Bacteria Release and Transport from Livestock Manure Applied to Pastureland

Michelle L. Soupir, *Virginia Polytechnic Institute and State University*
H. E. Saled Mostaghimi, *Virginia Polytechnic Institute and State University*
Elizabeth F. Alphin, *Virginia Polytechnic Institute and State University*

Available at: https://works.bepress.com/michelle_soupir/27/
Bacteria Release and Transport from Livestock Manure Applied to Pastureland

Michelle Soupir, Graduate Research Assistant
Virginia Tech, 201 Seitz Hall, Blacksburg, VA 24061, mpeterie@vt.edu

Saied Mostaghimi, H.E. and Elizabeth F. Alphin Professor
Virginia Tech, 308 Seitz Hall, Blacksburg, VA 24061, smostagh@vt.edu

Written for presentation at the
2003 ASAE Annual International Meeting
Sponsored by ASAE
Riviera Hotel and Convention Center
Las Vegas, Nevada, USA
27-30 July 2003

Abstract. A comparative field investigation was conducted on release and transport of bacteria from plots treated with cowpies, turkey litter, and liquid dairy manure. Rainfall conditions were simulated and runoff samples were collected to determine concentrations of E. coli, FC, and enterococcus present in runoff. The cowpie treatment had the highest percentage of source bacteria released by rainfall, ranging from 1.3% for enterococcus to 14.5% for FC. The cowpie follows with percentages ranging from 0.3 to 0.6%. Runoff samples collected from the transport plots treated with cowpies averaged 137,000 cfu/100 ml for E. coli and over 165,000 cfu/100 ml for FC during two rainfall simulations. Bacteria concentration in runoff from plots treated with liquid dairy manure decreased between the two simulations, while the bacteria concentration from the plots treated with turkey litter increased. The percent of the bacteria that is initially released by rainfall that is transported to the edge of the field in overland flow was highest for the cowpie treatment (95 to 121%), followed by the turkey (41 to 138%) and liquid dairy treatments (32 to 86%). Results indicated that among the animal waste types investigated, cowpies have the greatest potential to contributed E. coli, FC, and enterococcus to streams and waterways.

Keywords. Fecal Bacteria, Agricultural waste, Nonpoint pollution, Land Application, Bacteria Release and Transport

The Society for engineering in agricultural, food, and biological systems
Introduction

The transport of fecal bacteria from point and nonpoint sources to surface waters is becoming an increasing concern in the U.S. Elevated concentrations of fecal bacteria in drinking water can be detrimental to human health; potential diseases include Salmonellosis, Anthrax, Tuberculosis, Brucellosis, and Listeriosis (Azevedo and Stout, 1974). Approximately eight percent of U.S. river miles are impaired by pathogens (USEPA, 1998). A major source of fecal bacteria is runoff from agricultural land where manure has been applied or where animals are allowed to graze. Therefore, an understanding of the overland transport mechanisms for fecal bacteria can have a crucial role on the development of best management practices for reduction of pathogens concentration to surface water bodies.

The transport of bacteria in overland flow is affected by rainfall duration and intensity, method of manure application, fecal deposit age, and adsorption of cells to soil particles. Pathogenic organisms are largely retained at or near the soil surface (Faust, 1982), thus increasing the potential for pollution of surface runoff water. Because manure is less dense than soil, incorporating manure into soil increases the soil’s interrill erodibility and thus the amount of bacteria detached by overland flow (Khaleel et al., 1979). Runoff from snowmelt or rainfall can carry bacteria from fresh manure into the stream. Doran and Linn (1979) found that runoff from a grazed pasture had fecal coliform (FC) concentrations 5-10 times higher than from an ungrazed pasture, but the FC counts in runoff from both the grazed and ungrazed pastures exceeded the water quality standard of 200 CFU/100 ml more than 90% of the time.

