

## **Topical Session C: Pathogens**

# *Salmonella and Other Enterobacteriaceae in Dairy Cow Feed Ingredients and Their Antimicrobial Resistance*

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## **Introduction:**

Antimicrobial resistant Enterobacteriaceae might be introduced into dairy cows through the consumption of feeds, and the microbes may eventually enter the human food supply.

Members of the Enterobacteriaceae family are becoming more important in food safety and medicine. Estimates of medical and lost productivity costs associated with *Salmonella* species and *Escherichia coli* O157 ranged from \$0.2 to 3.5 billion in 1996. Additionally, *Escherichia*, *Klebsiella*, *Enterobacter*, *Serratia*, and *Citrobacter* are responsible for almost one-third of nosocomial infections in the United States (1990-92 data).

Several studies have suggested an association between antimicrobial use in animal feeds and the possible risk of humans contracting resistant bacterial strains such as *Salmonella* spp., *E. coli*, and other enteric infections from food-producing animals. Other studies have isolated different types of *Salmonella* spp. from animal feeds and other feed products. Veldman and others, for example, tested poultry feeds and feed components (fish meal, meat/bone meal, tapioca, maize grits) from 57 feed mills. Among the isolated bacteria, the most frequent serotype was *Salmonella hadar*. Harris and others tested swine feed and feed ingredients (grain, soybean meal, milk/whey, fats/oils, and protein products). The most frequent serotype isolated was *Salmonella worthington*.

Because of sparse data on antimicrobial resistant *Salmonella* spp. and other enteric bacteria in animal feeds and from dairy farms, the objectives of this study include the following:

1. Identify *Salmonella* spp. and other Enterobacteriaceae in dairy cow-feed-ingredient piles on the farms and their antimicrobial-resistance patterns.
2. Determine the prevalence of *Salmonella* spp. in the piles.
3. Determine whether the prevalence of *Salmonella* increases in individual piles over time.

## **Methods and Materials:**

Thirty-two farms were selected at random from 43 commodity dairy feeding farms. Of the 32 farms selected, 12 farms agreed to participate in the study. In the prevalence survey, 50 feed-ingredient piles were sampled for the presence of bacteria. In the repeated samples survey, 10 of the original 50 piles were sampled over time. Presumptive *Salmonella* positives (Assurance EIA *Salmonella* kit) were evaluated further using cultural methods and the Enterobacteriaceae Micro-ID system. A disk-diffusion method was used to identify ampicillin, chloramphenicol, ciprofloxacin, streptomycin, and tetracycline resistance.

## **Results:**

In the prevalence study, 42.0 % (21/50) of the 50 feed-ingredient piles were presumptive positive for *Salmonella*. By the culture method and Enterobacteriaceae Micro-ID system, 2.0 % (1/50) was confirmed as *Salmonella enteritidis* and serogrouped as poly Group B, Group C<sub>1</sub>. In the repeated samples study, 60.0 % (6/10) of the piles were presumptive positive for *Salmonella*. By the culture method and the Enterobacteriaceae Micro-ID system, 20.0 % (2/10) were confirmed as *Salmonella enteritidis* and serogrouped as poly Group B, Group C<sub>1</sub>. Fifty bacterial isolates were tested for antimicrobial resistance. Sixty-two percent (31/50) of the isolates demonstrated ampicillin resistance while 10.0 % (5/50) displayed tetracycline resistance.

## **Conclusions:**

The presence of antimicrobial resistant Enterobacteriaceae in feed ingredients raises concerns about health risks to food-producing animals such as dairy cows and subsequently to the consumer.

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# *Swine Hepatitis E Virus Contamination in Hog Operation Waste Streams-- An Emerging Infection?*

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Swine Hepatitis E Virus (sHEV) is a recently discovered virus endemic to Midwest hog herds. The proposed zoonotic nature of Asian strains of human HEV (hHEV) and the recent discovery of a clade of human HEV in the United States, with approximately 98% DNA and protein sequence homologies to sHEV, suggest the hypothesis that swine herds are a potential animal reservoir for hHEV. In order to determine whether sHEV is a potential environmental contaminant, we tested water samples collected downstream from hog-farm operations for sHEV by nested reverse transcription polymerase chain reaction amplification (RT-PCR). Thirty-three samples including pit slurries, lagoon influents, lagoons, tile inlets, drainage ditches, tile outlets, a draining creek, and a monitoring well were tested by RT-PCR. Three samples (9%) were positive, including two from waste lagoons and one from a tile outlet draining a field to which manure had been applied. Each sample was collected on a separate farm, two in Iowa and one in Missouri. We next identified three sHEV RT-PCR positive hog-stool samples out of 20 tested from a single Iowa farm. All three positive stools came from 3-month-old hogs. sHEV was confirmed by partial sequencing of RT-PCR amplicon. In order to model the duration of sHEV in the environment, 1% and 10% suspensions of sHEV positive stool were stored in water and phosphate buffered saline, respectively, at -85°C, 4°C, and room temperature. sHEV was detectable by RT-PCR under all conditions at 2 weeks of storage, the longest period tested to date. Therefore, sHEV is present in downstream water waste from hog-farming operations. sHEV may persist in the environment for at least 2 weeks and possibly longer.

