AgriLife Research 'genetically fingerprinting' E. coli from watersheds

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The Lampasas and Leon Rivers watersheds have been listed as impaired by the state due to high counts of *E. coli* and other bacteria taken in the late 1990s, but from whom, what and where the contamination originates is unclear, say Texas AgriLife Research experts.

Because the watersheds are located in a landscape that is predominately rural and agricultural, there has been some conjecture that the sources of *E. coli* are livestock related, said Dr. June Wolfe, a AgriLife Research scientist.

"However, the origin of the sources is unclear," said Wolfe, who is based at the Texas AgriLife Blackland Research and Extension Center at Temple.

And although routine sampling sometimes shows elevated bacteria levels in the watersheds, exactly how high are the levels throughout the year?

To identify the sources objectively, Wolfe and his research associate, Tony Owen, have been collecting water samples at 30 river sites – 15 in the Lampasas River watershed and 15 in the Leon River watershed – monthly since February. They've also been taking fecal samples from all over the watersheds of known possible sources: home septic systems, wildlife, livestock, pets and water-treatment plants.

The samples are then "genetically fingerprinted" to determine exactly what the source of *E. coli* is — or otherwise, Wolfe said.

It's all part of the "Bacterial Source Tracking" project, which was funded by a Section 319(h) Clean Water Act nonpoint source grant from the Texas State Soil and Water Conservation Board and U.S. Environmental Protection Agency. The grant was administered by the Texas Water Resource Institute in College Station.

"This approach will utilize proven scientific methods that will distinguish the various sources of bacteria," Wolfe said. The DNA fingerprinting is done by Dr. George Di Giovanni at the Texas AgriLife Research laboratory in El Paso.

E. coli are measured by the number of colonies cultured from a given volume of water. Here, the *E. coli* colonies show up as maroon spots because of a special growth medium. (Texas AgriLife Extension Service photo by Robert Burns)

Identifying the exact sources of contamination will allow the formation of a watershed protection plan that is fair, balanced and effective, Wolfe said.

The Lampasas River originates about 70 miles west of Waco and flows southeast for 75 miles, passing through Lampasas, Burnet and Bell counties. Land use within the watershed includes grazing for beef cattle and the production of hay, wheat, oats, sorghum, corn, cotton, peanuts and pecans, Wolfe said.

The Leon River has three primary forks that meet near Eastland, which is about 110 miles west of Fort Worth. From Eastland, the river runs about 185 miles south where it and the Lampasas River join with the Salado Creek near Belton in northern Bell County to form the Little River. Like the Lampasas, the Leon runs primarily through rural farmlands. But there is also considerable forestland and a significant amount of dairy production in the northern part of the watershed, he

said.

Parts of both the Lampasas and Leon watersheds have been listed by the Texas Commission of Environmental Quality as "impaired" for recreational use, Wolfe said.

"By impaired, it is meant that coliform bacterium levels exceed state and federal established criteria," Wolfe said. "Though these organisms are generally not harmful to human health, they may indicate the presence of pathogens that can cause disease or gastrointestinal illnesses."

The collection of water samples must be meticulous and meet stringent EPA procedural and documentation guidelines, Wolfe noted. When he and Owen collect and label water samples, they must also measure stream flow, water pH, dissolved oxygen and specific conductivity. And there is a strict time deadline, measured in hours, from when the water samples are collected and must be pre-processed by Wolfe at the Temple center's water science laboratory.

But collecting water samples is only half the project, Wolfe said. Without an *E. coli* library to compare the water samples, identifying the source of the contamination would be impossible. So in addition to taking water samples, their goal is to collect at least 100 known-sources fecal samples within each watershed.

"We are focusing on human, feral hog and cattle sources," Wolfe said. "Feral hogs are a potentially big contributor, but other wildlife sources, including small mammal and avian species will be collected as well."

Tony Owen, Texas AgriLife Research associate, collects a wastewater sample from the City of Lampasas processing plant in the spring. (Texas AgriLife Research photo by Dr. June Wolfe)

Sometimes their "poop-scooping" draws attention, Wolfe noted, as they are also interested in cataloging fecal samples from pets, a task that takes them into local city parks and other public areas.

At other times, the sampling has called for ingenuity. For example, to collect avian fecal samples, they draped large sheets of plastic under local bridges to catch droppings from birds roosting over the waterways.

As the fecal samples are collected, and the DNA fingerprinting completed by Di Giovanni, the results are included in the Texas *E. coli* bacterial source tracking library.

Wolfe said the development of the Lampasas River and Leon Rivers water protection plans are to proceed independent of his bacterial source tracking project.

"However, conclusions from this BST project will be integrated into the water protection plan through adaptive management," he said.

One issue the team has had to face this year is the drying up of rivers and streams because of the drought, Wolfe noted.

"The results will still be valid because droughts are a normal occurrence, and we need to get a data set during these times too," he said. "But ideally, we would like to be able to collect data during a normal year too."

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