MST in the MRG:

Identifying Sources of Fecal Coliform

Betsy Woodhouse *– Southwest Hydrology, University of Arizona*

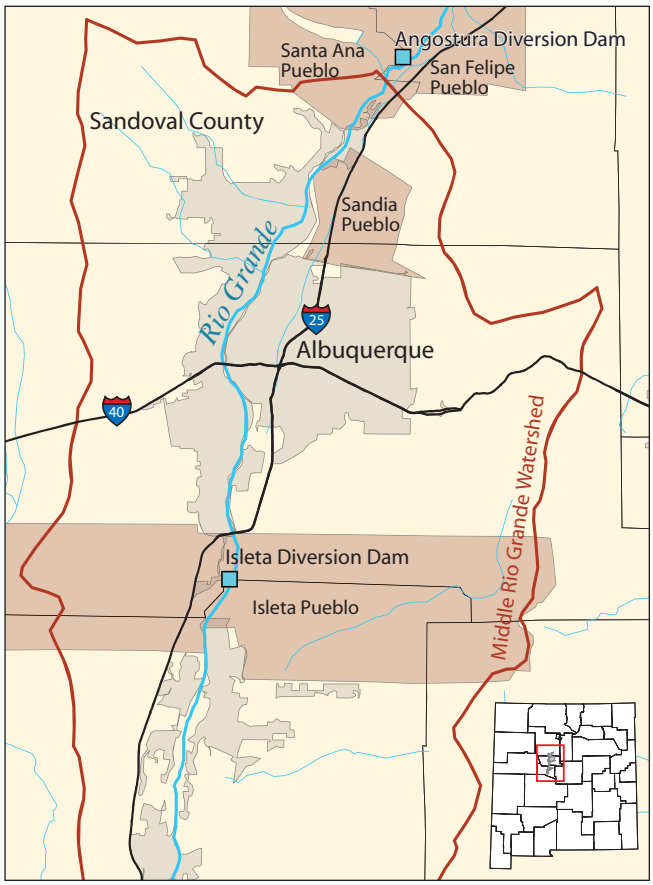
In 2002, high levels of fecal coliform in the Middle Rio Grande Basin of New Mexico were violating state and tribal water quality standards. Where was it coming from? Who or what was responsible? The New Mexico Environment Department, together with the Albuquerque Metropolitan Arroyo Flood Control Authority and Bernalillo County, hired Parsons Water and Infrastructure Inc. of Austin, Texas to find out. Only then could water managers begin to target solutions for reducing the fecal coliform.

The $340,000 investigation, initiated in February 2002, covered a 42-river-mile study area between Angostura Diversion Dam in southeastern Sandoval County south to the Isleta Diversion Dam just north of Isleta Pueblo (see map). Parsons' approach was to use microbial source tracking (MST) to try to differentiate among the *E. coli* secreted by various potential sources of fecal coliform in the study area. *E. coli* is a fecal coliform bacteria commonly found in the intestines of humans and animals.

How Does MST work?

MST is based on two principles. The first is that the genetic structure of the bacterial population is clonal: bacteria divide by binary fission, thus the daughter cells generated are virtually identical, and all descendents of a common ancestral cell are genetically related. Second, MST assumes that within a certain species of bacteria such as *E. coli*, members have adapted to conditions in specific hosts or environments to form resident strains of that bacteria that are regularly and abundantly shed from their hosts. Each resident strain has characteristics that allow it to be distinguished from other strains through ribotyping (DNA fingerprinting) or other methods. Thus, *E. coli* from birds in the Rio Grande watershed can be distinguished from *E. coli* from dogs, and if a strain of *E. coli* with an identical fingerprint is isolated from both a water sample and a suspected animal source, the animal is implicated as a contributor of that strain of the bacteria.

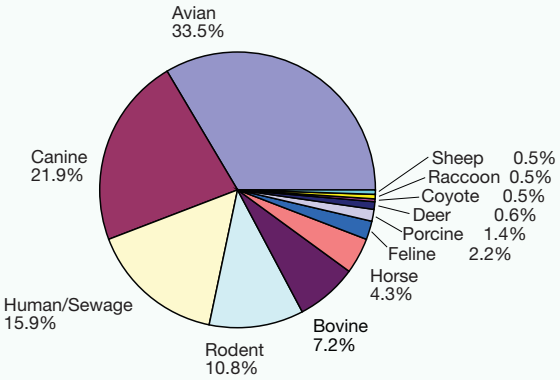
A less precise form of MST also was performed using antibiotic resistance analysis (ARA). ARA subjects the *E. coli* strain to various antibiotics, and the resulting profiles are compared to a library of known *E. coli* ARA profiles. The use of antibiotics in humans and animals results in development of resistance to certain antibiotics, and the microflora or bacteria of the intestines reflect that. However, a given profile is seldom unique to a specific animal or source, so the method uses statistics to determine the most probable category, such as human, wildlife, or livestock. This can be useful if a ribotype match between a water sample and a known source species is not possible: at least the general category of the source can be determined.



