U.S. Geological Survey Karst Interest Group Proceedings, Shepherdstown, West Virginia, August 20-22, 2002

Eve L. Kuniansky, *editor*

U.S. Geological Survey
Water-Resources Investigations Report 02-4174

Atlanta, Georgia
2002

Comparison of Methods to Source Track Bacteria in Ground Water in Karst Areas of Berkeley County, West Virginia

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Abstract

Escherichia coli, indicators of fecal contamination, were detected in 16 of 50 domestic water wells sampled during the summer of 2000 in Berkeley County, West Virginia, a region partially underlain by karstic limestone. The combination of diffuse and conduit ground-water flow, as well as the complex interaction of ground and surface water in this area make it difficult to link fecal contamination in ground water to aboveground sources. An emerging technology, bacteria source tracking, can likely identify the sources of fecal contamination in ground water of this region. Knowledge of the sources of fecal contamination will aid in the development of effective source control strategies. Although potentially powerful, the science of bacteria source tracking is relatively new, and no consensus is available regarding the best methods to identify fecal contamination sources in environmental settings. This study will compare seven bacteria source tracking methods for their ability to discriminate *Escherichia coli* isolates from feces of nine different source-animal categories in Berkeley County. The source tracking methods to be compared include ribotyping using HindIII, George Lukasik, University of Florida; ribotyping using EcoR1 and PvuII, Mansour Samadpour, University of Washington; antibiotic resistance analysis, Bruce Wiggins, James Madison University; pulsed-field gel electrophoresis using Not1, Kriston Strickler, West Virginia Department of Agriculture; sole source carbon utilization using BIOLOG, Charles Hagedorn, Virginia Polytechnic Institute and State University; rep-PCR using REP primers, Donald Stoeckel, U.S. Geological Survey; and rep-PCR using BOX primers, Howard Kator, College of William & Mary.

The study will be performed by challenging a library of known-source isolates with a library of blind isolates. The identities of the blind isolates are known only to the project manager. The known-source library will be developed using fecal samples that were collected from nine sources: humans, dogs, beef cows, dairy cows, swine, chickens, horses, deer, and geese. The blind collection of challenge isolates will include new isolates from the 9 sources represented in the known library, replicates from the original known-source library, and a collection of new sources that are not represented in the source library (such as mice, cats, goats, and llamas). Method performance will be assessed by the average rate of correct classification for isolates from each source group, the rate of false identification within each source group, and the ability of each method to handle the un-represented sources.

The library of known source isolates was constructed from a minimum of 20 fecal samples that were collected in August and September 2001 in Berkeley County for each of nine source-animal categories. Sterile toothpicks were used to pick apart fresh fecal samples and to extract a small portion from the center of each sample. The sterile toothpick containing the extracted feces was swirled in a vial of sterile buffer that was then wiped with alcohol, chilled, and shipped by overnight mail to the U.S. Geological Survey microbiology lab in Columbus, Ohio. Between 5 and 8 isolates of *Escherichia coli* were cultured and confirmed at the Columbus lab from each fecal sample to build a library of at least 100 isolates for each of the nine source-animal categories for a total known-source library of 900 isolates. The confirmed *Escherichia coli* isolates were distributed to the laboratories performing the seven bacteria source tracking methods. Currently, each lab is in the process of building a source library from these known source isolates.

At the time of writing, the collection of the feces for the challenge isolate set was scheduled for June 2002. Feces collection procedures will be identical to those used to collect feces for the known source isolate set. Approximately 15 feces samples will be collected for eight of the source-animal categories, and approximately 30 feces samples will be collected from human sources. Approximately 25 feces samples will be collected from new animal sources not in the known source isolate library. All feces sampled will be mailed without animal-source identification to the U.S. Geological Survey microbiology lab in Columbus, Ohio, for culturing and confirmation of bacteria isolates. The lab will culture and confirm one bacteria isolate from each fecal sample. These isolates plus selected replicates from the known source library will comprise the challenge set of isolates.

Each of the seven researchers will use his or her particular method of bacteria source tracking analysis to compare the challenge set of isolates to isolates in the known-source library. Each researcher will then offer a presumptive identification for each of the 200 isolates in the challenge set, using a direct match or statistical approach of their choice. The identification of the replicates will test the reproducibility of each method, and the identification of the unknowns will test the predictive accuracy of each method. A robust method of analysis will identify correctly all replicate isolates and all unknown isolates from the challenge set that are from sources included in the known-source library. A robust method of analysis will also correctly identify isolates as “unidentifiable” if they were from animal sources not in the source library.