator bacteria. Appl. Environ Microbiol. 53:523-526.

Coulson J.C., J. Butterfield, and C. Thomas. 1983. The herring gull Larsus Argentatus as a likely transmitting agent of *Salmonella* Montevideo to sheep and cattle. J. Hyg. Camb. 91:437-443.

Davies, R.H., and C. Wray. 1997. Distribution of *Salmonella* contamination in ten animal feedmills. Vet. Microbiol. 57:159-169.

Evans, S.J. 1996. A case control study of multiple resistant *Salmonella* Typhimurium DT104 infection of cattle in Great Britain. Cattle Practice, 4: 259-266.

FDA. 1998. Bacteriological Analytical Manual, FDA. 8th ed., AOAC International, Gaithersburg, MD.

Giacoboni, G.I. K. Itoh, K. Hirayama, E. Takahashi, and T. Mitsuoka. 1993. Comparison of fecal *Campylobacter* in calves and cattle of different ages and areas in Japan. J. Vet Med. Sci. 55:555-559.

Glickman, L.T., P.L. McDonough, S.J. Shin, J.M. Fairbrother. 1981. Bovine salmonellosis attributed to *Salmonella* anatum contaminated haylage and dietary stress. N. Engl. J. Med. 338:1333-1338.

Hancock, D.D., T.E. Besser, M.L. Kinsel, P.I., Tarr, D.H. Rice, and P.C. Paros. 1994. The prevalence of *Escherichia coli* O157:H7 in dairy and beef cattle in Washington state. Epidemiol. Infect. 113: 199-207.

Hancock, D.D., D.H. Rice, D.E., Herriott, T.E. Besser, E.D. Ebel, and L.V. Carpenter. 1997. Effects of farm manure handling practices on *Escherichia coli* O157:H7 in feedlot cattle. J. Food Prot. 60:462-465.

Hogan, J.S., K.L. Smith, D.A. Todhunter, P.S. Schoenberger, W.P. Weiss, and K.L. Smith. 1999. Bacterial counts in bedding materials used on nine commercial dairies. J. Dairy Sci. 72(1): 111-119.

Humphrey, T.J. and P. Beckett. 1987. *Campylobacter jejuni* in dairy cows and raw milk. Epidemiol. Infect. 98:263-269.

Jayarao, B.M. and D.R. Henning. 2001. Prevalence of foodborne pathogens in bulk tank milk. J. Dairy Sci. 84: 2157-2162.

Johnson, W.S. G.K.Maclachlan, G.F. Hopkins. 1979. The possible involvement of seagulls in the transmission of *Salmonella* in dairy cattle. Vet. Rec. 105:526-527.

Kabagambe, E.K., S.J. Wells, L.P. Garber, M.D. Salman, B. Wagner, and P.J. Fedorka-Cray. 2000. Risk factors for fecal shedding of *Salmonella* in 91 US dairy herds in 1996. Prev. Vet. Med. 43: 177-194.

Potter, M.E. 1994. The role of Epidemiology in risk assessment: a CDC perspective. Dairy, Food, and Env. Sanitation, 14(12): 738-741.

Rahkio, T.M., and Korkeala. 1997. Airborne bacteria and carcass contamination in slaughterhouses. J. Food Prot. 60(1): 38-42.

Rohrbach, R.W., F.A. Draughon, P.M. Davidson, and S.P. Oliver. 1992. Prevalence of Listeria monocytogenes, *Campylobacter jejuni*, Yersinia *enterocolitica*, and *Salmonella* in bulk tank milk; risk factors and risk of human exposure. J. Food Prot. 55:93-97.

Smith, B.P., G.W. Dilling, M.C. Thurmond, H. Konrad, J.A. Pelton, J.A. Picanso. 1994. Prevalence of *Salmonella* in cattle and in the environment on California dairies. J. Am. Vet. Med. Assoc. 205, 467-471.

Smith, K.L. and J.S. Hogan. 2000. Bedding's contribution to mastitis in dairy cows. Dairy Housing and Equipment Systems, Managing and Planning for Profitability. NRAES 129.

Tauxe, R.V. 1992. Epidemiology of *Campylobacter jejuni* infections in the United States and other industrial nations. In: *Campylobacter jejuni*: Current and future trends. American Society for Microbiology: Washington, D.C., p. 9 –12.

Wesley, I.V., S.J. Wells, K.M. Harmon, A. Green, L. Schroeder-Tucker, M. Glover, and I. Siddique. 2000. Fecal shedding of *Campylobacter* and *Arcobacter* spp. in dairy cattle. App. Environ. Microbiol. 66(5): 1994-2000.

Wray, C., R.H. Davies. 1996. A veterinary view of *Salmonella* in farm animals. Public Health Lab. System Microbiol. Digest, 13: 44-48.

Zehner, M.M., R.J. Farnsworth, R.D. Appleman, K. Larntz, and J.A. Springer. 1986. Growth of environmental pathogens in various bedding materials. J. Dairy Sci. 69(7): 525-230.

Zhao, J., M.P. Doyle, J. A. Shere, and L. Garber. 1995. Prevalence of enterohemorrhagic *Escherichia coli* O157:H7 in a survey of dairy herds. Appl. Envir. Microbiol. 61:1290-1293.

PART III

INCIDENCE OF *SALMONELLA*, *CAMPYLOBACTER JEJUNI*, AND *ESCHERICHIA COLI* O157:H7 IN THE TENNESSEE RIVER

ABSTRACT

Surface waters may play an important role in transmission of pathogenic agents via agricultural runoff, livestock, urban runoff, and illegal dumping or discharges from boats. These agents can return to humans by various routes, such as use of water for recreational sports, irrigation of crops, and as drinking water. Therefore, pollution of rivers is of particular importance. A microbiological survey of the Tennessee River, adjacent to The University of Tennessee research dairy farm, was conducted to determine the prevalence of *Salmonella*, *Campylobacter jejuni*, and *Escherichia coli* O157:H7. Fecal *coli*forms were also examined as possible indicators of the presence or absence of pathogens in the river. A Geographic Information System (GIS) was used to examine possible relationships between contamination with *Salmonella*, *C. jejuni*, and *E. coli* O157:H7 at the dairy farm and subsequent contamination of the Tennessee River.

Neither *C. jejuni* nor *E. coli* O157:H7 were recovered from the Tennessee River water samples, however, *Salmonella* (33%) was isolated from all sampling sites along the river. The concentration of fecal *coli*forms in river water samples ranged from 37 to > 2400 Most Probable Number per 100 ml water. No correlation ($P > 0.05$) was found between the indices of fecal contamination and *Salmonella* recovered from the Tennessee River.

GIS analysis revealed a decrease in isolation of *Salmonella* from sites directly across (11%) and directly downstream (11%) from the dairy farm (56%). However, *Salmonella* was isolated at a prevalence of 44% from sites upstream

from the farm. Based on the lack of bacterial species isolated from the river as opposed to those recovered from the dairy farm, and the variable pattern of pathogen isolation from the river, it can be concluded that The University of Tennessee research dairy farm did not contribute significantly to contamination of the Tennessee River.

INTRODUCTION

Surface waters, which include rivers, streams, lakes, and ponds, play an important role in the transmission of waterborne pathogens in the environment (Reinert and Hroncich, 1990). Sources of surface water contamination are classified as point or non-point. Point sources are known and can be documented, facilitating their control under food management practices. However, non-point sources of contamination are not known. They include, but are not limited to agricultural runoff, livestock, urban runoff, landfills, land development, recreational activities, and illegal dumping or discharges. Non-point sources are much more difficult to control than point sources, thus presenting a greater public health threat (Reinert and Hroncich, 1990).

Microbial waterborne pathogens of concern may enter water systems via fecal contamination. Waterborne enteric bacteria include both human-associated and zoonotic species, such as *Salmonella*, *Campylobacter jejuni*, and *Escherichia coli* O157:H7. These pathogens are ubiquitous in aquatic systems and have been isolated from various drinking, recreational, and surface water sources (Nataro and Levin, 1994). Outbreak cases of human and animal salmonellosis have been attributed to environmental contamination with sewage

effluent, septic tank effluent, and various wastewater sources (Reilly et al., 1981). In the United States, from 1978 to 1986, water was identified as the source of 19% of 57 reported *C. jejuni* outbreaks. Water-related outbreaks generally involved drinking untreated surface water or drinking inadequately treated water (Tauxe, 1992). The first reported outbreak of *E. coli* O157:H7 from water was reported in 1989. Water samples from 15 streams and reservoirs in Philadelphia, Pennsylvania were evaluated for the presence of the pathogen. Wildlife, especially deer, was implicated in the contamination of the water, as no dairy or cattle farms were located upstream. Human contamination of the water was considered unlikely (McGowan et al., 1989). In another waterborne *E. coli* O157:H7 outbreak in Africa, Isaacson et al. (1993) isolated *E. coli* O157:H7 from 18% of 76 river water samples, and one domestic water storage drum. Heavy rain, following a period of drought, was implicated as the cause of the water contamination. Contamination resulted in the flushing of contaminated cattle carcasses and manure into surface waters. This was verified through the testing of cattle manure and insects at the same time of water testing (Isaacson et al., 1993).

The Tennessee River is a popular recreational water source, which is used commonly for recreational fishing and boating activities. The river is also used both as a source for irrigation and as a source for local waterworks in Knoxville, Tennessee. The Tennessee River's main navigational channel is 652 miles long. It officially begins a mile above Knoxville, Tennessee, and eventually empties into the Ohio River at Paducah, Kentucky. The Tennessee River flows

East to West along the perimeter of the University of Tennessee Dairy farm. Agricultural runoff and livestock are common sources of contamination of surface water sources. Therefore, it was of particular interest to evaluate the prevalence of common foodborne pathogens in river water.

