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M.S. Coyne and J.M. Howell

Introduction
In the mid 70's, someone noticed that the ratio of two indicator bacteria in fecal wastes — fecal coliforms (FC) and fecal streptococci (FS) — was characteristic of particular animal wastes. In human wastes, the fecal coliform/fecal streptococci ratio (FC/FS ratio) was greater than 4. In domesticated animals, like cattle, the ratio was between 0.1 and 4.0. In wild animals, the ratio was less than 0.1. Since that time, many attempts have been made to use the ratio to determine the source of fecal bacteria in contaminated ground water.

If this ratio was reliable, it would have significant and practical applications. For example, if fecal bacteria in groundwater proved to come from wild animals, little could be done to control it, but it shouldn’t cause a lot of concern, because nature isn’t squeaky clean. However, if fecal bacteria were connected with human wastes, there would be very serious concern because those wastes could potentially carry human pathogens. Fecal bacteria from domesticated animals would still cause some potential health concerns. There would also be an opportunity to control the fecal contamination by managing the animals and their wastes.

Attempts to use the FC/FS ratio to determine contamination sources have not been very successful because the method has limits: (1) Sampling needs to occur soon after manure deposition (within 24 hours if possible) because the fecal bacteria die off at different rates; (2) It becomes difficult to distinguish fecal streptococci in wastes from fecal streptococci that are naturally present in soil and water when fewer than 100 fecal streptococci/100 mL of water are present; (3) The water pH needs to be between 4 and 9 because fecal coliforms die off quicker than fecal streptococci in acid or alkaline water.

We recently examined the microbial content of water samples from two watersheds in the Bluegrass region of Kentucky for which the land use and management inputs were documented, as well as the potential sources of fecal contamination. Our goal was to see whether the FC/FS ratio could be used to identify sources of fecal contamination in these environments when periodic monitoring of water quality was performed.

Study Sites and Sampling Methods
Study sites were watersheds typical of agricultural use in the Inner (site 1) and Outer (site 2) Bluegrass regions, respectively. Site 1 contained 1440 acres in a mixture of row crops, hay, and pasture. Four hundred beef cattle and one house that was sporadically occupied were at this site. Site 2 was located on a 358 acre watershed. Land use in the watershed included a mixture of tobacco, hay/pasture, and woods. Livestock in the watershed included 50 beef cattle and 85 dairy cattle. There were also several occupied homesteads.

We sampled springs, streams, and wells in sites 1 and 2 from January...
1992 to January 1993. Water samples (500 mL, about a pint) were collected in sterile plastic bags and stored at 39°F (4°C) until we could count the fecal coliforms and fecal streptococci. To count the bacteria, we filtered appropriate water volumes onto sterile membranes and placed these on growth media specific for either fecal coliforms or fecal streptococci. After incubating the filters one or two days, we counted any colonies growing on the filters that had the characteristic color and shape of either fecal coliforms or fecal streptococci. We then calculated the FC/FS ratio from the bacterial concentration in each water sample.

Results

When the mean FC/FS ratio was evaluated for springs, it usually indicated contamination by domestic animals (Table 1). The mean FC/FS ratio was about 6-fold higher in Spring 514 than the other springs in this study. Spring 514 was located below a house and was possibly contaminated by the septic field. Well 621 had a mean FC/FS ratio characteristic of domestic animal contamination, as did well 622 (although the maximum FC/FS ratio indicated human contamination). Both wells appeared to be affected by the presence of grazing cattle. All streams had mean FC/FS ratios over 4.5. Although this seemingly indicated human contamination, only stream 631 would have received human sewage directly. The only obvious sources of fecal contamination in streams 535 and 536 were cattle and wildlife.

The frequency of FC/FS ratios representative of each contamination source has also been used to characterize water samples. Figure 1 shows the percent of samples in each site with a FC/FS ratio indicative of a specific contamination source. Most samples at the two sites had FC/FS ratios indicative of domestic animal contamination. Twenty-two percent of the samples from spring 514 indicated human contamination, probably from septic field leachate. These occurred shortly after a nearby house was reoccupied following a period of vacancy.

This emphasizes the importance of the biological mat that surrounds the tile in septic leach fields. This mat helps to remove fecal bacteria in septic tank effluents. Without an efficient biological mat, ground water contamination is likely. Approximately 5 weeks after the house was reoccupied, the FC/FS ratio declined from greater than 4 indicating that the effectiveness of the septic leach field was improving with continual use.

The obvious sources of fecal contamination in wells 621 and 622 were cattle. None of the samples from well 621 indicated human contamination but 24% of the samples from well 622 indicated some human contamination. We believe that well 622 (which was hand dug, shallow, and lined with creek rock) received lateral flow from stream 631 (it had FC/FS ratios exceeding 4 approximately 40% of the time).

All streams frequently had FC/FS ratios indicative of human contamination. Approximately 23% of the samples from stream 535 and 33% of the samples from stream 536 indicated human contamination. These FC/FS ratios increased as temperatures rose during the springtime. They probably indicate greater fecal coliform growth, compared to fecal streptococci, in stream sediments following earlier contamination.

Conclusions

In typical agricultural settings, the FC/FS ratio from a single sample has little diagnostic use. The conclusions drawn must be carefully evaluated because so many environmental factors affect it. For example, warm shallow streams, high in organic carbon, permit fecal coliform regrowth and increase the FC/FS ratio. Samples taken in these conditions give misleading values. Consequently, the mean FC/FS ratio for a site is largely meaningless because the range of FC/FS ratios is so great.

Evaluating the frequency with which FC/FS ratios fall within certain indicative values is a more accurate predictor of fecal contamination source. However, it requires numerous samples and a thorough knowledge of the watershed under consideration. As indicated by our data, one spring (514), stream (631), and well (622) might have contained human sewage and these sites had the highest percentage of samples indicative of human contamination for a given water source. Likewise, some samples from streams 535 and 536 indicated human contamination (which was unlikely) but 77% and 63% of the samples, respectively, had FC/FS ratios representative of the land use, domestic animal grazing. The FC/FS ratio can suggest the probable source of fecal contamination, but since it relies on considerable educated guesswork, the conclusions drawn from it should not be considered absolute.

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Table 1. Minimum, mean, and maximum FC/FS ratios for springs, streams, and wells at two sites in the Inner and Outer Bluegrass region of Kentucky.

<table>
<thead>
<tr>
<th>Location</th>
<th>Sample</th>
<th>Minimum</th>
<th>Mean</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>Springs</td>
<td>0.02</td>
<td>0.9</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.07</td>
<td>6.2</td>
<td>68.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.03</td>
<td>0.8</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.14</td>
<td>1.2</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>Streams</td>
<td>0.20</td>
<td>11.0</td>
<td>73.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.60</td>
<td>10.0</td>
<td>84.0</td>
</tr>
<tr>
<td>Site 2</td>
<td>Springs</td>
<td>0.05</td>
<td>1.3</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.14</td>
<td>0.9</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>Stream</td>
<td>0.70</td>
<td>4.5</td>
<td>28.0</td>
</tr>
<tr>
<td></td>
<td>Wells</td>
<td>nd</td>
<td>0.4</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.20</td>
<td>2.7</td>
<td>7.7</td>
</tr>
</tbody>
</table>

nd = not determinable.
Figure 1. Percent of samples from each site with FC/FS ratios indicative of specific contamination sources.