Title: **Fecal-contaminant indicators in soils and water of a watershed producing swine and other livestock. NPB # 03-133**

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Abstract

The South Fork of the Iowa River drains a 215,000 acre watershed that contains nearly 100 confined livestock operations, mostly producing swine. Concerns about water quality in this watershed include bacterial pollution, indicated by the presence of *Escherichia coli* (*E. coli*), which could originate from manure. This study evaluated spatial and temporal patterns of *E. coli* in soil, field runoff, tile flow, and stream water during a 12-month study. After manure application, *E. coli* counts in soil were diminished to pre-application levels within 24 days, and indicated a 2.4 day half-life. A runoff event that occurred six and seven days after fall manure application showed large concentrations of *E. coli.* However, runoff from an adjacent, non-manured field also showed large concentrations, which were only 25-66% smaller, indicating wildlife as a possible significant *E. coli* source. Compared to runoff, tile flow had small *E. coli* concentrations, based on data from six county tiles. Only one of the six sites showed frequent exceedance of the applicable standard, probably because of surface inlet flows. Stream water monitoring over a 23-month period showed average *E. coli* counts in the three watersheds were similar, even though there are big differences in numbers of livestock facilites. There were distinct seasonal trends with greatest bacterial levels present in the summer. Runoff events immediately after manure application produce very high concentrations at field and watershed scales. Control of runoff through tillage practices, buffer strips or other means would help mitigate this risk. Finally, analytical methods were developed to detect the antibiotic tylosin in swine lagoon slurry, with concentrations of 0.065 to 1.97 ppm found at two facilities. This provides a possible range of concentrations that would be applied to soil.

I. Introduction

Fecal contamination of surface waters has drawn significant attention recently. Contaminant levels usually increase after runoff events, however, sources and pathways of movement remain a topic of uninformed debate. Attention has also been recently focused on trace organic compounds in the environment, (e.g., antibiotics, drugs, hormones), which have been documented at low concentrations in a range of streams across the U.S. (Kolpin et al. 2002). Coexistence of antibiotics and pathogens in environmental waters may contribute to increased antibiotic resistances in pathogens (Salyers and Amabile-Cuevas 1997). There is a need to understand if and how manure management practices can affect the movement of pathogens and antibiotics in the environment. Also, the co-distribution of pathogens and antibiotics in the environment under existing suites of land uses is not well understood. Runoff water can also transport *E. coli* across the soil surface (Abu-Ashour and Lee 2000). In-field practices that may reduce the movement of manure constituents in water would include application and tillage methods that reduce runoff across and preferential flow through soils. Management practices in riparian areas, such as constructed wetlands, can reduce counts of pathogenic bacteria (Neralla and Weaver 2000).

At watershed scales, the occurrence of bacterial contaminants may result from multiple sources and pathways, and their unraveling poses a significant problem for the environmental sciences. Encouragingly, one study found rates of *E. coli* die-off in small streams below wastewater treatment plant achieved 90% die-off in a median time of 10 hours, with the largest declines under low flows, warm temperatures, and clear stream conditions (Beaudeau et al. 2001). In contrast, other studies have found that E. coli can retain its metabolic activity and persist in the aquatic environment (e.g., Tanaka et al. 2000). Persistence of microbial contaminants is uncertain, with inconsistent temporal patterns reported in stream (Mallin et al. 2000) and lake-coastal (Whitman et al. 1999) environments. Where integrated assessments of bacteriological water quality have been carried out at watershed scales, a need to address multiple sources of contamination has been identified (e.g., Fisher 2000). While no one-year study can address all these issues, the research literature lacks reports on multi-scale investigations and this is a gap targeted by the research proposed here.

II. Objectives:

There were four objectives to this research, listed as follows:

Objective 1: Document changes in of *E. coli* bacteria populations and antibiotics in soils following spring and fall application of swine manure, compared to nearby, non-manured soils.

Objective 2: Measure concentrations of *E. coli* and antibiotics in surface runoff from two fields, one of which receives applications of swine manure.

Objective 3: Measure temporal changes in *E. coli* bacteria counts and antibiotics in tile-flow waters draining manured and non-manured agricultural areas.