Risk assessment approaches have been useful to systematically identify, analyze, quantify, and characterize the risk of specific waterborne illness (Sobsey et al., 1993). The use of a Geographic Information System (GIS), as a risk assessment tool, may be used in identifying areas with increases levels of pathogens as well as the transport of theses pathogens throughout the water system. GIS also simplifies the identification of infectious agents with multiple transmission routes.

A microbiological survey of the Tennessee River was conducted to determine the prevalence of *Salmonella*, *C. jejuni*, and *E. coli* O157:H7 at sampling sites upstream and downstream from the University of Tennessee dairy farm. Fecal *coliforms* were also examined as possible indicators of the presence or absence of these pathogens in the river. GIS was used in this study to examine possible relationships between contamination with *Salmonella*, *Campylobacter jejuni*, and *E. coli* O157:H7 at the dairy farm and subsequent contamination of the Tennessee River adjacent to the farm.

MATERIALS AND METHODS

River Sampling

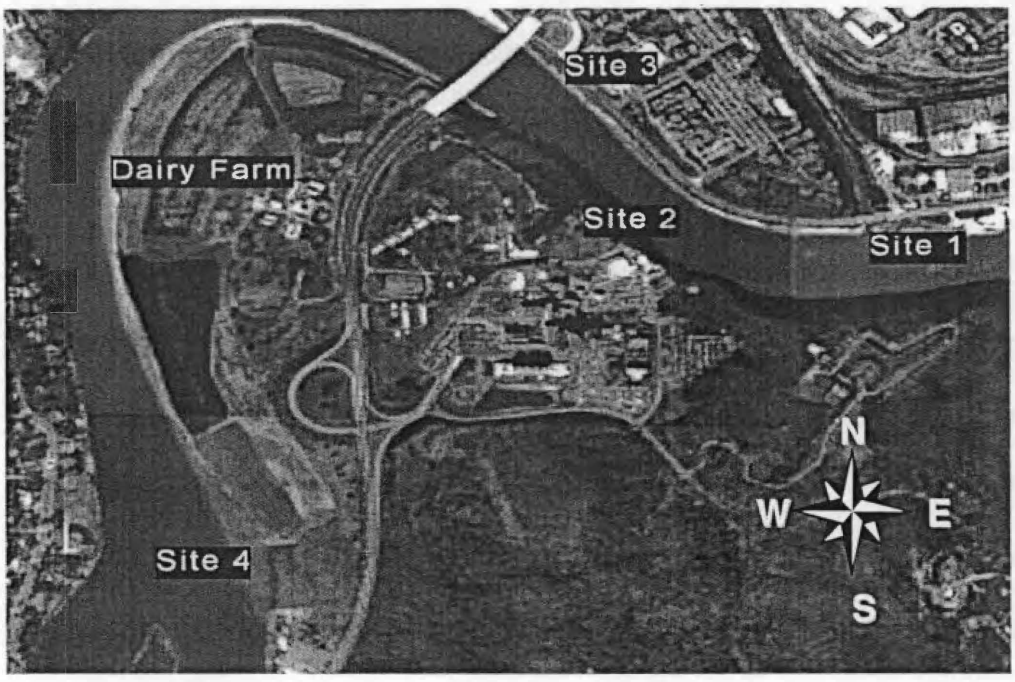
The Tennessee River, which runs along the perimeter of The University of Tennessee research dairy farm, was evaluated for the presence of *Salmonella*,

Campylobacter jejuni, *Escherichia coli* O157:H7, and fecal *coli*forms. Water samples were collected at five sites along the Tennessee River (Figure 3): The water treatment facility, poultry farm, dairy farm, naval reserve area, and the Agricultural Campus, directly across from the dairy farm.

Water sampling was conducted over a 6-month period (January – June). During each sampling period, three, 3 L samples per pathogen, were collected from each of the five river sampling sites. Samples were collected in sterile 1 L Nalgene bottles by submerging the bottles into the river at a depth of 3 ft, at a distance of 3 ft from the river shoreline at each sampling site. Samples were stored under refrigeration, and processed within 6 hours of collection. Isolation and identification of *Salmonella*, *C. jejuni* and *E. coli* O157:H7 was conducted in accordance with the procedures described in the Food and Drug Administration's Bacteriological Analytical Manual (FDA, 1998).

Isolation and Identification of Salmonella

River water sample (3 liters per sample) were filtered through 0.45 • m paper filters (Millipore Corp.,). Filters were added to 100 ml of lactose broth for pre-enrichment. Samples were pre-enriched for 24 h at 35°C. Following pre-enrichment 0.1 ml was transferred into 9.9 ml of Rappaport-Vassiliadis broth (Becton, Dickinson and Company, Franklin Lakes, NJ). Samples were then enriched in RV broth for 24 h at 42°C. After the enrichment phase, samples from each RV tube were streaked onto bismuth sulfite and XLT4 agar plates. All plates were incubated for 24 h at 35°C. Typical colonies were then picked from each plate and streaked onto a tryptic soy agar (Becton, Dickinson and



[a]Distance from Dairy Farm (km): Site 1 (E 4.2 km); Site 2 (E 3.6 km); Site 3 (NE 2.7 km); Site 4 (SW 4.5 km)
The Tennessee River flows from East to West.

Figure 3. The University of Tennessee research dairy farm and Tennessee River sampling sites.

Company, Franklin Lakes, NJ) slant to provide adequate growth of each isolate for further testing. All tryptic soy agar slants were incubated for 24 h at 35°C.

Each isolate was then inoculated into triple sugar iron agar (Becton, Dickinson and Company, Franklin Lakes, NJ) slants. All slants were incubated for 24 h at 35°C. In triple sugar iron agar, *Salmonella* typically produces an alkaline (red) slant and an acid (yellow) butt, with or without H_2S production. Samples testing positive for *Salmonella* in triple sugar iron agar were confirmed using the API 20E identification system for Enterobacteriaceae (bioMerieux Inc., Durham, NC). *Salmonella* isolates were confirmed serologically, by somatic (O) antigen analysis, using the *Salmonella* antisera poly A, B, C, D, E, F and G (Becton, Dickinson and Company, Franklin Lakes, NJ). All *Salmonella*-positive samples were inoculated onto fresh tryptic soy agar slants, incubated for 24 h at 35°C, and stored under refrigeration for further testing.

Isolation and Identification of Campylobacter jejuni

River water sample (3 liters per sample) were filtered through 0.45 •m paper filters (Cuno corporation, Meriden, CA). The filters were added to 100 ml of Bolton Broth (Oxoid Inc., New York, USA) for enrichment. Samples were incubated for 42 h at 42°C in an anaerobic jar under microaerophilic conditions generated by the CampyPak Microaerophilic System (Becton Dickinson Biosciences, Franklin Lakes, NJ). After enrichment, all samples were streaked onto *Campylobacter* blood-free selective agar plates, with supplements (Oxoid Inc., New York, USA). The plates were incubated under microaerophilic conditions for 24h at 42°C. Typical colonies were then subjected to oxidase,

catalase, and glucose (1%) tests. Samples testing positive for *Campylobacter* were then confirmed using API Campy strips to confirm that they were *Campylobacter* colonies. All *Campylobacter* isolates were confirmed serologically using latex agglutination tests (Integrated Diagnostic, Inc.) for *C. jejuni*, C. *coli*, and C. lardis. Procedure for the latex agglutination test involved adding a drop of latex solution to a sample card, mixing a loopful of sample with the latex solution (10 – 12 sec.) and observing for an agglutination reaction. Agglutination positive reactions resulted in a thick, stringy appearance to the sample mixture.

Isolation and Identification of Escherichia coli O157:H7

River water sample (3 liters per sample) were filtered through 0.45 •m paper filters (Millipore Corporation, Billerica, MA). The filters for each water sample were added to 100 ml of modified tryptic soy broth with added Novobiocin (Becton, Dickinson and Company, Franklin Lakes, NJ) and incubated for 18 h at 37°C in a shaking incubator. The samples were streaked onto sorbitol MacConkey agar (Becton, Dickinson and Company, Franklin Lakes, NJ) supplemented with sodium tellurite and cefixime (Becton, Dickinson and Company, Franklin Lakes, NJ) and incubated for 18 h at 37°C. After incubation, non sorbitol-fermenting colonies were picked from the plates and streaked onto tryptic soy agar slants. The slants were incubated for 18 h at 37°C. Colonies were biochemically confirmed by indole, citrate, and Methyl red, Vogues Proskauer tests. Isolates testing positive for *E. coli* were also confirmed biochemically using the API 20 E identification system for Enterobacteriaceae (bioMerieux). Isolates were tested for the O157 and H7 antigens using latex

agglutination. Procedure for the latex agglutination test involved adding a drop of latex solution to a sample card, mixing a loopful of sample with the latex solution (10 – 12 sec.) and observing for an agglutination reaction. Agglutination positive reactions resulted in a thick, stringy appearance to the sample mixture.

Fecal Coliform Analysis

Fecal *coli*forms (non- O157:H7 *E. coli*) were analyzed using the Most Probable Number (MPN) 3 tube, 3 dilution (0.1 ml, 1.0 ml, and 10 ml) method. River water samples, 0.1 ml, 1.o0 ml and 10 ml respectively, were added to tubes containing 9 ml of EC medium with 4-methylumbelliferyl-• -D-glucoronide (EC-MUG, Becton, Dickinson and Company, Franklin Lakes, NJ). Tubes were incubated for 24 h at 37°C. The tubes were examined under long-wave (365nm) ultraviolet light, and tubes showing • -glucoronidase activity (fluorescence) were considered positive for *E. coli*. The concentration of fecal *coli*forms (*E. coli*) per 100 ml of water was determined using the MPN table based on the number of fluorescent positive tubes.

