Middle Rio Grande Microbial Source Tracking Assessment Report

Prepared for

New Mexico Environment Department
Albuquerque Metropolitan Arroyo Flood Control Authority
Bernalillo County

Prepared by

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Austin, TX 78754

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EXECUTIVE SUMMARY

This Middle Rio Grande (MRG) Microbial Source Tracking Project was funded by the New Mexico Environmental Department, Albuquerque Metropolitan Arroyo Flood Control Authority, and Bernalillo County. The objective of this project was to identify specific sources of fecal coliform causing high levels of bacteria in the MRG. The three agencies recognized that this type of data was essential to provide water quality program managers and stakeholders the information necessary to target solutions for reducing fecal coliform concentrations/loadings within the surface waters of the study area. The study area lies within the MRG between Angostura Diversion Dam in southeastern Sandoval County to the Isleta Diversion Dam, at the northern border of Isleta Pueblo, a distance of approximately 42 river miles (Figure 2.1).

To identify specific sources of fecal coliform by subwatershed, this project involved several steps:

- Execution of a sanitary survey or reconnaissance tour of the watershed and a compilation of available data and literature to identify potential contributing sources of fecal bacteria to be considered.
- Development of watershed-specific libraries of ribotypes and antibiotic resistance profiles of *E. coli* isolated from fecal matter collected from known sources throughout the study area.
- Collection and culturing of a representative set of *E. coli* isolates from the waterbodies of concern under dry and wet-weather conditions.
- Determination of the ribotypes and antibiotic resistance profiles of these waterborne *E. coli* isolates, followed by matching to those from the known source library to identify the sources of each *E. coli* isolate.
- Quantification of the accuracy and precision of the ribotyping and ARA source determinations.
- Estimation of the relative source contributions of *E. coli* in the MRG watersheds, and the confidence of these estimates, based on the above measurements.

The sanitary survey proved valuable as it provided greater understanding of the diversity of animal species, location, and condition of wastewater infrastructure, and hydrology (pollutant loading pathways) throughout the MRG watershed. Examples of existing data and literature reviews used to identify potential sources of fecal coliform included land use data for each subwatershed from the National Land Cover Database; the number of people and their means of sewage disposal, such as a sewer connection or on-site disposal (septic systems); and estimates of cat and dog populations within each subwatershed using published per household data. Data were not available to determine the quantity of livestock or wildlife within each watershed. Countywide livestock populations were utilized.

Field collection of both ambient water samples and fecal coliform samples from known sources (local library samples) throughout the MRG study area were collected. Eight water sampling sites on the MRG were investigated. These sites are at the same locations where historical water quality sampling was performed. Another 22 sites within contributing subwatersheds were also selected for collection of ambient water

samples. All ambient water and local library samples were collected in accordance with an U.S. Environmental Protection Agency (USEPA) approved quality assurance project plan (QAPP). Two-hundred and six water samples were collected from the 30 sampling stations. Due to the lack of rain, only 10 sampling stations were sampled during both dry-weather and storm water runoff conditions. Concentrations of fecal coliform generally increased from the most upstream station to the most downstream station. By far, the highest fecal coliform concentrations were in surface waters under the influence of storm water runoff. Concentrations ranged from a low of 27 colony-forming units per 100 milliliters (cfu/100 mL) at Angostura Diversion Dam to over 1 million cfu/100 mL at the North Diversion Channel at Roy.

To identify the specific sources of fecal coliform from the water samples collected, this project utilized microbial source tracking (MST) technology. MST is based upon two principles. The first principle is that the bacterial population genetic structure is clonal. Therefore MST makes use of the clonal population structure of bacteria to classify organisms based on their genetic fingerprints into groups of clonal descent. The second principle behind the MST methodology is the assumption that within a given species of bacteria, various members have adapted to living/environmental conditions in specific hosts/environments. As a result, there is a high degree of host specificity among bacterial strains that are seen in the environment.

Knowing that in any watershed, there are multiple, contributing sources of animal microbial pollution, each of which has its own unique clones of bacteria that constitute their normal flora MST was recognized as an effective tool to achieve the goals and objectives of this study. When a strain of bacteria with an identical genetic fingerprint is isolated from both a water sample and a suspected animal source, the animal is implicated as a contributor of that specific strain of the bacteria to the polluted site.

