RESEARCH REPORT
ENVIRONMENT

Title: Pathogenic and Indicator Bacteria in Agricultural Watersheds
NPB# 04-070

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Abstract

Escherichia coli and *Enterococcus* are indicator bacteria that were measured in Iowa streams that drain land with different levels of swine production. We completed a study monitoring=g these bacteria over three and one-half years. Bacteria were readily transported from manured fields, reaching levels exceeding 10,000 per 100 ml of water. In contrast long-term averages showed only a few hundred bacteria per 100 ml and long-term averages do not correspond to the estimated densities of swine in the three catchments. This and other data obtained from manured and non-manured fields suggest that wildlife are also a source. Cattle are also a likely source. Regrowth of bacteria in water from stream sediments appears to contribute to the peak in populations observed in July, August and September that were seen in each year. Studies of *E coli* survival in soil suggest that avoiding manure application immediately before rainfall is a producer practice that will have immediate water quality benefits. This project also investigated the feasibility of using quantitative PCR to measure populations of *Salmonella* and *E coli* O157:H7, which are both human/livestock pathogens. While we were able to obtain qualitative measurements showing the presence of *E coli* O157:H7, the quantitation was not achieved.

Introduction

Concentrations of indicator bacteria indicate that many rivers and streams are contaminated with fecal bacteria. Survival of fecal bacteria in manure, soils and stream waters has been well researched and generally show that populations in soils and waters decrease fairly rapidly, although some fraction of the population can survive for several months in temperate climates (Cools et al., 2001; Santo Domingo et al., 2000). Contamination with fecal bacteria is generally thought to result from overland flow from grazing lands, direct runoff from animal feeding operations and runoff from manured lands. However, the relationships between runoff volumes and fecal bacteria concentrations is not straightforward (Baxter-Potter and Gilliand, 1988; Kistemann et al., 2002).

The impact of large-scale swine production at the watershed scale has not been fully investigated. In these systems, swine manure is stored in deep pits beneath the production houses or partially treated in lagoon systems. In fall, and to a somewhat lesser extent in spring, these wastes are land applied and fecal bacteria and pathogens present may be available for transport. In landscapes that have significant subsurface drainage, leaching of bacteria into these drainage systems may provide an additional mechanism of transport to surface waters (Jamieson, 2002).

Objectives

1. Continue our present water quality and watershed hydrology research in the South fork of the Iowa River and tributary watersheds, including expanded monitoring for *E coli* and *Enterococcus.*
2. Determine the population of pathogenic bacteria, such as *E coli* O157:H7, *Salmonella* and *Campylobacter* and relate those populations to of the indicator bacteria (*E. coli* and *Enterococcus*) populations in manure, freshly manured soils, and stream water.
3. Determine the survival of these pathogenic bacteria in stream sediments and waters.

Materials and Methods

Objective 1. We have continued the monitoring of *E. coli* and *Enterococcus* in the waters of Beaver Creek, South Fork and Tipton Creek. These two bacteria are replacing fecal coliforms as the standard indicator bacteria. These tests were performed with commercial kits (Colisure and Enterolert, IDEXX Laboratories). Samples were taken from a range of sampling sites on Beaver Creek, Tipton Creek, and the Southfork of the Iowa River. Samples consisted of 100 ml of unfiltered stream water or tile drainage waters. These samples were collected weekly in April through October and every two to three weeks at other times. At four locations at storm samples were obtained using automated samplers. Samples were generally analyzed within 6 hr of collection in accordance with standard E.P.A. procedures.

We used GIS analysis of aerial photographs to estimated the swine densities in these sub-basins and the percentage of land treated with swine manure. Swine numbers were estimated from the size of the house, average pen size and numbers of pigs per pen. These swine populations represent a maximum number, as we are unable to assess occupancy within the houses. The estimated areas of swine manure application were calculated using our swine numbers, estimated manure production, and application rates based on nitrogen for corn production. These are valid only at the watershed scale and are likely inaccurate at the local scale.