Thelin and Gifford (1983) placed cowpies on a platform and rained on them to determine the release of FC. Fecal deposits 5 days old or less released FC concentrations into the water on the order of millions of organisms per 100 ml. Fecal deposits that had not been rained on for up to 30 days released FC concentrations on the order of 40,000 per 100 ml. Larsen et al. (1994) placed bovine feces at 0.0, 0.61, 1.37, and 2.13 m from a runoff collection point to evaluate the release of FC. At the 0.0 m distance from the fecal deposit, the runoff bacteria concentrations corresponded to a release of 17% of the total FC in the manure, or between $40 \times 10^6$ and $115 \times 10^6$ organisms/ml. These values were significantly higher than those measured at the 2.13 m distance from the fecal deposit, where less than 5% of the organisms applied to the plots were present in runoff.

Computer simulation modeling is the primary approach used to develop Total Maximum Daily Loads (TMDL), even though insufficient data exist on several model input parameters related to the release and transport of fecal bacteria in runoff. Previous studies often focused on a single manure source and did not provide comparative results from different sources under similar climatic conditions. In addition, the detachment or release of fecal bacteria from land applied sources is not well-documented. Improvements in understanding the overland processes will improve modeling of fecal bacteria transport, and provide a basis for a more realistic evaluation of management practice implementation.

The overall goal of this study was to quantify the release and transport potential of three fecal bacteria indicators, \textit{E. coli}, enterococcus, and fecal coliform (FC), from land applied manure during runoff events. The specific objectives of this study were to identify differences in bacteria transport among various livestock manures by comparing edge of field bacteria levels in runoff from pasturelands treated with liquid dairy manure, poultry litter, and cowpies. In addition, this study evaluated bacteria release rates for different types of manure applied to pasturelands with different history of previous manure applications. The data from this study will serve as a baseline from which the release and transport of fecal bacteria from agricultural watersheds to surface waters can be modeled.
**Methodology**

Field plots were constructed on existing pastureland in and around Blacksburg, VA. Two sets of plots were established; one set for the study of in-field bacteria release and one set for the study of bacteria transport. Release plots were used to measure available fecal bacteria concentrations in runoff. Four manure treatments (turkey litter, liquid dairy manure, cowpies, and none) and three land type treatments: pasture with a history of poultry litter application (Turkey Farm), liquid dairy manure application (Dairy Farm), and no manure application (Tech Research Farm) were studied. A total of 36 release plots were constructed for three replications of the four manure treatments and three land type treatments.

The transport plots were used to measure the concentrations of fecal bacteria present in overland flow at the edge of the field. The transport plots were only constructed at the Tech research farm due to the labor intensiveness of this component of the research. The release of bacteria from plots applied with liquid dairy, dried poultry litter, and standard cowpies were compared to control plots on which no animal waste was applied. A total of eight transport plots were constructed; two replications of each treatment (turkey litter, liquid dairy manure, cowpies, and control).

**Plot Construction**

Twelve release plots were constructed at each of the three sites for measurement of fecal bacteria concentrations available to runoff. Each release plot had the dimensions of 1 m by 1 m. Pre-fabricated steel borders were placed into the soil along the plot boundaries to prevent water movement into or out of the plots. Runoff drained through a small flume and was collected down-slope in a bucket. The runoff volume was determined by weighing the bucket.

Eight transport plots were constructed at the Tech research farm. Each transport plot was 3 m wide by 18.3 m long on an approximate 5.5 percent slope. Plywood borders were placed to a depth of 15 cm along the plot boundaries to prevent water movement into or out of the plots. A “V” shaped outlet was placed at the down slope end of each plot to direct runoff into a 0.15 m (6-inch) H-flume equipped with an FW-1 stage recorder for flow measurement. The FW-1 stage recorder recorded runoff depth continuously.

**Animal Waste Collection and Application Methods**

The state of Virginia requires phosphorous-based application of manure on crop and pasture lands. This method uses the residual phosphorous levels in the soil and the phosphorous levels in the manure to determine the manure application rate. The $P_2O_5$ application rates recommended for Orchardgrass/Fescue-Clover Pastures on soil productivity groups I and II (DCR 1995) were 90.7 kg/acre (81 lbs/acre) at the Tech farm and 0 kg/ha (0 lbs/acre) at the turkey and dairy farms, respectively.

