WATER QUALITY

Microbiologists on the Trail Of Polluting Bacteria

Government officials hope scientists in the emerging field of bacterial source tracking can help them clean up polluted waterways

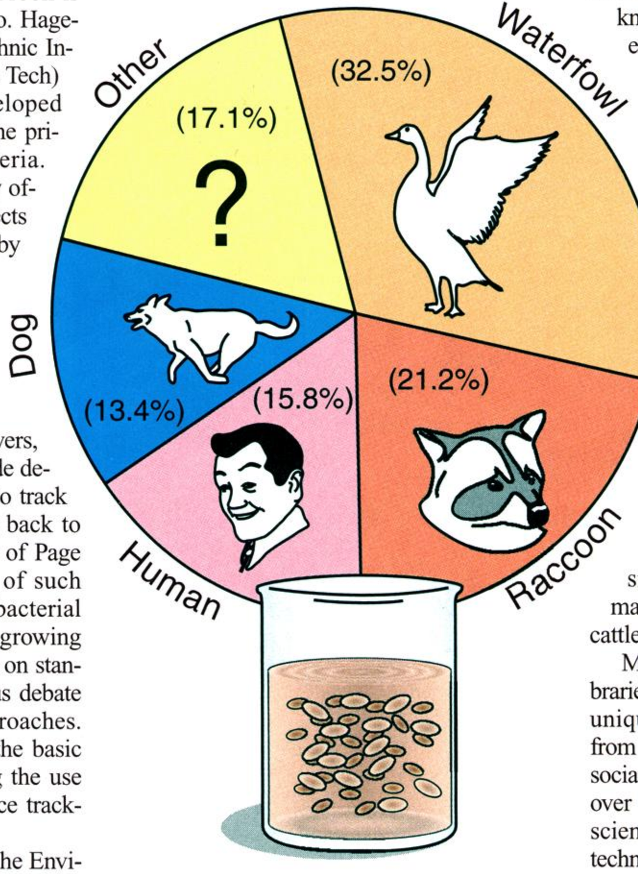
CLARKE COUNTY, VIRGINIA—It was an environmental mugging: Potentially dangerous levels of fecal bacteria were pouring down Page Brook, a picturesque local stream in this mostly rural enclave 100 kilometers west of Washington, D.C. But who was to blame? The leading suspects were human waste from faulty streamside septic systems, dung from cows grazing along the waterway, and droppings from a range of wildlife. But without firm evidence to identify the source, says county official Alison Teeter, it was impossible to know which sentence—new sewage controls for homeowners, fences to keep cows away from the stream, or stronger wildlife management—best fit the crime.

Thanks to some scientific sleuthing by Charles Hagedorn, however, Page Brook is cleaner today than it was 5 years ago. Hagedorn, a professor at Virginia Polytechnic Institute and State University (Virginia Tech) in Blacksburg, used a newly developed tracking method to indict cows as the primary source of the stream's bacteria. Armed with that information, county officials raised funds for fencing projects that have lowered bacterial counts by up to 98%. Without Hagedorn's 1997 study, Teeter says, "we might have wasted a lot of time and money on measures that didn't address the problem."

Court-ordered cleanups of thousands of similarly polluted lakes, rivers, and streams have created a nationwide demand for methods like Hagedorn's to track waterborne bacteria and pathogens back to their origins. But while the cleanup of Page Brook demonstrates the promise of such techniques, the infant science of bacterial source tracking (BST) is beset by growing pains. Researchers have yet to agree on standard methods, and there is rancorous debate over the soundness of certain approaches. One scientist has even challenged the basic biological assumption underpinning the use of one common bacterium for source tracking (see sidebar).

This week, scientists backed by the Environmental Protection Agency (EPA) released a report that calls for a sustained nationwide study to test, compare, and improve tracking methods. "We need to figure out which methods best answer which questions," says Bill Keeling of the Virginia Department of Conservation in Richmond. The report follows on the heels of the fledgling field's first national conference.[*]

Ending the blame game. For decades, public health officials have measured water quality by monitoring levels of fecal coliforms, bacteria that live in animal guts and survive in the environment when expelled along with feces. Although the coliforms may not cause disease, they can be accompanied by a rogue's gallery of pathogens, including microbes that cause hepatitis, cholera, and gastrointestinal illnesses. As a result, state officials have used them as a marker to routinely close swimming areas, wells, and shellfish beds when coliform counts rise above certain levels.