Objective 2. Quantitative PCR involves repeated replication of target DNA using the polymerize chain reaction (PCR). As the replication pattern is repeated fluorescent dye is released and quantitated. This reaction can be quantified by comparing experimental results to standards (DNA extracted from known amounts of cells). Quantitative PCR for E coli O157:H7 was performed as described by Ibekwe et al. (2002) with the following modifications. We extracted various amounts of stream water (10 to 100 ml) or stream water spiked with *E. coli* O157:H7. Extractions were performed after concentrating cells on filters or following centrifugation and clean-up was done using using a MoBio kit. We assayed the individual targets (genes coding for toxins stx1 and stx2 and the eae pathogenicity gene simultaneously rather than the multiplex method.

E. coli O157:H7 was also cultured on selective agar media, CT-SMAC and Chromagar O157 (Chromagar Inc.). Finally we compared these methods to the Neogen immunological test kit, which provides a plus/minus determination.

Objective 3. We developed a technique for obtaining stream sediment samples. Our previous data showed relatively high populations (>400 cells/100 ml water) of *E. coli* in streams weeks after any runoff event, which suggested the presence of in-stream sources, such as stream bottom sediments. Our method involves inserting a plastic pipe into the stream bottom and pumping the water out of the pipe. This method was applied at two different times at some of the same locations where stream water samples were obtained.

The survival of *E coli* O157:H7 was investigated using strain B6914 which was marked with green-fluorescent protein (Gagliardi and Karns, 2002). Bacteria were recovered on LB agar amended with cycloheximide and examined under UV light. The bacteria containing GFP fluoresce under these condition. Soils were placed in plastic cups and planted with corn, rye or left unplanted. A similar test was run with the inoculated soil flooded to simulate stream-bottom conditions.

Results

Objective 1. The mean stream water populations of E coli and *Enterococcus* over all sites and sampling times (2002-2005) are shown in Table 1. Swine densities and the fraction (%) of land in the sub-basin are lowest in Beaver Creek. Statistical analysis showed that the average population of *E coli* in Beaver Creek was greater than that in Tipton or South Fork, but the average population of *Enterococcus* was greater in South fork than in the other two basins. Figure 1 shows that populations are greatest in the summer months (July, August and September) and least in the first quarter (January, February, March). *Enterococcus* populations were generally greater than the *E coli* populations and follow the same seasonal pattern. *Enterococcus* and *E. coli* populations are correlated but the correlation is not strong. We also examined the data to determine if there were differences among the sites within the sub-basins. This approach would have indicated priority areas within the watershed for further study but few differences were found.

TABLE 1. Average populations of *E coli* and *Enterococcus* and land-use data.

	Beaver Creek	South Fork	Tipton Creek
E coli (cell/100ml)	340	232	160
Enterococcus	373	478	312
Manure (% of total acres)	22	49	71
Swine (average number/hectare)	4.1	13.7	11.3

Populations of these bacteria in tile drains are considerably smaller than stream water in some tile drains, but were present up to 1000 cells/100 ml , especially in larger tile drains. The tile drains TC4E and TC4W, which are the largest tiles have the greatest average populations. Surface inlets in these tile-drain systems may contribute fecal bacteria. The tile drains TC4E and TC4W were added to our study in aid the adoption of nutrient management standards by producers on these lands.