Geographic Information System (GIS) Analysis

An aerial photograph taken of the dairy farm and the Tennessee River was scanned into ArcView® GIS version 3.2 (Environmental Systems Research Institute, Redlands, CA) was used as the basemap onto which the spatial information was layered. Data generated from microbial analyses of river water samples (number of confirmed pathogens isolated at specific points along the river) were imported into the ArcView® project. Data were sorted and grouped by sampling site (water treatment facility, poultry farm, dairy farm, naval reserve

area, and the Agricultural Campus, directly across from the dairy farm), analyzed to give percent pathogen isolated at each site, and plotted onto the basemap. Cartographic software, Freehand 9.0® (Macromedia, NY, USA), was used to visually display data on the map. A series of colors and shapes were used for improved visualization of data.

Data Analysis

Data were stored and coded as positive or negative for the presence or absence of *Salmonella, C. jejuni,* and *E. coli* O157:H7, in river water samples. The prevalence of *E. coli* O157:H7 was calculated as the number of positive water samples divided by the total number of water samples tested. Data analyses were performed using SAS version 8.2 (SAS® Institute, Cary, NC, USA). A Chi-square test was used to test for associations between shedding of *Salmonella*, *C. jejuni*, and *E. coli* O157:H7 at the farm and isolation of the pathogens from the river. ArcView® GIS version 3.2 was used to perform advanced statistical analyses of spatial data, including cluster analyses, multiple regression, and Poisson probability distributions.

RESULTS

River water samples were collected from the Tennessee River at sites upstream and downstream from The University of Tennessee research dairy farm dairy farm. The Tennessee River flows from East to West. The results of the microbiological survey of the Tennessee River are shown in Table 5. Water samples (N = 135) were analyzed and *Salmonella* was recovered at an overall

Table 5. The prevalence of *Salmonella*, *Campylobacter jejuni* and *Escherichia coli* O157:H7 in the Tennessee River.

	% Pathogen isolated (N = 135)		
River sampling site[a]	***Salmonella*** (n = 45)	***C. jejuni*** (n = 45)	***E. coli* O157:H7** (n = 45)
Site 1	44	0	0
Site 2	44	0	0
Site 3	11	0	0
Site 4	11	0	0
Dairy Farm	56	0	0
TOTAL	33	0	0

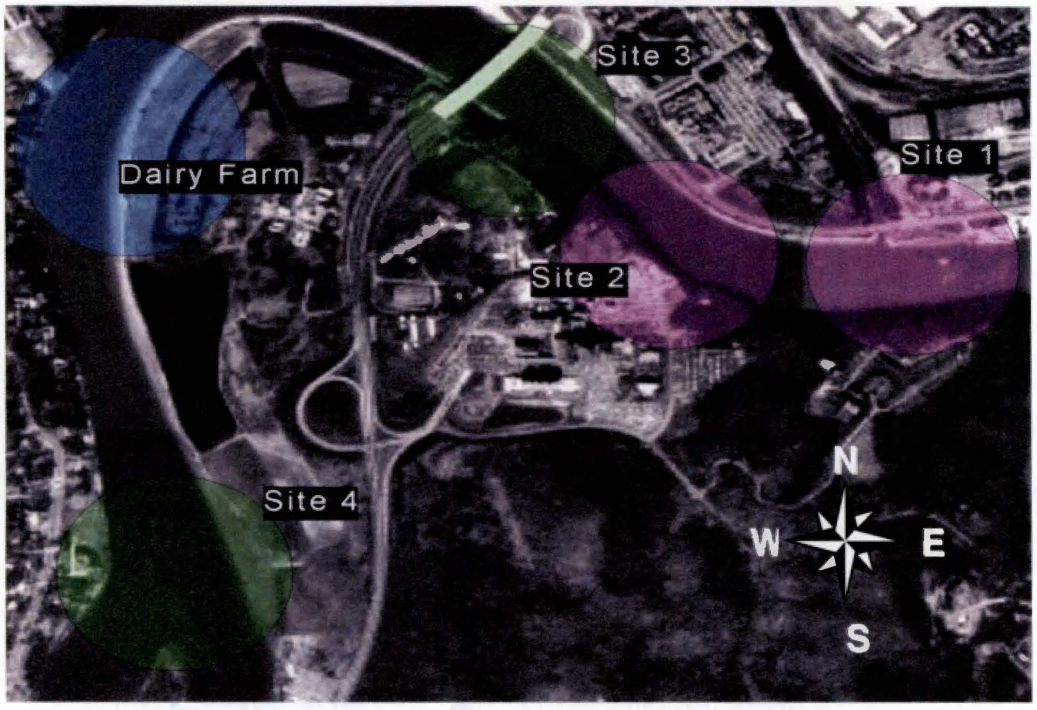
[a]Distance from Dairy Farm (km): Site 1 (E 4.2 km); Site 2 (E 3.6 km); Site 3 (NE 2.7 km); Site 4 (SW 4.5 km)

0 = not isolated

frequency of 33% from all sampling sites along the dairy farm. The highest levels of *Salmonella* recovered from the river were observed at the dairy farm (56%) and sampling sites 1 and 2 (44%) respectively. The lowest level of *Salmonella* (11%) was recovered from sampling sites 3 and 4.

Sampling of the Tennessee River was carried out over a period of 6 months (January – June). However, no significant seasonal variations were observed with isolation of *Salmonella*. Results of GIS analyses (Figure 4) indicate that high levels of *Salmonella* were observed at the dairy farm (56%) and sites 1 (44%) and site 2 (44%) upstream from the dairy farm. Lower levels of *Salmonella* were observed at sites 3 (11%), which is directly across from the farm, and site 4 (11%), downstream from the farm. Based on these results, it can therefore be suggested that a source other than the dairy farm may have contributed to the contamination of the Tennessee River.

Fecal *coli*forms were also evaluated as possible indicators of the presence or absence of *Salmonella, C. jejuni*, and *E. coli* O157:H7 in the Tennessee River. Fecal *coli*forms are defined as facultative anaerobic, Gram-negative, non-spore forming rods that ferment lactose, with acid production and gas formation occurring within 24 hours at 44.5°C (American Public Health Association, 1995). *Coli*forms are commonly used as indicators of fecal contamination or water pollution from sewage. In particular, *E. coli* has been demonstrated to be a more specific indicator for the presence of fecal contamination within the fecal *coli*form group of bacteria (Bej et al., 1991). Results of fecal *coli*form analysis and



% *Salmonella* isolated from river sampling site

11%

44%

56%

Distance from Dairy Farm (km): Site 1 (E 4.2 km); Site 2 (E 3.6 km); Site 3 (NE 2.7 km); Site 4 (SW 4.5 km)

The Tennessee River flows from East to West.

Figure 4. Geographic distribution of *Salmonella* isolated from the Tennessee River.

isolation from the Tennessee River of *Salmonella* are presented in Table 6. The concentration of fecal *coli*forms in river water samples ranged from 37 to > 2400 MPN per 100 ml water. No correlation ($P > 0.05$) was found between the indices of fecal contamination and *Salmonella* recovered from the Tennessee River.

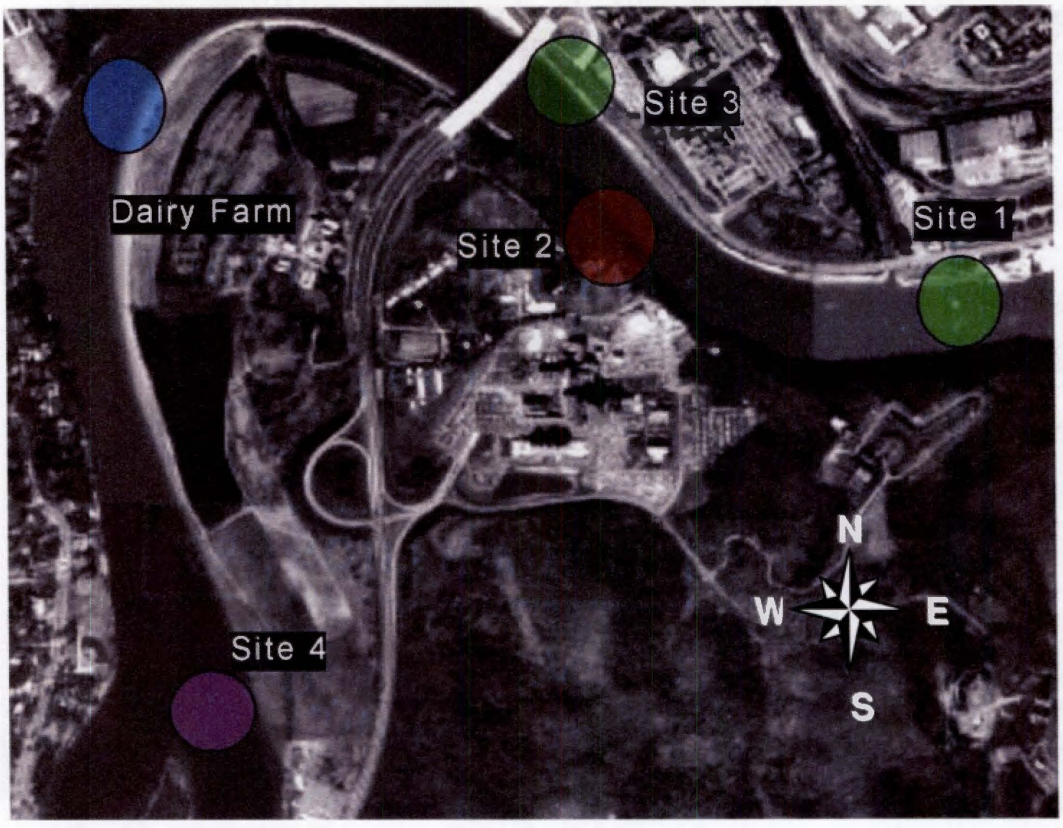
GIS analysis of fecal *coli*form data (Figure 5) indicate that the highest levels of fecal *coli*forms in the river were recovered from water samples taken at the dairy farm (570 MPN per 100 ml water), from site 2 (2000 MPN per 100 ml water), and from site 4 (220 MPN per 100 ml water). The high level of fecal *coli*forms directly downstream (site 4) from the dairy farm might be attributed to agricultural runoff from the dairy farm. However, higher counts of fecal *coli*forms were obtained directly upstream (site 2) from the dairy farm. Fecal *coli*form counts were lowest at this site (37 MPN per 100 ml water), however, *coli*form counts showed an increase from this site as water flowed downstream from East to West.

