LITTLE SAC RIVER WATERSHED

BACTERIAL SOURCE TRACKING ANALYSIS

FAPRI-UMC Report #06-05
May 2005

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Published by the Food and Agricultural Policy Research Institute (FAPRI), University of Missouri-Columbia, 2005
http://www.fapri.missouri.edu

This material is based upon work supported by the Environmental Protection Agency, region VII through a grant entitled “DNA Source Tracking of Fecal E. Coli in the Little Sac River, Missouri,” (grant X7-98740401-0). Any opinions, findings, conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Environmental Protection Agency.

The Food and Agricultural Policy Research Institute at the University of Missouri (FAPRI) is charged with providing objective, quantitative analysis to decision makers. Since 1984, this service has been provided to Congress and national trade associations, and has focused on commodity policy issues.

In 1995, the unit was asked to expand its focus and begin to bring the same level of effort to environmental issues, that of providing objective, analytical support. The unit spent considerable time examining the problems and determined the area most lacking analysis was at the local level; the farm, the watershed, and the local community.

Similar to the extensive peer-review effort the unit goes through on national commodity policy issues, the environmental analysis effort recognizes the strong need for local involvement. If the local people who must live with the analysis have doubts about the way the analysis was developed, then the effort is wasted. Consequently, the process FAPRI brings to the table also incorporates extensive local input.

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EXECUTIVE SUMMARY

Data collected from November 2003 to October 2004 at two sites on the Little Sac River show that the whole body contact water quality criteria was not met during this period. The year was divided in four periods: winter, spring, summer, and fall. Averages and geometric means were above the 200 colonies/100 ml for any of the winter, summer, and fall periods at both sites.

Dr. Charles Carson, professor of veterinary pathobiology at the University of Missouri directed the laboratory analyses of fecal material using repetitive extragenic palindromic polymerase chain reaction (rep-PCR) processing techniques to identify the sources of the bacteria found in the water. The data show that the highest fecal coliform loads come from unknown sources, geese, and human.

Data were analyzed by season and by flow condition. While there is a significant difference in the range of the fecal coliform concentrations between base flow and storm flow conditions, the sources of the contamination are similar. On the other hand, the sources of fecal contamination in winter are significantly different from what they are during the recreation season. The magnitude of the contamination is not significantly affected by the season.

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Background Information

The Little Sac River watershed spans both Greene and Polk Counties and covers a total of 643.7 km^2 (400 mi^2) miles (Figure 1). The river originates north of Springfield. It flows northwest 72.4 km (45 mi) to Stockton Lake, a water source for the city of Springfield. State Highway 13 runs north and south on the east side of the watershed.

Elevations in the watershed range from 270 m (885 ft) at the watershed outlet to 455 m (1490 ft) at the southeastern boundary. The major part of the watershed consists of rolling plains. On the east side, broad upland areas exist that divide the Little Sac watershed from the Pomme de Terre watershed.

The Little Sac River is used for:

- livestock and wildlife watering,
- protection of warm water aquatic life and human health associated with fish consumption,
- cool water fisheries,
- whole body contact (swimming), and
- boating and canoeing (MDNR, 2002).

Fecal coliform *Escherichia coli* (fecal *E. coli*) interferes with use of the Little Sac River as a place for recreational whole body contact or swimming. The 1998 303(d) list reported that 27 river-miles of the Little Sac River as impaired by fecal coliform (Figure 2). The 2002 303(d) recommendations included changing the impairment to 29 river-miles and the source of impairment to point and non-point sources (MDNR, 2002). The fecal coliform organism is used as an indicator of the presence of dangerous bacterial and viral pathogens.

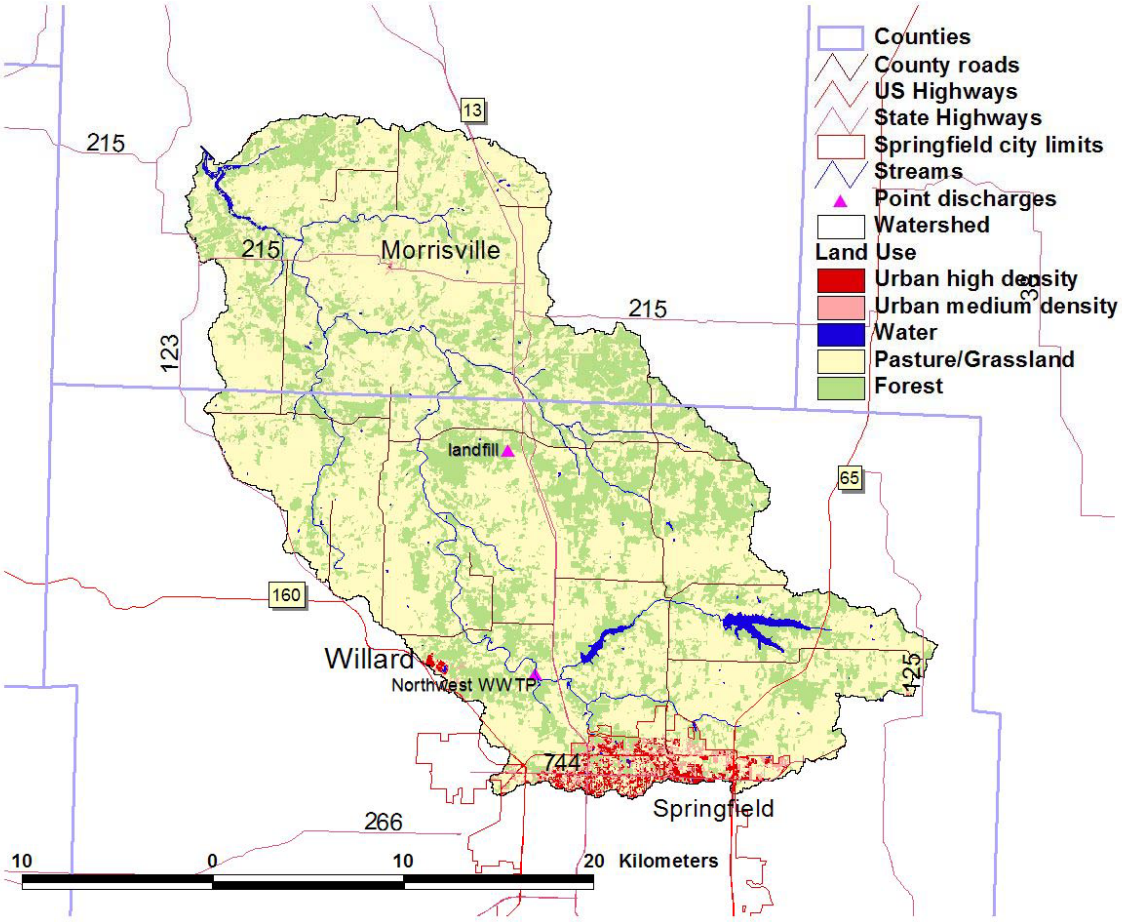

Figure 1. Little Sac River Watershed

The goal of this project is the identification of the main sources of the fecal *E. coli* impairment in the watershed through bacterial source tracking. Fecal coliform can originate from every land use in the Little Sac River drainage area including agriculture, recreation, and suburban development. Five most probable bacterial sources—cattle, horse, septic sewage, goose, and sewage plant waste—were selected after consulting a watershed group composed of concerned farmers, livestock producers, and other citizens.