Objective 4: Measure temporal changes in *E. coli* bacteria counts and antibiotics in stream waters of the Iowa River's South Fork, and tributaries draining sub-basins that are dominated by differing land uses.

V. Materials & Methods:

Objective 1. Two existing long-term experiments were utilized under this objective. The first is a long term manure-management plot trial operated by Heartland Pork. Manure was sampled for *E. coli* prior to application in Spring 2003. Subsequent to application, *E. coli* counts were tracked in soil of manured for one month to document die-off. This approach was also used at a second experimental site, (K farm), where runoff is being monitored from manured and non-manured fields. Manure applications at this site were in fall of 2003. Sampling at both sites was performed using a rectangular sheet metal square which was pressed into the soil. A sample approximately 5 cm in depth and 30 cm wide was recovered. This box was placed in the in the zone of surface application at the K farm. At the Buckeye site manure was injected followed by disking. Approximately 50 g of mixed soil sample was diluted in 200 ml water. The soil particles are briefly allowed to settle and the liquid is analyzed as described for water samples. We sampled both sites prior to application and sampled the non-manured soils at the Buckeye site. Because the *E. coli* populations in non-manured soils were consistently low these soils were not sampled over time.

Objective 2. Runoff was sampled from manured and non-manured fields being monitored at the K farm, and analyzed for *E. coli*. Rapid sample retrieval ensured followed by chilling, preservation, and rapid analysis ensured sample integrity. Results were obtained for one runoff event that occurred 6 days after manure application, in November of 2003. Populations of *Escherichia coli* in water samples were measured using a Colilert/Quantitray system (www.idexx.com), which relies upon a specific enzymatic activity of *E. coli* that produces fluorescence visible under ultraviolet light. The distribution of fluorescent responses on the Quantitray allows the *E. coli* population to be estimated as cells/100 ml of water.

Objective 3. Six tile-discharge monitoring sites were located**,** expanding the existing network for manual sampling of stream waters. Sampling of these sites commenced in early July 2003. Tile discharge was sampled on a weekly or biweekly basis (except during winter freeze). The samples were analyzed for E. coli. Tile sites were not monitored for flow rates.

Objective 4. Ongoing monitoring efforts within the stream network (13 sampling locations) of the South Fork were expanded to include analyses for *E. coli* (**Figure 1**). This sampling was also conducted at least biweekly from spring thaw through November. Figure 1 shows a greater density of swine production on Tipton Creek and the South Fork, while there are more cattle grazing in Beaver Creek. We used state aerial photos to identify and locate animal confinements and estimated the number of hogs produced based upon the size of the houses, then calculated a density for each watershed. Approximately 75% of swine production is concentrated in the area west of stations SF 400 in the South Fork and TC 13 in Tipton Creek. We stress these figures are estimates based on production building size, assuming full production capacity and 100% farrow to finish units.

Analytical methods for antibiotics given by Hirsch et al. (1998), and Lindsey et al. (2001) use high-pressure liquid chromatography (HPLC) and tandem mass spectrometry provides an accurate method to determine trace concentrations. The initial methods testing focused on sulfamethazine and tylosin, which are common feed additives (Shryock, 2000). The methods, involved concentration of the extracted antibiotics in a solid-phase cartridge, separation by liquid chromatography and mass spectrometry, and quantification on stable molecular fragments. We found that these methods described in the literature did not work particularly well for manure slurry or soil. Therefore we spent considerable time in improving methods for these matrices.