Use of *E. coli* as an indicator of the possible presence of pathogenic microorganisms is and has been extremely useful to protect public health. However, use of indicator bacteria, regardless of which ones are used, are only tools and have limitations. The presence of high levels of fecal *coli*forms in water systems may not always correlate with the presence of pathogens, such as *Salmonella*, *C. jejuni*, and *E. coli* O157:H7 (Bej et al., 1991).

Table 6. The relationship between fecal *coli*forms and the presence or absence of *Salmonella* in the Tennessee River.

Fecal *coli*forms (*E. coli*) MPN index per 100 ml water	*Salmonella*	
	Positive (n = 15)	Negative (n = 30)
3.7×10^1	4	5
8.9×10^1	1	8
2.2×10^2	1	8
5.7×10^2	5	4
2.0×10^3	4	5

No significant differences ($P > 0.05$)



Fecal coliforms
(*E. coli*)
MPN index per 100 ml water

- $0 < 1 \times 10^2$
- $1 \times 10^2 < 5 \times 10^2$
- $5 \times 10^2 < 1 \times 10^3$
- $1 \times 10^3 < 2 \times 10^3$

Distance from Dairy Farm (km): Site 1 (E 4.2 km); Site 2 (E 3.6 km); Site 3 (NE 2.7 km); Site 4 (SW 4.5 km)

The Tennessee River flows from East to West.

Figure 5. Geographic distribution of fecal *coli*forms isolated from the Tennessee River.
(MPN per 100 ml water)

DISCUSSION

Results of this research indicated that *Salmonella* was ubiquitous in the Tennessee River. Neither *C. jejuni* nor *E. coli* O157:H7 was recovered from the Tennessee River. According to a review by Roszak and Collwell (1987) the absence of *C. jejuni* may have in river water samples may be due to temporal physiological and morphological transitions in which *Campylobacter* spp. undergoes in aquatic systems. During these stages of transition, *Campylobacter* spp. may retain metabolic activity but demonstrate non-culturability on conventional growth media, resulting in a viable but non-culturable state (Roszak and Colwell, 1987). In aquatic environments *E. coli* O157:H7 is subjected to a variety of stresses, such as temperature, lack of nutrients, and oxidative stress. As result of exposure to these stresses, the organism may enter a sublethally injured state (Sinclair and Alexander, 1984). The inability to isolate *E. coli* O157:H7 from river water samples may be a consequence of the presence of the organism in low numbers in comparison with other microorganisms and the inability of the selective procedures employed to recover stressed cells (Pyle et al., 1995).

Due to the wide array of environmental reservoirs for *Salmonella*, the source of transmission of the pathogen in the Tennessee River was difficult to identify. At the research dairy farm, insects and bird droppings were identified as significant sources of transmission of pathogens to dairy animals and farm environments. Insects and birds may have been significant vectors of transmission of *Salmonella* along the Tennessee River. Birds are of particular

importance to isolation of *Salmonella* at sites upstream from the farm. River sampling site 2, in particular, was located directly beneath The University of Tennessee research poultry farm, also located upstream (east) of the dairy farm. High levels of *Salmonella* at sites 1 and 2 may also have been influenced by pathogen runoff due to rainfall from the poultry farm.

Both GIS and microbiological analyses revealed high prevalence of *Salmonella* in the Tennessee River at the dairy farm and at sites upstream from the farm. The Tennessee River flows east to west in direction. However, under currents, winds and other environmental factors may have resulted in shifts in the pattern of water flow. These factors may have contributed to the isolation pattern for *Salmonella* isolation from the river. Based on results of microbiological analysis at the dairy farm and the river water along the farm's perimeter, it was clear that significant effluent runoff of *Salmonella* occurred. However, based on the lack of other pathogens isolated at the farm and not isolated from the river, it can be concluded that The University of Tennessee research dairy farm did not contribute significantly to transmission of pathogens along the length of the river studied. It can therefore be suggested that sources other than the dairy farm contributed to contamination of the river.

LITERATURE CITED

American Public Health Association. 1995. Standard methods for the examination of water and wastewater, 19th ed. Section 9221 E, section 9060 A, section 9060 B. American Public Health Association, Washington, D.C.

Bej, A.K., S.W. McCarty, and R.M. Atlas. 1991. Detection of *coliforms* bacteria and *E. coli* by multiple PCR: comparison with defined substrate and plating methods for water quality monitoring. Appl. Environ. Microbiol. 57: 2429-2432.

Craun, G.F. 1988. Surface water supplies and health. J. Am. Water Works Assoc. 80:40-52.

FDA. 1998. Bacteriological Analytical Manual, FDA. 8th ed., AOAC International, Gaithersburg, MD.

Isaacson M., P.H. Canter, P. Effler, L. Arntzen, P. Bomans, and R. Heenan. 1993. Hemorrhagic *colitis* epidemic in Africa. Lancet 341: 961.

McGowan, K.L., E. Wickersham, and N.A. Strockbine. 1989. *Escherichia coli* O157:H7 from water. Lancet I: 967-968.

Nataro, J.P. and M.M. Levin. 1994. Bacterial diarrheas, p. 697-752. In A.Z. Kapikian (ed.), Viral Infections of the Gastrointestinal Tract. Marcel Dekker, New York.

Pyle, H.H., S.C. Broadway, and G.A. McFeters. 1995. A rapid, direct method for enumerating respiring enterohemorrhagic *Escherichia coli* O157:H7 in water. Appl. Environ. Microbiol. 61: 2614-2619.

Reilly, W.J., G.I. Forbes, G.M. Paterson, and J.C.M. Sharp. 1981. Human and animal salmonellosis in Scotland associated with environmental contamination, 1973-1979. Vet. Rec. 108: 553-555.

Reinert, P.E., and J.A. Hroncich. 1990. Source water quality management, p. 189-268. In F.W. Pontius (ed.), Water Quality and Treatment: a Handbook of Community Water Supplies. McGraw-Hill, Inc., New York.

Roszak, D.B. and R.R. Colwell. 1987. Survival strategies of bacteria in the natural environment. Microbiol. Rev. 51: 365-379.

Sinclair, J.L. and M. Alexander. 1984. Role of resistance to starvation in bacterial survival in sewage and lake water. Appl. Environ. Microbiol. 48: 410-415.

Sobsey, M.D., A.P. Dufour, C.P. Gerba, M.W. LeChevallier, and P. Payment. 1993. Using a conceptional framework for assessing risk to health from microbes in drinking water. J. Am. Water Works Assoc. 85: 44-48.

Tauxe, R.V. 1992. Epidemiology of *Campylobacter jejuni* infections in the United States and other industrial nations. In: *Campylobacter jejuni*: Current and future trends. American Society for Microbiology: Washington, D.C., p. 9 –12.

PART IV

AUTOMATED RIBOPRINTING OF *SALMONELLA* ISOLATES FROM DIFFERENT SPECIES, LOCATIONS, AND AGRICULTURAL RUNOFF

ABSTRACT

The Geographic Information System (GIS) and molecular characterization procedures were used to determine diversity and associations among *Salmonella* isolates from different species, locations, and agricultural runoff. Previously characterized *Salmonella* isolates were obtained following microbiological surveys of the University of Tennessee research dairy farm and the Tennessee River adjacent to the farm, and compared to isolates from various animal and environmental sources received from Washington State University. *Salmonella* isolates were further characterized using both the Analytical Profile Index (API) 20 E for Enterobacteriaceae and polyvalent somatic O *Salmonella* antiserum. Automated Riboprinting with the *PVU*II restriction enzyme was used to subtype the isolates.

Salmonella isolates (N = 190) were riboprinted using the Automated Riboprinter® Characterization System. The most frequently isolated *Salmonella* serotypes were *Salmonella* ser. Senftenberg (26), Typhimurium (25), Havana (8), and Newport (8). Comparison of *Salmonella* isolates recovered from Tennessee and Washington State revealed significant geographic correlations and similarities among isolates common to dairy cattle and farm environments.

INTRODUCTION

Molecular epidemiology is based on the use of a series of techniques of molecular biology to analyze microbiological traits that enable the differentiation of strains. Molecular typing methods may be categorized as phenotypic methods and genotypic methods. Phenotypic methods are those that detect

characteristics expressed by the organism. Genotypic methods are those that involve direct DNA analysis of chromosomal and extrachromosomal genetic elements. Molecular typing methods based on the analysis of chromosomal DNA or DNA fingerprinting has been shown to have broad applications in public health (Swaminathan and Matar, 1993).

The Automated Riboprinting Characterization System is a molecular typing technology capable of rapidly identifying and characterizing isolates of a variety of microorganisms. This automated system was designed to reduce the time involved in sample preparation and processing (Bruce, 1996). The Riboprinter uses standardized ribotyping procedures to generate riboprint patterns for foodborne pathogens. Identification of isolates is accomplished by band matching of the riboprint patterns in the database (Oscar, 1998). This comparison results in the identification of the target organism at a genus, species and strain level. The automated system also compares patterns of each new sample against all other patterns run on the system (Bruce, 1996). Automated Riboprinting has useful applications in Epidemiology as well as in areas of food processing. Wiedmann et al. (1995) evaluated the use of the automated Riboprinter to compare *Listeria* isolates from 4 separate outbreaks involving cattle, sheep, and goats. In 3 of 4 outbreaks, the same strain of *L. monocytogenes* appeared in silage and isolates from infected animals. Results from this study strongly implicated silage as the source of infection (Wiedmann et al., 1995).

