



Epidemiologic Approaches to Food Safety

1999-2002



*National Research Initiative
Cooperative State Research, Education, and
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USDA*



Epidemiology..... is **the study** of the occurrence and the risk factors of disease or health-related events in a population and the **application of this knowledge** to the control of that disease or event. It incorporates population medicine, critical reasoning, is interdisciplinary in nature, and lends itself to establishing prevention and control programs and then measuring the impact of these programs. This discipline is ideal for the study of disease processes or risk factors in real world settings.

Epidemiology is an integrative science that approaches problems using real world data but is anchored in the scientific approach. Epidemiology, through identifying risk factors and determining cause of disease, has been instrumental in developing health policy for the United States with pivotal studies on tobacco use and cancer, the spread of AIDS, nutrition and heart disease, and food safety. Food safety research is the necessary foundation for providing a safe and healthy food for the U.S. and the world's population, and epidemiology is providing the scientific underpinning for our food safety policy. Projects in this USDA program integrate research involving epidemiologists, microbiologists, statisticians, animal scientists, food scientists, geographers, and animal industry groups. These types of projects are the essential step in understanding and addressing food safety issues.

Epidemiologic approaches for food safety provides a model for use of epidemiologic research to address other important agricultural and public health issues such as animal health, zoonoses, and biosecurity.

Unique Strengths of the USDA NRI Program: Epidemiologic Approaches to Food Safety

This is the only existing **research** program to fund large epidemiologic (population-based) studies in food safety. These projects span the food safety continuum: from farm to fork; food animals, aquaculture, and produce; and various foodborne pathogens in different regions of the U.S.

The program addresses several concerns outlined in the recent National Academy Report (2003) entitled, “**Frontiers in Agricultural Research: Food, Health, Environment, and Communities**”, that calls for more multi-disciplined, flexible research and for increasing research in the understanding the epidemiology of microbial pathogens.

The program has resulted in the development of a cadre of epidemiologists and microbiologists. These scientists communicate regularly and present in mass at scientific meetings. As a result, innovative collaborations have emerged to address new problems. The researchers funded by this program have developed networks that foster the trust and willingness among members to share methods, samples, and cross-train graduate students in their laboratories.

Outcomes of the Program.....

Scientific Presentations

- Whole day research sessions at the annual meeting of the American Veterinary Medical Association (2001-2003)
 - 2003
 - ½ day - Epidemiologic Research Providing Food Safety Intervention and Control Strategies
 - ½ day - Practical Recommendations on Antimicrobial Resistance in Agriculture
 - 2002
 - ½ day - Food Safety Research and Management Practices in Feedlots
 - ½ day - Antimicrobial resistance: From Farm to Fork
- Doubled the number of presentations at the food safety session of the annual meeting of the Conference of Research Workers in Animal Disease. Over 70% of the current presentations in the last 2 years have been supported by the NRI and this program
- Individual research presentations
- Multiple presentations at the American Society of Microbiology Annual Meeting (2002-2003)

Enhanced Communications

- List serve maintained at the University of Nebraska provides the opportunity to talk about funding, new methods, and management issues
- Annual meeting of grantees to discuss emerging issues, the development of new methods, potential collaborations, and other scientific issues

Advancement of the Science

- Workshop on Epidemiologic Approaches for Food Safety was convened in 2000 as a result of this program.

This workshop was supported by CSREES, CDC, Association of Teachers of Veterinary Preventive Medicine and Public Health, and the American College of Veterinary Preventive Medicine and was held in conjunction with the U.S. Animal Health Association. This was the first workshop to specifically address epidemiologic methods and their application in food safety research. This workshop served as a catalyst for new food safety research and technology transfer by: 1) strengthening the existing network of epidemiologists, microbiologists, and other food safety specialists; 2) increasing awareness about epidemiological approaches that integrate with other scientific disciplines; and 3) building consensus about the significant food safety research questions facing the U.S. and approaches to address those questions.

Leveraging of Resources

- Because the nature of this research is large population studies, other Federal agencies have been able to use existing research studies in order to collect additional data for their missions.
 - CVM (FDA) added funds to an existing microbial contamination in produce study so that *Enterococci* samples could be collected and tested for antimicrobial resistance

History and Background of 32.1

NRI's 32.1 Epidemiological Approaches for Food Safety became a program area for funding in 1999. The program's mission was to augment other food safety research programs by providing funds for large-scale population-based studies. Since 1999, the program has provided approximately \$5 million per year (as of 2002 a total of \$20,410,000) to fund epidemiologic studies. The topics for these funded grants include a number of foodborne pathogens including *Salmonella*, *E. coli*, *Campylobacter*, and *Vibrio*; different food animal species (cattle, pigs, oysters) and produce (lettuce, melons); and steps along the food production chain (on-farm to processed food). The funded projects involve multiple institutions and geographic regions.

The program's objective is to enhance the understanding of the multiple factors involved in food safety and provide the science-based data for policy decisions. Significant gaps exist in the knowledge base for food safety and the risk factors involved in the entire continuum of food production, from farm to table. Epidemiologic studies of pre- and post-harvest areas are vital to identify and characterize pathogenic organisms, including their sources and reservoirs; and to understand the transmission of the pathogen along the entire continuum. The identification of risk factors for exposure to and infection by these pathogens can be accomplished by several different epidemiologic research methodologies. These methodologies can be applied at any stage of food production. Environmental and ecological data are needed to increase our understanding of disease-causing microorganisms, their products, and naturally occurring contaminants in meats, poultry, seafood, and fresh fruits and vegetables. Studies that elucidate these data or information, or provide a "value of information" analysis, can provide important information on where research is needed and could be useful for risk assessments.

The current emphasis of this program is any population-based epidemiologic study that involves identifying risk factors and evaluating strategies to reduce foodborne contamination specifically in areas of (a) determination of the levels of microbial contamination in finished food products; (b) identification of risk factors and possible intervention or management strategies; (c) identification of farm-based solutions that contribute to decreased prevalence of foodborne pathogens; (d) development of outcome measures for the impact of intervention or management strategies on microbial contamination or food safety; (e) identification of potential sites of contamination in the processing, transportation, retail setting, and consumer use of food products; and (f) development of sampling strategies to optimize the detection level of foodborne pathogens/toxins throughout the food production chain.

Highlights from Funded Research.....