Area Characteristics

Karst Terrain

Karst features such as sinkholes, caves, losing streams, and springs are present throughout the watershed. Karst results from acidic water percolating through limestone or dolostone. The water dissolves the carbonate bedrock forming open spaces in the rock. Karst terrain provides direct connections for surface water to groundwater. Karst connections also link septic systems to ground water (Waite and Thomson, 1993)

Sinkholes are subsidence of the surface, generally in areas with shallow soil surface cover. Waite and Thomson (1993) included 2,500 sink holes in their Greene County study. The sinkholes ranged from 3 m (10 ft) diameter to 445 ha (180 ac) and anywhere from 3 m (10 ft) to 148 m (60 ft) deep. Sinkholes often provide the major access to caves.

A losing stream has many cracks, small openings, or sink-points in its bed and banks that allow water to directly recharge the ground water aquifer. Some losing streams can spend a large part of the year as a dry stream channel, only flowing when the subsurface karst system backs up and overflows during major storm events. When these types of streams flow, they can contribute major sources of sediment, bacteria, organic material, and other debris that are readily transported underground. Water that enters the groundwater aquifer through sinkholes and losing streams often resurfaces at springs.

Springs represent the surface emergence of ground water. The ease in which water travels through karst terrain causes rapid movement of water and any pollutants it carries between the surface and the ground water (Waite and Thomson, 1993). Figure 2 shows the sinkholes, losing streams, and springs identified in the Little Sac River watershed.

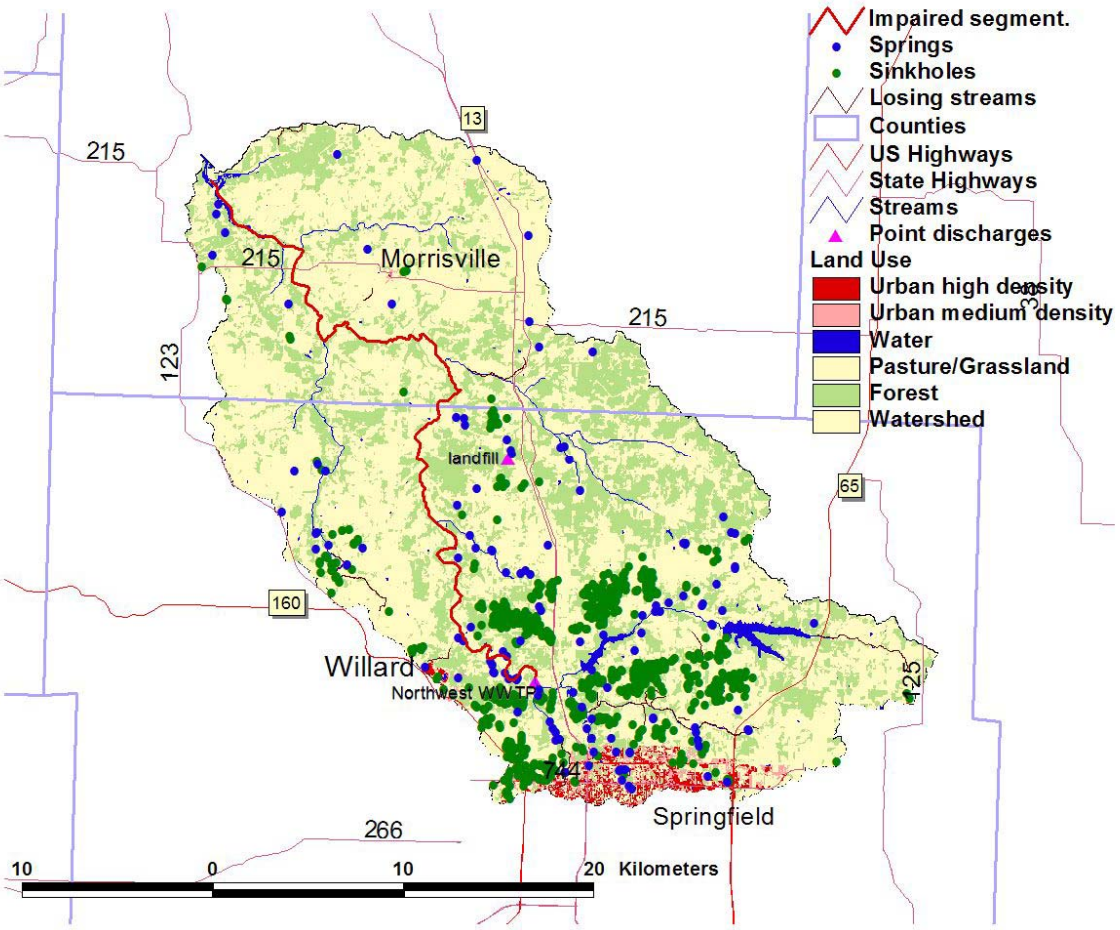

Figure 2. Springs, sinkholes, and losing streams in the Little Sac River watershed

Land Use and Potential Sources

A review of potential sources helped determine what host sources to include in the database of DNA patterns used to identify *E. coli* sources. Agricultural production is the primary land management enterprise of this watershed. Grassland covers 66.8 percent of the area and forests cover 30.2 percent. The remaining land is surface water (0.6 percent) and residential areas (2.4 percent) (Figure 1). Farms in Greene and Polk counties range from traditional cow-calf operations to vegetable farms producing crops such as broccoli, sweet corn, and hot peppers. In 2002, Polk and Greene Counties ranked 55th and 88th, respectively, out of 3,078 counties nation-wide in acres of land used for forage harvested. Polk County reported the second largest hay harvest in Missouri and ranked first for cattle and calves. Greene County ranked 11th for cattle and calves. (Missouri Agricultural Statistics Service, 2003).

Livestock

Livestock in the Little Sac River Watershed include mainly beef cattle. The "cattle and calves" 2002 census for Polk County was 109,365 and Greene County reported 73,560. These animals consume biomass and produce manure that is deposited on the grass.

A weighted average based on the fraction of watershed area in Greene and Polk counties and acreage of grassland is utilized to estimate the number and types of agricultural operations and animals in the watershed. Greene and Polk counties agricultural facts adjusted for the size of the Little Sac River watershed indicate that there were about 9,296 and 21,700 cow/calf pairs in the portion of Polk and Greene counties in the watershed, respectively, in 2002.

Horses.

In the 2002 U.S. agricultural census, Missouri ranked second in the country, behind Texas, in horse population (USDA). The census ranks Greene County 2nd in the state with 3,789 horses and Polk County 6th with 2,824 horses. These are horses on farms defined as entities reporting a net agricultural income greater than $1,000. Greene and Polk counties agricultural statistics adjusted for the size of the Little Sac watershed indicate that 2,235 horses live in the watershed (Missouri Agricultural Statistics Service, 2003). Except for a few larger ranches in Greene County, small numbers of horses are found on any one farm. These animals are here year-round.

Septic tanks.

The 2000 census (U.S. Census Bureau) indicates that there were 11,183 and 104,517 housing units in Polk and Greene counties, respectively. Out of these, 89 percent and 93 percent are occupied in Polk and Greene counties, with an average of 2.5 and 2.4 people per household, respectively. Excluding the households in Springfield (69,650) and Willard (1,226), because both towns have a waste water treatment system, that leaves 31,299 occupied households in Greene County that, in all likelihood, have a septic tank. Assuming that the distribution of units that use a septic tank is uniform across the rural areas of Polk and Greene counties, the number of septic tanks of occupied housing units in the Little Sac River Watershed is estimated to be 1, 691 and 18,466 in Polk and Greene counties, respectively.