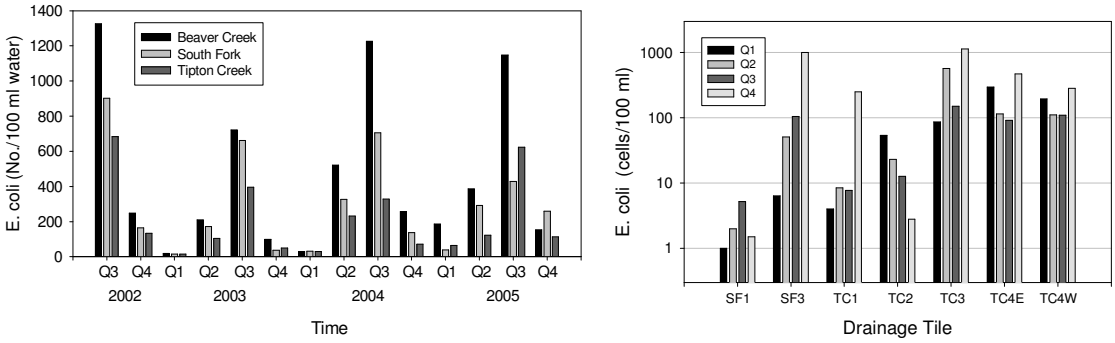

Figure 1. Seasonal variation of *E. coli* in the South Fork of the Iowa River. Data on the left are from drainage tiles averaged over 3 month intervals (quarterly). On the right are average stream water populations over 3 month intervals for the Tipton Creek, South Fork, and Beaver Creek sub-basins.

We obtained storm runoff data in April of 2005. The runoff event occurred immediately after a period where farmers had been land-applying manure in the watershed. Results for Beaver Creek and South Fork are shown in Figure 2. Tipton Creek data are similar. At the peak of the runoff event populations exceed 10,000 bacteria/100 ml for both *E. coli* and *Enterococcus*. Figure 2 shows that these high concentrations persist for about 36 hr then concentrations begin to decline. In the runoff *E. coli* populations tend to be greater than the *Enterococcus* populations. We attribute the bulk of these bacteria to animal manure, but there is not as much difference between Beaver Creek and South Fork as might be expected based on the greater estimated hog density in South Fork compared to Beaver Creek. Similar results were obtained in November 2003 when runoff occurred during the manure application season. Populations (cells/100 ml) of *Enterococcus* ranged from 120,000 to 250,000 in lagoon liquid and from 50,000 to 250,000 in lagoon solids found at the bottom. The *E coli* populations were similar 92,000 to 250,000 in liquids and 10,000,000 in solids. Clearly, populations of these indicator bacteria are large enough to account for the fecal bacteria we observed in runoff. Previously, our work and other researchers have shown that *E coli*, *Enterococcus* and *Salmonella* all die off rapidly in soils with half lives (times for 50% loss) of three to five days.

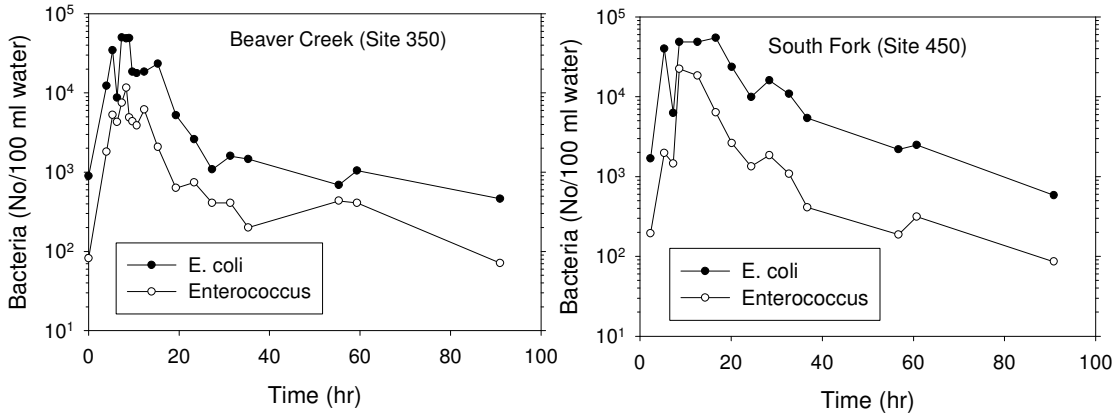

Figure 2. Runoff concentrations of E. coli and Enterococcus in Beaver Creek and South Fork of the Iowa River in April of 2005.