Results from the study on microbial contamination of produce will be relevant for resolving the current embargo on the importation of Mexican cantaloupe into the US.

Results from the *Campylobacter* study suggest that effective control of *Campylobacter* in poultry should use multiple approaches (including immunology-based and management-based strategies) that target different segments of the poultry production system.

A food safety course was taught in Chile that was based on current research of *Salmonella* in dairy cattle.

Prevention of fecal contamination of water tanks and control of vermin in cattle pens may reduce *E. coli* O157. However, control of management factors alone will not completely prevent fecal shedding of *E. coli* O157 in feedlot cattle.

Research of *Campylobacter* in poultry in Iceland has produced the potential to create a standard for allowable levels of *Campylobacter* in poultry rinses supported by both FSIS and the North American poultry industry.

Development of a statistically validated method for spatial sampling of abattoir holding pens.

Presentation of data on *Salmonella* spread on farms to dairy industry groups and to veterinarian and producer extension programs. Consequently, these researchers have been involved with producers to reduce on-farm *Salmonella* contamination by changing water use practices.

Participation in several workshops on the role of on-farm use of antibiotics and providing guidance for prudent use of antibiotics.

Research has resulted in the participation of several investigators in two American Academy of Microbiology research colloquiums- preharvest food safety and the impact of antimicrobial resistance in agriculture.

Several investigators have contributed their research results in chapters in a new microbial food safety book.

Abstracts of Funded Research

Awards in 1999

Ecologic Assessment of *Salmonella enteritidis* var *Typhimurium* in a Dairy Milk Shed

Sischo, W.M.; Adaska, J.; Atwill, E.R.; Kirk, J.Jeffrey; Moore, D.A.; Wilson, D.; White, D.G. University of California; Department of Population Health and Reproduction; Tulare, CA 93274; \$600,000; 3 Years

The objective of this research is to specifically identify the diversity of *S. typhimurium* in a geographic community with a high concentration of dairy cattle and describe the medical ecology of the bacteria relative to the diversity. We believe that only a portion of the known *S. typhimurium* is associated with dairy cattle and can be identified in milk or on cull animals and some of these may be associated with food-borne and human diseases. In addition, there are specific inputs, reservoirs, and animal management practices that are responsible for the persistence, spread, and expression of this subset of *S. typhimurium* on California dairies. Specifically, we will monitor changes in *S. typhimurium* isolated from a two county, dairy milk shed in central California (400,000 dairy cattle) and identify the genetic variants of these isolates. We will determine the spatial patterns of *S. typhimurium* variants in the milk shed (that is determine where they move in the milk shed) and assess the pathogen, environment, and management factors associated with persistent dairy herd infection with *S. typhimurium*.

New Methods for Risk Analysis of Infectious Animal Diseases Affecting Food Safety

Salman, M., Colorado State University; Department of Environmental Health; Fort Collins, CO 80523-2002; \$359,515; 2 Years

Current risk analysis procedures for food-borne diseases are inadequate. The long-term goal of our multidisciplinary research is to refine existing and develop new methodologies to allow for risk analysis procedures for food-borne diseases that are more efficient but still scientifically-based. We will apply the methods to toxoplasmosis in swine, *Escherichia coli* 0157:H7 in feedlot cattle, and *Salmonella enteritidis* in shell eggs, but the methods will be broadly applicable to other food-borne diseases.

Following Resistant *Salmonella* Through the Food Chain: a Molecular Ecology Approach

Maurer, J.; Summers, A.; White, D.; University of Georgia; Department of Avian Medicine; Athens, GA 30602; 814,564; 3 Years

The emergence of multiple-drug resistant *Salmonella* has made antibiotic resistance an important health issue in medicine and food safety. Once antibiotics are introduced, antimicrobial resistance quickly develops, spreads, and persists, even when antibiotic usage is discontinued. The current model is that food-borne pathogens obtain most antibiotic resistance from a pre-existing pool of drug resistance genes in resident benign bacteria of healthy animals. To assess how rapidly drug resistance develops and how long it persists in animals colonized with *Salmonella*, we will examine the distribution of drug resistance genes among the microbial fauna present on poultry farms.

We will identify by PCR and DNA-DNA hybridization, drug resistance genes common to both *Salmonella* and resident bacteria in chickens. Specific emphasis will be placed on tracking integrons, mobile genetic elements responsible for the diversity and rapid evolution of multiple drug resistant bacteria. A novel "integron capture" plasmid will be introduced to the chicken's microflora and followed during the normal rearing period for broiler chickens. The plasmid will be periodically isolated to examine the drug resistance genes acquired by the plasmid's resident integron during the vector's passage among the microbial community of the chicken's gastrointestinal tract. Experiments will be performed in a high-security level, biocontainment facility. Clear definition of the genetic elements within the chicken normal flora that facilitate genetic exchange and persistence of multiple antibiotic resistance will permit realistic risk assessment of the role of poultry *Salmonella* as reservoirs of antibiotic resistance and agents in its transmission to other animals and humans.

Molecular Epidemiology of *Salmonella* Transmission in Swine Production Systems

Weigel, R.M.; White, B.A.; University of Illinois, Urbana-Champaign; Department of Veterinary Pathobiology; Urbana, IL 61801; \$885,294; 3 Years

The goal of the proposed research is to identify critical control points for interventions to reduce *Salmonella* infection in modern swine production facilities. Intensive ecological sampling will be conducted in long term studies of 8 large, multi-site, modern swine production facilities. Each farm will be visited monthly for 14 months. At each visit, samples will be obtained from various suspected biological and environmental reservoirs of *Salmonella*. This includes fecal samples from a cross-section of age classes of swine and from rodents, birds, and medium-sized mammals, as well as samples of insects, feed, water, and pen floor contents. These samples will be cultured for *Salmonella*. The *Salmonella* isolates will be characterized genetically, using modern molecular biological techniques. Using information on genetic similarity and proximity in location and time of sampling, inferences will be made regarding probable modes of transmission of *Salmonella* to swine. It will be possible to identify likely targets for intervention if (a) particular biological and environmental sources of *Salmonella* are consistently linked (genetically, temporally, and spatially) to swine, (b) during times of low prevalence, *Salmonella* is maintained primarily in one reservoir, or (c) new genotypes entering the farm are consistently identified first in a specific reservoir.