Wildlife

Wildlife in the Little Sac River watershed includes many species, most of them difficult to inventory. There is no wildlife inventory at the county level in Missouri. Only one set of patterns from wildlife were included in the DNA source-tracking database: migratory geese. Estimates from the Missouri Department of Conservation about waterfowl population and densities in 2004 can help quantify the Canada goose population. Their density around Springfield is thought to be medium (Brad Jump, personal communication) and equal to 2.15 geese per square miles in the spring of 2004 (Raedeke, and Graber, 2004). A small winter population of resident Canada geese exists that is difficult to estimate.

The Northwest waste water treatment plant.

The Northwest waste water treatment plant (WWTP) has had occasional problems with disinfection of its wastewater that have led to the discharge of large amounts of bacteria into the Little Sac River. Under normal conditions, the plant discharges low levels of bacteria. The permit includes fecal coliform daily maximum limits of 400 colonies per 100 ml for a design flow of 23,700 m^3/d (6.4 million gallons per day), or 0.274 m^3/s (9.67 ft^3/s). During the last three years, the average actual fecal coliform concentration reported by the plant between April 1 and October 31 is lower: less than 10 colonies/100ml. Data collected from April to June 2004 show an average effluent concentration of 72 colonies/100ml.

Climate

Two weather stations characterize the watershed: the Bolivar station in Polk County and the Springfield station located at the Springfield airport in Greene County. Bolivar received an average annual precipitation of 113.9 cm (44.8 in) from 1960 to 2003. Sixty-four percent, 72.5 cm (28.5 in), fell during the growing season April to September. The Springfield Airport received an average annual precipitation of 116 cm (45.8 in) from 1960 to 2003. Sixty-two percent, 71.9 cm (28.3 in), of the average precipitation fell between April 1 and October 31.

Methodology

Sampling

After a tour of the watershed and consideration of the locations of the United States Geological Survey (USGS) flow gauges and the sampling points for a 319 project conducted by the Watershed Committee of the Ozarks, two sampling points were selected. These are indicated on the watershed map (Figure 3). The stream at these points is well mixed and easy to access. The upstream point helps characterize the impact of the upstream urban areas and the waste water treatment plant. Water is collected from the bridge on Farm Road 129 (FR 129) 1 mile downstream of the Northwest Springfield waste water treatment plant. The location is accessible under all weather conditions. The other point characterizes the whole watershed and is downstream of several known swimming/wading areas. The water is collected from the bridge on Route 215 (RD 215), about 2 miles west of Morrisville. This site is also accessible under all weather conditions. Both locations correspond to sampling points for the 319 project. The collection of water samples started in November 2003 at these two sites.

A continuously recording streamflow USGS gauging station is maintained at a site approximately 0.25 river miles downstream from the bridge on RD 215 (station 06918740). Continuous streamflows have been made since September 1968. The Missouri Department of Natural Resources (MDNR) has monitored *E. coli* concentrations at this U. S. Geological Survey gauge station in 1999-2000. U.S. Geological Survey monitored both fecal coliform and *E. coli* there in 1999-2000 (Smith, 2002). FR129 is one sampling point in a current study conducted by the Watershed Committee of the Ozarks.

Because the part of the river that is impaired by high bacteria counts is not a losing stream, the whole body contact water quality standard currently applies April 1 to October 31. Samples were collected only once a month in November and December 2003 and in January and February 2004. From the first Tuesday in March to the last Tuesday in October 2004, river water samples were collected once a week from both locations, yielding 52 samples. Samples were also collected in a non-systematic way at the outlet of the City of Springfield's Northwest Wastewater Treatment Plant (WWTP).

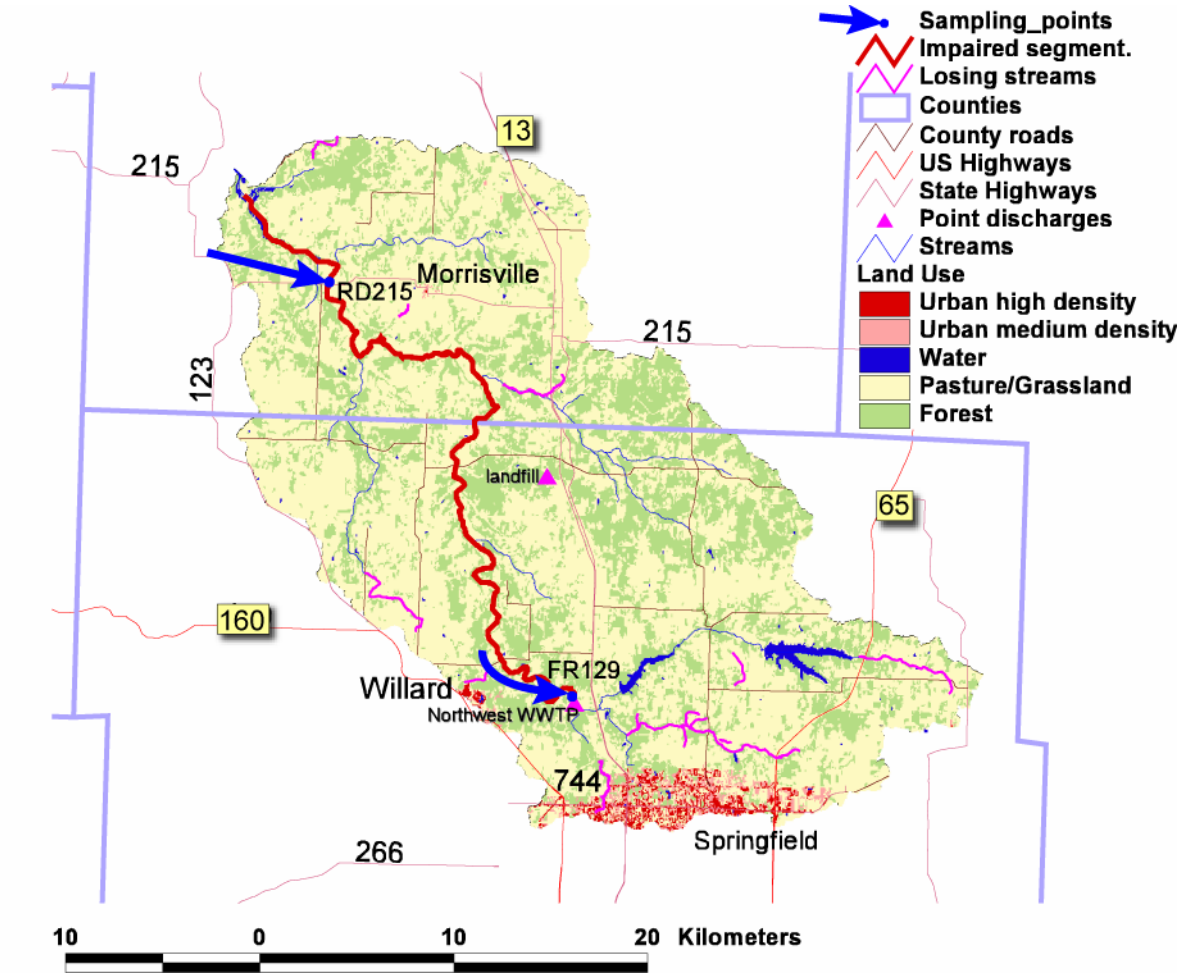

Figure 3. Sampling points on the Little Sac River

Sample Collection and Analysis

A 14.2 L (15 U.S. qt) bucket was lowered from the center and upstream side of each bridge and rinsed three times before sampling. A sterile 500 ml (16 oz) bottle was filled with water drawn with that bucket. The river water samples were placed in a cooler with ice-substitute packs and immediately driven to the water quality laboratory at the University of Missouri - Columbia to be processed no later than 6 hours after collection, as determined by EPA guidelines. Thirty-eight samples were collected at each site, twenty-nine samples from April 1 to October 31, 2004.

