# IOWA'S WATER
Ambient Monitoring Program

Fecal Source Tracking in Iowa

Elevated fecal indicator bacteria levels in Iowa's surface waters are of concern to recreational users because these bacteria indicate the presence of fecal material in the water. Monitoring by Iowa's Ambient Water Monitoring Program has shown the prevalence of fecal indicator bacteria in surface waters, but has not been able to determine the source(s) of these bacteria. New technologies called source tracking methods have the possibility of scientifically determining the source of fecal contamination in water. However, these technologies are in early stages of development and are expensive and time consuming. Thus, few projects have been undertaken at this time using these methods.

The Water Monitoring Program and the University of Iowa Hygienic Laboratory have been involved with two projects focused on exploring these different methods of bacterial and chemical source tracking (see insert). Each source tracking method provides only one line of evidence in determining the source(s) of fecal contamination in a watershed. Caution should be exercised in

Fecal matter

Bacteria

Any warm-blooded animal

E. coli bacteria   DNA pattern of *E. coli*
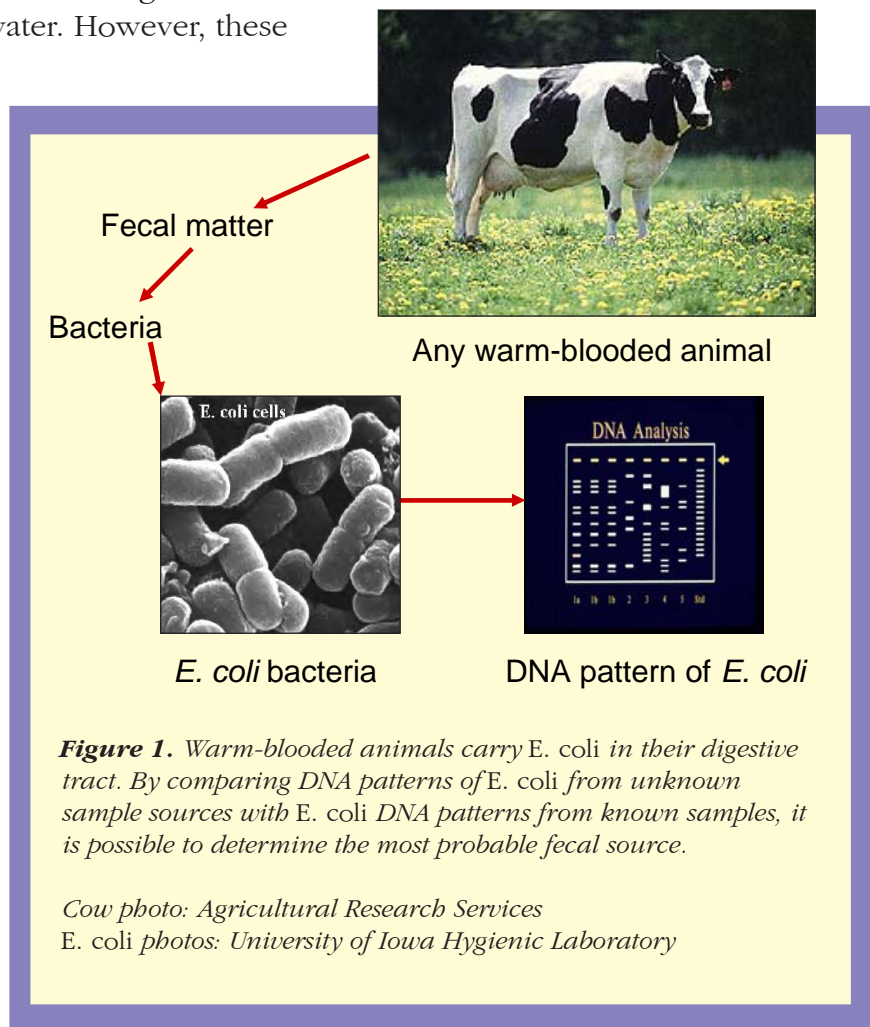
Figure 1. *Warm-blooded animals carry* E. coli *in their digestive tract. By comparing DNA patterns of* E. coli *from unknown sample sources with* E. coli *DNA patterns from known samples, it is possible to determine the most probable fecal source.*

