

***Escherichia coli* Source Tracking by DNA Fingerprinting
In
Diverse Watersheds of Northern New Mexico**

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Introduction

The purpose of this project is to identify sources of fecal contamination in surface waters as human or nonhuman by analyzing DNA fingerprints generated using the polymerase chain reaction (PCR) and whole *E. coli* cells. Four stream monitoring sites surrounding the city of Las Vegas, NM support unique watershed characteristics and are currently monitored for fecal coliform concentrations: a section of catastrophically burned forest (Cow Creek near Pecos, NM), a mixed conifer forest (Gallinas River near Montezuma, NM), rangeland prairie (Spring Arroyo NE of Las Vegas) and commercial/urban land use (Gallinas River below Las Vegas).

The forested Gallinas River watershed, upstream from the village of Montezuma, provides 95% of the water supply for 18,000 residents of the City of Las Vegas, and identification of *E. coli* sources as human or nonhuman will allow for better site-specific management practices by the city to better protect the water source. Currently, there are approximately 200 full-time and seasonal residences, a dormitory-style summer camp, several recreational areas and two campgrounds in the watershed, all of which utilize either pit latrines or individual septic systems. There is also a variety of input from nonhuman sources throughout the watershed, including intensive wildlife and livestock grazing in the flood plain.

The Gallinas River downstream from Las Vegas extends approximately 20 miles before joining the Pecos River and flowing to the Santa Rosa Lake State Park, a recreational reservoir. Several small communities reside on land grants and private property near the rivers between Las Vegas and Santa Rosa, using the surface water for irrigation and as a residential water source.

The City of Las Vegas has established separate sanitary and storm water sewer systems to minimize the chance for contamination of the Gallinas River with raw sewage. However, there is still potential for introduction of human waste into the river. Fecal coliform levels are generally much lower in steam baseflow than during storms, unless an

inappropriate sewage discharge is present upstream. Even with separate sewer systems, human sewage can be introduced into surface waters during sanitary sewer overflows (SSOs). Leaks and overflows are common in many older sanitary sewers where capacity is exceeded, high rates of infiltration and inflow occur (i.e., outside waters get into pipes, reducing capacity), frequent blockages occur, or lines are simply falling apart due to poor joints or pipe materials. Power failures at pumping stations are also a common cause of SSOs. The greatest risk of a SSO occurs during storm events; however, little comprehensive data is available to quantify SSO frequency and bacteria loads in most watersheds. The Association of Metropolitan Sewage Agencies (AMSA) estimates that about 140 overflows occur per one thousand miles of sanitary sewer lines each year (1,000 miles of sewer serves a population of 250,000), proportionately, that equates to 10.8 assumed annual overflows for the population of Las Vegas.

Sewage may be introduced into storm sewers via illicit connections by accident or design. The hundreds of miles of storm and sanitary sewer pipes in a community create a confusing underground network of utilities, making it possible for improper connections to go unnoticed. Illegal transient dumping of raw sewage from septage vacuum trucks, recreational vehicles and portable toilets may also be a source of contamination.

About one-fourth of all American households rely on on-site septic systems to dispose of their wastewater, which translates to about 20 million individual systems. After solids are trapped in a septic tank, wastewater is distributed through a subsurface drain field and allowed to percolate through the soil. Bacteria are effectively removed by filtering and straining water through the soil profile, if the system is properly located, installed and maintained. The system may fail when wastewater breaks out or passes through the soil profile without adequate treatment. The assumed rate of septic system failure is reported to range from five to nearly 40% with an average of about 10%. Of special concern are systems that experience location in inadequate soils, poor design, siting, testing or inspection, hydraulic overloading, tree growth in the drain field, old age, and a failure to conduct scheduled cleaning. To identify potentially failing systems, inspectors are trained to consider septic systems that are older than 20 years, situated on smaller lots, service multiple homes or provide seasonal treatment, adjacent to shorelines

or ditches, located on thin or excessively permeable soils, or are close to bedrock or the water table (Schueler, 1999).

There is a potential for all of these sources of contamination to be found throughout the city of Las Vegas and the upper watershed of the Gallinas Canyon. There is also a dispersed agricultural population distributed on the prairie to the east of Las Vegas, which is drained by the Spring Arroyo, a tributary to the Pecos Arroyo that joins the Gallinas River immediately south of Las Vegas and upstream from the sampling location. This population is assumed to depend exclusively on septic systems for their household waste. Therefore, waste from the eastern prairie may be identified at either the Spring Arroyo monitoring site or south of Las Vegas at the Lower Gallinas monitoring site. The burned Cow Creek watershed also supports a scattered population of residences in its upper reaches that depends on septic systems and pit latrines.