Risk Factors for *Salmonella* and *Campylobacter* Infections and Drug Resistance in Dairy Cattle

Kaneene, J.B.; Michigan State University; Population Medicine Center; East Lansing, MI 48824-1314; Ruegg, P.; University of Wisconsin; Warnick, L.; Cornell University; Wells, S.; University of Minnesota; Saeed, M.; Purdue University. \$765,447; 3 Years

A three year longitudinal study of 130 dairy herds is proposed to: 1) identify the patterns of occurrence and shedding of *Salmonella spp.* and *Campylobacter jejuni*, and associated risk factors, on dairy farms in the Midwest and Northeast; 2) evaluate the relative sensitivity and specificity of different sampling frequencies for detection of *Salmonella* and *C. jejuni* in fecal and environmental samples; 3) determine susceptibility profiles of *Salmonella* and *C. jejuni* isolates to a number of antimicrobial agents; 4) compare conventional and organic dairy farms in the Midwest and Northeast with respect

to: a) shedding, and b) susceptibility and resistance profiles of the two organisms to specific antimicrobial agents; and 5) determine molecular mechanisms involved in reduced susceptibility and/or development of resistance of *Salmonella* and *C. jejuni* isolates to antimicrobial agents. Dairy farms will be selected from Michigan, Minnesota, New York, and Wisconsin. Fecal, water, milk, feed, and environmental samples will be collected and tested for the presence of specific strains of *Salmonella* and *Campylobacter* using standard molecular techniques. Further typing will be done by a modified Taqman procedure for *Campylobacter*, and phage molecular typing of *Salmonella*. In vitro susceptibility testing of the strains to a number of antimicrobial agents will be conducted. Risk factors associated with bacterial shedding and the development of resistance to selected antimicrobial agents will be evaluated using multi-variable statistical methods. Results from this study will provide ways to improve pre-harvest pathogen reduction by identifying factors related to shedding and antimicrobial resistance that can be changed by dairy producers.

Microbial Contamination of Produce: A Field Study in the Lower Rio Grande Valley, TX

Moe, C.L.; Jaykus, L.; Moll, D.; Sobsey, M.D.; Brandenberger, L.; Hart, R.; Kieszak, S.; Backer, L.; Moore, D.A."; University of North Carolina, Chapel Hill; Department of Epidemiology; Chapel Hill, NC 27599-7400. \$416,572; 3 Years

In recent years, new and emerging foodborne pathogens have been described and changes in food production have led to new food safety concerns. Foodborne diseases have been associated with many different foods, including recent outbreaks linked to contaminated fresh fruits and vegetables. This study will examine how specific environmental conditions and farming and produce processing practices are associated with fecal contamination of selected produce groups. Some growing, handling and processing methods used in the produce industry may increase the risk that these foods will become contaminated. The study will describe farming and shipping practices on approximately 12 farms in the lower Rio Grande Valley of Texas with three vulnerable produce groups that are minimally processed and eaten raw (leaf lettuce/spinach, parsley/cilantro/basil, green onions). Key agricultural practices where contamination may occur will be identified by measuring the microbial quality of produce at each step from planting to harvesting, processing and shipping. Sources of fecal contamination will be determined by periodically measuring microbial quality of irrigation and process water, measuring fecal indicator organisms on hand rinses from farm laborers and handlers, and conducting sanitary surveys of sources of human and animal feces in and around the farms and processing areas. Information gained through this study will be used to formulate recommendations for the produce industry to focus resources on the most critical risk factors for microbial contamination of produce and design effective intervention measures to prevent contamination and subsequent foodborne disease.

Ecology of Antimicrobial Resistance of Enteric *Salmonella* and *E. coli* in Cattle Operations; Wittum, T.E; Morley, P.S.; Love, B.C.; Saville, W.J.

The Ohio State University; Department of Veterinary Preventive Medicine; Columbus, OH 43210; \$771,868; 3 Years

Antimicrobial drugs are commonly used in food animal production for the treatment of clinical disease and for the enhancement of animal performance. Recently, the use of antimicrobial drugs in food animals has been implicated as an important cause for the emergence of antimicrobial-resistant bacteria of public health concern. However, there is little more than circumstantial evidence that this use of antimicrobial drugs poses a serious public health risk. In particular, there is little evidence upon which to base changes in food animal production practices which might reduce any potential public health risk. Our goal is to describe the on-farm ecology of *Salmonella spp.* and generic *E. coli* antimicrobial resistance patterns in intensively managed cattle operations. We have chosen this goal because beef products are an important vehicle for the food-borne transmission of bacteria. *Salmonella* and generic *E. coli* represent an important food-borne pathogen and a normal inhabitant of the bovine intestinal tract, respectively. Our objectives are to describe the antimicrobial resistance patterns of *Salmonella spp.* and generic *E. coli* recovered from intensively managed cattle operations, and to identify patterns of antimicrobial use and other factors which promote the development of antimicrobial resistance of bacteria in these operations. We will accomplish these objectives using intensive monitoring of dairy and feedlot cattle populations. By accomplishing these objectives, we expect to obtain detailed information which will help to fill the void of information regarding the development of antimicrobial-resistant bacteria in cattle operations, and lead to the development of practical intervention programs.

Dynamics of *Campylobacter* Transmission on Poultry Farms

Zhang, Q.; Morishita, T.Y., The Ohio State University; Department of Veterinary Preventive Medicine; Wooster, OH 44691; \$384,284; 3 Years

Campylobacter jejuni has emerged as the leading bacterial cause of food-borne human enteritis. Many studies have revealed that poultry products are the major source of human exposure to the pathogen. The high prevalence of this pathogen on poultry farms, especially in broiler chickens, provides a strong rationale for reducing the pathogen level at the preharvest stage. Although many farm-based epidemiological studies have been conducted, the manner in which *C. jejuni* enters a flock and how it is maintained within a flock are poorly understood. Lack of this information has hampered the design of effective intervention strategies on farms. The major goals of this multidisciplinary project are to elucidate the sources of *C. jejuni* infection in broiler chickens and to examine the host and environmental factors that affect the transmission of the organism on broiler farms. Our hypothesis is that transmission of the organism is likely a complex and dynamic process, which is mediated by both vertical and horizontal transmission and affected by the host's immune status and the environmental conditions in the production system. The specific aims of this project are to conduct both laboratory and field studies: i) to assess the effect of host immune status on horizontal spread of the organism in young chicks; ii) to determine if vertical transmission of *C. jejuni* occurs from breeders to progenies; and iii) to identify the potential sources and routes for horizontal spread of *C. jejuni* on poultry farms. These studies address key issues in transmission of *C. jejuni* and should yield information useful for controlling this important foodborne pathogen.