Cow photo: Agricultural Research Services
E. coli *photos: University of Iowa Hygienic Laboratory*

Figure 2. *DNA ribotyping is performed at the University of Iowa Hygienic Laboratory using the Riboprinter® Microbial Characterization System.*

Photo by University of Iowa Hygienic Laboratory.

evaluating a watershed based on data from one type of source tracking method. A toolbox approach with many "tools" or lines of evidence (i.e., watershed evaluation, bacterial and chemical source tracking methods) are necessary to accurately determine the animal or human source of fecal contamination in a watershed.

Bacterial Source Tracking Methods

DNA ribotyping involves comparing DNA patterns or "fingerprints" of *E. coli* bacteria from affected waters to DNA fingerprints of *E. coli* from known sources of fecal material in the watershed. Researchers believe that the DNA of bacteria taken from fecal matter may vary substantially from one watershed to the next. Therefore, the collection of known sources of fecal material in a particular watershed is necessary to generate a DNA fingerprint database or library for the watershed for comparison with unknown bacteria in the water.

At the University of Iowa Hygienic Laboratory, DNA ribotyping is performed using the Riboprinter® Microbial Characterization System (Qualicon, Wilmington, DE). Once the *E. coli* bacteria are isolated from other types of bacteria, restriction enzymes cut the cell's DNA into pieces and a process called electrophoresis separates the pieces by size through a gel. The DNA fragments are then transferred onto a membrane and specific fragments are detected by using a labeled piece of DNA as a probe. The resulting banding pattern of DNA fragments corresponding to the relevant rRNA is known as a ribotype. The ribotype patterns of the DNA fingerprint are imported into a statistical analysis software program called BioNumerics® (Applied Maths). Patterns for each bacterium coming from the animal or human category are grouped into libraries for comparison and identification purposes. By comparing DNA patterns or ribotypes of *E. coli* from unknown sample sources with *E. coli* DNA patterns or ribotypes of *E. coli* from known sources, it is possible to determine the most probable fecal source.

Figure 3. The type of fecal source can be determined by analyzing the type of antibiotic resistance demonstrated by the E. coli *of known sources and comparing that resistance profile with the profile from unknown* E. coli *sources.*

Photos by Maureen Clayton (upper right) and Yijun Deng (lower left), University of Northern Iowa.

Multiple antibiotic resistance analysis uses several common antibiotics to determine the probable source of bacteria. Human *E. coli* bacteria can be distinguished because they typically have the greatest resistance to antibiotics when compared with domestic and wildlife fecal bacteria. While domestic and wildlife *E. coli* bacteria have significantly less resistance to antibiotics, the type of animal can often be determined by analyzing the type of antibiotic resistance and the concentration of antibiotic necessary to cause resistance. This method also uses *E. coli* from known fecal samples for comparison with unknown *E. coli* bacteria in water samples. To determine the particular resistance profile, the *E. coli* bacteria is exposed to numerous antibiotics at different concentrations and its susceptibility (growth or lack of growth) to the antibiotic is noted and a resistance pattern emerges that can be used to identify the source.