Pointing fingers. New microbial tracking methods enabled researchers to uncover sources of bacterial pollution in Virginia's Four Mile Run.

ILLUSTRATION: C. SLAYDEN

Unfortunately, the approach hasn't kept waterways clean. Although modern sewage treatment has eradicated the worst problems, EPA estimates that at least 40,000 kilometers of streams and coastal waters still carry bacterial loads that exceed health standards. Environmentalists have won a string of court victories in the last decade that require states to set goals for reducing bacteria counts. But efforts to set these targets—called Total Maximum Daily Loads (TMDLs)—often have become exercises in finger-pointing, as farmers, ranchers, and homeowners have argued that someone else is to blame. Desperate to calm such conflicts, TMDL specialists asked microbe scientists to come up with objective ways to pinpoint bacteria sources. Despite sparse funding, a handful of researchers have proposed ideas that range from DNA fingerprinting of different species of bacteria to techniques that identify viruses unique to specific animals.

The most prominent techniques are so-called "library-dependent" methods, which require researchers to match a bacterium found in a waterway to one included in a previously created library of bacteria from known sources. The Page Brook study, for example, used a library-dependent technique called antibiotic resistance analysis (ARA) that was developed by Bruce Wiggins of James Madison University in Harrisonburg, Virginia.

ARA assumes that the strains of bacteria in people, farm animals, and wildlife respond differently to antibiotics. To catalog these signatures, Hagedorn's team first combed the small watershed for excrement from every potential source, including people, cows, and deer. The researchers then cultured *Enterococcus* bacteria from the samples, exposed the microbes to a battery of antibiotics, and recorded the results. Later, they found that *Enterococcus* taken from the stream had signatures that—statistically, at least—matched cataloged signatures produced by cattle-borne bacteria.

Many genetic approaches also require libraries, with researchers seeking to match the unique DNA or RNA profiles of bacteria from waterways with those from bacteria associated with different sources. For example, over the past 5 years, another Virginia Tech scientist, George Simmons, used a genetic technique called pulsed-field gel electrophore-

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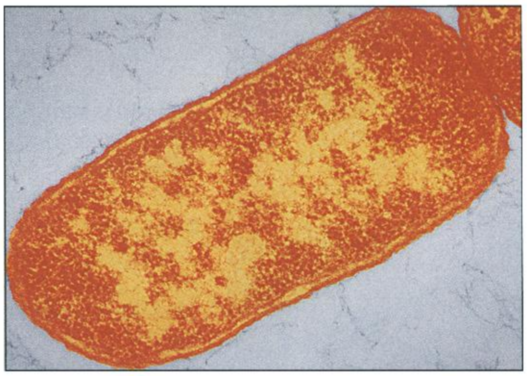
[*] U.S. EPA workshop on microbial source tracking, held 5–7 February 2002 in Irvine, California (www.sccwrp.org/tools/workshops/source_tracking_workshop.html).

Is *E. coli* Distinct Enough to Join the Hunt?

Underlying many bacterial source-tracking techniques is the assumption that some strains of bacteria are found only within a single kind or group of animals. But the idea of host specificity may be wrong when it comes to the common fecal bacteria *Escherichia coli*, argues David Gordon, a microbiologist at the Australian National University in Canberra. *E. coli* populations are so mercurial, he says, that scientists should think twice before using them for source tracking. But others say that new techniques may still make the bacterium a tracking star.



Fuzzy trail. *E. coli* may not be a good bacterium for source trackers.

E. coli is popular with public health scientists because it is common, is easy to culture, and can be deadly in some variants. Until recently, however, few researchers had studied how populations of the bacterium varied by geography and host.

The answer, Gordon says in a May 2001 *Microbiology* review, is that host and place count for little in explaining *E. coli*'s genetic variation. Geographic separation, for instance, could explain just 10% of the differences found in bacteria hosted by rodents in Mexico and Australia; the rest, he says, are due to diet and other factors. Similarly, Australian mammals shared many strains. "There is some host specificity," he says, "but the patterns aren't strong enough to unambiguously assign a strain to a source." Other microbes, says Gordon, may produce better results.