Because the turkey and dairy farms have a history of receiving land applications of manure, the phosphorous levels were much higher in these fields. The Department of Conservation and Recreation (DCR) Standards and Criteria (1995) recommendation is that no additional phosphorous be applied to the pasture. Currently, the best solution is to apply the manure at a rate slightly lower than the estimated crop uptake, or to restrict manure applications to every other or every third year. Based on this approach, the experimental design was adjusted so that the manure would be applied to the plots at the rate of 56 kg $P_2O_5$ per hectare (50 lbs $P_2O_5$ per acre). Farm equipment used to spread manure cannot spread evenly or accurately if the application rates are too low.
Previous animal waste analysis reports were obtained from the DCR and from the farm managers. The previous analyses were used to estimate the phosphorous content in the dairy and turkey manure that would be applied to the plots. Based on the previous year’s manure samples, the waste was applied to the plots at a rate of 56 kg $P_2O_5$ per hectare (50 lbs $P_2O_5$ per acre). Table 1 compares the results from the previous manure tests to those for the manure samples collected prior to their application to the plots.

Table 1. Concentrations of $P_2O_5$ in manure and the application rate and volume of the manure applied to the transport and release plots.

<table>
<thead>
<tr>
<th>Manure type</th>
<th>$P_2O_5$ estimate based on samples from previous years</th>
<th>$P_2O_5$ estimate based on current waste samples</th>
<th>$P_2O_5$ applied to the plots</th>
<th>Application Rate</th>
<th>Transport Plots</th>
<th>Release Plots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid Dairy</td>
<td>0.67 kg/1000 L</td>
<td>0.67 kg/1000 L</td>
<td>56 kg/ha</td>
<td>81,958.5 L/ha</td>
<td>450.1 L/plot</td>
<td>8.2 L/plot</td>
</tr>
<tr>
<td>Cowpie</td>
<td>2.0 kg/t</td>
<td>1.7 kg/t</td>
<td>50 kg/ha</td>
<td>29.4 t/ha</td>
<td>161.6 kg/plot</td>
<td>3.0 kg/plot</td>
</tr>
<tr>
<td>(180 cowpies)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turkey</td>
<td>20.4 kg/t</td>
<td>19.9 kg/t</td>
<td>54.7 kg/ha</td>
<td>2.8 t/ha</td>
<td>15.1 kg/plot</td>
<td>0.28 kg/plot</td>
</tr>
</tbody>
</table>

The dried turkey litter was collected from the Virginia Tech turkey barns. The litter, comprised of pine shavings and manure, was collected after a flock of turkeys were sent to market. The litter was stacked under a covered shed for a time period varying between 3 and 6 weeks before it was applied to the plots. The litter was uniformly broadcast onto the plots using small buckets.

The liquid dairy manure applied to the plots was obtained from the Virginia Tech Dairy manure storage pond. The storage pond contents are agitated twice a year, to suspend the solids that accumulate on the bottom of the pond. The manure was pumped into a tank and stored throughout the duration of the field experiment. The liquid manure was mixed in the tank before being drained into buckets and applied to the field plots.

“Standard” cowpies were constructed from fresh dairy cow deposits. Each cowpie was standardized by weight and shape, and randomly positioned by project personnel at various locations in the “cowpie” treatment plots. The size and shape of the “standard cowpies” was based on research by Thelin and Gifford (1983), who developed standard cowpies to study FC release patterns. The fresh deposits were formed by taking fresh manure and mixing it in a cement mixer for approximately 15 minutes. The manure was then placed in a mold with a diameter of 20.3 cm and a depth of 2.54 cm. Fecal deposits were placed in the mold until a weight of 0.9 kg was reached. The transport plots were divided into 1 m by 3 m sections. Approximately 9 cowpies were placed in each of the sections. A total of 360 cowpies were applied to the two transport plots. The three cowpies were randomly placed in each of the 1 m by 1 m release plots.

Rainfall simulation on Release Plots

A Tlaloc 3000 portable rainfall simulator, based on the design of Miller (1987), with a ½ 50WSQ Tee Jet nozzle was used to apply rain to the release plots. Rainfall simulations were conducted within 24 hours of the manure application. The plot was rained on until runoff occurred for 30 minutes. After 30 minutes, the rainfall simulation ended and the runoff sample was collected.
This rainfall simulator has been developed as the standard simulator used to test the phosphorous index in various states.