To further understand the relationships controlling E coli populations in stream water we examined the relationship between stream flow, temperature and cell populations. Figure 3 shows the relationship between stream flow and *E coli*.

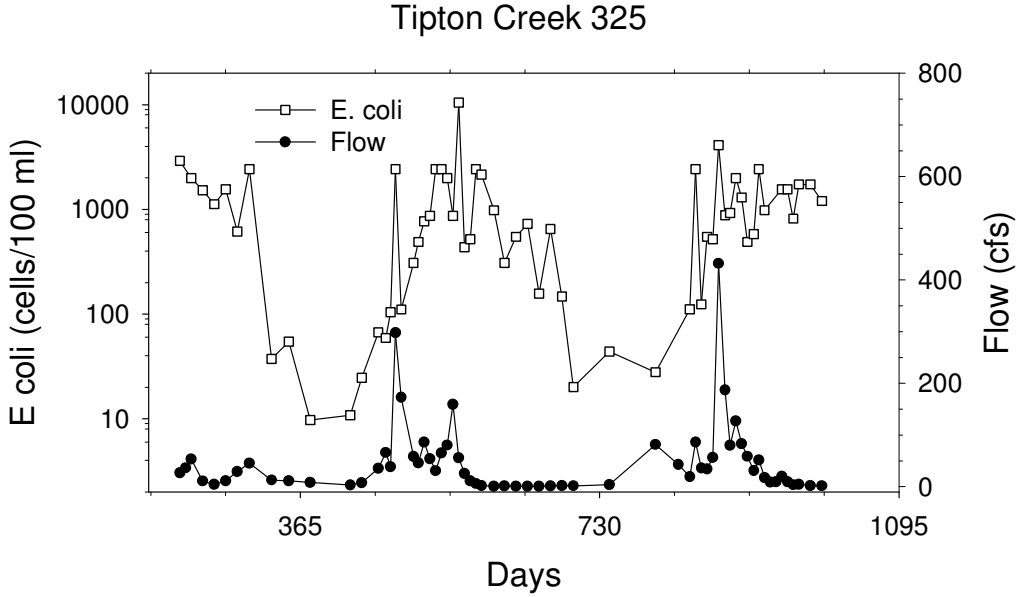

Figure 3. *E coli* (left axis) and stream flow (cubic feet per second) over a three year period in Tipton Creek. Note periods at when populations of E coli remain high (> 200 cells/100 ml) while stream flows are extremely small.

Figure 1 also shows that populations peak in the months of July, August and September, which suggests a possible temperature effect. Using both stream flow and temperature in a statistical model we can explain approximately 50% of the variability in the *E coli* data.

Objective 2. Our activities focused on developing and adapting the methods for PCR-based quantification of E. coli O157:H7. Selected PCR primers (from candidates described in the literature) were tested. By spiking samples with the target organism, extracting the DNA, performing the PCR. Our experience with this has been inconsistent, due to several factors. Extraction of stream water to obtain sufficient cell numbers for detection, which is about 300 cells. Below this level we had problems with sample clean-up which in turn has lead to artifacts (false positive detections). Using enrichment (growing cells in media prior to performing the PCR) results in lower detection levels, but the original population cannot be quantified.

Cultural methods have shown us that *E coli* O157:H7 is present in very low densities, but is present in about the same frequency (50 to 60% of the samples) that we found previously using a commercial ELISA test. Some of our isolates previously obtained were re-tested with PCR techniques and confirmed as positive for the Styx genes (1 and 2) and the ease gene which is consistent with their identification as *E coli*. In summary, we found that the quantitative PCR methodology needed further development before application to the large number of samples necessary for watershed studies. The limited presence/absence results that we obtained generally agree with the results we obtained previously with the Neogen and Chromagar assays, but quantitation was not obtained.