There are currently more than 2500 serovars of *Salmonella* (Swaminathan and Matar, 1993). *Salmonella* serovars are classified within two species: *Salmonella* enterica and *Salmonella* bongori. All *Salmonella* species are potentially pathogenic to humans (D'Aoust, 2001). The majority (59%) of the 2500 *Salmonella* serovars belong to *S.* enterica subspecies. Within the *S.* enterica subspecies, the most common O-antigen serogroups are A, B, C1, C2, D, and E. Strains in these serogroups cause approximately 99% of *Salmonella* infections in humans and warm-blooded animals (Brenner et al., 2000).

Studies of animals have shown that certain *Salmonella* serotypes are more virulent than others, and that certain serotypes are more "human adapted" and more likely to cause invasive disease (Taylor et al., 1993). The difference in ability to cause human illness may be determined by segregation of isolate populations among humans and animals. In other words, certain serotypes may be transmitted preferentially within human populations, whereas other serotypes may be limited primarily to animal populations (Sarwari et al., 2001).

According to a review by Olsen et al. (2001), from 1987 to 1997, 441,863 humans *Salmonella* isolates of known serotype were reported to CDC related to human illness. The top 5 reported *Salmonella* serotypes were *S.* Typhimurium (24%), Enteritidis (22%), Heidelberg, Newport, and Hadar (Olsen et al., 2001). The United States Department of Agriculture, Plant Health Inspection Service, conducted a two-year study, from 1990 to 19991 to identify *Salmonella* serotypes commonly associated with healthy and ill feedlot cattle (Centers for Epidemiology and Animal Health, 1995). Serotypes most commonly recovered from healthy

cattle were *S.* Anatum, Montevideo, Muenster, Kentucky, and Newington. *Salmonella* serotypes commonly recovered from ill cattle were *S.* Typhimurim, Dublin, Typhimurium, Cerro, and Newport (Centers for Epidemiology and Animal Health, 1995). Data from these studies suggest commonalities among *Salmonella* serotypes isolated from cattle and those isolated from humans, thereby indicating a potential risk to human health.

During 1999, Centers for Disease Control and Prevention reported 10,697 laboratory-confirmed cases of nine diseases under surveillance by the Foodborne Disease Active Surveillance Network (CDC, 2001). Among the 4000 *Salmonella* isolates serotyped, the top five serotypes identified from human cases were *S.* Typhimurium (24%), Enteritidis (10%), Newport (9%), Heidelberg (7%) and Muenchen (6%) (CDC, 2000). In 2000, 12,631 laboratory-confirmed cases of nine diseases were identified under FoodNet surveillance. *Salmonella* Typhimurium (23%), *Salmonella* Enteritidis (15%), *Salmonella* Newport (11%) and Heidelberg (7%) were also the most commonly identified *Salmonella* serotypes identified in 2000 (CDC, 2001a).

Salmonella Enteritidis and *S.* Typhimurium have both gained significant public health attention with regards to numerous foodborne illness outbreaks being linked to the two pathogens. Illness resulting from *S.* Enteritidis has long been linked to consumption of fresh shell eggs and egg products (D'Aoust, 2001). *S.* Enteritidis presents a unique public health concern because of its transovarian transmission and localization within the egg magma. Commercial egg sanitizing practices targeting the surface of the egg are ineffective in

elimination internal contamination (D'Aoust, 2001). Multiple strains of *Salmonella* ser. Typhimurium DT104 should be of great public health concern due to resistance of the pathogen to multiple antibiotics used commonly in medical and veterinary practices. Also, illness due to S. Typhimurium DT104 is generally more severe than illness associated with other *Salmonella* species (Doyle, 1997).

The purpose of this study was to use the Geographic Information System (GIS) and automated Riboprinting to examine relationships that exist between animals and their environments. A representative sample of *Salmonella* isolates, from The University of Tennessee research dairy farm, the Tennessee River, and Washington State University, were riboprinted to determine diversity and associations among isolates from different species, locations, and runoff.

MATERIALS AND METHODS

Salmonella Isolates

For comparison, 55 previously characterized *Salmonella* isolates were obtained following a 12-month microbiological survey of The University of Tennessee research dairy farm animals and farm environments. Also, *Salmonella* isolates (n = 31) were obtained following a 3-month survey of the Tennessee River adjacent to the University of Tennessee dairy farm. These *Salmonella* isolates were compared to isolates of *Salmonella* received from Washington State University. *Salmonella* in Washington State (n = 104) were isolated from various sources including animals, feed, water, and soil.

Sample Preparation

Salmonella samples were streaked onto brain heart infusion agar (BHI) plates. BHI plates were incubated for 18 h at 37°C. A Gram stain of each isolate was performed prior to preparation of the samples for Riboprinting. An isolated bacterial colony was picked from the agar plate and added to 200 ml of sample buffer. The sample was vortexed for 5 sec. A 30 ml sample suspension was pipetted into the appropriate well of the sample carrier. The Riboprinter® system can automatically process up to 32 samples in 8 h. The sample carrier was placed into the heat treatment station to undergo a series of heating and cooling phases. Afterwards, 5 ml of lysing agents A and B were added to each sample well in the carrier.

Automated* Riboprint*ing

The Riboprinter Microbial Characterization System® (Qualicon, Inc., Wilmington, DE) was used to generate a standardized digital Riboprint pattern for the confirmed *Salmonella* isolates. Single *Pvu*II (restriction enzyme) identification Riboprint patterns were obtained for all *Salmonella* isolates. The sample carrier, containing 8 prepared samples, was loaded into the instrument along with the required consumables for that batch.

Once strain-tracking and related sample information was entered, the instrument automatically processed strains. The instrument carried out cell lysis and restriction digestion and loaded the restricted DNA onto an agarose gel. Electrophoresis and direct blotting onto a nylon membrane were carried out, followed by hybridization of the membrane with a labeled probe. Following

development of the image by use of a chemiluminescent substrate, the image was digitized using a low light camera. The software extracted information from the image. It recognized data lanes on the image and distinguished between the reference marker and sample lanes. The pattern for each lane consisted of a series of light and dark bands. The system automatically compared the Riboprint pattern generated for each new sample to the patterns stored for all other samples in the database.

Serotyping

Confirmation of *Salmonella* isolates using polyvalent somatic (O) antiserum (Bacto *Salmonella* Antisera Poly A, B, C, D, E, F and G; Becton, Dickinson and Company, Franklin Lakes, NJ) was performed on isolates identified as *Salmonella* by riboprinting.

Geographic Information System (GIS) Analysis

An aerial photograph taken of the dairy farm and the Tennessee River was scanned into ArcView® GIS version 3.2 (Environmental Systems Research Institute, Redlands, CA) and used as the basemap onto which the spatial information will be layered. Data generated from Riboprint analyses of *Salmonella* isolates from The University of Tennessee research dairy farm and the Tennessee River were imported into the ArcView® project and plotted onto the basemap. Cartographic software, Freehand 9.0® (Macromedia, NY, USA), was used to visually display data on the map. A series of colors and shapes were used for improved data visualization.

Data Analysis

SAS GIS version 8.2 was used to generate GIS maps for comparison of *Salmonella* isolates recovered from the Tennessee dairy farm animals, farm environments, and the Tennessee River to those recovered from animals, farm environments, and human clinical isolates from Washington State.

The Riboprinter Microbial Characterization System enabled comparison, correlation, and generation of similarity indices among *Salmonella* isolates. ArcView® GIS version 3.2 was used to perform advanced statistical analyses of spatial data, including cluster analyses, and Poisson probability distributions.

RESULTS

The Riboprinter® system extracts patterns that are like fingerprints of individual bacterial samples. These patterns can be used to characterize or group samples, since similarities and differences in patterns revealed similarities and differences among the bacteria themselves. Patterns can be used also to identify the genus and species (or serotype for *Salmonella*) of samples. A total of 190 *Salmonella* isolates were Riboprinted, using the Automated Riboprinter® Characterization System, to compare isolates from various species, locations, and sample types. *Salmonella* isolates were obtained from epidemiological surveys of The University of Tennessee dairy farm and the Tennessee River, as well as from Washington State University.

A total of 55 *Salmonella* isolates from the research dairy farm survey were riboprinted (Table 7). Dairy farm *Salmonella* isolates riboprinted as *Salmonella* ser. Senftenberg (23), Typhimurium (5), Havana (4), Infantis (3) Harfort (2), and

Table 7. Serotypes of *Salmonella* isolated from dairy farm animal and environmental samples.

***Salmonella* serotype (number)**	**Species/sample type**
Senftenberg (23)	Silage
Typhimurium (5)	Insects Soil
Havana (3)	Cow mouth Cow bedding
Infantis (3)	Trough water
Hartfort (2)	Soil

"other "*Salmonella* (18). *Salmonella* ser. Senftenberg was isolated predominantly from silage samples. *Salmonella* ser. Senftenberg isolates from silage samples were compared (Figure 6) and all isolates (lanes 1 - 8) showed very similar riboprint patterns (comparison $\geq$ 97%). These results indicate that *Salmonella* contamination may have been from a common source. *Salmonella* ser. Havana, which was isolated from cow oral and bedding samples, also showed similar riboprint patterns(Figure 7). Patterns 1-4 from cow mouth samples, displayed similar riboprint patterns (comparison $\geq$ 98%). Lanes 5-8, riboprint patterns of *Salmonella* ser. Havana isolates from cow bedding samples, were not similar (comparison $\leq$ 75%). However, 1 of the 4 bedding isolates showed similar riboprints to *Salmonella* ser. Havana isolated from cow mouth samples (comparison > 98%).