**Rainfall simulation on Transport Plots**

Due to the unreliability of natural precipitation for short-term field research, the Department of Biological Systems Engineering’s rainfall simulator (Dillaha et al., 1987) was used to generate storm events to produce runoff from the field plots. Rainfall was applied at a uniform rate (approximately 4.45 cm/hour) to all pasture plots. A series of rainfall simulations was conducted within 24 hours after manure application. The first simulation (S1) lasted approximately 3 hours. The rainfall continued until a steady state runoff resulted. The S1 simulation represented the bacteria transport during dry field conditions. Before the second simulation (S2) began (approximately 22 hours after the end of the first simulation, S1), soils were saturated. This was due to an overnight natural rainfall of approximately 2.9 cm (1.15 in) and the long simulated rainfall event during the first simulation. Therefore, the second rainfall simulation represented the transport characteristics of bacteria under saturated soil conditions.

The uniformity of rainfall applications was measured using a network of volumetric rain gauges in and around each plot. The uniformity coefficient was determined for both rainfall simulations. The uniformity coefficients for the first and second rainfall events were 93% and 95.5%, respectively.

**Sampling and Analysis**

The total runoff volume was collected from each of the release plots and weighed to determine the volume. The runoff was collected in buckets and a single sample was taken from the total runoff volume. A total of 32 runoff samples were collected from the release plots. Grab samples of runoff water were collected from the transport plots every 3 to 9 minutes during both simulated storm events. A total of 68 samples were collected during S1 and 68 samples were collected during S2.

Samples were analyzed, immediately after collection, for FC, *E. coli*, and enterococcus concentrations in runoff. The samples were analyzed using the Spread Plate (Clesceri et al., 1998) and membrane filtration methods (Clesceri et al., 1998 and EPA, 2000).

**Statistical Analysis**

The release plots were analyzed using a Generalized Randomized Block Design procedure. Tukey’s pairwise comparison was used to test significance between the treatments at the P<0.05 significance level. Transport plots were analyzed using the repeated measure method (Ott and Longnecker, 2001). The response variable was the concentration of bacteria in the runoff leaving the plot. Tukey’s pairwise comparison was used to find significance between the treatments at the P<0.05 significance level. The null hypothesis tested for both the release and transport plots was that there was no difference in the concentrations of the bacteria in surface runoff among the treatments.

\[ \mu_{\text{turkey}} = \mu_{\text{cowpie}} = \mu_{\text{liquid dairy}} = \mu_{\text{control}} \]
Results and Discussion

Release Plots

The concentrations of bacteria and TSS in runoff from the release plots are presented in Table 2. The results from the Tech Research Farm are quite different than expected. The Tech Research Farm, which in the past had not received manure applications, had much higher concentrations in the runoff from the control plots compared with the other farms. This could be due to a higher wildlife population in the area and the lack of cattle to discourage wildlife, or to the build up of stable populations in the soil (Faust, 1982). The plots with the liquid dairy and turkey manure applications had lower concentrations of bacteria than the control. The cowpie plots, consistent with the other sites, had the highest concentrations of bacteria in the runoff. The turkey plots resulted in less suspended solids in the runoff than the control, which may partially explain the reason for reduced bacterial loading from these plots.

Table 2. Concentrations of enterococcus, fecal coliform, E. coli, and Total Suspended Solids from the release plots.