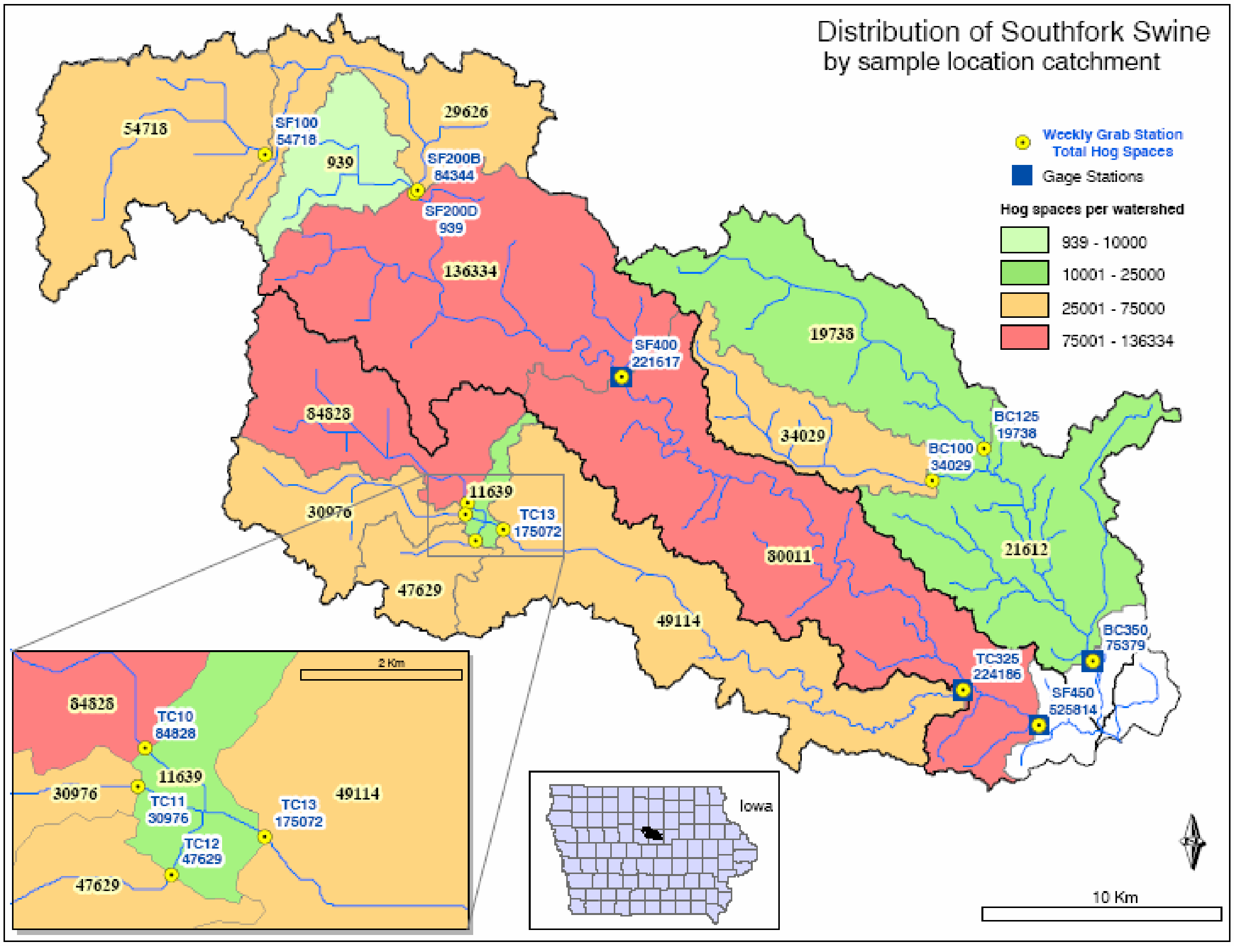


Figure 1. The study watersheds: Tipton Creek (TC), beaver Creek (BC) and Southfork (SF). Swine densities were estimated from the number and size of confinement buildings. Numbers on gold background show numbers of swine for the colored area immediately upstream from sampling location. Blue numbers below the sampling station show the total number of animals potentially present for all areas upstream of that location. Tile drainage sampling sites are not shown.

VI. Results:

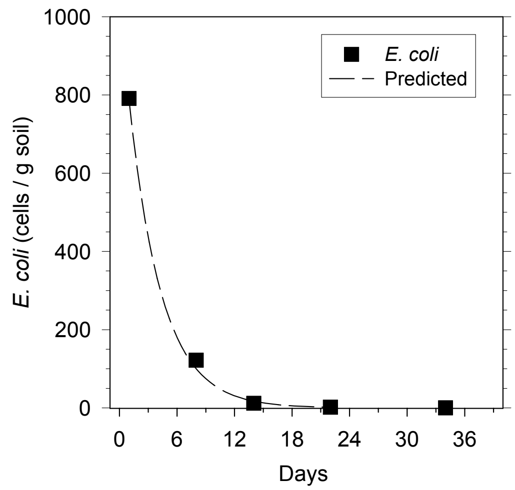
Objective 1: Soil populations of *E. coli* were measured in two different experiments using a modified Colilert most-probable-number procedure. At the Heartland site, the mean (n=8) *E. coli* populations in the commercial fertilizer and annual manure plots prior to manure application were both less than one cell per gram of soil. Swine manure slurry was injection-applied at the Buckeye site at a rate of approximately 4000 gallons per acre using commercial equipment on November 11, 2003. Based on nine samples of the manure, we determined that the mean *E. coli* population in the manure was 3.2 x 10^6 cells/ ml of slurry. Soil populations 7 and 12 days after manure application were 255 and 45 cells/g soil, respectively.