Using a dilution method, three volumes of river water (0.5 ml, 5 ml, and 50 ml) were extracted from the bottle and diluted to form 50 ml samples. These samples were filtered and cultured overnight on selective media at 44.5 °C. After incubation the colonies on the plates were

counted with the naked eye and the count corrected to reflect the number of fecal coliform colonies in 100 ml of water. The counts from the three dilutions were averaged.

Because the bacterial source tracking is based on fecal *E. coli*, not fecal coliform, several steps are taken to isolate the fecal *E. coli* colonies. Correct identification of fecal *E. coli* isolates was assured by the use of a battery of biochemical tests with results of growth scored by an automated reader. Final confirmation of isolates as fecal *E. coli* was accomplished with a BBL Crystal Identification Systems Enteric/Nonfermenter system (Becton Dickinson) with indole and oxidase tests.

Bacterial Source Tracking

All water samples sent to the University of Missouri were processed for bacterial source tracking. This technique predicts the source of the contamination by linking the DNA of the bacteria isolated from the environmental samples to the DNA database/library of known host fecal *E. coli* isolates. The method relies on the fact that each animal species hosts unique strains of fecal bacteria that are adapted to the intestinal characteristics and the diet of that particular host.

Confirmed *E. coli* isolates (single *E.coli* strains) were selected for repetitive extragenic palindromic polymerase chain reaction (rep-PCR) processing. The DNA were extracted from the bacteria cells. Primers were then used to target specific DNA sequences. PCR amplified these small amounts of genetic information and obtained large numbers of copies of this DNA sequence from the isolates. PCR can rapidly amplify a single DNA molecule into many billions of molecules. The amplified DNA products were then separated using electrophoresis (Hager, 2002). The genetic material is loaded into a gel that is exposed to an electric field. The various sizes of negatively charged fragments of DNA move toward the anode, resulting in the formation of a bar-code-like pattern. This pattern represents the genomic "signature" of each associated *E. coli* isolate. The large amount of genetic material obtained with PCR enables the visualization of these bands. The details of the rep-PCR methodology are specified in Carson et al. (2003) and Dombeck et al. (2000).

Gel images of DNA fingerprints were captured with a Kodak EDAS 290 system (Kodak Co., Rochester, NY). Fingerprint patterns were analyzed with Bionumerics software, version 3.0 (Applied Maths, Kortrijk, Belgium), using rep-PCR bands between 300 bp and 10.0 Kb (Carson et al., 2003). The pattern analysis software also compensates for variation within the gel by normalization, background measurement, and spectral analysis. These latter functions compensate for error related to gel-to-gel variation.

Figure 4 shows fingerprints obtained from a water sample. A variety of different patterns are detectable visually–putatively indicative of the various sources of pollution. The number of times particular host associated patterns are represented is related to the relative contribution of the corresponding pollution source. DNA size markers are in the two outside lanes.

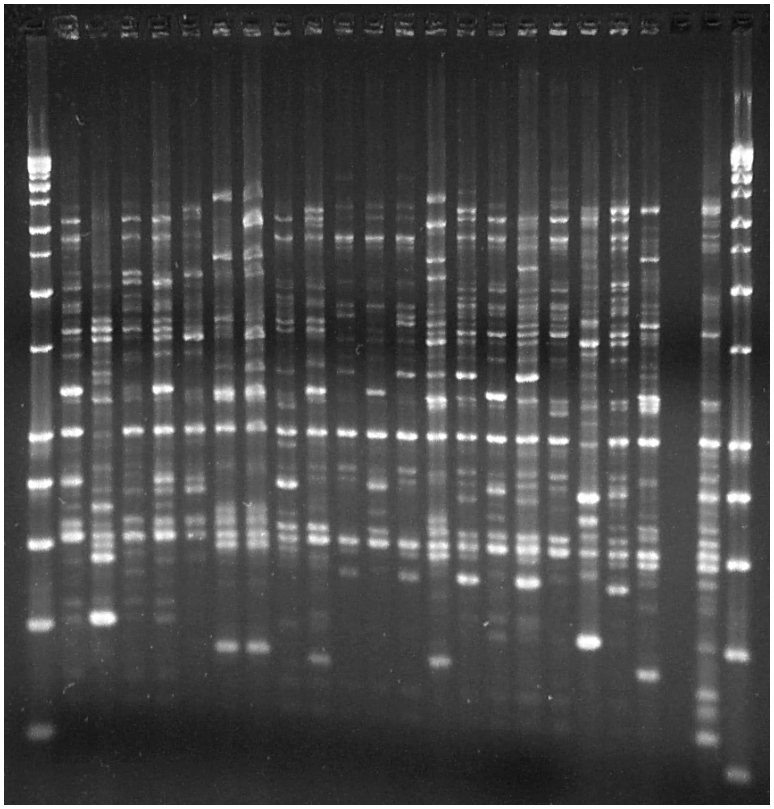

Figure 4. Electrophoresis gel image of fecal *E. coli* DNA patterns in a Little Sac River water sample.

Unknown patterns of *E. coli* isolated from water samples were associated with a host source by comparison with library patterns of known-host isolates (cow, horse, sewage, septic, and geese).

DNA Database

To realize the identification of the Little Sac River's fecal *E. coli* sources, fecal material from within the watershed was first collected. The establishment of a locally representative library of known-host DNA patterns requires 200-300 individual patterns or isolates per host class. Ten grams of fecal material or a 500 ml sewage sample serve as the source of 20 patterns/isolates. A steering committee of watershed residents established to assist with the Little Sac River TMDL project proposed cattle, horses, migratory geese, septic tanks, and sewage as the probable main sources of local fecal contamination. With the help of employees from the Natural Resources Conservation Service (NRCS), landscape samples were collected, analyzed, and processed to build a database specific to this watershed. Fresh fecal samples were collected and double bagged in resealable plastic bags or, in the case of the septic tank, a 500 ml bottle. The samples were transported in a cooler with ice-substitute packs by over-night mail or hand-delivered to the water quality laboratory at the University of Missouri - Columbia. Raw sewage entering Springfield's Northwest WWTP and sewage effluent exiting the plant were sampled from November 2003 until the end of June 2004 during the river water sampling trips.

Recent research results (Ritter and Robinson, 2004) showed it is important that each host class be represented by the same number of fecal *E. coli* isolates. To achieve this, we selected an equal number of isolates from the cattle, goose, and horse feces samples, from the septic tank samples, and from the treatment plant outflow samples. Table 1 shows the number of isolates available and included in the database.

Between 7 and 20 isolates were cultured from each water sample and processed to obtain DNA patterns. Using pattern recognition software, the method estimated the similarity between the unknown patterns and the patterns in the database. Even though the software always matches the unknown pattern with a known pattern, a threshold of 80 percent similarity between the

patterns of unknown origin and a database pattern and quality grades of a, b, or c were selected to determine the host of an *E. coli* colony. Patterns that could not be associated to any host class with sufficient certainty were qualified as unidentified or "others." The causes for uncertainty of the host class for these isolates include insufficient library size, missing potential sources in the database (rodents, dogs, etc.), or technological errors in processing the isolates.

Table 1. Number of isolates included in the Little Sac database

Host	Number of samples	Number of isolates	Number of isolates included
Cattle	10	227	206
Horse	9	228	209
Septic tanks	7	198	198
Migratory goose	9	209	209
Northwest Waste Water Treatment Plant	40	355	207

Flow Data

Flows values are needed for the purpose of differentiating base flow conditions from storm events. Flows are recorded on a continuous basis at the USGS flow gauge close to RD 215 and the data have been obtained from the USGS web site. These data are not yet the official flow data from USGS because they have not gone through the quality control process. This does not have any impact in this situation because we only seek to characterize the flow type on the sampling days.