At the outset of the project it was decided that two different MST methods would be used: ribotyping and Antibiotic Resistance Analysis (ARA) to quantify and compare the precision, accuracy, and specificity of ARA and ribotyping. In addition to the usefulness of the direct head-to-head comparison, application of two methods would provide benefits to NMED and stakeholders in other ways. First, application of two independent methods can validate the results, increasing stakeholder confidence in the outcome. Second, because any one method may not perform completely successfully in all samples of a given study, a second method provides back-up to ensure the study will generate useful results. For instance, if an *E. coli* ribotype from a water sample does not match a ribotype from a known source species, ARA may be able to at least indicate whether the source was wildlife, livestock, or human. A third benefit of using two methods is that the results may be more directly compared to other studies, including the 2002 ARA project conducted by the City of Albuquerque.

Although the New Mexico and Pueblo surface water quality standards specify fecal coliform concentration limits, *Escherichia coli* (*E. coli*) was selected for MST analysis because they are exclusively endemic to the mammalian intestinal tract and comprise a substantial fraction of the fecal coliform microbial group. Studies show that the *E. coli* strain that inhabits the intestines of one species is genetically different from the strain that inhabits another. *E. coli* strains collected from water samples are compared to a library

of known *E. coli* strains to determine the contributing species. Development of an *E. coli* strain library for local species was part of this MST project.

E. coli strains were also analyzed using the Antibiotic Resistance Analysis (ARA) method, which subjects the *E. coli* strain to various antibiotics. The resulting profiles are compared to a library of known *E. coli* ARA profiles. A given antibiotic resistance profile is seldom unique to a given animal or source. The ARA method uses statistics to determine the most probable mammal category, such as human, livestock, and wildlife.

The results using ribotyping indicate that a variety of sources appear to contribute to the fecal coliform levels in the MRG, as illustrated in Table E.1 below.

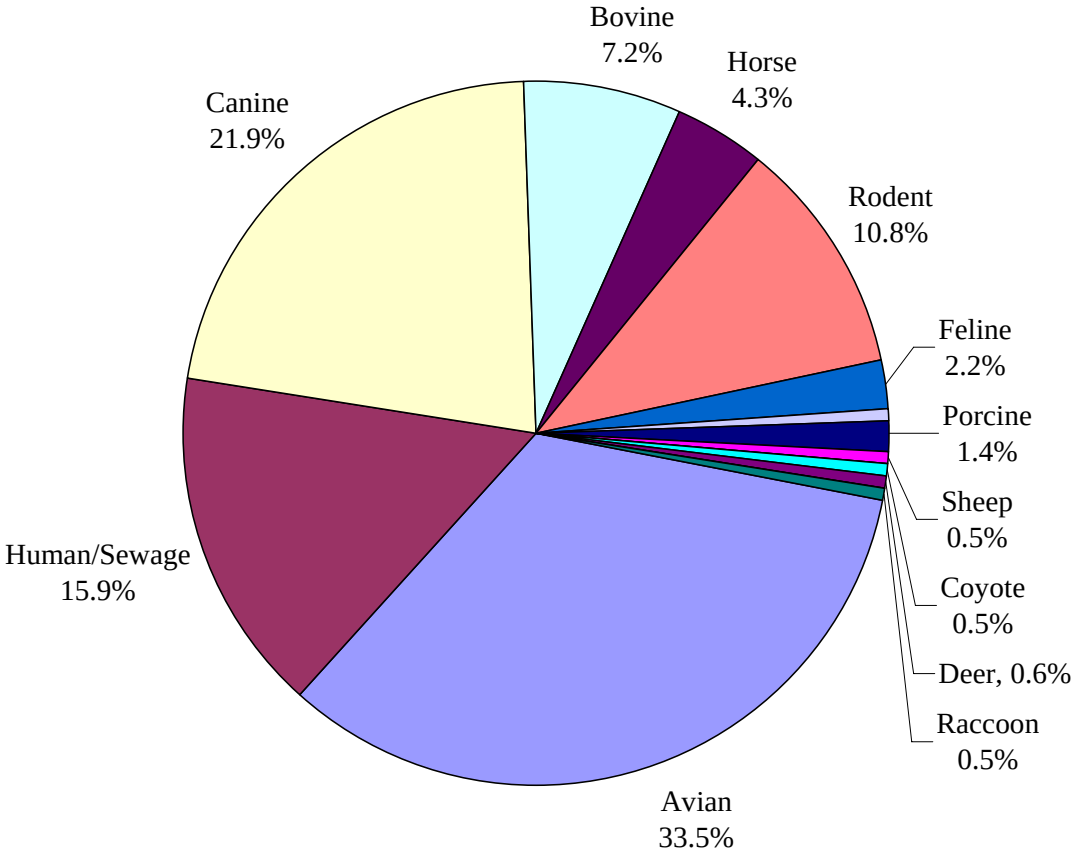
Table E.1 Fecal Source Estimate using Ribotyping for the Entire MRG Project Area

