

DNA whodunit

Microbiologists use genetic fingerprinting to identify sources of water pollution

By Steve Werblow

Imagine fabled detective Sherlock Holmes transported through time, strolling along the shore of a small Pennsylvania lake in 1996, chewing his pipe and puzzling over a case of contaminated water. High fecal coliform levels have forced park officials to close Deep Creek Lake to swimmers. Despite attempts to reduce the contamination—removing pit toilets near the lake, persuading some homeowners to replace failed septic systems, looking at farms—coliform levels remain high.

Where is the contamination coming from? How can the Philadelphia Suburban Water Company and Montgomery County reduce the pollution?

Chances are, Holmes would send his faithful sidekick, Watson, out to pick up a few Philly cheese steaks so the great detective could compare notes over lunch with a new generation of sleuths — biologists who track microbial contamination to its source using genetic fingerprinting.

The Usual Suspects

Park managers had bacterial test results in hand that indicated high levels of fecal coliforms — in this case, *Escherichia coli*, a common indicator of fecal contamination. Holmes might well recognize the bacterial tests: using indicator organisms such as fecal coliforms to ascertain water quality goes back to the late 19th Century. The problem is that common bacterial tests indicate only that fecal matter is present, but give no clues to the source of the contamination.

E. coli is present in most fecal matter, regardless of the source. In most contamination cases, communities find fecal



Canadian Geese—regulars on Deep Creek Lake.

Photo: Philadelphia Suburban Water Company

coliform in their water and round up the usual suspects — often local farmers. "But just because the numbers are higher below a dairy farm doesn't mean that's the source," says Preston Luitweiler, manager of research and environmental affairs for the Philadelphia Suburban Water Company in Bryn Mawr, Penn. "The farmer says, 'it's not us, it's the geese, it's the septic systems, it's the deer.' The homeowner says, 'it's the farmers.' There can be a lot of finger-pointing before anything gets done."

Microbial Source Tracking (MST)

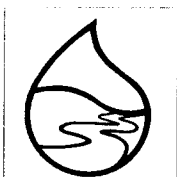
Faced with this coliform conundrum, Luitweiler called in two microbiologists using separate genetic fingerprinting techniques and asked them to find the culprit — a process called Microbial Source Tracking (MST). At the heart of MST is the fact that although *E. coli* is common to animals and birds, different animals host different strains of the bacterium. "Each source is a kind of specialized ecosystem," says Mansour

Samadpour, assistant professor of environmental health at the University of Washington School of Public Health and Community Medicine in Seattle and one of the sleuths hired by Luitweiler. "The environment in the intestine of a human is substantially different than the environment inside the intestine of a cow or duck." The trick: figuring out which strains thrive in which host, then matching the strains of *E. coli* in the water to the animals that carry them.

Samadpour and molecular biologists Vito Delveccio and Sharon Rose of the Institute of Microbiology and Medicine at the University of Scranton received *E. coli* samples taken from the lake and from feces found nearby. The two labs tested the samples using separate DNA fingerprinting techniques, zeroing in on different portions of the bacterial chromosomes in search of telltale patterns.

Samadpour used ribotyping — focusing on characteristics in a gene in the *E. coli* that codes for ribosomal (Continued, next page)

The cost of conducting a Microbial Source Tracking study can range from around \$10,000 to hundreds of thousands of dollars, depending on variables such as the size of the water body, the duration of the sampling period, and whether you're studying a fast-moving river or a placid pond.

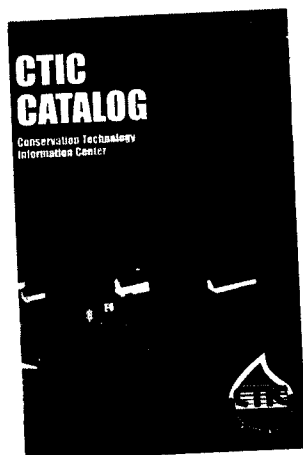


Know Your Watershed

Different-sized strands of DNA are arrayed on a gel plate, forming distinctive "genetic fingerprints." Samples 3, 6, 9 and 11 contain the same strain of *E. coli*; that information helps scientists match coliforms in water samples with the sources of the fecal contamination.