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## *A System to Describe Antimicrobial Resistance Among Human and Animal Populations*

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Global concerns about antimicrobial resistance have grown in recent years and include the agricultural and human-health care arenas. The World Health Organization has seated several consultancy groups to examine the implications of antimicrobial use and resistance development. The National Academy of Sciences also has taken up the issue of antimicrobial use and resistance. Numerous other groups have held public and private meetings to discuss various aspects of antimicrobial resistance. Though there is little consensus regarding the roles of various antimicrobial-use practices in the development of resistance that can impact public health, there is widespread recognition that the issue merits further study and that there is a sense of urgency in our need for more data and information.

To track emerging resistance, the National Antimicrobial Resistance Monitoring System - Enteric Bacteria (NARMS-EB) was established in 1996. The overall system is comprised of two separate components for antimicrobial-susceptibility testing of veterinary and human isolates. Testing of the human isolates component of the system is done at the Center for Disease Control in Atlanta. Testing of the veterinary isolates is conducted by the U.S. Department of Agriculture at the Agricultural Research Service Richard Russell Research Center in Athens, Georgia. *Salmonella* was chosen as the sentinel organism to describe levels of resistance and monitor trends in both systems. Currently, *Campylobacter* and *Escherichia coli* O157 (when available) also are tested in both systems. Testing for the veterinary NARMS-EB *Salmonella* isolates is conducted using a semi-automated system (Sensititre<sup>TM</sup>, Trek Diagnostics). Plates are custom made with 17 antimicrobials in an MIC format. This system is also used for the *E. coli* O157 isolates. *Campylobacter* susceptibility testing to seven antimicrobial drugs is done using the E-test (AB BIODISK). Testing for the human NARMS-EB isolates is conducted using the same testing methodologies and antimicrobials as those used for the veterinary isolates. Veterinary isolates represent a broad range of species and come from diagnostic laboratories, healthy animals on farms, and raw product collected in slaughter or processing plants. The samples from farms are collected as part of the National Animal Health Monitoring System (NAHMS) and represent dairy, beef cow-calf, beef-feedlot, and swine operations.

The goals and objectives of the monitoring program are to (1) provide descriptive data on the extent and temporal trends of antimicrobial susceptibility in *Salmonella* and other enteric organisms from the human and animal populations; (2) facilitate the identification of resistance in humans and animals as it arises; (3) provide timely information to veterinarians, physicians, and others; (4) prolong the life span of approved drugs by promoting prudent and judicious use of antimicrobials; and (5) identify areas for more detailed investigation. Information resulting from the monitoring program and follow-up outbreak investigations will be distributed to veterinarians, physicians, and food animal producer groups in a timely manner. Use of the information will be targeted to redirecting drug use so as to diminish the development and spread of resistance over the short term with directives involving long-term use developed in collaboration with the appropriate professional practitioner groups. Outbreak investigations and field studies will be initiated as a result of major shifts or changes in resistance patterns in either animal or human isolates.

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# *Microbial Sources Tracking*

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The lack of appropriate methodology for tracing bacterial contamination in the environment is a major impediment in identification and control of the sources of these pollutants and adversely affects the decision-making process in water-quality and fisheries-resources management. Several methods for tracking genetically engineered microorganisms have been used, but their utility is limited to the detection of organisms carrying reporter genes or their products. Limited efforts to track sources of natural bacterial populations have been made; the approach used was based on quantification of indicator organisms at various sites. These studies invariably have raised more questions than answers. I have developed and tested a tracking system for identification of sources of microbial pollution. The methodology can be used to identify and assess the impact and contribution of nonpoint sources of microbial pollution and to establish and characterize the impact of the point sources of microbial pollution in fecal runoff. The method can be used to identify the sources of fecal coliforms at the species level and map their distribution, transport, and movement in watersheds, rivers, lakes, and drinking-water-distribution systems. Microbial sources tracking studies conducted in a closed watershed, a swimming beach, and an industrial wastewater-treatment plant will be presented and discussed.