Category	Number of Isolates	% Source Contribution	95% Confidence Interval
Human/ Sewage Subtotal	235	15.9 %	14.0 – 17.8 %
Bovine	106	7.2%	5.9 – 8.5 %
Equine	63	4.3%	3.3 – 5.3 %
Porcine	21	1.4%	0.8 – 2.0 %
Sheep	8	0.5%	0.1 – 0.9%
Goats	5	0.3%	0 – 0.6 %
Livestock Subtotal	203	13.7%	11.9 – 15.5 %
Avian Subtotal	496	33.5%	31.1 – 35.9 %
Canine	324	21.9%	19.8 – 24.0 %
Feline	33	2.2%	1.4 – 3.0 %
Canine & Feline Subtotal	357	24.1%	21.9 – 26.3 %
Rodent	164	10.8%	9.2 – 12.4 %
Deer & elk	9	0.6%	0.2 – 1.0 %
Coyote	7	0.5%	0.2 – 0.8 %
Raccoon	8	0.5%	0.1 – 0.9 %
Bear	1	0.1%	0 – 0.2%
Opossum	1	0.1%	0 – 0.2%
Non-Avian Wildlife Subtotal	190	12.8%	11.1 – 14.5 %
Unknown	139	8.6 %	7.2 – 9.9 %
Grand Total	1620		

Overall, ribotyping results show, the largest fraction of *E. coli* matched those found in avian sources, followed by canine, human/sewage, rodents, bovine, and equine. The source of approximately 9 percent of the *E. coli* could not be identified. With the exception of rodents, only a few species of wild mammals were identified as sources of fecal coliform found in water: deer or elk, raccoon, coyote, bear, and opossum. It should be noted that an unknown fraction of the canine isolates may be from coyotes and foxes, as many *E. coli* strains are resident both in domestic dogs and wild canines. Figure E.1

provides a graphical representation of the percentage contribution of the identified sources of fecal coliform based on ribotyping.

Figure E.1 Sources of *E. coli* in the Entire MRG Study Area using Ribotyping



The percentage contribution of each species will vary with time and conditions. The percentages shown in the table and figure measure the quantity of *E. coli* strains in the water at that particular station and time. The larger the number of *E. coli* strains identified, the more confidence there is that the measured percent contribution is close to the actual percent contribution.

Forty-seven (2.9%) of the 1,635 *E. coli* isolated and purified from ambient water samples were hemolytic strains of *E. coli*. Hemolytic *E. coli* such as strain O157:H7 can cause serious illness in humans. These hemolytic *E. coli* were found in 29 water samples under runoff and non-runoff conditions from 15 sites, and thus generally were in low abundance compared to non-hemolytic strains in all samples. The ribotypes of these hemolytic bacteria indicated that 21 were resident in canine hosts, eight were from cats, seven were from human or sewage sources, five were avian-resident species, one each were from equine and rodent sources, and four were from unknown sources. As a percentage of *E. coli* by source, hemolytic *E. coli* were most abundant in canines (45%), cats (17%), humans (15%), and avian (11%). Only one hemolytic *E. coli* was observed in livestock-resident strains, and few were also observed in wildlife-resident strains.