Chemical Source Tracking Methods

Sterols, caffeine, and cotinine (nicotine by-product) are types of chemical source tracking methods. Sterols are chemical markers found in the fatty acids of cell walls and membranes. They are involved in the life processes of organisms and are thus a potential marker for living organisms and their impact on environmental systems. A number of variations on the basic steroidal molecular framework occur in animals, and distinctions among them may provide differences among different types of animals. Thus, sterols have the potential to yield information as indicators of human and animal waste. The 'key' sterols are coprostanol, cholesterol, and dihydrocholesterol. Coprostanol is normally only produced in the digestive system of humans and higher mammals. It is a useful sewage tracer even when fecal bacteria populations have diminished. Cholesterol is present in human feces but is also common in aquatic environments. A calculated con-
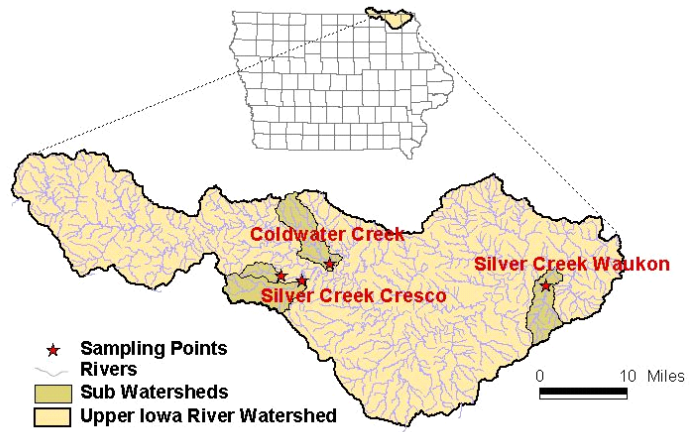
centration ratio of coprostanol (representing sewage contamination) versus the sum of cholesterol and dihydrocholesterol (representing a rough estimate of sewage and non-sewage sources) has been utilized to measure the source of fecal contamination. Variables such as diet, environmental processing, and the presence of plant sterols may impact this measurement.

Caffeine and cotinine (nicotine by-product) have been utilized as source tracking markers to indicate the presence (or absence) of human waste. Some caution must be used when interpreting caffeine analytical results because this compound has become quite common in the environment, although finding this compound can be significant in specific situations.

Sterols analysis can distinguish between different types of bacteria sources. Photo by University of Iowa Hygienic Laboratory.

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Fecal Source Tracking Projects

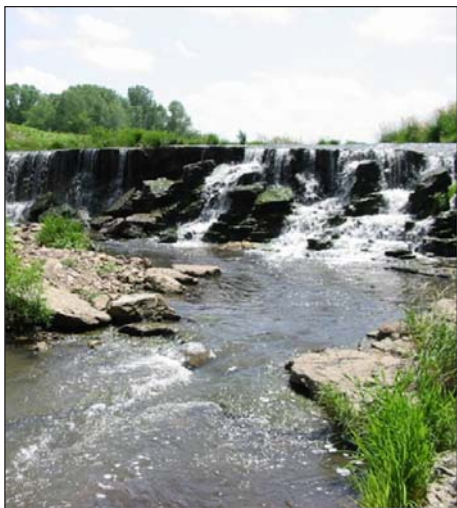**Upper Iowa Watershed Bacteria Source Tracking Project**

The Upper Iowa River and its watershed are valuable natural and economic resources located in extreme northeast Iowa and southeast Minnesota. The Upper Iowa River watershed is a 1,005 square mile watershed recognized by the U.S. Environmental Protection Agency and the State of Iowa as a priority watershed for water

Figure 1 insert. *Water sampling sites for the Upper Iowa Watershed Bacteria Source Tracking Project.*