The USGS HYSEP method (Sloto and Crouse, 1996) adapted to a spreadsheet program (Pettyjohn and Henning, 1979) was applied to the daily flow values to separate hydrographs into surface runoff and baseflow. The Little Sac River is a stream that is mostly fed by groundwater flow. On average from 1969 to 2003, the average annual ratio of base flow to total flow of the Little Sac River was 52 percent. Using that ratio, by definition storm flow conditions existed when more than 48 percent of the stream flow came from storm flow. During base flow conditions, 52 percent or more of the flow was from groundwater. This resulted in the definition

of nine storm events during which samples were collected. Flow values at RD215 and flow conditions are included in Table A.1 in Appendix A.

Results

Fecal *E. coli* concentrations

The fecal coliform concentrations vary mostly between 100 and 2000 colonies/100ml. Concentrations higher than 2000 colonies/100 ml are frequently associated with increased flow, even when the flow increase is small or moderate. The total fecal *E. coli* concentration at FR129 fluctuated between 85 colonies/100 ml on June 6, 2004 and 14,800 colonies/100 ml on October 12, 2004. During the same time period, at RD 215 the total fecal *E. coli* concentration ranged from 11 colonies/100 ml on February 17, 2004 and 5,200 colonies/100 ml on August 24, 2004. On March 9, 2004, the concentration measured at FR 129 was 12,000 colonies/100 ml.

Average bacteria concentrations for the two monitoring sites are summarized in Tables 2 and 3. To analyze the temporal variations, four seasons were defined: winter, November 1 to March 15; spring, March 16 to June 15; summer, June 16 to August 15; and fall, August 16 to October 31. The values in Tables 2 and 3 show that the bacteria limit for whole body contact recreation waters was exceeded in 2004, for any of the seasons.

Statistical analyses (t-test and f-test) show that there is no significant difference in the average fecal coliform concentrations of each site. However, the range of values measured at the site closer to Springfield is larger than it is at the downstream site. Statistical analyses also show that there are no significant seasonal differences in the fecal coliform concentrations between the two sites.

Table 2. Fecal coliform concentrations in the Little Sac River at Farm Road 129, by season

	November to mid-March	Mid-March to mid-June	Mid-June to mid-August	Mid-August to October
Maximum	12000	2700	850	14800
Minimum	90	85	160	210
Mean	2632	478	456	2089
Standard deviation	4642	676	275	4249
Geomean	797	314	383	951

Table 3. Fecal coliform concentrations in the Little Sac River at Road 215, by season

	November to mid-March	Mid-March to mid-June	Mid-June to mid-August	Mid-August to October
Maximum	1700	2200	1120	5200
Minimum	11	84	230	180
Mean	369	500	490	1368
Standard deviation	663	592	312	1951
Geomean	99	312	423	625

Influence of Dry and Wet Weather

Figure 5 shows the fecal coliform concentrations measured at each site along with the flow values measured at the USGS gauge. For reasons of scale, the two highest fecal coliform concentrations are not shown.

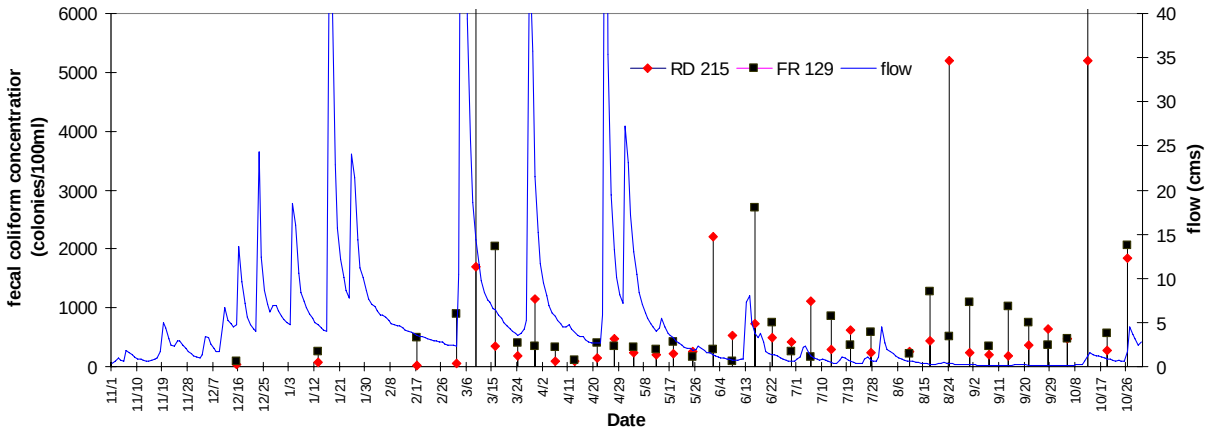

Figure 5. Weekly fecal *E. coli* concentrations in the Little Sac River

The two highest concentrations at FR 129 are obtained on March 9 and October 12 during or just after storm events that followed a dry period. The October 12 event was modest but nevertheless produced the highest fecal coliform concentration recorded during this study at that location, possibly because it followed six weeks of dry weather. The two highest concentrations at RD215 were recorded on October 12 and August 24. A very small rise in flow values from August 20 to August 24 indicates some increased contributions through groundwater.

While the lowest fecal coliform concentrations were obtained during base flow conditions at both sites, some high concentrations were also obtained when the proportion of surface runoff cannot explain the high values. On August 17, for example, the fecal coliform concentrations were 1280 and 430 colonies/100ml at FR129 and RD215, respectively. The previous event was on July 31 and the flow on August 17 was, therefore, entirely from groundwater contributions. High concentrations under similar flow conditions were also measured on August 31, September 14 and 21. Results relative to flow conditions are presented in Tables 4 and 5. They point out the similarity of the contamination at each site under base flow conditions during the recreation season.

Table 4. Average fecal coliform concentrations in the Little Sac River at Farm Road 129, by flow condition

	All-year storm flow conditions	April-October storm flow conditions	All-year base flow conditions	April-October base flow conditions
Maximum	14,800	14,800	2,050	1,280
Minimum	320	320	85	85
Mean	3,741	3,046	529	482
Standard deviation	5,585	5,267	437	336
Geomean	1,309	1,152	394	379

Table 5. Average fecal coliform concentrations in the Little Sac River at Road 215, by flow condition

	All-year storm flow conditions	April-October storm flow conditions	All-year base flow conditions	April-October base flow conditions
Maximum	5,200	5,200	2,200	2,200
Min	230	230	11	84
Mean	1,863	1,990	372	442
Standard deviation	1,979	2,260	428	456
Geomean	1,072	995	232	325

Fecal *E. coli* Sources

The contribution of each potential source is indicated by the relative presence of that particular pattern in the total array of water isolates and expressed as a percentage. DNA analyses of the samples determine what proportion of fecal coliform comes from each source: sewage, cattle, horses, septic tanks, and migratory geese. By prorating these percentages to the concentrations of fecal coliform in the water samples, the contribution from each source is determined. Results are presented for each water sample in the Appendix B.