Table E.2 compares *E. coli* data collected under runoff (storm event) or non-runoff weather conditions. Although not shown in this table, the number of *E. coli* strains

increased during runoff conditions for within each of the species categories. The proportion of *E. coli* relative to the total number increased or decreased for each species. The most striking difference was the proportional increase in canine *E. coli*. Dog scat left in yards, parks, and along sidewalks or trails appears to be responsible for approximately one-quarter of the *E. coli* in storm induced surface waters. It should be noted that wastewater treatment facilities treating human sewage are typically permitted to discharge up to the most stringent applicable water quality standard. Typical permit limits are 100 colony-forming units per 100 milliliter (cfu/100 mL) of water discharge. Dilution by the receiving stream can significantly reduce the discharged concentration.

The scope of this study did not permit field investigations upstream of each sampling station to identify where various sources of *E. coli* were entering the drainage ways or river. Nevertheless, some sources of excessive human E. coli were identified and include inadequately treated wastewater discharges and improperly disposed dirty diapers. Other sources of human *E. coli* that are typical across the U.S. include broken underground sewer pipes that leak into the storm water collection system and sewer system overflows (SSO). Although local authorities have had success in significantly reducing SSOs, it is a constant battle and no large city has completely eliminated them. SSOs are typically caused by a blockage in the pipeline or a pump station failure. Collapsed pipes or tree-roots and grease are common problems. Routine sewer system cleaning of the sewer system has been shown to reduce SSOs and identify potential problem areas. Aggressive enforcement of grease trap ordinances has also been shown to reduce SSOs.

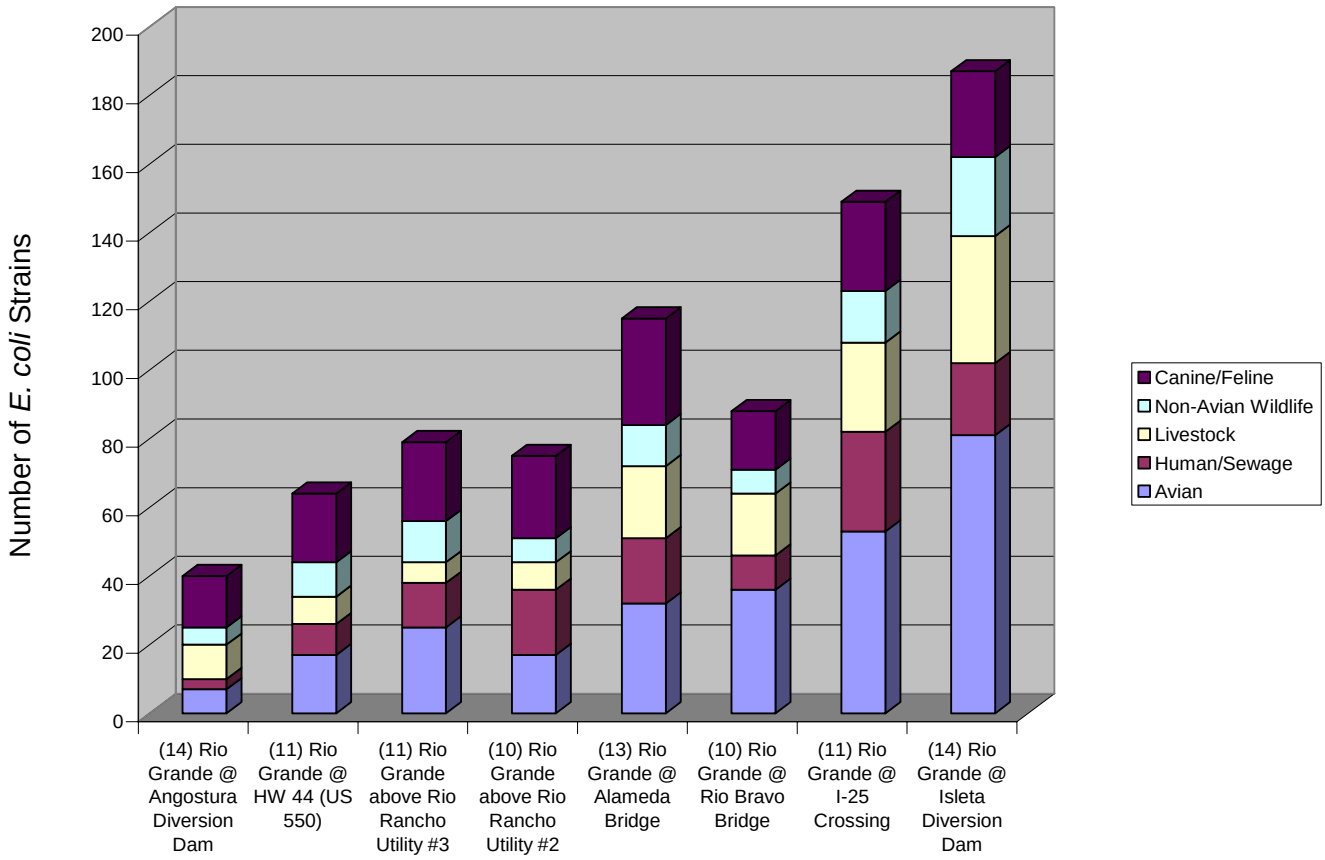
Table E.2 Comparison of Sources under Runoff vs. Non-Runoff Conditions