<table>
<thead>
<tr>
<th></th>
<th>Enterococcus (cfu/100 ml)</th>
<th>Fecal Coliform (cfu/100 ml)</th>
<th>E. coli (cfu/100 ml)</th>
<th>TSS* (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tech Research Farm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liquid Dairy</td>
<td>17,000</td>
<td>35,000</td>
<td>23,000</td>
<td>86.0</td>
</tr>
<tr>
<td>Cowpie</td>
<td>285,000</td>
<td>159,500</td>
<td>152,500</td>
<td>71.5</td>
</tr>
<tr>
<td>Turkey</td>
<td>12,075</td>
<td>29,050</td>
<td>18,550</td>
<td>31.5</td>
</tr>
<tr>
<td>Control</td>
<td>23,000</td>
<td>29,350</td>
<td>21,300</td>
<td>37.5</td>
</tr>
<tr>
<td><strong>Dairy Farm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liquid Dairy</td>
<td>3,067</td>
<td>300,000</td>
<td>55,000</td>
<td>166.0</td>
</tr>
<tr>
<td>Cowpie</td>
<td>8,133</td>
<td>300,000</td>
<td>300,000</td>
<td>189.3</td>
</tr>
<tr>
<td>Turkey</td>
<td>1,880</td>
<td>92,000</td>
<td>28,000</td>
<td>134.7</td>
</tr>
<tr>
<td>Control</td>
<td>1</td>
<td>134</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Turkey Farm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liquid Dairy</td>
<td>1,867</td>
<td>47,667</td>
<td>30,667</td>
<td>110.7</td>
</tr>
<tr>
<td>Cowpie</td>
<td>1,007</td>
<td>65,000</td>
<td>37,000</td>
<td>131.0</td>
</tr>
<tr>
<td>Turkey</td>
<td>507</td>
<td>9,000</td>
<td>4,733</td>
<td>146.7</td>
</tr>
<tr>
<td>Control</td>
<td>23</td>
<td>167</td>
<td>1</td>
<td>51.7</td>
</tr>
</tbody>
</table>

*Total Suspended Solids

The plots at the Turkey Farm and the Dairy Farm had more consistent results. Figure 1 compares the average concentrations of E. coli in the runoff at the three different farms. The plots treated with cowpies had the highest E. coli concentrations in the runoff. In general, the plots treated with liquid dairy manure had higher E. coli concentrations than the plots treated with turkey litter. Statistical analysis performed on the treatments, which accounted for the different site locations, found statistical differences among all treatments except for the turkey treatment, which was not statistically different from the liquid dairy or control treatments.
Figure 1. *E. coli* present in runoff from release plots

Figure 2 shows the concentration of FC in the runoff at the three different farms. The plots treated with cowpies, again, had higher FC concentrations in the runoff followed by the liquid dairy and turkey litter. Statistical analysis indicated significant differences among all treatments except for the turkey treatment, which was not statistically different from the control treatment. The FC release concentrations from the plots treated with cowpies ranged from $6.5 \times 10^4$ CFU/100 ml to $30 \times 10^4$ CFU/100 ml, which corresponds with the values reported in the study by Larsen et al. (1994) who reported the FC release concentrations from bovine feces were between $40 \times 10^4$ and $115 \times 10^4$ organisms/100 ml.

In summary, the results from the release plots indicate that during a short but intense rainfall event, the cowpie treatment has the highest bacteria release rate. The liquid dairy treatment had a slightly lower release rate, followed by the turkey litter treatment.
By comparing the bacteria concentrations in the source manure to the average concentrations from the release plots, we were able to determine the percent of the source bacteria that is initially released by rainfall and would potentially be available to be transported to the edge of the field in overland flow. The bacteria concentration in the source manure is initially measured in CFU/gram. This was converted to CFU/100 ml to make the comparison. Table 3 shows the percent of bacteria released from the manure.

Table 3. Percent of bacteria that are released from the manure.

<table>
<thead>
<tr>
<th>Manure Treatment</th>
<th>% Fecal Coliform released from waste</th>
<th>% E. coli released from waste</th>
<th>% Enterococcus released from waste</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid Dairy</td>
<td>0.3%</td>
<td>0.1%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Cowpie</td>
<td>0.6%</td>
<td>0.5%</td>
<td>0.3%</td>
</tr>
<tr>
<td>Turkey</td>
<td>14.5%</td>
<td>5.7%</td>
<td>1.3%</td>
</tr>
</tbody>
</table>

The turkey treatment had the highest percentage of source bacteria released by rainfall, ranging from 1.3% for enterococcus to 14.5% for FC. The cowpie follows with percentages ranging from 0.3 to 0.6%.

**Transport Plots**

Runoff from the transport plots was measured continuously using FW-1 stage recorders. Figure 3 shows the runoff volume from each of the transport plots. Runoff volume increased during S2, due to the saturated ground conditions before the simulation began. The runoff from the plots varies due to differing soil conditions or compaction levels in the soil prior to the rainfall simulation. Runoff volumes also varied because the time at which runoff began differed among the plots. During S1, the plots treated with liquid dairy had the highest runoff volume, followed by the cowpie, turkey litter, and control treatments. During S2, the plots treated with cowpies had the highest runoff volume, followed by the control, liquid dairy, and turkey litter treatments. The predominant factor affecting runoff volume appears to be the time of between the beginning of the rainfall simulation and when runoff first occurred. The plots with earlier runoff times also had higher runoff volumes.