Results have been analyzed by season and flow conditions. Seasonal variations of the percentages of isolates identified in each host class are summarized in Tables 6 and 7 and illustrated in Figures 6 and 7. When taking all the samples into consideration, the proportions of isolates associated with the WWTP are 16 percent for the upstream site (1 mile downstream of

the WWTP) compared to 13 percent for the downstream site. At the upstream site, 15 percent of the isolates are associated with geese; they represent 27 percent of the isolates at the downstream site. The unexpected result is in accord with results obtained in other watersheds where geese are abundant. Cattle and horses represent 9 and 7 percent respectively of the isolates at the upstream site, and 14 and 10 percent at the downstream site. Very few isolates are associated with septic tank effluent (2 percent at each site). The largest source is categorized as unknown others: 51 percent at the upstream site and 34 percent at the downstream site.

Table 6. Percentage of isolates identified in each host class at Farm Road 129, by season

	Cattle	Horses	Geese	Sewage	Septic	Others
November to mid-March	4%	8%	1%	27%	2%	59%
Mid-March to mid-June	12%	9%	16%	19%	4%	40%
Mid-June to mid-August	9%	5%	30%	11%	1%	44%
Mid-August to October	7%	4%	15%	10%	2%	62%

Table 7. Percentage of isolates identified in each host class at Road 215, by season

	Cattle	Horses	Geese	Sewage	Septic	Others
November to mid-March	10%	20%	10%	14%	1%	46%
Mid-March to mid-June	13%	10%	27%	17%	3%	31%
Mid-June to mid-August	14%	10%	38%	13%	2%	22%
Mid-August to October	18%	4%	31%	9%	2%	36%

The results are consistent with the location of the sites and the surrounding land use: proportionally more WWTP isolates and less cattle and horse at the upstream site. Chronologically, we also see more WWTP associated isolates at the upstream site in the winter (mid-November to mid-March) when the effluent is not disinfected. After April 1, less WWTP associated isolates are detected. At the downstream site, the seasonal variations of the sewage contribution are not significant. Cattle and horses contribute evenly through the year and in

similar amounts in spite of a cattle population that is larger than the equine population. Finally, the proportion of isolates that cannot be matched with one of our databases is higher upstream than it is downstream. It is also higher in winter than during the recreation season.

While at the upstream site there is a statistically significant difference of the percentage of isolates associated to sewage in winter compared to any of the other seasons, there is no seasonal variation at the downstream site. This could indicate that there are two sources of bacteria associated with sewage. One is the treatment plant outflow and is found to be significant in winter at FR 129. By the times the flow arrives at RD215, the bacteria have decayed and are not detectable anymore. The unidentified other source or sources contribute bacteria associated to sewage during all seasons, all over the watershed, and in similar proportions. Hypotheses include illegal discharges throughout the watershed, leaks from the Springfield sewer system, and the contamination of springs and groundwater. It is also possible that the bacteria are from malfunctioning septic tanks, although, given our database they were not recognized as such.

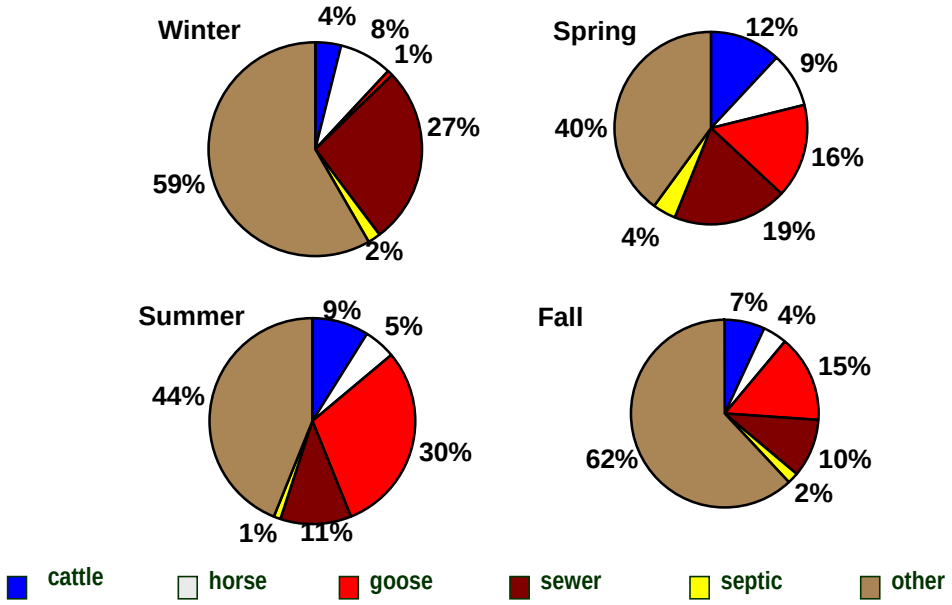

Figure 6. Percentage of isolates identified in each host class at Farm Road 129, by season

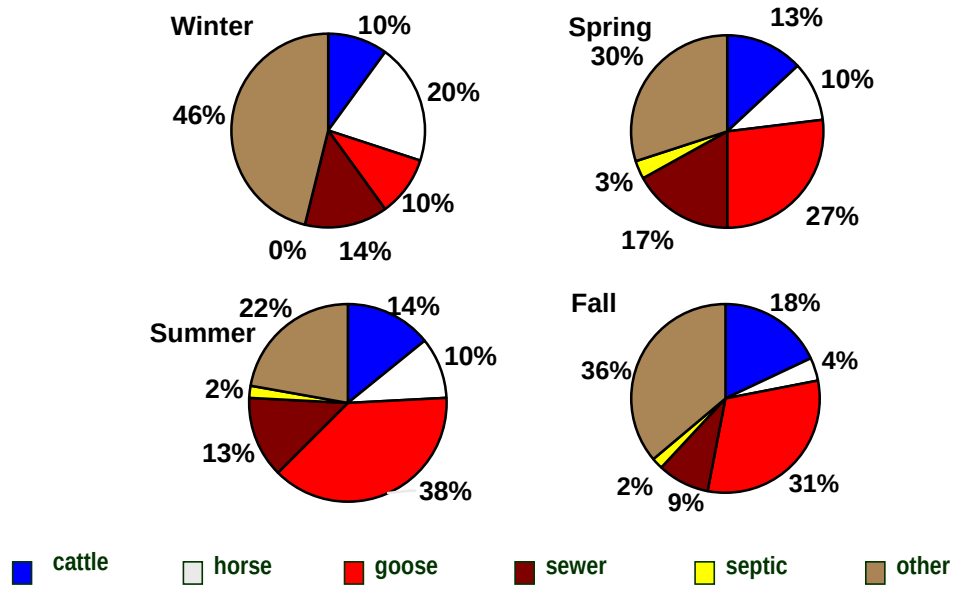

Figure 7. Percentage of isolates identified in each host class at Road 215, by season

Geese seem to be everywhere in the watershed; they explain 30 percent to 40 percent of the loading at the downstream site during the recreation season. The percentages of isolates matched to goose correspond to the goose life cycle. In winter, only a limited number of geese remain in the watershed. In spring, they arrive in greater numbers and prepare to nest. Not only are they in greater number but each female will eat more and, therefore, produce more droppings. In summer, the young are born and grow, which leads to an even greater consumption of food and amount of droppings. Numbers go down in the fall as geese depart.

Sources were analyzed as a function of the flow condition using daily flow values measured at the USGS flow gauge located at the upstream site. Results are presented in Table 8 and illustrated in Figure 8. Although the results appear to be impacted by the flow conditions, the magnitude of the difference is not significant given the number of samples available and the variation from sample to sample. In summary, these results indicate the following.

- The cattle and horse contributions seem to increase when there is storm runoff. However, the differences in percentages are not statistically significant for any of the sources.