A total of 31 *Salmonella* isolates from the Tennessee River survey were Riboprinted (Table 8). A variety of *Salmonella* serotypes were detected in Tennessee River water samples including, *Salmonella* ser. Newport (4), Senftenberg (3), Waycross (3), Berkeley (3) and Havana (2). The serotype distribution of *Salmonella* isolates from the Tennessee River is displayed in Figure 8. *Salmonella* ser. Berkeley, which was isolated from the river at the dairy farm and at site 1 (4.2km East, upstream), displayed similar riboprint patterns (comparison $\geq$95%; Figure 9).

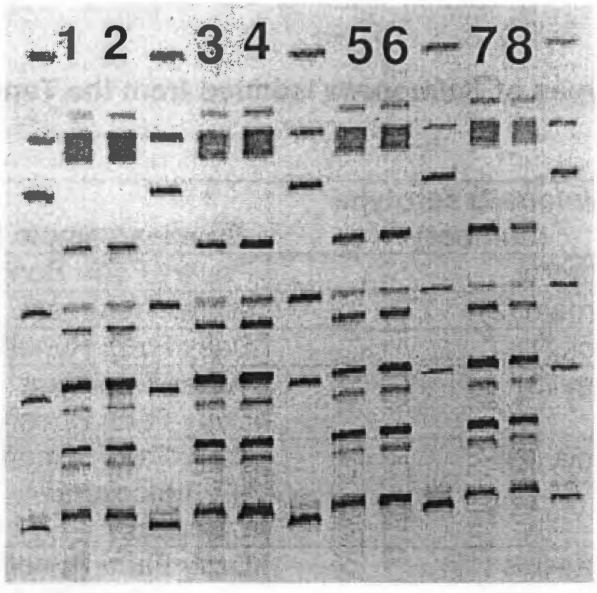


Figure 6. Riboprint patterns of *Salmonella* ser. Senftenberg isolated from dairy farm environments.

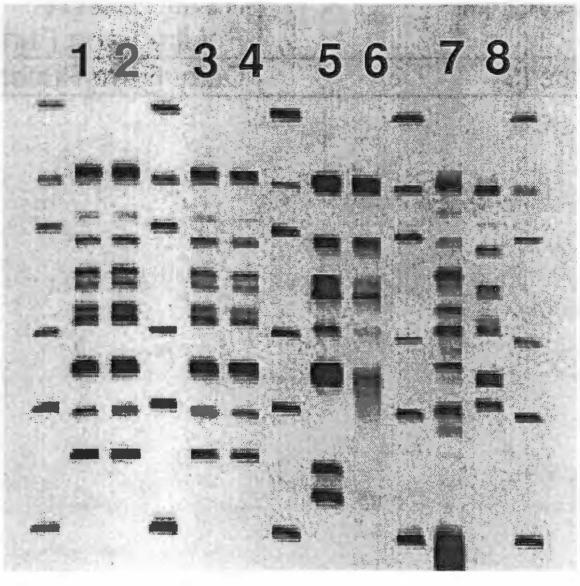
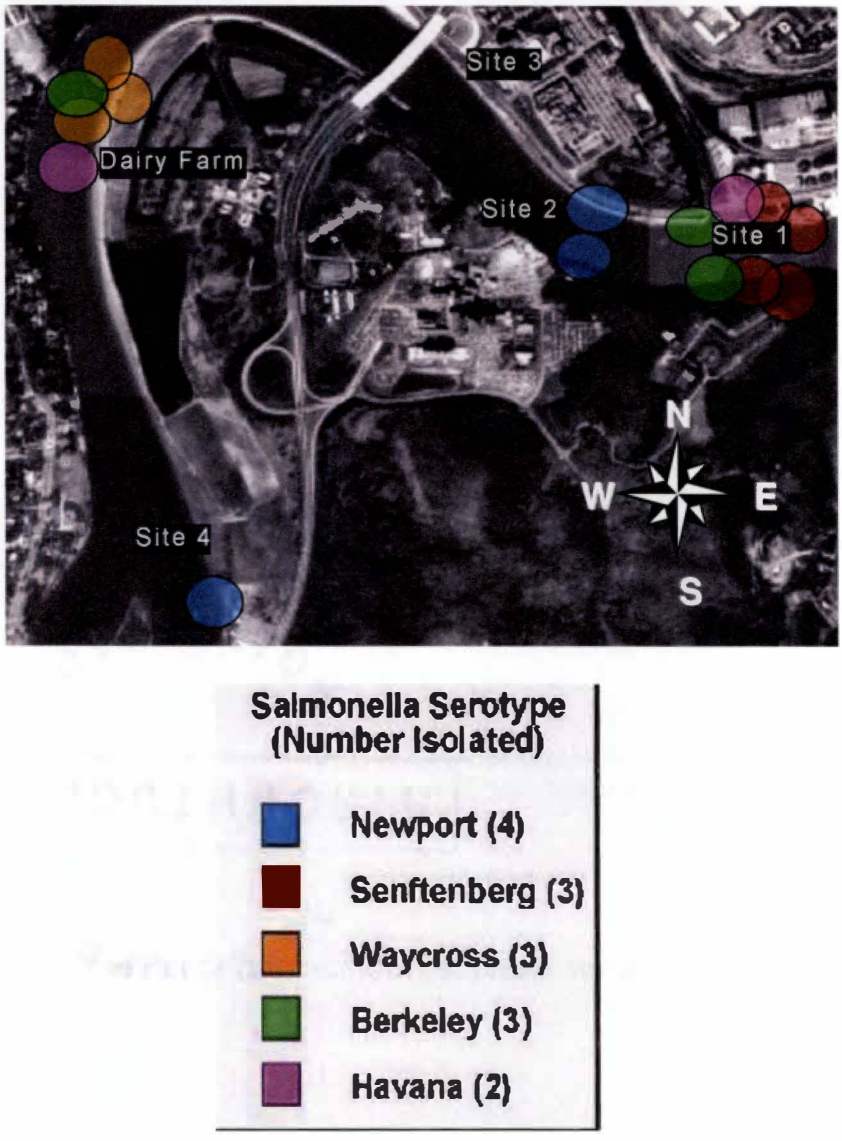


Figure 7. Riboprint patterns of *Salmonella* ser. Havana isolated from dairy farm environments.

Table 8. Serotypes of *Salmonella* isolated from the Tennessee River.

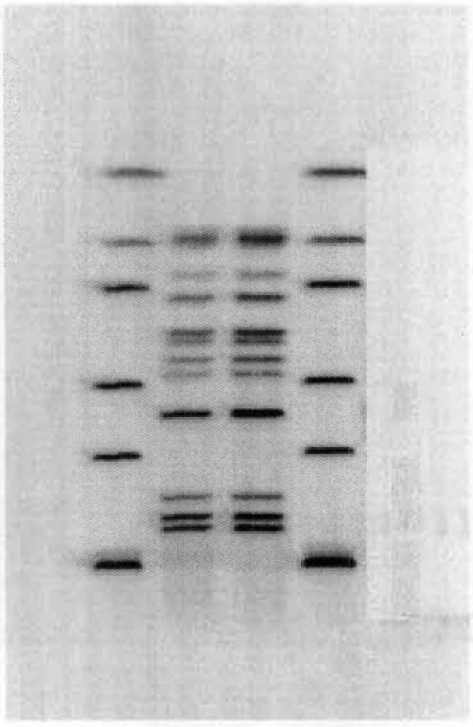
Salmonella serotype (number)	Species/sample type
Adelaide (1)	Poultry Farm Runoff
Amsterdam (2)	Water Treatment
Bangkok (1)	Dairy Farm Runoff
Berkeley (3)	Water Treatment Dairy Farm Runoff
California (2)	Dairy Farm Runoff UT Agricultural Campus
Choleraesuis (1)	Dairy Farm Runoff
Enteritidis (1)	Poultry Farm Runoff
Gombe (1)	Dairy Farm Runoff
Havana (2)	Water Treatment Dairy Farm Runoff
Kottbus (1)	Poultry Farm Runoff
Newport (4)	Water Treatment
Pullorium (1)	Poultry Farm Runoff
Saintpaul (1)	Water Treatment
Senftenberg (3)	Naval Reserve Poultry Farm Runoff
Waycross (3)	Dairy Farm Runoff



Distance from Dairy Farm (km): Site 1 (E 4.2 km); Site 2 (E 3.6 km); Site 3 (NE 2.7 km); Site 4 (SW 4.5 km)

The Tennessee River flows from East to West.

Figure 8. Serotype distribution of *Salmonella* isolated from the Tennessee River.



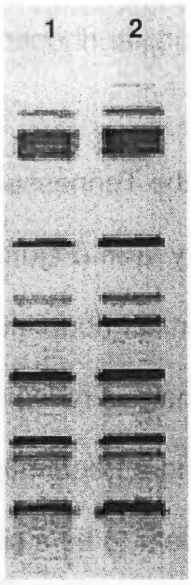
Lane 1: *Salmonella* ser. Berkeley isolated from Dairy Farm
Lane 2: *Salmonella* ser. Berkeley isolated from Tennessee River

Figure 9. *Salmonella* ser. Berkeley isolated from the Tennessee River.

Salmonella ser. Havana also isolated from river water samples collected at the dairy farm and site 1, displayed similar riboprint patterns (comparison ≥ 95%; Figure 10).