![Figure 3. Runoff Volumes from the transport plots.](image-url)
Statistical analysis was performed on the runoff volumes using the repeated measure method and Tukey’s pairwise comparison. No statistical differences in the runoff volumes from the different treatments were found. There were also no significant differences between the runoff volumes during S1 and S2 simulations at the 0.05 error level.

The flow-weighted concentration (FWC) was calculated for the total suspended solids (TSS) in runoff from each of the transport plots (Table 4). The FWC was calculated by multiplying the sample concentration by the volume of runoff that occurred during that time period. These values were then summed and divided by the total volume of runoff from the plot. The addition of the manure to the plots decreased TSS concentrations from the liquid dairy and turkey plots when compared to the control. Gerba et al. (1975) reported that as bacteria and organic substances accumulate on the soil surface, the trapped bacteria become part of the filtration system, and increase the filtration properties of the soil. This may explain the decrease in TSS concentrations from the liquid dairy and turkey litter plots during the first simulation. The cowpie treatment covered just the areas where the fecal deposits were located, but not the entire plot area. The cowpies had higher moisture content than the other waste types, therefore it is possible that after the raindrop impacts disintegrated the cowpies, they were more readily carried off the plots by runoff.

Table 4. Total Suspended Solid concentrations present in runoff from the transport plots.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Suspended Solids – FWC</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Simulation 1 (mg/L)</td>
<td>Simulation 2 (mg/L)</td>
<td>Average (mg/L)</td>
</tr>
<tr>
<td>Liquid Dairy</td>
<td>59.9</td>
<td>83.4</td>
<td>71.7</td>
</tr>
<tr>
<td>Cowpie</td>
<td>176.7</td>
<td>54.6</td>
<td>115.7</td>
</tr>
<tr>
<td>Turkey</td>
<td>37.3</td>
<td>22.5</td>
<td>29.9</td>
</tr>
<tr>
<td>Control</td>
<td>85.2</td>
<td>29.1</td>
<td>57.1</td>
</tr>
</tbody>
</table>

Flow Weighted Concentration

The trends in the TSS concentrations were compared to the trends for the bacteria concentrations in the runoff from the transport plots (Table 5). In general, the plots treated with cowpies and liquid dairy manure had lower bacteria concentrations in the runoff during S2 than S1. The opposite occurred for the turkey litter, except for the enterococcus concentrations. The TSS concentrations, however, decreased during S2 simulations compared with S1 for the cowpies and turkey litter treatments (Table 4), but they increased for the plots treated with liquid dairy manure. These results indicate that higher TSS concentrations in runoff do not necessarily correspond with higher bacteria concentrations. The proportions of bacteria transported in the dissolved form and attached to suspended solids may differ among the different treatments.

Runoff data and sample concentrations from the transport plots were used to calculate the bacteria flow weighted concentrations. Table 5 presents the bacteria FWC for the transport plots for both S1 and S2 simulations.

Enterococcus concentrations in runoff were slightly lower than the *E. coli* and FC concentrations for all treatments. Enterococci are a subgroup of fecal streptococcus. Enterococcus is used as an indicator bacteria because it is often present in recreational water bodies when human illness occurs (USDA, 2000) and is most often used as a fecal indicator in marine waters. Federal standards for primary contact enterococcus is 33 CFU/100 ml. The concentrations reported in this study are much greater since they represent the edge of the field levels as opposed to in-stream concentrations. In-stream concentrations are expected to be lower due to dilution effects and die-off. The cowpie treatment had the highest FWC for both S1 and S2 events. The enterococcus levels in the runoff from the liquid dairy and turkey plots were slightly lower during
S2 compared with S1. Statistical analysis was performed using the repeated measure method and Tukey’s pairwise comparison. No statistical differences in the enterococcus concentrations in the runoff from the different treatments were found. There were also no significant differences between the concentrations measured during S1 and S2 simulations at the 0.05 error level.