- At RD 215, the goose contribution seems to be relatively more important during base flow conditions.
- The sewage contribution at the upstream site (FR 129) seems to decrease when there is storm runoff. This could indicates that this type of discharge, whether it comes from the treatment plant or from leaks of the sewage system, tends to be diluted by cleaner runoff.
- There is less unidentified others at FR 129 when it rains than when it does not.

Table 8. Percentage of isolates identified in each host class, by flow condition

	Cattle	Horses	Geese	Sewage	Septic	Others
FR129						
Base flow conditions	8%	5%	15%	16%	3%	53%
Storm flow conditions	12%	11%	18%	13%	2%	44%
RD215						
Base flow conditions	13%	8%	29%	14%	2%	34%
Storm flow conditions	17%	13%	23%	12%	3%	32%

In the future, additional host classes can be added to the existing database to help reduce the number of isolates that cannot be classified with the current database. In particular, it might be worth including urban storm runoff and other wildlife species.

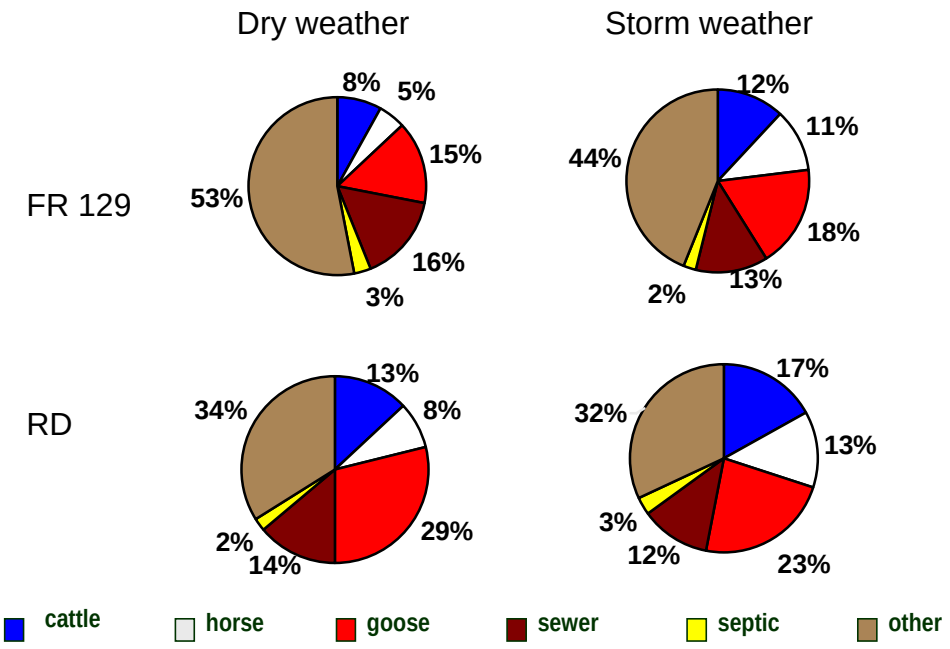

Figure 8. Percentage of isolates identified in each host class, by flow condition

Potential Pathogens

To isolate fecal *E. coli* from water and fecal samples, the methodology includes a sequence of procedures, the last of which is a final identification process via the BD BBL Crystal Identification Enteric/Nonfermenter ID kit (Becton, Dickinson and Company; Sparks, MD 21152). This kit contains 30 dehydrated compounds to test a particular bacterial isolate for such processes as degradation and hydrolysis of various substrates, fermentation, and oxidation. It was designed for the identification of aerobic gram-negative bacteria belonging to the family *Enterobacteriaceae*. There are thousands of known profiles, corresponding to specific strains of bacteria, saved in the BBL Crystal's database that are used as a reference when identifying an unknown isolate. When an unknown isolate is scanned, the BBL system will match the profile to the most similar profile in its memory and list the bacterial specie with a confidence level of accuracy. If the isolate is pathogenic, the BBL system warns us. It is extremely rare to find pathogenic *E. coli* in the environment, as most fecal *E. coli* strains are non-pathogenic. Based on the BBL Crystal system, we have isolated a number of potential pathogenic *E. coli* strains (as well as other pathogenic bacterial species) detected in the Little Sac watershed samples.

Other pathogens were suspected to be present in the Little Sac River when numerous "abnormal" (pink in color as opposed to the normal blue observed with fecal coliforms) colonies were detected in several water samples. A few of these pink isolates were identified by Dr. Fales at the veterinary diagnostic laboratory at the University of Missouri - Columbia. They include *Plesiomonas shigelloides*, *Aeromonas caviae*, and *Enterobacter cloacae*. Table 9 lists the potential pathogens isolated in the course of this study.

Table 9. Pathogens identified in the Little Sac River watershed.

Isolate(s)	Date	Location / Specie	BBL Confidence Level	Fecal Coliform Count	Identification source
2- *E. coli* 0157:H7	4/21/04	beef cattle	.9361 / .9063	NA	
2-enteropathogenic *E.coli*-AD	4/21/04	Rd 215; water	.9499 / .9684	148	
1-enteropathogenic *E.coli*-AD	4/21/04	septic tank	0.9972	NA	BBL-Crystal Identification Enteric/ Non-fermenter ID kit
1-enteropathogenic *E.coli*-AD	4/22/04	horse	0.9831	NA	
1-*Klebsiella pneumoniae*	4/22/04	septic tank	0.98	NA	
1-enteropathogenic *E.coli*-AD	4/27/04	Rd 215; water	0.9852	480	
1-enteropathogenic *E.coli*-AD	5/4/04	Fr 129; water	0.9768	320	
1-enteropathogenic *E.coli*-AD	5/4/04	Doling Park geese	0.9928	NA	
Plesiomonas shigelloides	5/12/04	Rd 215; water	NA	200	
Plesiomonas shigelloides	5/25/04	Rd 215; water	NA	260	
Plesiomonas shigelloides	5/25/04	FR 129; water	NA	160	veterinary diagnostic laboratory
Plesiomonas shigelloides	6/1/04	Rd 215; water	NA	2200	
Aeromonas caviae	7/27/04	Rd 215; water	NA	230	
Enterobacter cloacae	8/10/04	FR 129; water	NA	210	

Conclusions

Bacterial source tracking was used to identify the sources of contamination of the Little Sac River at two sites from November 2003 to October 2004. Monthly samples were collected from November through February. Weekly samples were collected thereafter.

Fecal coliform concentrations were analyzed according to season and flow conditions. They show that the water quality limit for whole body contact recreation was exceeded at both sites during the recreation season. When analyzed by season, there was no difference in fecal coliform concentrations between any of the four seasons. The fecal coliform concentrations were significantly higher in water samples collected during storm events compared with samples collected during dry weather periods.

A database of 5 classes (containing approximately 200 isolates in each class) was developed specifically for the Little Sac River watershed. It included cattle, horse, goose, septic tank, and sewage from the Northwest WWTP. About 40 to 70 percent of the isolates found in the river were identified using the rep-PCR method. In those identified, we found a high proportion of goose isolates, especially in the summer. Cattle and horse were found equally throughout the year and in higher proportion at RD 215. Cattle and horse proportions were similar in spite of a higher proportion of cattle in the watershed. Very few isolates were associated with septic tanks. The proportion of isolates associated with the Northwest WWTP was high at the upstream site (FR 129) in winter. In summer, the proportions of WWTP associated isolates were similar at both sites.

In the future, some classes of isolates can be added to the database or the database enlarged to help determine the source of isolates that could not be associated with any of these five classes. Potential sources could be urban runoff and other wildlife species.