Methods

Water samples were collected during an array of environmental conditions. Samples were collected manually during periods of base flow. During storm events, flow based samples were collected using automated sampling equipment established at each site prior to the start of the project. Several samples were collected manually during a period of increased discharge following a severe spring snowstorm.

E. coli concentrations were quantified using IDEXX Colilert® fluorescence and reported as a most probable number (MPN) of colony forming units (CFU) per 100ml (CFU/100ml).

E. coli sources are identified using DNA fingerprinting (ribotyping), which is outsourced to Source Molecular, Inc. in Gainesville, Florida. This process determines if *E. coli* is produced from human or nonhuman sources by analyzing a DNA “fingerprint” from genes that code for ribosomal nucleic acids. A database of these fingerprints allows for comparison of the minute differences between bacterial cells that have adapted to the internal environments of the varying sources. In summary, the company has compiled a database of fingerprints for the variations of cells that have adapted to the antibiotics developed in the human body, and those of many other sources, and can identify these

adaptations in other cells that have experienced the same conditions. Five to seven isolates were examined from each sample that was submitted for testing.

Occasionally, there is no comparable fingerprint in the database to match the cells being tested. These isolates are listed as “indeterminate” and cells from known sources within the specific region where sources originate must be tested to expand the database. Likewise, cells listed as “animal” can be specified if matches are detected from specific nonhuman animal samples (<http://www.sourcemolecular.com/index.html>).

Results

Five water samples were submitted for fingerprinting from the Montezuma Monitoring site (Gallinas River upstream from the Village of Montezuma). Two of these were collected during spring snowmelt and three were collected during storm events in the month of July 04. One of the July samples, while supporting a viable population of *E. coli* at collection (88.20), experienced die off during shipping and contained <3 CFU/100ml upon arrival for testing. This sample was not processed. All isolates processed from this location (n=20) were found to be from Non-Human sources.

Five water samples were submitted for fingerprinting from the Lower Gallinas Monitoring Site (Gallinas River downstream from the City of Las Vegas). Two of these were collected during spring snowmelt and three were collected during storm events in the month of July 04. Twenty-nine isolates were processed from this location. Of these, 25 were found to be non-human, three were indeterminate and one was determined to be from a human source.

Two water samples were submitted for fingerprinting from the Spring Arroyo Monitoring Site (Spring Arroyo in the prairie NE of the City of Las Vegas). These were collected during spring snowmelt and the ten isolates were found to be from non-human sources.

Two samples were submitted for testing from the Cow Creek Monitoring Site (Burned forest in the Upper Pecos watershed). These samples held low, though viable, populations of *E. coli* at collection (5.00 and 10.00); however, upon delivery for DNA fingerprinting, the populations had decreased below 3 CFU/100ml and were not processed for analysis.

There is a difference in the numbers of samples submitted from each site due to the relevance of the Gallinas River for human interaction. Therefore, emphasis was placed on the Montezuma and Lower Gallinas monitoring sites. In the future, New Mexico Highlands University intends to perform DNA fingerprinting on campus. At that time, additional testing may be performed on all locations. This will be more economical and allow for decreased holding time of water samples and consequently, less die off of cells prior to testing..

Montezuma Monitoring Site

Gallinas River Upstream from the Village of Montezuma

Date	CFU/100ml	Isolate #	Flow Conditions	Source
4/06/04	88.80	1	Spring Snow Melt	Non-Human
4/06/04	88.80	2	Spring Snow Melt	Non-Human
4/06/04	88.80	3	Spring Snow Melt	Non-Human
4/06/04	88.80	4	Spring Snow Melt	Non-Human
4/06/04	88.80	5	Spring Snow Melt	Non-Human
4/08/04	119.00	1	Spring Snow Melt	Non-Human
4/08/04	119.00	2	Spring Snow Melt	Non-Human
4/08/04	119.00	3	Spring Snow Melt	Non-Human
4/08/04	119.00	4	Spring Snow Melt	Non-Human
4/08/04	119.00	5	Spring Snow Melt	Non-Human
7/24/04	88.20	N/A ***	Storm Event	N/A
7/24/04	1,989.00	1	Storm Event	Non-Human
7/24/04	1,989.00	2	Storm Event	Non-Human
7/24/04	1,989.00	3	Storm Event	Non-Human
7/24/04	1,989.00	4	Storm Event	Non-Human
7/24/04	1,989.00	5	Storm Event	Non-Human
7/25/04	963.55	1	Storm Event	Non-Human
7/25/04	963.55	2	Storm Event	Non-Human
7/25/04	963.55	3	Storm Event	Non-Human
7/25/04	963.55	4	Storm Event	Non-Human
7/25/04	963.55	5	Storm Event	Non-Human