Table 5. Flow weighted bacteria concentrations in runoff from the transport plots for rainfall simulations S1 and S2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Enterococcus (cfu/100 ml)</th>
<th>FC* (cfu/100 ml)</th>
<th>E. coli (cfu/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S1† S2‡ Average</td>
<td>S1 S2 Average</td>
<td>S1 S2 Average</td>
</tr>
<tr>
<td>Liquid Dairy</td>
<td>9,341 3,179 6,260 74,073 6,817 40,445 31,294 5,526 18,410</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cowpie</td>
<td>187,406 50,465 118,936 234,288 96,045 165,166 200,047 73,235 136,641</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turkey</td>
<td>6,757 6,521 6,639 16,719 18,953 17,836 9,275 16,450 12,863</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6 2 4 51 36 43 16 11 13</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Fecal coliforms; †Simulation 1; ‡Simulation 2

Figure 4 presents the E. coli results in a graphical form. The E. coli FWC decreased for the liquid dairy and cowpie treatments during S2, compared with the S1 values. For the turkey treatment, however, the E-coli concentrations increased during S2. This increase can be partly due to the nature of the poultry waste. The liquid dairy and cowpies wastes are more easily transported, while the turkey litter is dry and may require a more significant runoff event to transport the litter off of the plots. The runoff from the cowpie plots clearly had the highest E-coli FWC for both simulations. Statistical differences were only found between the E. coli concentrations in the runoff from the cowpie and the control plots. There were no statistical differences in E. coli concentrations between the two rainfall events.

![Figure 4](image-url)  
Figure 4. Flow weighted concentrations of E. coli in runoff samples from transport plots.

The concentrations of FC in runoff exhibited similar patterns as the E-coli among the different treatments. During S1, the liquid dairy and cowpie treatments had the highest average FC FWC. During S2, the cowpie continued to produce the highest FWC of FC, but the runoff from the plots treated with turkey litter had the second highest FWC, followed by the liquid dairy. The runoff FWC of FC from cowpie treatment were statistically different from all of the other
treatments. There were no statistical differences in FC FWC for each treatment between the two rainfall simulations.

Federal standards for primary contact for FC is 200 CFU/100 ml, much less than the levels present in runoff from the manure treated plots. Baxter-Potter and Gilliland (1988) reported that the typical range of FC present in runoff from pastureland were 1,000 to 57,000 CFU/100 ml. The average value for the two simulations from the pasture treated with cowpies in this study was $1.65 \times 10^5$ CFU/100 ml. The cattle stocking density is not provided in the previous studies, therefore it is not possible to compare the results. Furthermore, this study was designed to evaluate bacteria losses from edge of the field in small plots under intensive rainfall conditions. The bacteria concentrations reported in this study are expected to be much higher than those produced under natural rainfall from large pasture fields or watersheds. FC concentrations from grazed pasture in south central Nebraska contained concentrations of $1.21 \times 10^6$ CFU/100 ml (Schepers and Doran, 1980), which is similar to the results obtained from this study. Fecal bacteria concentration in runoff from grazed pasture is dependent on both the stocking density and the proximity of the cattle to streams. Cattle loafing in shaded or feeding areas produce high concentrations of cowpies in a smaller area and therefore higher bacteria concentrations in runoff. McCaskey et al. (1971) found FC concentrations to range from 1.4 to $21.7 \times 10^6$ CFU/100 ml in runoff from dairy waste applied to pasture plots by a tank wagon. They also reported that runoff from the control area had FC concentrations of $9.9 \times 10^5$ CFU/100ml. These values are much greater than the concentrations of $4.0 \times 10^4$ CFU/100 ml measured in our study.

To determine a relationship between the bacteria release and transport, the average concentrations from the release plots were compared to the average FWC from the transport plots. By comparing the concentrations from the release plots to the concentrations from the transport plots, we were able to determine the percent of the bacteria initially released by rainfall that is transported to the edge of the field in overland flow. Table 6 shows the percent of the released bacteria that is transported in overland flow.

Table 6. Percent of released bacteria that are present in overland flow.