Effect of Water Chlorination on Prevalence of *Escherichia coli* O157:H7 and *Campylobacter* in Feedlot Cattle; Besser, T.E.; Hancock, D.D.; Bohach, C.H.; Washington State University; Department of Veterinary Microbiology and Pathology; Pullman, WA 99164-7040; \$325,528; 2 Years

Cattle slaughtered while infected with bacteria such as *E. coli* O157:H7 or *Campylobacter spp.* are a probable source of these bacteria in contaminated beef products, and eventually in humans consuming these food products. Our preliminary data leads us to believe that infection of cattle with these agents, like human infections, most often follows their eating contaminated feeds or drinking contaminated water. *E. coli* O157:H7 can be frequently isolated from cattle water troughs, and has been shown to persist in troughs for months, so water is a logical starting point for control of these infections. Our research question is: "Does providing cattle with a cleaner water supply reduce the number of cattle infected with *E. coli* O157:H7 and *Campylobacter*?" We will chlorinate the water supplies of 32 pens of feedlot cattle in two feedlots through the feeding period and compare the infection rate in these cattle to that of 32 pens of cattle consuming non-chlorinated water. At the same time, we will evaluate the effect of chlorination on water consumption and weight gains. If chlorination of cattle water supplies reduces cattle infections with bacteria that cause human disease, but is neutral or beneficial on cattle welfare and weight gain, this will be the first proven management practice enabling beef producers to reduce the risk of human disease due to these food-borne enteropathogenic bacteria.

Awards in 2000

Epidemiology and Ecology of Antibiotic Resistance Determinants on Dairy Farms. Singer, R., University of Illinois, Department of Veterinary Pathobiology; Urbana, IL 61801 \$1,391,326; 4 years

The increasing rate of development of bacterial resistance to antibiotics has been well-documented, and this has major consequences for human and animal health. There is a considerable lack of data regarding the ecologic and epidemiologic forces that drive the spread and persistence of antibiotic resistance genes in agricultural settings. Therefore, through a 3-year prospective study in 6 Illinois dairies, we have the following specific objectives: 1) To evaluate antibiotic resistance profiles of commensal and pathogenic bacteria over time in order to assess factors that influence changes in antimicrobial susceptibility; 2) To study the bacterial genes that confer antimicrobial resistance in order to elucidate the ecology of these genes in the natural environment; and 3) To develop and validate quantitative sampling methods for monitoring antimicrobial resistance and to build mathematical models that predict the spread and persistence of resistance genes on the dairy. Feces from all cows in the study will be cultured for bacterial organisms in the genera *E. coli*, *Salmonella*, *Enterococcus*, and *Prevotella*. Milk samples will also be cultured. The results of this study, descriptive and analytical, will greatly increase our understanding of the epidemiology and ecology of antibiotic resistance. On the dairy farm, critical points for controlling resistance will be identified. Efficient monitoring and sampling strategies will be identified. The risk of gene transfer between commensal and pathogenic bacteria will be estimated.

A Novel Strategy to Test and Monitor Beef Feedlot Food-Safety Control Points

Smith, D.R.; Hungerford, L.L.; Gray, J.T.; Moxley, R.A.; Klopfenstein, T.; Milton, C.T., University of Nebraska, Lincoln; Institute of Agriculture and Natural Resources; Department of Veterinary and Biomedical Sciences; Lincoln, NE 68583 \$953,735; 3 years.

The goal of this project is to improve food safety by developing efficient, effective methods to determine the *Escherichia coli* O157:H7 and *Salmonella* status of pens of feedlot cattle and to reduce the potential that these foodborne pathogens leave the feedlot. Pen testing will reduce time, cost, and labor and avoid detrimental animal welfare effects of handling finished cattle prior to shipping (shrink, dark cutters, and bruising). Additionally, most conceivable interventions to reduce human foodborne pathogens in feedlot cattle are directed towards pens. Based on preliminary data, a pen-testing protocol has been identified that shows promise as a monitoring tool for feedlot production HACCP programs and as a research tool to identify and test potential HACCP control points. Objectives of this project are to complete validation of this pen-test for *E. coli* O157:H7 and *Salmonella* shedding; determine what a positive (or negative) pen test means in terms of length of time cattle may be shedding *E. coli* O157:H7 or *Salmonella* in commercial feedlots; identify potential control points using risk factor analyses; and disseminate results to researchers, industry partners and consumers. This research will provide the beef production industry with effective monitoring tools to be used in HACCP programs to reduce the risk of cattle leaving the feedlot carrying foodborne pathogens.

National Pediatric Diarrhea Surveillance Study

Acheson David WK, New England Medical Center, Division of Infectious Diseases, 750 Washington, Street, Boston, MA 02111 (now at University of Maryland, Baltimore) \$937,473; 2 years.

The major goals of this project are to gain a better understanding of the causes, risk factors and outcomes of foodborne disease related diarrhea in children under the age of 3 in the U.S. The first goal of this proposal is to determine a) the prevalence of specific bacterial, viral and protozoal pathogens in the stools of 1000 healthy children between 6 months and 3 years of age who are representative of U.S. children with respect to geography, family income, and ethnicity, b) the incidence of acute diarrheal illness occurring among these children and c) the incidence of infection with specific bacterial, viral and protozoal foodborne pathogens associated with clinical illness. The second goal is to characterize the type of gastrointestinal illnesses that occur in these children, the duration, treatment, and sequelae occurring within two months of acute illness. The third goal is to identify risk factors for diarrheal illness and infection with specific foodborne pathogens in these children. Risk factors to be studied include consumer practices related to food (and water) purchasing, handling, storage, and cooking in the home. This study we will obtain unique data on the causes and outcomes of diarrheal disease in young children in relation to foodborne pathogens. Consumer data will define potential sites and routes of contamination within the home environment. These data will be of great value in understanding foodborne disease in relation to agricultural products in a vulnerable section of the population that is thoroughly under investigated.

Epidemiological aspects of combining *E. coli* O157:H7 control programs and feedlot performance.