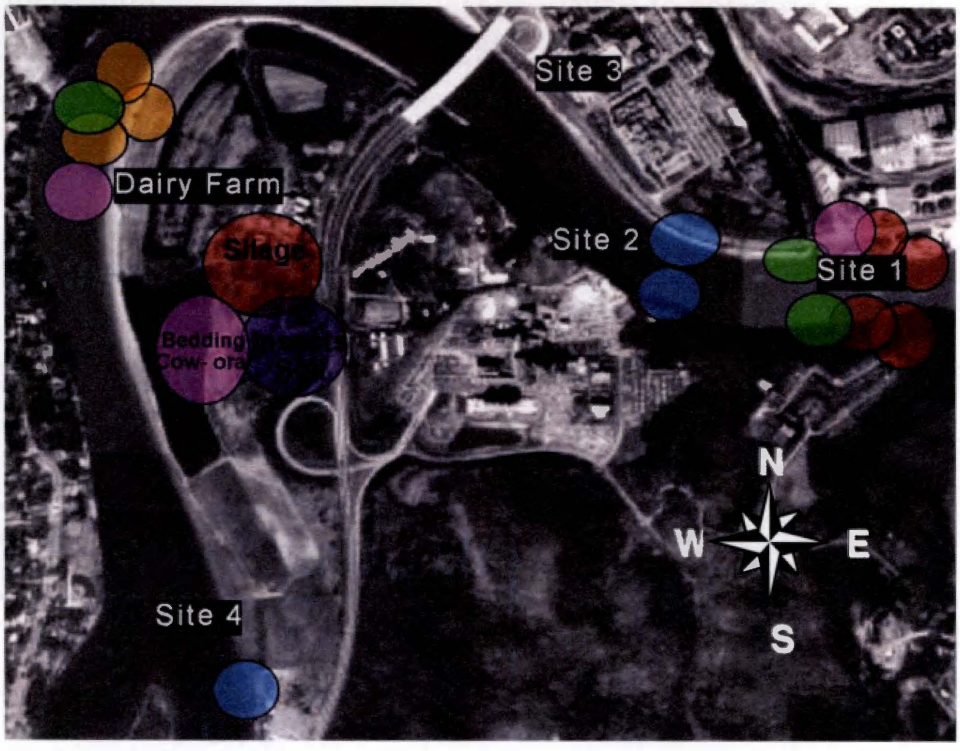
Salmonella Isolated from the Tennessee River were compared to *Salmonella* isolates from the dairy farm (Figure 11). *Salmonella* isolates from the Tennessee River did not show similar riboprint patterns to *Salmonella* isolated at the dairy farm. Results of GIS analysis revealed that serotypes showing similar riboprint patterns were isolated more frequently at sites upstream from the dairy farm. This dairy farm did not appear to be a significant source of contamination of the Tennessee River. It can be suggested that a source other than The University of Tennessee research dairy farm may be sources of these specific *Salmonella* serotypes isolated from the Tennessee River.

Washington State University provided The University of Tennessee Food Safety Research Group with *Salmonella* isolates various animal species and sample types isolated over a 15-year period (Table 9). Although a variety of *Salmonella* Serotypes were detected amongst the Washington State isolates, *Salmonella* ser. Typhimurium (20), Cerro (6), Anatum (4), Hadar (4), Meleagridis (4), and Newport (4) serotypes most frequently detected by riboprinting. *Salmonella* ser. Typhimurium was commonly isolated from bovine feces and feed samples (Figure 12). Bovine feces samples collected in 1995, 1998, 1999 and 2000 showed similar Riboprint patterns (comparison ≥ 95%).



Lane 1: *Salmonella* ser. Havana isolated from Dairy Farm
Lane 2: *Salmonella* ser. Havana isolated from Tennessee River

Figure 10. *Salmonella* ser. Havana isolated from the Tennessee River.



Salmonella Serotype (Number Isolated)

- **Newport (4)**
- **Senftenberg (3)**
- **Waycross (3)**
- **Berkeley (3)**
- **Havana (2)**
- **Typhimurium (25)**

Distance from Dairy Farm (km): Site 1 (E 4.2 km); Site 2 (E 3.6 km); Site 3 (NE 2.7 km); Site 4 (SW 4.5 km)

The Tennessee River flows from East to West.

Figure 11. Serotype distribution of *Salmonella* isolated from dairy cows, farm environments and the Tennessee River.

Table 9. Serotypes of *Salmonella* isolated from Washington State University.

***Salmonella* serotype (number)**	**Species/sample type**
Arizonae (2)	Reptile feces Ovine intestine
Adelaide (1)	Mink necroscopy
Albany (1)	Mink necroscopy
Anatum (5)	Bovine feces Emu feces
Bareilly (1)	Reptile oral
Bellvue (1)	Cheetah feces
Blikwa (2)	Bovine feces Ocelot culture
California (1)	Bovine feces
Cerro (6)	Bovine feces
Chandans (1)	Avian cloaca
Drypool (1)	Bovine intestine
Ealing (1)	Iguana feces
Enteritidis (1)	Bovine necroscopy
Give (2)	Bovine feces
Godesberg (1)	Reptile feces
Haardt (1)	Bovine feces
Hadar (4)	Bovine feces
Havana (2)	Bovine feces Avian necroscopy
Infantis (2)	Canine bronci Avian
Isang (1)	Reptile feces
Jangwani (1)	Bovine feces
Kentucky (2)	Bovine feces
Lanka (1)	Bovine feces
Lille (1)	Equine feces
Mbandaka (3)	Bovine serum
Meleagridis (4)	Bovine feces
Miami (1)	Feline feces
Montevideo (3)	Bovine feces Reptile feces
Newport (4)	Bovine feces Bovine necroscopy
Oranienburg (2)	Bovine feces
Paratyphi (1)	Avian feces
Salmonella Serotype (number)	Species/Sample Type
Pomona (3)	Rodent colon
Reading (1)	
Rubislaw (1)	Bovine necroscopy
Siantpaul (1)	Bovine feces
Schwarzengrund/Bredeney (1)	Moose necroscopy
Seminole (1)	Bovine feces
Tennessee (2)	Bovine feces Canine feces
Tilene (1)	Cheetah necroscopy
Typhimurium (20)	Bovine feces Feed (bovine) Mink necroscopy Reptile feces Bovine necroscopy
Weltevreden (2)	Bovine feces

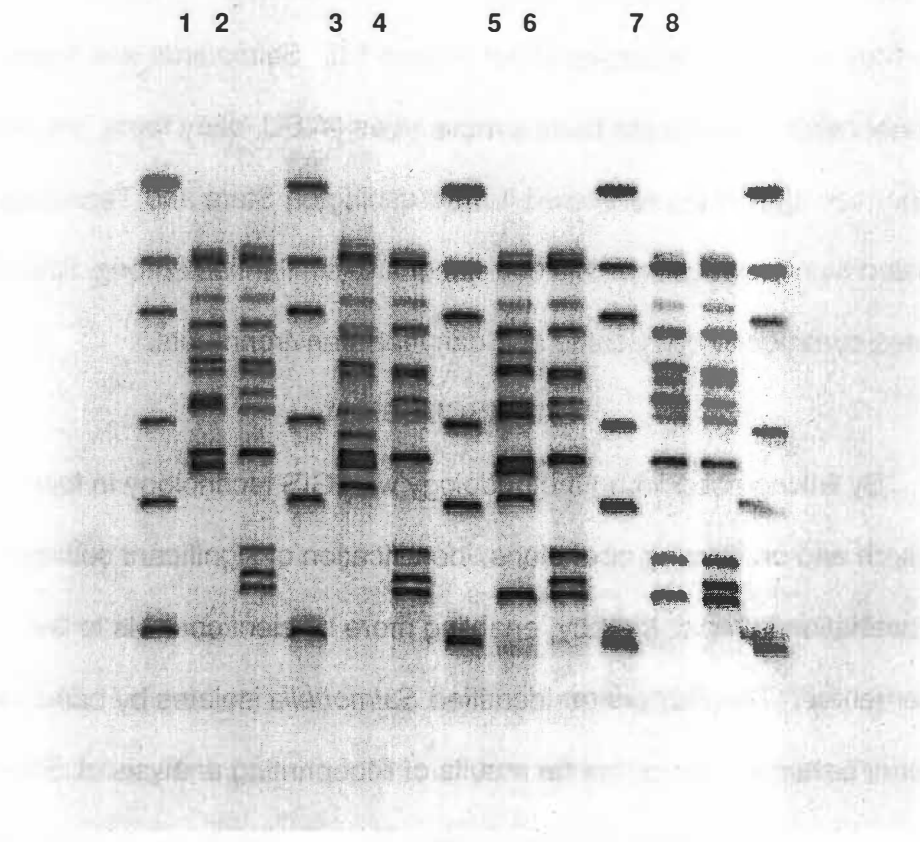
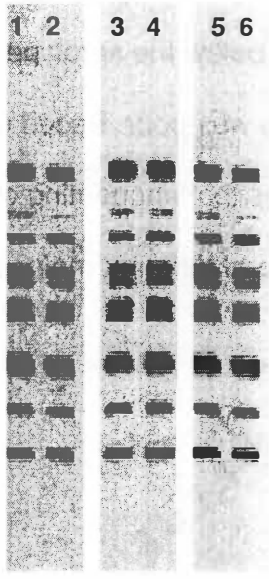


Figure 12. *Salmonella* ser. Typhimurium isolated from Washington State University bovine feces samples.

Riboprints of Washington University *Salmonella* isolates were compared to *Salmonella* isolates from surveys of The University of Tennessee research dairy farm and the Tennessee River (Figure 13). *Salmonella* ser. Havana was the only common serotype to all sample types (WSU, dairy farm, TN River). The comparison of isolates recovered from Washington State and Tennessee revealed significant geographic correlations or similarities among *Salmonella* isolates common to dairy cattle and dairy farm environments.