<table>
<thead>
<tr>
<th>Manure Treatment</th>
<th>% Released fecal coliform present in overland flow</th>
<th>% Released E. coli present in overland flow</th>
<th>% Released enterococcus present in overland flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid Dairy</td>
<td>31.7%</td>
<td>50.8%</td>
<td>85.6%</td>
</tr>
<tr>
<td>Cowpie</td>
<td>94.5%</td>
<td>83.7%</td>
<td>121.3%</td>
</tr>
<tr>
<td>Turkey</td>
<td>41.1%</td>
<td>75.2%</td>
<td>137.7%</td>
</tr>
<tr>
<td>Control</td>
<td>0.4%</td>
<td>0.2%</td>
<td>0.1%</td>
</tr>
</tbody>
</table>

The cowpie treatment had the highest percentage of released bacteria present in overland flow with percentages ranging from 95 % for FC to 121% for enterococcus. The turkey treatment follows with percentages ranging from 41% to 138%. The differences between the three species are related to the survival characteristics of the bacteria. Enterococcus is able to survive longer in the environment than FC and *E. coli*. The transport concentrations may be higher than the release concentrations because of background bacteria present in the soil.

In recent years significant changes have occurred in the livestock industry. Animal production areas are highly concentrated, resulting in more manure applications to the fields. In addition, the indicator organisms have changed over the years. Many previous studies provided information on total coliforms, fecal streptococcus, and FC concentrations in runoff. The State of Virginia is currently using *E. coli* as the primary indicator organism in fresh water and enterococcus as the primary indicator organism in marine waters (Virginia DEQ, 2002).
Previous studies rarely provide information on \textit{E. coli} or enterococcus. Runoff from the transport plots treated with manure greatly exceeds the Federal Standards for primary contact.

**Summary and Conclusions**

Field plots were constructed on existing pastureland in southwest Virginia. Two sets of plots were established; one set for the study of in-field bacteria release and another set for the study of bacteria transport. The plots were treated with turkey litter, liquid dairy manure, and standard cowpies. Rainfall was simulated and runoff samples were collected to determine concentrations of \textit{E. coli}, FC, and enterococcus present in runoff.

The runoff collected from the release plot treated with cowpies had higher concentrations of fecal bacteria indicators than those treated with liquid dairy manure or turkey litter. The turkey treatment had the highest percentage of source bacteria released by rainfall, ranging from 1.3\% for Enterococcus to 14.5\% for FC. The cowpie follows with percentages ranging from 0.3 to 0.6\%.

The bacteria flow weighted concentrations in runoff samples from the plots treated with cowpies were over 200,000 CFU/100 ml of \textit{E. coli} and almost 235,000 CFU/100 ml of FC. Runoff from plots treated with liquid dairy treatment had greater fecal bacteria concentrations in runoff during the first rainfall event (S1), which was applied within 24 hours after manure application. These concentrations however were reduced during the second rainfall event (S2), which occurred one day after the initial rainfall. During S1, the concentrations were 31,000 CFU/100 ml for \textit{E. coli} and 74,000 CFU/100 ml for FC, but they decreased to much lower levels during S2 (5,500 CFU/100 ml for \textit{E. coli} and 6,800 CFU/100 ml for FC). The turkey treatment resulted in the opposite effect. During S1, the bacteria concentrations remained low (9,300 CFU/100 ml for \textit{E. coli} and 17,000 CFU/100 ml for FC, but increased during S2 (17,000 CFU/100 ml for \textit{E. coli} and 19,000 CFU/100 ml for FC). This is most likely explained by the composition and transport characteristics of the waste. The percent of the bacteria that is initially released by rainfall that is transported to the edge of the field in overland flow was highest for the cowpie treatment (95 to 121\%), followed by the turkey (41 to 138 \%) and liquid dairy treatments (32 to 86\%).

This comparative study clearly indicates that the cowpies have a greater potential to contribute fecal bacteria into streams than the land application of liquid dairy manure or turkey litter; although, runoff from all treatments exceed federal standards for primary contact. These results imply that areas where cattle may congregate, such as in watering or feeding areas, should be moved away from streams, and the buffer zone between grazing cattle and streams should be increased to reduce the loading of fecal bacteria.

**Acknowledgements**

This study was supported, in part, by the Division of Soil and Water Conservation, Virginia Department of Conservation and Recreation, Richmond, Virginia.

**References**