Sargeant, J.M.; Sanderson, M.W.; Zhang, P., Kansas State University; Food Animal Health and Management Center (Sargeant); Department of Clinical Sciences (Sanderson); Department of Agricultural Economics (Zhang); Manhattan, KS 66506 \$231,483; 1 year

Escherichia coli O157:H7 has emerged as a significant public health concern over the past decade. The source of this pathogen in cases of human illness is usually related to contaminated feeds, including beef products. Control of *E. coli* O157:H7 in the beef industry will require a concerted effort from all segments of the industry, including the farm level. The goal of the current project is to determine the extent to which specific management practices concomitantly affect cattle performance and fecal shedding of *E. coli* O157:H7 in feedlot cattle. Our hypothesis is that inappropriate management practices, such as poor pen hygiene and feed and water management, not only predispose cattle to shed *E. coli* O157:H7, but that these same management practices reduce performance in feedlot cattle, thereby affecting feedlot revenues. We will use an observational study design to identify specific management factors which are associated with *E. coli* O157:H7, providing the factual information required to design on-farm control programs. We will also investigate the relationship between these management factors and average daily gain and feed efficiency, thereby providing the motivation for feedlot producers to implement control schemes. Our specific research objectives are as follows: 1) To determine which management practices are related to fecal shedding of *E. coli* O157:H7 in feedlot cattle; 2) To quantify the relations between management practices that foster the shedding of *E. coli* O157:H7 and feedlot cattle performance, as defined by average daily gain and feed efficiency.

Food-borne Antibiotic-Resistant and Extraintestinal Pathogenic *E. coli*.

Johnson, J.R.; Tatini, S.R., University of Minnesota, Minneapolis, MN 55455-2070 \$542,357; 3 years

Antibiotic-resistant *Escherichia coli* (ABREC) and extraintestinal pathogenic *E. coli* (ExPEC) are significant health threats and may be transmitted through the food supply. However, little is known regarding the prevalence or distribution of ABREC or ExPEC in retail foods. We will conduct a 2-year prospective microbiological survey of diverse food items from diverse retail markets to determine (1) whether the commercial food supply constitutes a significant reservoir of ABREC and ExPEC for potential acquisition by humans and their household pets; (2) which types of foods and stores are associated with the highest prevalence and concentration of such organisms; (3) whether the prevalence of ABREC or ExPEC in retail foods varies according to season or increases over time; and (4) what proportion of food-borne ABREC strains exhibit multiple or transferable antimicrobial resistance. Retail foods will be selectively cultured to isolate and enumerate total *E. coli* and ABREC. ABREC and ExPEC will be detected and extensively characterized using state-of-the-art methods. Statistical analyses will be performed by an experienced statistician with input from a public health epidemiologist. The project will (a) identify sources and reservoirs of pathogenic organisms in food, and (b) determine the levels of microbial contamination in finished food products. This will allow consumers to modify their shopping or food preparation practices, and will identify for producers and the food safety

community specific foods or types of markets deserving of further investigation, regulation, and/or modifications in food production and handling practices.

Awards in 2001

Enteric Pathogens in Oysters

Joens, L.; Levine, J. University of Arizona; Department of Veterinary Science; Tucson, AZ 85722-3308, \$1,100,000; 3 Years

Although *Campylobacter spp.*, *Salmonella spp.*, and Norwalk-like viruses have been detected in shellfish and shellfish beds and have been associated with foodborne outbreaks, there is limited information on the prevalence of these pathogens in U.S. market oysters. These research studies will attempt to assess the relationship between fecal coliforms present in the water column and the prevalence of pathogens in oysters sold in retail markets. This will include determining the primary sources (human versus agricultural) of fecal contamination. In addition, this project will evaluate current potential health hazard identification techniques used by State Shellfish Sanitation agencies and assess the validity of using automated conductivity-based microbial growth monitoring for the classification of shellfish growing areas.

Poultry: A Food Animal Model for Following Antimicrobial Resistant *Enterococci*

Hofacre, C.; Maurer, J.; White, D.; Hudson, C.; Angulo, F.; Headrick, M.
University of Georgia; Department of Avian Medicine; College of Veterinary Medicine; Athens, GA 30602-4875; \$786,350; 3 Years

There is continued concern about the use of antibiotics as growth promoting agents in food animals and the potential for development of antibiotic resistance in human pathogens. The long term goal of this study is to understand the processes involved in the development and spread of resistance in gram positive bacterial flora of poultry. This study will collect microflora samples from commercial poultry farms and processing/slaughter plants for one year. The farms will have one house using growth promoting antibiotics throughout the flocks' life and one house with no antibiotics used. Comparisons of drug resistance genes and plasmids will be made between poultry gram-positive commensals and human enterococci. The human samples will be obtained from the National Antimicrobial Resistance Monitoring System.

Perimarketing Control Strategies to Reduce *Salmonella* Contamination of Pork Carcasses

Hurd, H.S.; McKean, J.D.; Griffith, R.W.; O'Connor, A.M.; Wesley, I.V.; Prusa, K.J.
USDA Agricultural Research Service; National Animal Disease Center; Ames, IA 50010; \$783,400; 4 Years

Recent research has shown substantive evidence that abattoir holding pens are a significant source of *Salmonella* infection in swine. In commercial abattoirs, *Salmonella* prevalence can increase five to sevenfold during the routine pre-slaughter rest period. On average, 50% of pigs entering the slaughter process may be carrying *Salmonella* in their internal organs and gastrointestinal tract. The objectives of this study are to better understand the *Salmonella* infection in the holding of market swine and to evaluate cost-effective strategies to reduce the number of *Salmonella*-infected pigs entering the

slaughter process. Studies will be conducted in three commercial abattoirs. Pen floor samples will be collected over time to estimate seasonal changes in *Salmonella*. Various treatment strategies will be evaluated. The *Salmonella* prevalence in various tissues and meat quality will be compared between treatment and control pigs. This project will provide some information about pen ecology as influenced by environmental factors.

Retail Meat Survey for *Toxoplasma gondii*

Dubey, J.P.; Gamble, H.R.; Jones, J.L.; Hightower, A.W. USDA Agricultural Research Service; Beltsville, MD 20705-2350

\$853,106; 3 Years

Toxoplasma gondii is an obligate intracellular protozoan parasite which causes mental retardation, loss of vision, and other congenital health problems in humans and is an increasingly important cause of mortality and morbidity in the immuno-suppressed. The Centers for Disease Control and Prevention list *T. gondii* as one of three pathogens which account for over 75% of all deaths due to foodborne disease in the U.S.. Although cats have long been thought to be the main source of human exposure, the CDC now estimates that up to 50% of all human infections result from the ingestion of infected meat. However, little is known about the prevalence of this parasite in retail meats. The goal of this proposed research is to determine the prevalence of *T. gondii* in three major commodity meats (beef, chicken, and pork). Samples will be collected over an 18 month period from a sampling method representing 80% of the U.S. population. Strains of *T. gondii* will be isolated from meat and be genetically typed and compared with isolates from humans.