Objective 3. Stream bottom sediments sampled to a depth of 2.5 cm showed large and highly variable populations of E coli (Figure 4). These data showed much larger populations than in 2004, when E coli did not exceed 2,500 cells/g of sediment. Therefore, these results support the idea that *E. coli* establish resident environmental populations in these sediments during summer months.

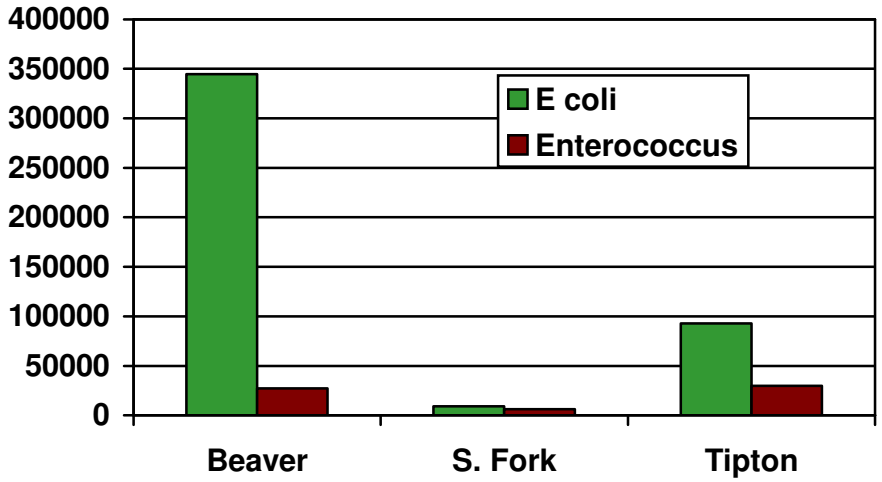

Figure 4. Stream bottom populations of *E coli* in September of 2005. Values shown are means of at least four samples taken at least two locations.

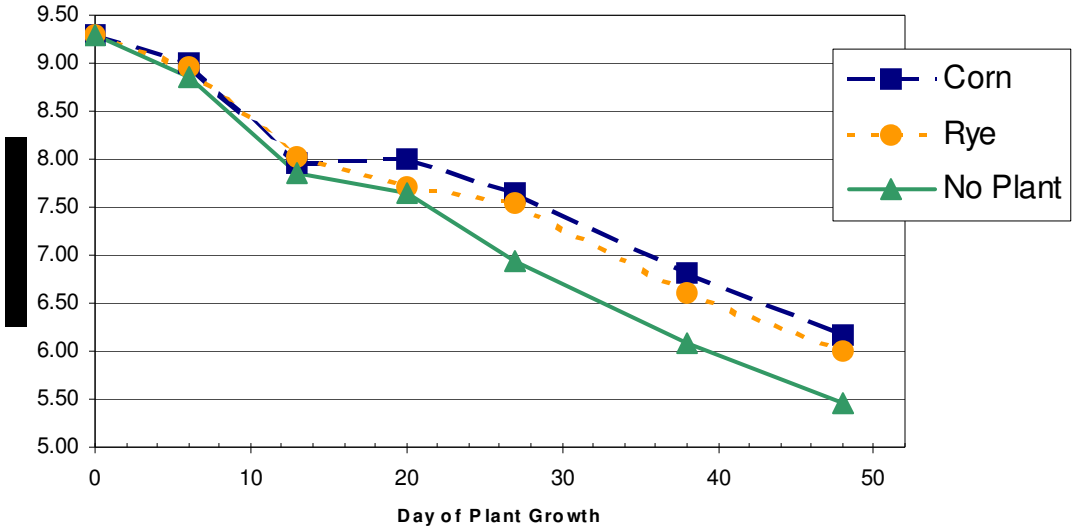

Figure 5. Survival of E coli 0157:H7 in soil in the presence of corn, rye or without a plant. Plants and soils were kept in growth chambers with 16 hours of daylight.