Category	Runoff		Non-Runoff	
	% Contribution	95% Confidence Interval	% Contribution	95% Confidence Interval
Human/Sewage Subtotal	15.5 %	13.5 – 17.4 %	18.5 %	13.6 – 23.5 %
Bovine	7.2 %	5.8 – 8.5 %	6.9 %	3.7 – 10.1 %
Equine	4.4 %	3.3 – 5.5 %	3.4 %	1.1 – 5.8 %
Porcine	1.2 %	0.6 – 1.7 %	3.0 %	0.9 – 5.2 %
Sheep	0.6 %	0.2 – 1.0 %	0.4 %	0 – 1.3 %
Goats	0.4 %	0.1 – 0.8 %	0.0 %	0 – <1.3 %
Livestock Subtotal	13.7 %	11.9 – 15.6 %	13.8 %	9.4 – 18.2 %
Avian Subtotal	31.9 %	29.4 – 34.4 %	38.8 %	32.6 – 45.0 %
Canine	**23.8 %**	**21.5 – 26.1 %**	**13.4 %**	**9.1 – 17.7 %**
Feline	2.1 %	1.3 – 2.8 %	3.4 %	1.1 – 5.8 %
Canine & Feline Subtotal	**25.9 %**	**23.5 – 28.2 %**	**16.8 %**	**12.1 – 21.5 %**
Rodent	11.1 %	9.5 – 12.8 %	10.8 %	6.9 – 14.7 %
Deer & Elk	0.5 %	0.1 – 0.9 %	1.3 %	0 – 2.7 %
Coyote	0.6 %	0.2 – 1.0 %	0.0 %	0 – <1.3 %
Raccoon	0.7 %	0.2 – 1.1 %	0.0 %	0 – <1.3 %
Bear	0.1 %	0 – 0.2 %	0.0 %	0 – <1.3 %
Opossum	0.1 %	0 – 0.2 %	0.0 %	0 – <1.3 %
Non-Avian Wildlife Subtotal	13.1 %	11.2 – 14.9 %	12.1 %	7.9 – 16.2 %
Unknown	**9.1 %**	**7.6 - 10.7 %**	**3.3 %**	**1.1 – 5.6 %**

The report provides a table like Table E.1 and a pie-chart like Figure E.1 for most of the 30 sampling stations. A table similar to Table E.2 is provided for certain sampling stations with storm water runoff data.

Figure E.2 below graphically shows the proportional contribution of the species categories for the eight main stations and an increasing upstream-downstream trend in the number of *E. coli* collected (Y-axis) at each station.