Prevalence, Strain Types and Antibiotic Resistance of *Campylobacter* in Turkey

Grow-out Farms; Kathariou, S.; Carver, D.; North Carolina State University; Department of Food Science; Raleigh, NC 27695; \$668,263; 3 Years

Campylobacter is a leading cause of human food-borne illness in the U.S. Transmission involved primarily poultry, although pork, beef, raw milk, and other sources have also been identified. Resistance to several antibiotics, including fluoroquinolones, commonly used for treatment of human infections, is increasing in *Campylobacter*. Extensive studies with broilers suggest that birds become colonized in the farm, usually without symptoms, and that meat becomes contaminated during slaughter and processing. This study will investigate the prevalence of *Campylobacter* in 60 turkey grow-out farms in Eastern North Carolina. It will evaluate the impact of distinct turkey husbandry practices in the grow-out turkey farms, and of antibiotic use for veterinary purposes, on *Campylobacter* prevalence, strain types, and antibiotic resistance profiles. The results from this study will provide a currently unavailable database on *Campylobacter* colonization, subtypes and antibiotic resistance in turkeys. This data will be useful for designing possible intervention strategies for reducing the prevalence of *Campylobacter* in poultry and the reduced incidence of resistance to antimicrobials.

Clonal Dissemination of Antimicrobial Resistant *Campylobacter jejuni* and *Escherichia coli*

Besser, T.E.; Sischo, W.M.; Hancock, D.D.; Washington State University, Department of Veterinary Microbiology and Pathology, Pullman WA 99164-7040; \$1,295,036; 3 Years

There is an increasing concern that antibiotic resistance in both pathogenic bacteria and in the normal flora present a risk to public health, and reduction in the degree of antibiotic resistance is an important public health goal. The antibiotic resistant flora that appear after antibiotic exposure of cattle and other food animals may be ‘new’ antibiotic resistant strains originating on the farm, or may be pre-adapted strains that originated elsewhere and were transferred to the farm by animals, feed, water, wildlife, humans, or other mechanisms. The origin is important, since different origins require different control measures. For *Salmonella typhimurium*, wide dissemination of antibiotic resistant strains is the predominant process. This study will look at whether wide dissemination of antibiotic resistant strains is also important in *Campylobacter jejuni* and *E.coli* in the bovine intestine. In addition, this study will determine whether antibiotic resistant *E.coli* can be competitively displaced by non- antibiotic resistant strains. These studies will provide basic information about the role of clonal dissemination in the epidemiology of antibiotic resistance on cattle farms, and so aid risk assessment of commensal flora with antibiotic resistance and prediction of effective interventions to reduce antibiotic resistance frequency.

Awards in 2002

Risk Factor Analysis of *Salmonella* and *Campylobacter* Flock Status in Broiler Production and Processing.

Wills, R.W., Bailey, R.H.; Mississippi State University; Department of Pathobiology and Population Medicine; College of Veterinary Medicine; Mississippi State, MS 39762-6100; \$1,018,600; 3 years.

The goal of this study is to identify measurable risk factors in the poultry production process and determine the impact they have on the occurrence of *Salmonella* and *Campylobacter* on broiler carcasses at the end of processing. This research will include 4 segments: breeder/hatchery, grow-out, transportation from farm to plant, and processing. This study will look at the risk factors associated with each segment as well as risk factors that will affect the final outcome (end of processing). Sampling strategies will be evaluated that optimize the classification of *Salmonella* and *Campylobacter* status of flocks as they move through production and processing. Finally, this research will help identify the production segments, and management and intervention strategies most likely to impact *Salmonella* and *Campylobacter* status.

Sources and Risk Factors for *Campylobacter* in Poultry and Impact of Human Disease in a Closed System. Stern, N.J., Lowman, R., Hiatt, K.L.

USDA, Agricultural Research Service; Poultry Microbial Safety Research Unit; Russell Research Center; Athens, GA 30604-5677; \$900,000; 3 years.

This research project provides the unique opportunity to use a closed production system to study risk factors for *Campylobacter* in poultry and its impact on human health. This study will look at 3 potential sources of *Campylobacter* and measure the risk factors for contamination of broiler flocks. The sources include the external environment of the broiler house, egg-borne, and water-borne transmission. Iceland provides the opportunity for longitudinal research along multiple stages of broiler production. This specific broiler

system provides the sole source of poultry meat for the isolated human population. This study will follow the poultry from hatcheries, breeder production, through broiler hatcheries, production, processing, and retail. Isolates from human campylobacteriosis will also be analyzed for similarity to poultry.

Diarrhea Etiology and Risk Factor Study

Tarr, P.I.; Koehler, J. Children's Hospital and Regional Medical Center, University of Washington, Department of Gastroenterology, Seattle, WA 98105; \$1,299,314; 42 months.

Knowledge is lacking about specific causes for diarrheal episodes in children and whether they are related to specific foods and whether they are bacterial, viral, protozoal, or from other causes. This study will investigate childhood diarrheal episodes that remain unexplained after medical evaluation. Diarrheal episodes will be analyzed for bacterial, viral, and protozoal pathogens and matched with age, season, and geographic controls. These data will be used to confirm or refute the presence of *E. coli* and *Listeria*. This study will evaluate risk factors including foodborne, environmental, and personal contact, in a prospective controlled study.

Risk Factors for Microbiological Contamination of Produce: A Field Study of Domestic and Imported Produce in Packing Sheds. Moe, C.L.; Jaykus, L; Moll, D; Kieszak-Holloway, S; Anciso, J; Backer, L; Hart McElroy, R.; Emory University, Department of International Health, Atlanta, GA 30322; \$274,816; 18 months.