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# *Investigation of the Chemical and Microbial Constituents of Ground and Surface Water Proximal to Large-Scale Swine Operations*

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Continued expansion and intensification of large-scale swine operations in the United States have brought about some important environmental, agricultural, and public-health issues. Waste-management practices for these operations commonly utilize open earthen lagoons, ponds, or slurry tanks for the temporary storage of manure in a liquefied form, which is subsequently applied as fertilizer on agricultural fields. This practice, under certain conditions, may contaminate the ground and surface water in the surrounding area. Research on the direct and indirect human-health effects of this contamination is very limited. We conducted a pilot investigation on the chemical and microbial constituents of ground and surface water proximal to large-scale swine operations in the State of Iowa. We measured potential chemical (pesticides, antibiotics, heavy metals, minerals, and nutrients) and microbial (*Escherichia coli*, *Salmonella sp.*, *Enterococcus sp.*, *Yersinia sp.*, *Campylobacter sp.*, *Cryptosporidium parvum*) contaminants that may be hazardous to human health. The study accomplished its primary goal of obtaining a broad profile of the chemical and microbial constituents of both ground and surface water proximal to large-scale swine operations. We identified chemical pollutants and zoonotic pathogens in the environment on and proximal to these operations. However, the sample-collection sites were not in locations that could pose a direct threat to human health. More research is needed to accurately determine the level of risk, pathways of exposure, and critical control points to avoid any potential exposure; follow-up investigations are being considered in the near future.

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# *Identification of Sources of Fecal Coliform Bacteria and Nutrient Contamination in the Shoal Creek Basin, Southwestern Missouri*

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Missouri is a leader in the Nation in livestock and poultry production. According to the U.S. Department of Agriculture (USDA) 1997 Census of Agriculture, Missouri ranks second in the Nation in the number of beef cattle, sixth in the Nation in the number of hogs and horses, and 11<sup>th</sup> in the Nation in the number of broilers, pullet chicks, and pullets sold. Much of the beef and poultry production is concentrated in the southwestern part of the State in Barry and Lawrence Counties. From 1992 to 1997, beef-cattle production in Barry County increased in rank from 154<sup>th</sup> to 92<sup>nd</sup> in the Nation with more than 41,000 beef cattle inventoried in 1997. Broiler production increased during this same period, and Barry County increased in rank from 32<sup>nd</sup> to 20<sup>th</sup> in the Nation with more than 56 million broilers sold during 1997. Recent (1998) estimates place the number of broilers in Barry County between 90 and 100 million.

The rapid growth in the livestock and poultry industries has caused concern about impacts on surface- and ground-water quality in southwestern Missouri. Shoal Creek drains much of the intense beef-cattle and poultry-producing areas of Barry and adjacent counties, and more than 500 poultry houses are located within the upper 233 mi<sup>2</sup> (square miles) of the basin. Between 1992 and 1999, concentrations of fecal coliform bacteria in water samples collected by the Missouri Department of Natural Resources from the upper reach of Shoal Creek averaged more than 5,000 colonies per 100 mL (milliliters). These concentrations greatly exceed the Missouri limit of 200 colonies per 100 mL for the stated uses of Shoal Creek and have resulted in the upper Shoal Creek basin being placed on the 303(d) list of impaired water bodies in Missouri. The U.S. Geological Survey, U.S. Environmental Protection Agency, Region VII, and Missouri Department of Natural Resources recently (1999) initiated a cooperative study to identify the sources of bacterial contamination in Shoal Creek. This multi-discipline investigation combines standard water-quality assessment tools with emerging techniques, including microbial source tracking of *Echerichia coli* using ribotyping and pulse-field electrophoresis; identification of *Salmonella* by culture; and the determination of concentrations of optical brighteners, antibiotics, and hormones in water samples. A network of stream and tributary sites is being monitored monthly for discharge, field parameters, distribution of indicator bacteria, nutrients, and optical brighteners. An expanded suite of analytes including hormones, antibiotics, and major ions are being collected quarterly from all surface-water sites, four springs, and selected sites during storm events. Preliminary results suggest that the largest bacteria densities are not associated with known sewage treatment plant effluents. Of the one dozen *Escherechia coli* isolates initially examined, a single isolate of *E. coli* O157:H7 has been identified from a tributary site outside the Shoal Creek basin.

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