The second site is a corn-soybean field at the K Farm. This field is equipped with equipment to measure runoff and to obtain water samples. Prior to manure application, soil populations were similar to the pre-application populations at the Buckeye site. Based on 20 samples taken from the applicator in the field, the mean *E. coli* population in the manure was 1.50 x 10^6 cells/g soil. The manure slurry was applied at 4000 gallons per acre to the soil surface with shallow incorporation using a commercial applicator (see **Figure 2**).

Manure was applied on October 28, 2003. The *E. coli* survival in the soil following application is shown in **Figure 3**. The half-life (time for 50% reduction) for the bacteria is estimated to be 2.4 days. By 24 days after application, the population had returned to levels that were similar to populations prior to manure application.

Figure 3. Photo of liquid swine manure at the K farm immediately after application

Figure 3. Survival of *E coli* in field soil following manure appilcation.



Progress towards measuring antibiotics in soil was slower than anticipated. We have succeeded in developing a method for analyzing tylosin swine lagoon slurry. Laboratory studies showed that the method could recover >90% of the tylosin added to the lagoon material. HPLC and mass spectrometry allowed the quantification of tylosin A, B and D. Concentrations of tylosin in two producer lagoons were 65 and 1970 μg/L (ppb). Further laboratory studies on the degradation of tylosin in swine lagoon slurries is described in a paper submitted to *Water Resources Research* (Kolz et al. 2004). Several different solvents have been tested for extracting tylosin from soil, but recoveries are generally below 75%. We are continuing to improve this method. We have also developed methods for the analysis of sulfamethazine. Testing this method for soil and manure slurry is continuing.

Objective 2. Runoff from two small within-field catchments was examined at the K Farm. On November 3 and 4, 2003, six and seven days after manure application, a fall rain produced runoff at the site. *E. coli* in the runoff water ranged between 4,611 and 5,172 cells/100. At the "control" site (a cropped field without manure application) runoff contained 1,565 to 3,873 cells/100 ml. We presume that the *E coli* delivered from this non-manured field is from wildlife sources, such as deer, mice, weasels and raccoons. This is the only runoff event obtained at these sites to this point in a year when manure was applied.