This study builds on a previously funded study involving microbial contamination of produce and the identification of specific farming and processing (packing shed) practices that are associated with fecal contamination of domestically grown produce. Preliminary data showed that contamination often occurs during processing in packing sheds and that packing sheds handle both domestic and imported produce. This newly funded study will compare the prevalence of microbial contamination (fecal indicators and pathogens) between domestically grown produce and Mexican produce and will identify specific processing practices that contribute to increased contamination in such products. This research will help design effective intervention measures to reduce produce contamination during processing and packing and to design exposure assessment models to compare foodborne disease risks associated with domestic versus imported produce.

Ecology of Intermittent *Salmonella* Infections in a Dairy Milk Shed

Sischo, W.M.; Atwill, E.R.; Moore, D.A; University of California VMTRC, Dept. of Population Health and Reproduction, Veterinary Medicine Extension, California Animal Health and Food Safety Laboratory; Tulare, CA 93274; \$602, 628; 24 months.

This research specifically identifies intermittent *Salmonella* infections in dairy cattle. The goals are to determine the spatial and temporal attributes of the *Salmonella* by serotype and molecular types in a dairy milk shed; compare on-farm attributes of herds with and without intermittent *Salmonella* infections; and determine intervention strategies to reduce the spread of *Salmonella* within a dairy milk shed. This project concentrates on the medical ecology of intermittent *Salmonella* infections on dairies to help determine the risk factors for circulation of organisms between dairies. This study

builds on a previously funded study involving persistent *Salmonella* infections in California dairy milk sheds.

Role of Pest Filth Flies in the Agro-Ecology and Epidemiology of Shiga-toxicogenic *E. coli* 0157,0111, and 026 in Cattle. Keen, J. E.; Gerhardt, R. R.; Jones, C. J.

USDA Agricultural Research Service, US Meat Animal Research Center, Clay Center, NE 68933 and University of Tennessee-Knoxville, Department of Entomology and Plant Pathology, Knoxville, TN 37996-4508;\$1,100,000; 4 years.

This research looks at the role of pest filth flies as reservoirs and vectors for shiga-toxicogenic *E. coli* bovine infections on dairy and beef cattle operations. This research will measure the prevalence of STEC 0157, 0111, and 026 in the feces and on hides of cattle and in fly populations. Through a cohort study, fly densities and fly and cattle STEC status over 2 years in 8 cattle operations will be monitored. An intervention study will evaluate the effects of total pest fly exclusion on natural STEC 0157 intestinal infection and hide contamination rates in small groups of confined beef cattle through finishing will be quantified.

Epidemiologic Risk Assessment of *Cryptosporidium* spp. in Watersheds. Mohammed, H.; Wade, S; Chang, Y; Nydam, D.; Cornell University; College of Veterinary Medicine; Population Medicine and Diagnostic Sciences; Ithaca, NY 14853; \$349,670; 26 months.

The long term goal is to understand the epidemiology of *Cryptosporidium parvum* in animals and its impact on the environment. The prevalence of 5 animal and human species and genotypes of *Cryptosporidium* on dairy farms in a New York City Watershed will be measured. Researchers will evaluate risk factors that lead to the predominance of certain genotypes and describe the mechanism by which each of these genotypes is transmitted from source to waterways. Data from this study will be put into a risk model to design intervention strategies that will help reduce occurrence in animals and consequent transmission to the environment.

Awards in 2003

Transmission Dynamics of Antimicrobial Resistance in Integrated Animal and Human Populations. Scott, H.M.; Anderson, R; Barling, K; Bischoff, K; Harvey, R; Sheffield, C.; Texas A&M University, Department of Veterinary Anatomy and Public Health; College Station, TX 77843-4458; \$1,500,000; 4 years

Scientists continue to argue about the role of the use of antimicrobials in food animals and the potential for increased infectious bacterial infections in humans that are difficult to treat because of antibiotic resistance. This study has identified a unique, vertically integrated and semi-closed population of swine and humans to test hypotheses concerning the transmission of antimicrobial resistance among animals and humans. The primary hypothesis is that the strains of antimicrobial resistant enteric bacteria are transmitted in both directions between host populations. This study will address this hypothesis through two objectives: 1) Establish the baseline prevalence of host-specific and host-common antimicrobial –resistant enteric bacteria (*E.coli* and *Enterococcus* spp.) and then conduct a 3 year longitudinal study tracking group-level, housing-defined

cohorts of swine, swine barn workers, slaughter plant workers, and pork-product consumers in order to quantify the transmission of resistant strains; 2) Develop and empirically assess mathematical models of the transmission dynamics of antimicrobial-resistant commensal bacteria within the multi-site, multi-stage swine production system, and among the groups of swine, swine workers, slaughter plant workers, and consumers.

Ecological Fitness of Antibiotic Resistant *Campylobacter* in Poultry. Zhang, Q.; Morishita, T.; Iowa State University, Department of Veterinary Microbiology; Ames, IA 50011; \$768,992; 3 years.

Campylobacter jejuni is the most commonly recognized bacterial cause of foodborne illnesses in the US, and poultry are considered the major reservoir for this organism. The emergence of antibiotic resistant *Campylobacter* in poultry is a potential threat to public health. This study addresses key issues concerning the spread and persistence of FQ-resistant *Campylobacter* in poultry. The objectives of this research are to: 1) examine the ecological fitness of FQ-resistant *Campylobacter* in broiler chickens using laboratory model systems; 2) conduct an epidemiological survey of broiler farms to monitor the dissemination and fitness of FQ-resistant *Campylobacter* in the absence of antibiotic selection pressure; and 3) determine the role of natural transformation in mediating the dissemination of antibiotic resistance determinants in *Campylobacter*.

Impact of Intertwined Turkey –Hog Production on the Species, Strains, and Antibiotic Resistance of *Campylobacter* in Turkeys. Kathariou, S.; Carver, D; Morrow, M; North Carolina State University; Department of Food Science; Raleigh, NC 27695-7624; \$570,963; 3 years.

Campylobacter is a leading agent of human foodborne illness in the US, and poultry are considered a major reservoir. The ability of the organism to colonize in multiple animal species suggest the potential for transmission from one type of meat animal to another pre-harvest. *C.coli* appears to be more readily acquire antibiotic resistance than *C.jejuni*. In eastern North Carolina, a unique situation exists in the turkey production is located in a hog-dense region, and the turkey integrated industry is tightly intertwined with hog production. The hypothesis of this research is that the intertwined turkey/hog husbandry systems promote transmission of *C.coli* from hogs to turkeys, where the bacteria acquire resistance to ciprofloxacin that can be transferred to other *Campylobacter*s in the turkey flock, including *C.jejuni*. This research will help better understand the epidemiology of *Campylobacter* species and strains colonizing turkeys, especially in terms of the transmission and establishment of antibiotic resistant strains.