The study area extended from Angostura Diversion Dam south to Isleta Diversion Dam, a distance of 42 river miles.

Building the Library

The first step in the MST investigation was to identify all potential sources of fecal coliform in the watershed that could be contributing to the Rio Grande concentrations, and the approximate population of each. Available data included land use for each subwatershed, population from the 2000 federal census, means of sewage disposal (sewer connection or on-site septic), and dog and cat populations on a national per-household basis. Data on the quantity of livestock or wildlife within each watershed were not available, but countywide livestock populations were used to develop estimates. Wild birds, which have been found to be significant sources of bacterial contamination in many U.S. watersheds, are difficult to count, but bird lists available for several regions within the study area provided indications of relative seasonal abundance in specific geographic areas.



Identified sources of E. coli *in the entire Middle Rio Grande study area based on ribotyping. The source of nine percent of the samples could not be identified.*

Once the suspects were identified, samples of their fecal matter were collected to build watershed-specific libraries of ribotypes and antibiotic resistance profiles of *E. coli* isolates from them. The investigators obtained about 900 *E. coli* isolates from 580 samples. Sources included sewage, wildlife, birds, pets, livestock (including bovine, equine, poultry, goats, sheep, hogs, rabbits, and alpacas), and exotic species from the Albuquerque Zoo. These locally collected isolates were supplemented by a library of more than 65,000 isolates ribotyped by Mansour Samadpour at MEI Inc. in Seattle, where the MST analyses were performed.

Next, *E. coli* isolates from the water bodies were needed to compare to the possible suspects. The investigators collected ambient water samples and fecal coliform samples over two years. Eight water sampling sites were selected on the Middle Rio Grande at locations where historical water quality sampling was performed. An additional 22 sites within contributing watersheds were also sampled. A total of 206 water samples were collected from the 30 locations. Due to lack of rain, only 10 stations were sampled during both wet and stormwater runoff conditions. Concentrations of fecal coliform generally increased downstream.

And the Culprits Are…

A variety of sources appear to contribute to fecal coliform levels in the Middle Rio Grande. According to the ribotyping, wildlife (primarily birds) and pets collectively account for 70 percent of the *E. coli* detected in the water samples. Humans and livestock accounted for 16 and 14 percent, respectively (see chart). The source of about 9 percent could not be identified. With the exception of rodents, only a few species of wild animals were identified as sources: deer or elk, raccoon, coyote, bear, and opossum. However, an unknown fraction of the canine isolates could have been from coyotes and foxes, since many *E. coli* strains exist in both domestic dogs and wild canines.

A very small fraction (3 percent) of the *E. coli* samples isolated from ambient water samples were hemolytic strains that can cause serious illness in humans, but they were found in 29 water samples (15%). Ribotypes indicated that of the 47 hemolytic *E. coli* strains, 21 (45%) were resident in canine hosts, eight (17%) from cats, seven (15%) from human or sewage sources, five (11%) from avian residents, one each from equine and rodent sources, and four were unknown.

The number of *E. coli* strains increased during runoff conditions within each of the species categories, but especially for canine *E. coli*. Dog scat appears to be responsible for about a quarter of the *E. coli* in stormwater flows, as opposed to around 13 percent under non-runoff conditions.

What's the Solution?

Now that the culprits have been identified, state and regional governments are developing action strategies for managing fecal coliform. Individual distribution charts for each of the 30 water sampling locations were developed that will enable water managers to separate what are considered to be natural background sources of fecal coliform—birds and other wildlife—from sources that could be better controlled, such as septic systems, pets, and livestock. Also, outflow from wastewater treatment plants will be better managed to reduce their fecal coliform contributions. An education/outreach program is being developed to educate pet owners and ranchers on best management practices. Although both Albuquerque and Bernalillo County have ordinances limiting the number of dogs at a residence, it is harder to make owners pick up after them regularly on public and private property. Education, it is hoped, will bring attention to and mitigate this health problem.

The 331-page report, "Middle Rio Grande Microbial Source Tracking Assessment Report," prepared by Parsons Water & Infrastructure (Oct. 2005) is available at www.nmenv.state.nm.us/swqb/Rio_Grande/Middle/MST/.