In the course of this study, several pathogenic *E. coli* strains pathogens and other pathogenic bacteria were identified in water samples from the Little Sac River and in watershed landscape samples.

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Appendix A:

Water Quality Data

Table A.1. Fecal coliform counts at the WWTP outlet and in Little Sac at Farm Road 129 and Road 215

Date	FC at WWTP outlet	FC at FR129 (1 mile from WWTP)	FC at Rd 215 (Morrisville)	Flow in Morrisville (m^3/s)	Flow condition*
11-19-03		NA	NA	5.02	1
12-15-03		90	38	4.73	0
1-13-04		260	69	4.73	0
2-17-04		500	11	3.57	0
3-2-04		890	55	2.35	0
3-9-04		12,000	1,700	14.34	1
3-16-04		2,050	340	6.49	0
3-23-04		410	180	3.57	0
3-30-04	No growth	350	1,140	21.54	1
4-6-04	7,500	320	84	5.67	0
4-13-04	340	102	94	3.94	0
4-20-04	34	410	148	2.69	0
4-27-04	2	340	480	13.26	1
5-4-04	50	320	240	13.01	1
5-12-04	100	300	200	4.02	0
5-18-04	74	420	220	3.26	0
5-25-04	2 (not *E. coli*)	160	260	1.87	0
6-1-04	34	300	2,200	1.30	0
6-8-04	20	85	520	0.74	0
6-16-04	65	2,700	730	3.74	1
6-22-04	NA	750	500	1.39	0
6-29-04		260	420	0.60	0
7-6-04		160	215	1.16	0
7-13-04		850	290	0.45	0
7-20-04		370	620	0.57	0
7-27-04		590	230	0.74	1
8-10-04		210	250	0.60	0
8-17-04		1,280	430	0.28	0
8-24-04		210	5,200	0.43	1
8-31-04		1,090	240	0.21	0
9-7-04		340	200	0.16	0
9-14-04		1,030	180	0.12	0
9-21-04		750	360	0.16	0
9-28-04		370	640	0.16	0
10-5-04		480	470	0.12	0
10-12-04		14,800	5,200	1.19	1
10-19-04		570	280	0.88	0
10-26-04		2,060	1,850	1.64	1

* 1: storm flow, 0: base flow

Appendix B:

Bacterial Source Tracking Data

Table B1. Sources of fecal coliform at Farm Road 129

Date	Fecal coliform concentration colonies/100ml	Cattle	Horse	Goose	Sewage	Septic	Other
11-19-03	NA	0%	8%	0%	58%	0%	33%
12-15-03	90	0%	11%	0%	11%	11%	67%
01-13-04	260	0%	0%	0%	17%	0%	83%
02-17-04	500	25%	25%	0%	0%	0%	50%
03-02-04	890	0%	0%	0%	33%	0%	67%
03-09-04	12,000	0%	0%	0%	24%	0%	76%
03-16-04	2,050	0%	11%	6%	44%	6%	33%
03-23-04	410	17%	0%	0%	50%	8%	25%
03-30-04	350	6%	22%	39%	17%	6%	11%
04-06-04	320	6%	0%	17%	22%	11%	44%
04-13-04	102	5%	5%	5%	25%	0%	60%
04-20-04	410	12%	0%	0%	24%	6%	59%
04-27-04	340	18%	24%	29%	24%	0%	6%
05-04-04	320	22%	11%	6%	11%	11%	39%
05-12-04	300	0%	0%	0%	0%	0%	100%
05-18-04	420	13%	19%	19%	19%	6%	25%
05-25-04	160	35%	0%	15%	15%	0%	35%
06-01-04	300	17%	6%	28%	17%	0%	33%
06-08-04	85	5%	15%	20%	20%	0%	40%
06-16-04	2,700	7%	20%	27%	0%	0%	47%
06-22-04	750	15%	0%	10%	10%	5%	60%
06-29-04	260	21%	5%	37%	0%	0%	37%
07-06-04	160	10%	0%	45%	20%	0%	25%
07-13-04	850	0%	5%	30%	20%	0%	45%
07-20-04	370	0%	10%	50%	10%	0%	30%
07-27-04	590	11%	11%	11%	5%	0%	63%
08-10-04	210	5%	5%	25%	15%	0%	50%
08-17-04	1,280	5%	10%	30%	5%	10%	40%
08-24-04	510	15%	5%	25%	15%	0%	40%
08-31-04	1,090	0%	0%	0%	10%	0%	90%
09-07-04	340	11%	0%	26%	32%	5%	26%
09-14-04	1,030	5%	5%	11%	5%	0%	74%
09-21-04	750	11%	11%	11%	0%	0%	67%
09-28-04	370	0%	0%	15%	15%	0%	70%
10-05-04	480	0%	7%	0%	0%	0%	93%
10-12-04	14,800	0%	5%	10%	15%	0%	70%
10-19-04	570	0%	0%	21%	11%	5%	63%
10-26-04	2,060	29%	6%	12%	6%	0%	47%

Table B2. Sources of fecal coliform at Road 215

Date	Fecal coliform concentration colonies/100ml	Cattle	Horse	Goose	Sewage	Septic	Other
11-19-03	NA	20%	35%	5%	0%	5%	35%
12-15-03	90	14%	14%	14%	0%	0%	57%
01-13-04	260	0%	18%	0%	18%	0%	65%
02-17-04	500	0%	0%	13%	56%	0%	31%
03-02-04	890	7%	7%	13%	0%	0%	73%
03-09-04	12,000	10%	25%	5%	10%	0%	50%
03-16-04	2,050	17%	44%	17%	11%	0%	11%
03-23-04	410	11%	0%	0%	74%	0%	16%
03-30-04	350	17%	17%	22%	22%	0%	22%
04-06-04	320	6%	12%	41%	12%	0%	29%
04-13-04	102	5%	5%	5%	25%	5%	55%
04-20-04	410	13%	6%	19%	19%	6%	38%
04-27-04	340	21%	21%	21%	7%	7%	21%
05-04-04	320	20%	10%	10%	10%	5%	45%
05-12-04	300	5%	5%	45%	5%	5%	35%
05-18-04	420	14%	14%	29%	14%	0%	29%
05-25-04	160	5%	5%	20%	20%	0%	50%
06-01-04	300	10%	0%	90%	0%	0%	0%
06-08-04	85	15%	5%	20%	10%	5%	45%
06-16-04	2,700	24%	29%	24%	0%	0%	24%
06-22-04	750	5%	0%	53%	16%	0%	26%
06-29-04	260	16%	11%	37%	11%	0%	26%
07-06-04	160	15%	25%	50%	0%	0%	10%
07-13-04	850	15%	25%	45%	5%	0%	10%
07-20-04	370	18%	12%	29%	29%	6%	6%
07-27-04	590	11%	0%	37%	11%	11%	32%
08-10-04	210	16%	0%	16%	21%	0%	47%
08-17-04	1,280	5%	10%	35%	10%	5%	35%
08-24-04	510	10%	5%	45%	15%	0%	25%
08-31-04	1,090	22%	0%	22%	0%	11%	44%
09-07-04	340	5%	5%	35%	10%	0%	45%
09-14-04	1,030	32%	0%	37%	5%	5%	21%
09-21-04	750	24%	6%	12%	24%	0%	35%
09-28-04	370	13%	0%	50%	0%	0%	38%
10-05-04	480	21%	5%	47%	0%	0%	26%
10-12-04	14,800	32%	11%	26%	5%	0%	26%
10-19-04	570	25%	0%	15%	5%	0%	55%
10-26-04	2,060	10%	0%	15%	30%	0%	45%