Publications

Goldstein, C., M. D. Lee, S. Sanchez, C. R. Hudson, B. Phillips, B. Register, M. Grady, C. Liebert, A. O. Summers, D. G. White, and J. J. Maurer. 2001. Incidence of class 1 and 2 integrases in clinical and normal flora bacteria from livestock, companion animals, and exotics. *Antimicrob. Agents Chemother.* 45:723-726.

Hofacre, C. L., D. G. White, J. J. Maurer, C. Morales, C. Lobsinger, C. R. Hudson and S. G. Thayer. 2001. Antibiotic resistant bacteria in rendered animal products. *Avian Dis.* 45:953-961.

Hong, Y., T. Liu, C. Hofacre, M. Maier, S. Ayers, D. G. White, L. Wang, and J. J. Maurer. 2003. A restriction fragment length polymorphism based polymerase chain reaction as an alternative to serotyping for identifying *Salmonella* serotypes. *Avian Dis.* In Press.

Lu, J., S. Sanchez, M. D. Lee, C. Hofacre, B. G. Harmon, and J. J. Maurer. 2003. Evaluation of broiler litter with reference to the microbial composition as assessed using 16S rDNA and functional gene markers. *Appl. Environ. Microbiol.* 69:901-908.

Liu, T., K. Liljebjelke, E. Bartlett, C. L. Hofacre, S. Sanchez, and J. J. Maurer. 2002. Application of nested PCR to detection of *Salmonella* in poultry environments. *J. Food Prot.* 65:1227-1232.

Keyes, K., M. D. Lee, and J. J. Maurer. 2003. Antibiotics: mode of action, mechanism of resistance and transfer. In M. Torrence and R. Isaacson Eds., *Current Topics in Food Safety in Animal Agriculture*, Iowa State University Press, Ames, IA. (In Press).

Sanchez, S., C. L. Hofacre, M. D. Lee, J. J. Maurer, and M. P. Doyle. 2002. Animals sources of human salmonellosis. *J. Am. Vet. Assoc.* 221:492-497.

Sanchez, S., M. A. McCrackin Stevenson, C. R. Hudson, M. Maier, T. Buffington, Q. Dam, and J. J. Maurer. 2002. Characterization of multi-drug resistant *Escherichia coli* associated with nosocomial infections in dogs. *J. Clin. Microbiol.* 40:3586-3595.

White, D. G., S. Ayers, J. J. Maurer, S. G. Thayer and C. Hofacre. 2003. Antimicrobial susceptibilities of *Staphylococcus aureus* isolated from commercial broilers in northeast Georgia. *Avian Dis.* 47:203-210.

Zhang, Q., Jerrel C. Meitzler, Shouxiong Huang, and Teresa Morishita. 2000. Sequence polymorphism, predicted secondary structures, and surface-exposed conformational epitopes of *Campylobacter* major outer membrane protein. *Infect. Immun.* 68:5679-5689.

Sahin, O., Q. Zhang, J. C. Meitzler, R. Mohan, B. Harr, and T.Y. Morishita. 2001. Prevalence, antigenic specificity, and bactericidal activity of poultry anti-*Campylobacter* maternal antibodies. *Applied and Environmental Microbiology.* 67:3951-3957.

Sahin, O., T. Y. Morishita, and Q. Zhang. 2002. Campylobacter colonization in poultry: sources of infection and modes of transmission. *Animal Health Research Reviews* 3:95-105.

Sahin, O., Q. Zhang, and T. Y. Morishita. 2003. Detection of Campylobacter . In *Microbial Food Safety in Animal Agriculture*. Ed. R. E. Isaacson and M. E. Torrence. In press.

Sahin, O., N. Luo, S. Huang, J. Meitzler, and Q. Zhang. 2003. Effects of anti-Campylobacter Maternal Antibody on the Colonization of Campylobacter jejuni in Poultry. Submitted to *Applied and Environmental Microbiology*

Sahin, O., P. Kobalka, and Q. Zhang. 2003. Transmission and Survivability of Campylobacter jejuni in chicken eggs. Submitted to *Journal of Applied Microbiology*.

Ebako, G.M., T. Y. Morishita, and Q. Zhang. Potential sources and routes for horizontal spread of Campylobacter jejuni on poultry farms. Manuscript in preparation.

Lipsitch, M., R.S. Singer, and B.R. Levin. 2002. Antibiotics in agriculture: When is it time to close the barn door? *Proceedings of the National Academy of Sciences*. 99:5752-5754.

Singer, R.S., R. Finch, H.C. Wegener, R. Bywater, J. Walters, and M. Lipsitch. 2003. Antibiotic resistance--the interplay between antibiotic use in animals and human beings. *The Lancet: Infectious Diseases*. 3:47-51 NOTE: Order of authors in this Forum article was based on logical progression of ideas, not on amount of contribution.

Singer, R.S., C.L. Cooke, C.W. Maddox, R.I. Isaacson, and R.L. Wallace. 2003. A PCR screen using pooled samples for the detection of Salmonella in feces. *Journal of Veterinary Diagnostic Investigation* (Accepted).

Singer, R.S. 2002. Epidemiology and Ecology of Antibiotic Resistance in Agriculture. In: M. Torrence and R. Isaacson (eds.), *Current Topics in Microbial Food Safety in Animal Agriculture*. Iowa State Press (In Press).

Sargeant, J.M., Sanderson, M.W., Smith, R.A., Griffin, D.D. *Escherichia coli* O157 in feedlot cattle feces and water in four major feeder cattle states in the USA. Submitted to *Prev. Vet. Med*.

Johnson JR, Murray AC, Sullivan M, Snippes P, Kuskowski MA, Smith KE. Isolation and molecular characterization of nalidixic acid-resistant extraintestinal pathogenic *Escherichia coli* from retail chicken products.(in press)

Johnson JR, Van der Schee C, Kuskowski MA, Goessens W, Van Belkum A. Phylogenetic background and virulence profiles of fluoroquinolone-resistant clinical *Escherichia coli* isolates from the Netherlands. *J Infect Dis* 2002; 186:1852-6.