Scientists familiar with bacterial source tracking say that Gordon's caution is justified. But powerful genetic fingerprinting techniques may overcome such problems, says Mansour Samadpour, a University of Washington, Seattle, public health specialist who has pioneered molecular source-tracking methods using *E. coli.* "Sensitive methods can identify differences in bacteria that may otherwise appear identical," he notes.

Valerie Jody Harwood, of the University of South Florida in Tampa, urges source trackers to adopt "a strong population biology perspective." It may be impractical to prove that a specific bacterium came from a specific source, she says. "But we probably can show that one set of strains is more likely to come from one host than another"—or at least determine if they came from a human source. That ability, she says, would be enough to keep *E. coli* in the source trackers' toolbox.

–D.M.

sis to fingerprint bacteria sources in Four Mile Run, an urbanized stream near Washington, D.C. Simmons, now retired, found that it carried bacteria that matched those from several hosts (see graphic), including significant contributions from hard-to-control wild sources such as raccoons and geese.

The new EPA report, however, cautions that such findings are typically based on small studies and use methods not yet widely replicated. Various source-tracking methods have been applied to fewer than 125 waterways, and reports in peer-reviewed journals are scarce. The report calls for a four-phase national study to find out what really works, starting with basic experiments to see if single laboratories can replicate their own results. The study would end with a real-world test designed to compare different methods in a single, complex watershed.

The report is silent on how much such studies might cost and where funding might come from. The U.S. Geological Survey and Orange County, California, are funding small comparison studies, and California is considering a multimillion-dollar effort. Virginia, meanwhile, is the first state to require that BST be used in the development of TMDLs, giving researchers a chance to refine their techniques.

Testy talk. Such studies could help settle several simmering technical debates. The validity of ARA, for instance, has come under attack from Mansour Samadpour, a University of Washington, Seattle, public health specialist who has pioneered molecular BST techniques and started a company that specializes in them. "ARA doesn't work—it has nothing to offer," Samadpour told *Science*. At the recent BST meeting, he presented unpublished data suggesting that a standard ARA method was too crude to reliably segregate a group of *Escherichia coli* bacteria—the most common fecal bacteria—into useful groups. "It just doesn't provide the resolution you need to see important distinctions," he says.

ARA developer Wiggins notes that his method has passed muster in peer-reviewed studies and claims that Samadpour's study compared apples and oranges. "He tested something, but it wasn't ARA as we know it." Wiggins agrees that his method can be improved, and although it may not match the power of genetic approaches, he says it can be faster and cheaper.

Other BST researchers, meanwhile, complain that Samadpour has not shared enough information to allow them to evaluate his RNA ribotyping approach. Although Samadpour's company, the Institute for Environmental Health in Seattle, has been involved in more than 80 source-tracking studies, few have been reported in peer-reviewed journals. But Samadpour says he is ready to share the firm's bacteria library, which contains genetic profiles of more than 70,000 strains of *E. coli*, with other researchers who copy his methods. But because most don't, "What use would they have for [the library]?" he asks.

Potentially a great deal, suggests the new report. That's because one of the most pressing questions facing BST researchers is whether a library constructed for one study can be used for others. Waterborne bacteria can vary widely by place, season, and time of day, and even diet can shift the dominant strains found within an individual.

Given this variability, some BST researchers fear that they will have to construct new libraries for every new watershed, driving up expenses. But Samadpour believes that with a big enough library and extensive sampling programs, powerful molecular techniques can do the trick. "For the moment, there is no way around brute-force methods," he says. He figures that 500,000 *E. coli* strains, taken from a range of vertebrates, could cover the country if the data were updated regularly. Such a database might cost at least $10 million, he says.

Such cost estimates are driving researchers toward techniques that wouldn't require expensive libraries. Microbiologist Katharine Field of Oregon State University in Corvallis, for instance, is looking at using certain *Bacteroides* bacteria, which may carry host-specific genetic markers that vary little from place to place. Other techniques seek to detect widespread, species-specific antibodies that adhere to shed bacteria.

Fully developing and testing such methods, however, will require researchers to adopt standard methods and share data. In the end, it's likely that more than one method will be needed to track down solutions.

–DAVID MALAKOFF

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