Figure E.2 Comparison of Fecal Coliform Sources for 8 Monitoring Sites on the Rio Grande



Conclusion

The overall top *E. coli* contributors are wildlife (primarily avian) at 46 percent and pets at 24 percent. These two groups account for 70 percent of the *E. coli* detected in all the water samples. Humans and livestock contributed 16 and 14 percent, respectively.

The estimated percentages of *E. coli* sources are inversely proportional to man's ability to control these sources. *E. coli* contributions from wildlife are the highest, but the most difficult to control, particularly given the large population of waterfowl that frequent the MRG watershed. Pet waste throughout the MRG watershed may be more controllable. Both the City of Albuquerque and Bernalillo County have ordinances limiting the number of dogs at a residence. Nevertheless, it will take a significant behavior change by pet owners to regularly pick up after their dogs on both public and private property.

E. coli contributions from humans are more easily reduced through reduction of sewer system overflows (SSO) and leaks, compliance with wastewater treatment permit limits, and identifying and repairing failing septic systems. SSOs are caused by pipeline blockage and/or lack of adequate pipeline capacity. A wastewater conveyance system capacity management and operations and maintenance program was implemented by the City of Albuquerque in the 1990s which significantly reduced SSOs. Improvements or plans for improvements to the Rio Rancho area wastewater treatment facilities are unknown at this time. Bernalillo County passed an ordinance requiring all residences or businesses using a septic system within 200 feet of a sanitary sewer to connect to the sewer. Three-thousand new sewer connections have been made since 1990.

Permitted concentrated animal feeding operations (CAFO), such as dairies, are required to capture manure and water in contact with the manure. Three medium size dairies are located in the Isleta Drain watershed. The bovine *E. coli* contribution detected at the Isleta Drain sampling station was much less than the average bovine contribution at all the sampling stations. Therefore, CAFOs may not be the major source of bovine *E. coli*. Some residential areas do have significant livestock populations – predominantly horses and cattle. While there are limitations on the number of head per acre by local ordinance, manure management at these facilities is not specifically regulated.

This study has accomplished the state intent. As a result of this study, sources of fecal coliform in the MRG and in the contributing subwatersheds have been documented. The results of this study will enable water resource managers and stakeholders in the region to undertake appropriate, geographically specific control actions and management measures to improve storm water and surface water quality.

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ACRONYMS AND ABBREVIATIONS

µL	Microliter
4Q3	Minimum 4-day, 3-year discharge
AMAFCA	Albuquerque Metropolitan Arroyo Flood Control Authority
ARA	Antibiotic Resistance Analysis
ARP	Antibiotic Resistance Profile
BISON-M	NM Biota Information System
BMP	Best Management Practice
CAFO	Confined Animal Feeding Operation
CFU	Colony-Forming Unit
CFR	Code of Federal Regulations
CMOM	Capacity Management, Operations, and Maintenance
CWA	Clean Water Act
DNA	Deoxyribonucleic Acid
DQO	Data Quality Objective
dH_2O	Distilled water
LA	Load Allocation
MAL	Minimum analytical level
MEI	Molecular Epidemiology, Inc.
mFC	A nutrient agar (growth medium) used in the isolation and enumeration of fecal coliform bacteria
MOS	Margin of Safety
MRG	Middle Rio Grande
MS4	Municipal Separate Storm System
MST	Microbial Source Tracking
NM	New Mexico
NMAC	New Mexico Administrative Code
NMED	New Mexico Environment Department
NPDES	National Pollutant Discharge Elimination System
PCS	Permit Compliance System
QA/QC	Quality Assurance/Quality Control
QAO	QA Officer
QAPP	Quality Assurance Project Plan
RFLP	Restriction Fragment Length Polymorphism
rRNA	ribosomal Ribonucleic Acid
SDS	Sodium dodecyl sulfate
SSO	Sanitary Sewer Overflow
SWMP	Storm Water Management Program
SWQB	Surface Water Quality Bureau
TMDL	Total Maximum Daily Load
USEPA	U.S. Environmental Protection Agency
USGS	United States Geological Survey
WLA	Wasteload Allocation
WQA	Water Quality Act

WQCC	Water Quality Control Commission
WQS	Water Quality Standards
WWTF	Wastewater Treatment Facility