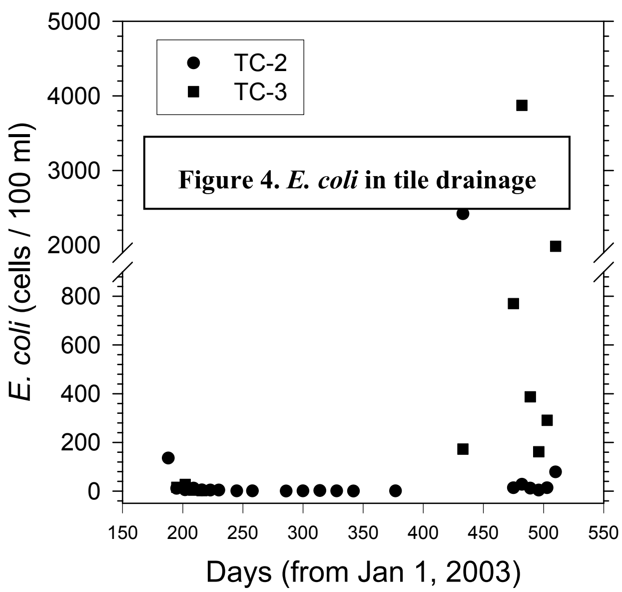


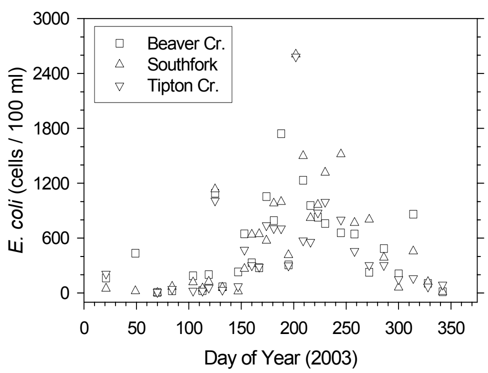
Figure 4. *E. coli* in tile drainage

Objective 3. Densities of *E. coli* in tile drainage were much lower than in stream waters. Six tile discharges were monitored starting in mid-year of 2003 and continuing to present. We assume that all tiles drain some manured land, but the proportion of manured land is not known. All the discharges are from county (drainage district) mains, and certainly contain drainage from multiple fields.

The variation in *E coli* concentrations over time is shown in **Figure 4**. Almost all samples from tile drain TC-2 are below 100 cell/ 100ml, but elevated levels of *E. coli* are more common in TC-3. We suspect that TC-3 contains a surface inlet that receives runoff water carrying *E. coli*. Of the 91 samples taken from all six sites only 6% would exceed a single sample standard of 487 *E. coli* per 100 ml.

Objective 4. Spatial and temporal trends in *E. coli* populations in stream water were examined. Our report is based on data collected beginning in August of 2002 through May of 2004. Three sub-watersheds were examined: Tipton and Beaver Creeks, and the Southfork of the Iowa River.

Figure 5. Mean *E. coli* populations for the three watersheds during 2003.



The average *E. coli* densities in the three sub-watersheds have a general seasonal trend with a peak in the July and August period (as shown in **Figure 5**). The geometric mean populations of *E. coli* for the entire 23 month period was 267 cells / 100 ml for Beaver Creek (142 samples), 203 for Southfork (236 samples) and 146 for Tipton Creek (235 samples). The relevant EPA standard (geometric mean) for comparison is 126 *E. coli* per 100 ml water. This standard was most frequently achieved during winter periods.

Stream water .*E coli* populations averaged over the 23 month time period were similar among the three sites within Beaver Creek and Southfork. There was a tendency towards larger populations at the TC325 site at the base of Tipton Creek compared to the upstream sites.

In November 2003 widespread swine manure application was followed by a runoff-producing rainstorm. This rainfall event produced the largest *E. coli* densities yet measured in these watersheds, reaching above 240,000 *E coli* per 100 ml (**Figure 6**). The largest *E. coli* concentrations are from the Tipton Creek watershed. The high populations in water at SF 450 appear to due to the entry of Tipton Creek water into the Southfork upstream from this station. At SF 400 *E coli* populations are lowest among the four automated stations. These elevated *E. coli* populations in Tipton Creek probably represent the result of a worst-case scenario where large amounts of manure were transported in runoff. Significant quantities of E coli are also found in Beaver Creek.

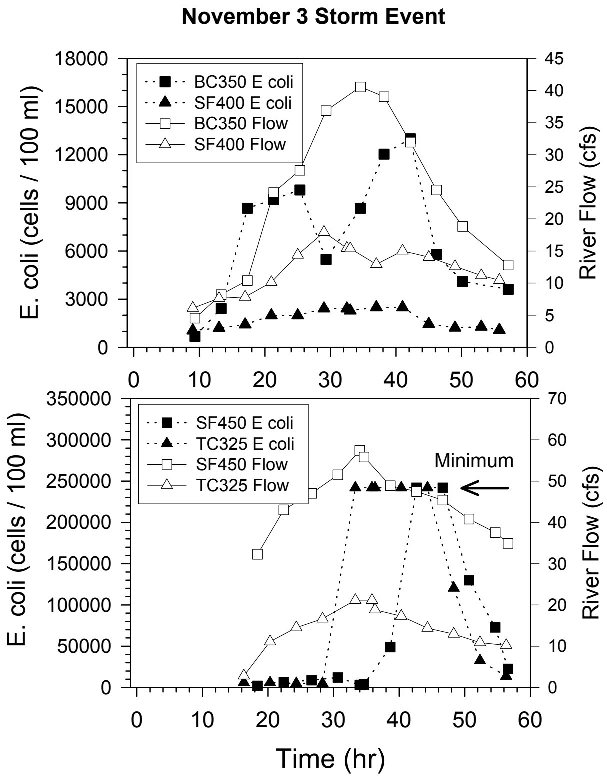


Figure 6. Stream water populations of *E. coli* during a runoff event. River flow is shown in cubic ft per second.