*** Upon arrival for testing, the sample contained >3 cfu/100ml.

Lower Gallinas Monitoring Site

Gallinas River Downstream from the City of Las Vegas

Date	CFU/100ml	Isolate #	Flow Conditions	Source
4/06/04	260.00	1	Spring Snow Melt	Non-Human
4/06/04	260.00	2	Spring Snow Melt	Non-Human
4/06/04	260.00	3	Spring Snow Melt	Non-Human
4/06/04	260.00	4	Spring Snow Melt	Non-Human
4/06/04	260.00	5	Spring Snow Melt	Non-Human
4/08/04	3,090.00	1	Spring Snow Melt	Non-Human
4/08/04	3,090.00	2	Spring Snow Melt	Non-Human
4/08/04	3,090.00	3	Spring Snow Melt	Non-Human
4/08/04	3,090.00	4	Spring Snow Melt	Non-Human
4/08/04	3,090.00	5	Spring Snow Melt	Non-Human
7/24/04	2,400.00	1	Storm Event	Non-Human
7/24/04	2,400.00	2	Storm Event	Non-Human
7/24/04	2,400.00	3	Storm Event	Non-Human
7/24/04	2,400.00	4	Storm Event	Non-Human
7/24/04	2,400.00	5	Storm Event	Non-Human
8/13/04	48,392.00	1	Storm Event	Non-Human
8/13/04	48,392.00	2	Storm Event	Non-Human
8/13/04	48,392.00	3	Storm Event	Non-Human
8/13/04	48,392.00	4	Storm Event	Non-Human
8/13/04	48,392.00	5	Storm Event	Non-Human
8/13/04	48,392.00	6	Storm Event	HUMAN
8/13/04	48,392.00	7	Storm Event	Indeterminate
8/13/04	2,736.00	8	Storm Event	Non-Human
8/13/04	2,736.00	9	Storm Event	Non-Human
8/13/04	2,736.00	10	Storm Event	Non-Human
8/13/04	2,736.00	11	Storm Event	Non-Human
8/13/04	2,736.00	12	Storm Event	Non-Human
8/13/04	2,736.00	13	Storm Event	Indeterminate
8/13/04	2,736.00	14	Storm Event	Indeterminate

Spring Arroyo Monitoring Site

Spring Arroyo in the Prairie NE of the City of Las Vegas

Date	CFU/100ml	Isolate #	Flow Conditions	Source
4/06/04	86.00	1	Spring Snow Melt	Non-Human
4/06/04	86.00	2	Spring Snow Melt	Non-Human
4/06/04	86.00	3	Spring Snow Melt	Non-Human
4/06/04	86.00	4	Spring Snow Melt	Non-Human
4/06/04	86.00	5	Spring Snow Melt	Non-Human
4/08/04	959.00	1	Spring Snow Melt	Non-Human
4/08/04	959.00	2	Spring Snow Melt	Non-Human
4/08/04	959.00	3	Spring Snow Melt	Non-Human
4/08/04	959.00	4	Spring Snow Melt	Non-Human
4/08/04	959.00	5	Spring Snow Melt	Non-Human

Conclusions

These results suggest that there is no human input of fecal coliform bacteria to surface waters from the upper Gallinas watershed or the Spring Arroyo NE of Las Vegas.

Twenty-nine isolates were tested from the Gallinas River downstream of the City of Las Vegas. Nineteen of these were from increased discharge during storm events. Of these nineteen, one to four isolates were from human origin. This suggests that the City of Las Vegas may be experiencing sanitary sewer overflows or unauthorized drainage during storm events. Further testing of this phenomenon is strongly suggested.

The Cow Creek watershed experiences low levels of *E. coli* bacteria and source testing was not possible with minimal bacteria levels and current funding.

This project may continue under funding from New Mexico Highlands University in the next twelve months, when the university acquires the resources to perform DNA fingerprinting in its on-campus facilities.

Literature Cited

Schueler, T.R. 1999. "Microbes and Urban Watersheds: Concentrations, Sources and Pathways." *Watershed Protection Techniques*. 3(1): 554-565.

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<http://www.sourcemolecular.com/index.html>.