Project Title: Microbiological Impact of Agricultural and CAFO Activities on Surface and Ground Water Quality (GWERD Task 12465)

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Long-Term Goal/APM: Long Term Goal 3 - Provide the tools to restore and protect aquatic systems and to forecast the ecological, economic, and human health outcomes of alternative solutions/APM 144 (2004) - Develop a strategy to evaluate BMP performance via molecular based methods in watersheds impaired by fecal contamination *(contributes to this APM)*

Abstract: Previous evidence of microbial contamination of natural waters by agriculture and Concentrated Animal Feeding Operations (CAFOs) has created the necessity to further evaluate the situation. Pathogenic bacteria and pathogenic viruses are often present in the intestinal microflora of humans, as well as animals associated with CAFOs. This creates the potential for pathogen transfer into nearby watersheds, which in term poses a public health threat. The objective of this research is to provide a microbiological evaluation of the Turkey Creek Watershed located in northwest central Oklahoma. This is a priority watershed listed under Section 303D of the Clean Water Act, and is currently the focus of a USGS study on sources of impairment. Nitrogen-isotope ratios of nitrate and detection of organic compounds typical of human wastewater previously indicated that animal and/or human wastes were the major sources of nitrate in Turkey Creek. Therefore, it is important to identify the species source of fecal contamination as well as specific pathogens that may be present in the watershed. This research project consists of two phases. The first phase is dedicated to bacterial source tracking using the bacterium E. coli as the indicator organism. The methodology is based on antibiotic resistance analysis in combination with a statistical discriminant analysis. The second phase will involve identification of specific viral and bacterial pathogens in the water. The target microorganisms of this project are the human enteroviruses (poliovirus, coxsackievirus, echovirus) and the bacteria *Salmonella spp.*, *Yersinia enterocolitica*, and *Campylobacter jejuni*. The methodology will be based on hollow-fiber ultra-filtration and real-time PCR technology. The application of the results will aid in the development of TMDLs and risk management strategies for optimizing land use practices in the animal industry.

Status: The first phase of this project is complete. The antibiotic resistance patterns for the fecal sources and the water *Escherichia coli* isolates have been developed, and statistically analyzed. Additionally, the seasonal trends of *E. coli* has been determined. Preparative work on the second phase is currently taking place to develop pathogen recovery by ultra-filtration and PCR procedures. Pathogen recovery from environmental water and identification work will commence September 2005.

Products: (1) Antibiotic resistance analysis for microbial source tracking on a watershed where sources exhibit low resistance (under clearance), (2) paper on recovery and identification of pathogens in a TMDL-listed watershed *(planned)*.