VII. Discussion:

This research shows that two distinct processes affect indicator bacteria (*E. coli*) populations. Clearly the transport in runoff leads to *E. coli* concentrations that exceed relevant water quality standards. Our data also shows that the risk of transporting *E. coli* depends upon the timing and amount of rainfall following manure application. Because *E. coli* populations rapidly decline in soil, rainfall in the two weeks following manure application is most likely to transport these bacteria. Surprisingly, survival of *E coli* following manure injection and disking was about equivalent to surface spreading with less intensive incorporation.

While runoff events transport *E. coli* periodically, the nearly ubiquitous presence of these bacteria during the summer months many days after any runoff event indicate that the bacteria have become resident in the environment or that there are persistent inputs to streams from wildlife, cattle, or septic systems. The comparison of *E. coli* in runoff from the manured field and non-manured field at the K farm suggests that wildlife-derived *E. coli* populations could be more substantial than generally recognized. We performed some preliminary experiments (data not shown) showing that *E. coli* in stream water dies off (< 200 cells/ 100 ml) within about four days. This suggests the presence of a continuous source of *E. coli* in the environment during summer months. Tile drainage does not appear to be that source, based on the tiles we monitored. While we found that tile drainage can transport *E. coli*, average concentrations were much reduced from those in stream water. However, transport of manure-derived *E. coli* through surface inlets is a likely route of transport. In ongoing NPB-funded research we hope to determine if streambed sediments are a possible source of *E. coli.*

Elevated levels of *E. coli* indicate the presence of more serious pathogens, particularly enteric pathogens such as *E. coli* O157:H7, *Salmonella*, and *Camplylobacter*. Enteric bacteria and viral pathogens increase the risk of intestinal disease to people involved in recreational water sports in the affected waters. The waters in these watersheds feed into the Iowa river where some of these activities occur. We performed some limited testing (Neogen™ ELISA) that showed the presence of *E. coli* O157:H7 in about two-thirds of our samples. We were able to confirm the presence of O157:H7 by isolating typical colonies on selective media.

VIII. Lay Interpretation:

This study reports on the presence of the bacterium *E. coli* in agricultural watersheds, which indicates fecal pollution. We examined its survival in soil and its transport in tile drainage and runoff. We also report on the presence of tylosin, an antibiotic administered in swine. *E. coli* is presently one of two indicator bacteria that EPA uses to assess the potential presence of fecal pathogens. Water quality standards have been issued for *E. coli*.

In summary we found that

1. The water quality in these watersheds does not meet applicable water quality standards for *E. coli* the EPA indicator bacterium.
2. Swine and cattle production almost certainly contribute to the bacterial concentrations found in these streams, but at the watershed scale individual sources cannot be identified. In addition to the contributions of swine and cattle, we found evidence that wildlife may be significant source of *E. coli* in runoff water.
3. *E. coli* applied in swine manure rapidly decline in soil reaching baseline levels about 24 days after application in the field. Producers can minimize risk be applying manure in periods when dry weather is expected.
4. The risk of *E. coli* transport in tile drainage appears to be much smaller than through overland runoff. Occasional samples with elevated concentrations of E. coli are likely due to runoff entering through surface inlet drains.
5. Long-term monitoring over a 23 month period (12 months funded by this grant) showed that *E. coli* averages in the three watersheds were similar. There were distinct seasonal trends with greatest bacterial levels present in the summer. Runoff events at the field and watershed scale immediately after manure application produce very high concentrations. Control of runoff through tillage practices, buffer strips or other means would help mitigate this risk.
6. The antibiotic tylosin (Tylan™) was found in swine lagoon slurry in concentrations of 0.065 to 1.97 ppm. This is an expected range of concentrations that would be applied to soil.

IX. Acknowledgements:

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