quality protection. This river system is heavily utilized for swimming, tubing, and canoeing. The Upper Iowa River Watershed Alliance has monitored 39 stream sites throughout the Upper Iowa River Watershed since 1999 in an effort to identify sub-watersheds that are contributing elevated levels of fecal indicator bacteria to the Upper Iowa River. The water quality monitoring identified six sub-watershed tributaries that had elevated bacteria levels. Three of the six tributaries were selected for a bacteria source tracking project; Coldwater Creek, Silver Creek near Cresco, and Silver Creek near Waukon. Potential bacteria sources in these sub-watersheds include runoff from feedlot and manure-amended agricultural lands, inadequate septic systems, and wildlife.
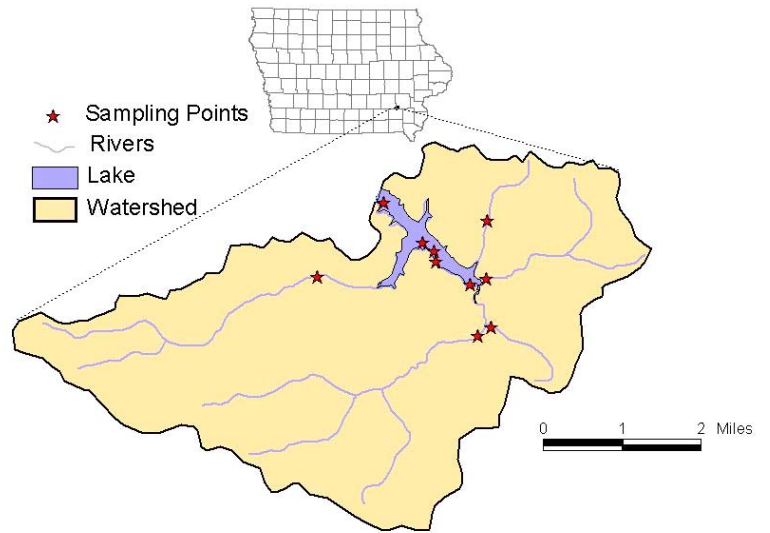
The Upper Iowa Bacteria Source Tracking Project, begun in 2002, used DNA ribotyping to identify sources in the Upper Iowa River Watershed and initiated the establishment of a statewide *E. coli* bacteria DNA database. A total of 259 *E. coli* strains from known manure sources (e.g., hog, cattle, sheep, goose, raccoon, deer, and human) were collected and analyzed to build a statewide ribotyping library with patterns from known Iowa strains. After obvious outliers were removed, the following *E. coli* strains were used in the identification of sources in the three Upper Iowa sub-watersheds: cattle (88), deer (35), human (27), geese (26), and swine (24). DNA ribotyping was performed on 50 *E. coli* strains from water samples taken from the three sub-watersheds in Coldwater Creek, Silver Creek near Cresco and Silver Creek near Waukon (Figure 1 insert). DNA ribotyping successfully discriminated between human and cattle bacterial sources. However, the number of *E. coli* strains was insufficient to distinguish between the other animal sources.

Upper Iowa River. Photo by Lora Friest.

Figure 2 insert. Water sampling sites for the Lake Darling Bacteria Source Tracking Project.

Lake Darling Bacteria Source Tracking Project

Since 2000, Iowa's Ambient Water Monitoring Program has conducted monitoring for fecal bacteria at the state-owned beaches. Most beaches have variable levels of bacteria that may be high one week and low the next. However, a few beaches show chronically high levels of bacteria. The beach monitoring program has classified these beaches as "vulnerable." Lake Darling Beach (located in Washington County) is one of those "vulnerable" beaches and was considered a good candidate for a bacteria source tracking project due to its small watershed size and relatively small number of possible sources.

In 2003, the Lake Darling Bacteria Source Tracking Project was initiated and work began to obtain the necessary fecal and water samples. The methods used include DNA ribotyping, multiple antibiotic resistance analysis, pathogens analysis for *Salmonella spp.* and *E. coli* O157:H7, and sterols, caffeine, and cotinine analyses. Fecal samples were taken from five potential source categories: cattle, swine, human, deer, and goose. Ten sampling events will be completed across all flow regimes – four high-flow, two mid-flow, and four low-flow. This sampling frequency will determine if different bacteria sources are present during different times of the year. Six sampling events were completed in 2003 (two mid-flow and four low-flow) and the remaining four high-flow sampling events will occur in spring of 2004. Water samples are taken at ten locations: four in the lake, one at the beach, and five in the surrounding watershed (Figure 2 insert). Results from the project will be available by fall 2004.

Lake Darling.   *Photo by Clay Smith.*

Acknowledgements