(Photo: Dr. Mansour Samadpour)

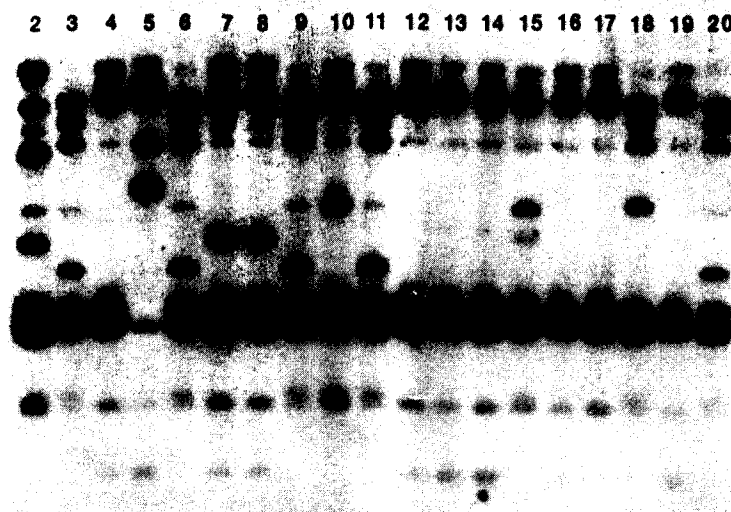
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(Continued from previous page) RNA. With restriction enzymes, he broke the genetic material into fragments. Using radioactive markers, Samadpour highlighted the ribosomal RNA-coding genes among the fragments, which are distinctive for each strain of *E. coli*. In Scranton, Rose employed a process called Rep-PCR: she looked for specific, repeated sequences in the DNA, then used primers to amplify the genetic material between the repeats. Because the locations of the repeating sequences differ in each strain of *E. coli*, the length of the fragments between the repeating codes differs, too.

To examine the lengths of the genetic materials and reveal the crucial patterns, the researchers put the fragments on gel plates. An electrical field attracted the negatively-charged DNA fragments toward the positive end of the gel. Weighed down along the way, the bits of genetic material arrayed themselves along the plate in size order, forming "fingerprints" that look like bar codes.

By matching the patterns between water and fecal samples, both scientists concluded that it looked like resident geese and ducks were to blame for 70 percent of the *E. coli* samples found in the lake. "That doesn't mean [waterfowl] are the only problem," says Luitweiler,

who notes that Samadpour found matches with deer and horses to account for some of the lake's *E. coli*, too. "But if you have limited resources to spend to try to solve the problem, where are you going to spend them?" Luitweiler and his colleagues are currently focusing on controlling the population of Deep Creek Lake's resident Canada geese; continued Microbial Source Tracking will help chart their success.

Building A Bigger Rogues Gallery

Samadpour took his research a step further and compared the Pennsylvania samples to DNA fingerprints (also called ribotypes) in his library of

15,000 strains of the bacteria. There were a number of matches, indicating that a collection of ribotypes — a sort of rogues gallery of bacterial strains — could be employed across a wide geography.

Samadpour and Luitweiler have since teamed up on a grant project to collect and fingerprint *E. coli* samples from a broad range of sources, building Samadpour's library and extending its utility. Says Luitweiler, "Right now, it's like sending in a fingerprint to the FBI if they have a file that has one million fingerprints. If there's 250 million people, you've got a one-in-250 chance of getting a match. If the FBI had 250 million fingerprints in its file — or even 100 million — your chances of finding a match would be much better."

The Washington biologist is also organizing other, similar projects around the nation. "The idea is to turn this into an international database of source-specific genetic fingerprinting," Samadpour says. Sherlock Holmes, master of deduction, would be impressed.

—For more information on microbial source tracking, contact Mansour Samadpour at (206) 543-5120, Preston Luitweiler at (610) 645-1132 or Vito Delveccio and Sharon Rose at (717) 941-6353.

Photo: Philadelphia Suburban Water Company



Scientists used two genetic fingerprinting techniques to identify resident geese as the culprits in fecal contamination that has kept Deep Creek Lake closed to swimmers for years.