DISCUSSION

By linking riboprinting methodology with GIS technology in food safety research and on farming operations, identification of significant sources of contamination is rapid; thereby, enabling more efficient controls to be implemented. The Riboprinter identified *Salmonella* isolates by band matching of riboprint patterns. Based on the results of Riboprinting analysis of *Salmonella* isolates from Tennessee and Washington State, it was found that the Riboprinter was an efficient method in its ability to identify *Salmonella* serovars from a variety of sources. Of the 190 *Salmonella* isolates riboprinted, 158 (83%) were identified by the system at or below the serotype level. Only 32 (16%) of the *Salmonella* isolates were not characterized via riboprinting. These *Salmonella* isolates were classified as "other" *Salmonella* and were placed in 1 of 5 similarity groups for *Salmonella* in the system's database. According to Oscar (1998), the Riboprinter occasionally cannot match a riboprint pattern of an isolate to other patterns in the database. In this case, the Riboprinter makes no positive identification, but places the pattern in one or more similarity groups of isolates showing that



Lanes 1 and 2: *Salmonella* ser. Havana isolated from dairy farm
Lanes 3 and 4: *Salmonella* ser. Havana isolated from Tennessee River
Lanes 5 and 6: *Salmonella* ser. Havana isolated from Washington State

Figure 13. *Salmonella* ser. Havana isolated from Tennessee and Washington State.

particular pattern. Expansion of the Riboprinter database will improve the Riboprinter's identification capabilities. The automated riboprinting system did, however, have some limitations. Although the Riboprinter successfully identified most *Salmonella* isolates at or below the serotype level, the repeatability of riboprint patterns between runs was poor. Based on results of this study, it is recommended for future studies that riboprinting of these *Salmonella* isolates be compared to results using several different molecular methods of characterizing foodborne isolates.

LITERATURE CITED

Brenner, F.W., R.G. Villar, F.J. Angulo, R. Tauxe, and B. Swaminathan. 2000. *Salmonella* nomenclature. J. Clinical Micro. 38(7): 2465-2467.

Bruce, J. 1996. Automated system rapidly identifies and characterizes microorganisms in food. Food Tech. 1: 77-81.

CDC. 2000. Preliminary FoodNet data on the incidence of foodborne illness: Selected sites, United States, 1999. Morbidity and Mortality Weekly Reports, 49(10): 201-205.

CDC. 2001a. Preliminary FoodNet data on the incidence of foodborne illness: Selected sites, United States, 2000. Morbidity and Mortality Weekly Reports, 50: 241-246.

Centers for Epidemiology and Animal Health. 1995. *Salmonella* shedding by feedlot cattle. U. S. Department of Agriculture, Animal and Plant Health Inspection Service, Fort Collins, Colorado.

D'Aoust, J.Y. 2001. Foodborne salmonellosis: Current international concerns. Food Safety Magazine, 7(2): 10-16.

Doyle, M.E. 1997. Emergence of highly virulent strains of *Salmonella* Typhimurium. Internet location: http:// www. Wisc.edu/fri/dt104.htm. Accessed 03 March, 2003.

Jayarao, B.M. and D.R. Henning. 2001. Prevalence of foodborne pathogens in bulk tank milk. J. Dairy Sci. 84: 2157-2162.

Murinda, S.E., L.T. Nguyen, S.J. Ivey, B.E. Gillespie, R.A. Almeida, F.A. Draughon, and S.P. Oliver. 2002. Molecular characterization of *Salmonella* spp. isolated from bulk tank milk and cull dairy cow fecal samples. J. Food Prot. 65(7): 1100-1105.

Olsen, S.J., P. Bishop, F.W. Brenner, T.H. Roels, N. Bean, R.V. Tauxe, and L. Slutsker. 2001. The changing Epidemiology of *Salmonella*: Trends in serotypes isolated from humans in the United States, 1987-1997. J Infect Dis 183: 753-761.

Oscar, T.P. 1998. Identification and characterization of *Salmonella* isolates by automated ribotyping. J. Food Prot. 61(5): 519-524.

Swaminathan, B., and G.M. Matar. 1993. Molecular typing methods. In: D.H. Persing, T.F. Smith, F.C. Tenover, and T.J.White eds. Diagnostic molecular microbiology: principles and applications. Washington, DC: American Society for Microbiolgy, pp. 26-45.

Sarwari, A.R., L.S. Magder, P. Levine, A.M. McNamara, S. Knower, G.L. Armstrong, R. Etzel, J. Hollingsworth, and J.G. Morris. 2001. Serotype distribution of *Salmonella* isolates from animals after slaughter differs from that of isolates found in humans. J Infect Dis 183: 1295-1299.

Taylor, J.L., D.M. Dwyer, and C. Groves. 1993. Simultaneous outbreak of *Salmonella* enteritidis and *Salmonella* schwarzengrund in a nursing home: association of S. enteritidis with bacteremia and hospitalization. J Infect Dis 167: 781-782.

PART V

SUMMARY AND CONCLUSIONS

SUMMARY

In part 1 of the study, a comprehensive epidemiological survey was conducted to determine the prevalence of *Salmonella, Campylobacter jejuni*, and *Escherichia coli* O157:H7 in dairy animals and dairy farm environments. The experimental design included 321 dairy cows (lactating and non-lactating) and calves from The University of Tennessee, Knoxville Experiment Station research dairy farm. Samples were collected monthly for 12 months from dairy cows, calves, feed and the farm environment, which included pastures, barns, bedding, soil, bulk tank milk, milking equipment, air, insects, and wild birds. A Geographic Information System (GIS) was used to examine relationships that exist between animals and their environments.

A strong seasonal influence occurred with the isolation of *Salmonella* and *C. jejuni* at the farm. The prevalence of *Salmonella* isolated from dairy animals, feed, and dairy farm environments was 32%. *Salmonella* isolation was highest in the summer (48%) in both cows and calves. *Salmonella* isolation during winter, spring, and summer months was between 24% and 26%. Feed (59%), bedding materials (47%), water (40%), bird droppings (34%) and insects (39%) were identified as significant sources of *Salmonella*. The prevalence of *C. jejuni* isolated at the farm was 21%. Isolation of *C. jejuni* was significantly higher during winter (28%) and fall (29%). Bird droppings (33%) were strongly correlated ($P \leq 0.05$) with contamination of feed (40%) and water (60%) sources. The prevalence of *E. coli* O157:H7 at the farm was only 2 %. Isolation was very infrequent throughout the entire study period. No statistical differences ($P \geq$

0.05) were observed among sample type. Data generated from the current research were used to devise strategies to reduce pathogen contamination at the farm through environmental management and risk

In part 2 of the study, a microbiological survey of the Tennessee River, adjacent to The University of Tennessee research dairy farm, was conducted to determine the prevalence of *Salmonella*, *C. jejuni*, and *E. coli* O157:H7. Fecal *coli*forms were also examined as possible indicators of the presence or absence of pathogens in the river. GIS was used to examine possible relationships between contamination with *Salmonella*, *C. jejuni*, and *E. coli* O157:H7 at the dairy farm and subsequent contamination of the Tennessee River.

Neither *C. jejuni* nor *E. coli* O157:H7 were recovered from the Tennessee River water samples, however, *Salmonella* (33%) was isolated from all sampling sites along the river. The concentration of fecal *coli*forms in river water samples ranged from 37 to > 2400 Most Probable Number per 100 ml water. No correlation ($P > 0.05$) was found between the indices of fecal contamination and *Salmonella* recovered from the Tennessee River.

GIS analysis revealed a decrease in isolation of *Salmonella* from sites directly across (11%) and directly downstream (11%) from the dairy farm (56%). However, *Salmonella* was isolated at a prevalence of 44% from sites upstream from the farm. Based on the lack of bacterial species isolated from the river as opposed to those recovered from the dairy farm, and the variable pattern of pathogen isolation from the river, it can be concluded that The University of

Tennessee research dairy farm did not contribute significantly to contamination of the Tennessee River.

Finally, in part 3 of the study, GIS and molecular characterization procedures were used to determine diversity and associations among *Salmonella* isolates from different species, locations, and agricultural runoff. Previously characterized *Salmonella* isolates were obtained following microbiological surveys of the University of Tennessee research dairy farm and the Tennessee River adjacent to the farm, and compared to isolates from various animal and environmental sources received from Washington State University. *Salmonella* isolates were further characterized using both the Analytical Profile Index (API) 20 E for Enterobacteriaceae and polyvalent somatic O *Salmonella* antiserum. Automated Riboprinting with the *PVU*II restriction enzyme was used to subtype the isolates.

Salmonella isolates (N = 190) were Riboprinted using the Automated Riboprinter® Characterization System. The most frequently isolated *Salmonella* serotypes were *Salmonella* ser. Senftenberg (26), Typhimurium (25), Havana (8), and Newport (8). Comparison of *Salmonella* isolates recovered from Tennessee and Washington State revealed significant geographic correlations and similarities among isolates common to dairy cattle and farm environments.

CONCLUSIONS

In conclusion, the full potential of GIS as a surveillance tool is not yet realized. However, advances in GIS may prove valuable to food safety research in the future. Use of GIS allows for improved monitoring and instantaneous

visualization of data. Using GIS reduces time required to analyze numerical data. GIS is useful for identifying critical control points and aids the decision-making process regarding control of foodborne pathogens in the environment.

The dairy farm environment represents an array of reservoirs of foodborne pathogens. The development of efficient, on-farm management strategies to control transmission of foodborne pathogens requires knowledge of the prevalence and distribution of these pathogens on animals and in the environment. The results of this study indicated that the risk of foodborne disease may be reduced by targeting controls in animal production environments. It can be concluded from this research that epidemiological knowledge of factors affecting shedding of pathogens by food animals may reduce transmission of pathogens at the farm and throughout the food chain.

VITA

Kimberly Denise Lamar was born in Memphis, Tennessee on December 27, 1975. She graduated from Southern Baptist Educational Center in Olive Branch, Mississippi in May of 1993.

Kimberly attended Tulane University in New Orleans, Louisiana, where she received a Bachelor of Science degree in Psychology in May of 1997. In August of 1999, she completed her Masters of Public Health from East Tennessee University. That same year, in December of 1999, she completed a Masters of Science in Environmental Health from that same university. Kimberly will earn the Ph.D. degree in Food Science and Technology from the University of Tennessee in August 2003. Kimberly is presently working for the United States Department of Agriculture, National Agricultural Statistics Service in Albany, New York.