The effect of plants on the survival of *E coli* O157:H7 in soil is shown in Figure 5. The half-life in the presence of corn is 4.6 days and in the treatment without plants it is 3.8 days. These half-lives are only slightly longer than the three day half-life obtained in

field studies in Tipton Creek. Over the 48 day period more than 99.9 % of the pathogen died. The original inoculum level used in these studies was much larger than would normally be found, except in the feces of highly infected animals. When *E coli* O157:H7 was inoculated in soil that was saturated with water to simulate a stream bottom sediment, the half-life was 5.7 days. In our study, *E coli* O157:H7 was more persistent in soil than that reported by Gagliardi and Karns (2002), but similar to that of Natvig et al. (2002).

Discussion

Stream water monitoring in this project and our previous NPB project show that the waters of this agricultural watershed are contaminated with *E. coli* and *Enterococcus*, which are fecal bacteria. To a limited extent, we have detected the pathogen *E. coli* O157:H7, but its populations are apparently too low for us to quantify effectively with PCR. While *E. coli* O157:H7 is considered a very minor pathogen in U.S. swine herds, other pathogenic *E coli* are present in swine, and other animal hosts are present in the watershed. The generally similar behavior of *E coli* and *Enterococcus* indicate that these indicator bacteria are both responding to the same controlling factors.

Our findings allow several statements to be made regarding sources of fecal bacteria. First, rainfall during, or within two weeks of manure application are likely to produce runoff containing large populations of fecal bacteria originating from swine manure. Previously we showed that some of these bacteria could be attributed to wildlife by measuring *E coli* in runoff from a non-manured site, but we have not obtained runoff again at that site. Two of our sub-basins (Tipton Creek and South Fork) have larger hog populations and a greater fraction of their land treated with manure than the third (Beaver Creek). These differences are not reflected in patterns of *E coli* or *Enterococcus* carried in stream water over a wide range of low conditions. This is further evidence that other animals than swine are contributing to the stream water populations.

Bacterial populations in tile drainage were variable with respect to both location and time. Some tiles had relatively clean water, while others were not that different from stream water. Our results are generally consistent with findings from other corn-belt locations (reviewed by Jamison et al., 2002). We have installed flow monitors in some tiles that will alalow us to dtermine if bacteria are transportedrapidly in response to storm events.

Stream water populations of bacteria showed strong seasonal trends, with the greatest populations developing in late summer. Fortunately, this period of time corresponds to the lowest flows in the watershed, so downstream transport to the Iowa River is minimized. The source of these populations appears to be stream bottom sediments, which were detected in all samples. Some populations were quite large, exceeding 50,000 cells per gram of sediment. We still need to measure these sediment populations in early spring to understand if they persist over the winter months.

Finally we examined the survival of *E. coli* O157:H7 in soil and showed similar survival patterns to that of *E coli* obtained in earlier field studies after swine manure application. This result also supports the notion that non-pathogenic *E coli* are an effective surrogate for pathogenic *E. coli* and *Salmonella*. These data also show that if *E coli* populations in manure can be managed to achieve populations of 10,000/g soil immediately after application, then >99% reduction will be obtained within 30 days.

Lay Interpretation

Escherichia coli and *Enterococcus* are indicator bacteria that were measured in Iowa streams that drain land with different levels of swine production. We completed a study monitoring=g these bacteria over three and one-half years. Bacteria were readily transported from manured fields, reaching levels exceeding 10,000 per 100 ml of water. In contrast, long-term averages showed only a few hundred bacteria per 100 ml and long-term averages do not correspond to the estimated densities of swine in the three catchments. This and other data obtained from manured and non-manured fields suggest that wildlife are also a source. Cattle are also a likely source. Studies of *E coli* survival in soil suggest that avoiding manure application immediately before rainfall is a producer practice that will have immediate water quality benefits. This project also investigated the feasibility of using quantitative PCR to measure populations of *Salmonella* and *E coli* O157:H7, which are both human/livestock pathogens. While we were able to obtain qualitative measurements showing the presence of *E coli* O157:H7, the quantitation was not achieved.

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