

TRANSPORT OF FECAL BACTERIA FROM POULTRY LITTER AND CATTLE MANURES APPLIED TO PASTURELAND

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Abstract. Land applications of manure from confined animal systems and direct deposit by grazing animals are both major sources of bacteria in streams. An understanding of the overland transport mechanisms from land applied waste is needed to improve design of best management practices (BMPs) and modeling of nonpoint source (NPS) pollution. Plots were established on pasturelands receiving phosphorus-based livestock waste applications to measure the concentrations of *Escherichia coli* (*E. coli*), fecal coliform (FC), and *Enterococcus* present in overland flow at the edge of the field. The flow-weighted bacteria concentrations were highest in runoff samples from the plots treated with cowpies (1.37×10^5 colony forming units (cfu)/100 ml of *E. coli*) followed by liquid dairy manure (1.84×10^4 cfu/100 ml of *E. coli*) and turkey litter (1.29×10^4 cfu/100 ml of *E. coli*). The temporal distribution of fecal bacterial concentrations appeared to be dependent upon both the animal waste treatment and the indicator species, with peak concentrations occurring either at the beginning of the runoff event or during peak flow rates. BMPs could be selected to reduce peak flows or first flush effects depending upon the litter or manure applied to the land. The commercial Biolog System was used to identify the dominant species of *Enterococcus* present in the cowpie source manure (*Enterococcus mundtii* 55%) and in the runoff collected from the transport plots treated with cowpies (*Enterococcus faecalis* 37%). The identification of predominant species of *Enterococcus* that are associated with specific sources of fecal pollution could greatly assist with identifying the origins of NPS pollution.

Keywords: bacteria, Biolog, *E. coli*, *Enterococcus*, fecal coliform, land application of waste, nonpoint pollution

1. Introduction

Land application of waste from confined animal production facilities is an effective method of disposing of animal waste while supplying nutrients to crops and pastureland. However, it has been well-documented that runoff from agricultural livestock and poultry litter applied areas is a source of fecal contamination in water (Crowther *et al.*, 2002; Edwards *et al.*, 1994, 2000; Gerba and Smith, 2005; Tian *et al.*, 2002). The EPA's National Water Quality Inventory report (USEPA, 2000) identified bacteria as the leading cause of impairments in rivers and streams in the United States and agricultural practices were identified as the leading source of all bacterial impairments.

Transport of animal manures into surface water bodies can be detrimental to the health of humans, animals, and the ecosystem (USEPA, 2003). Animal waste contains many different types of organisms pathogenic to humans and animals which could be transported into streams when over-applied to agricultural lands. More than 150 pathogens found in livestock manure are associated with risks to humans, including *Campylobacter spp.*, *Salmonella spp.*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Cryptosporidium parvum* and *Giardia lamblia*, which account for over 90% of food and waterborne diseases in humans (USEPA, 2003).

An understanding of the overland transport mechanisms from land applied waste is needed to improve design of BMPs and modeling of NPS pollution for development and implementation of Total Maximum Daily Loads (TMDL). The process of classifying sources of NSP pollution could be greatly simplified by identifying the predominant species of *Enterococcus* that are associated with specific sources of fecal pollution. The Biolog System identifies microorganisms based on carbon source utilization (Biolog, 2003). Hagedorn *et al.* (2003) employed carbon source utilization as a form of phenotypic fingerprinting to classify enterococcal isolates from known fecal sources in four different geographical regions. The study concluded that the commercial Biolog System provided levels of correct classification from the enterococcal library that were in the upper range of those reported in the literature.

The first objective of this study was to quantify the transport potential of three fecal bacterial indicators: *E. coli*, *Enterococcus*, and FC from livestock manure (turkey litter, liquid dairy manure, and cowpies) applied to pastureland; the second objective was to identify temporal trends of fecal bacteria concentrations during the runoff event; and the third objective was to use the Biolog System to identify the enterococcal species present in dairy cowpies and determine which species have the highest potential to be transported by runoff.

2. Materials and Methods

Field plots were constructed on existing pastureland at the Virginia Tech Research Farm, Blacksburg, Virginia (37°12.95'N, 80°29.52'W) to measure the concentrations of bacteria present in overland flow at the edge of the field. The transport of bacteria from plots treated with turkey litter, liquid dairy manure, and cowpies were compared to control plots on which no animal waste was applied. Two replications of each treatment resulted in the construction of a total of eight transport plots.

2.1. PLOT CONSTRUCTION

The 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. A "V" shaped outlet was placed at the down slope end of each plot to direct runoff into

TABLE I
Manure characteristics and application rates of P₂O₅

Manure characteristics	Liquid dairy	Cowpie	Turkey
P ₂ O ₅ concentration in waste samples	0.68 kg/1000 L	1.8 kg/t	19.9 kg/t
P ₂ O ₅ application rate (kg/ha)	56	50	54.7
Animal waste application rate	81,958.5 L/ha	29.4 t/ha	2.8 t/ha
<i>E. coli</i> (cfu/g)	410,000	>300,000	3,000
Fecal coliform (cfu/g)	490,000	>300,000	3,000
<i>Enterococcus</i> (cfu/g)	670,000	>300,000	3,600
Moisture content	96%	83%	49%

a 0.15 m (6-inch) H-flume equipped with an FW-1 stage recorder for continuous flow measurement (Soupir, 2003).

2.2. SOIL CHARACTERISTICS

The soils at the Virginia Tech Research Farm were evaluated for particle size distribution and residual phosphorus (P) levels. Soils were identified as Grosclouse and Poplimento silt loams with a particle composition of 34% sand, 61% silt and 5% clay. The residual P levels in the soil were 8.8 ppm. Based on these concentrations, the recommended P₂O₅ application rate was 100.9 kg/ha (VADCR, 1995). The pasturelands were dominated by fescue and red clover in addition to some orchardgrass and broadleaf weeds.

2.3. MANURE APPLICATION RATES AND CHARACTERISTICS

Often pasturelands have a history of receiving land applications of manure, resulting in high soil P levels. In this situation, animal waste should be applied at a rate slightly lower than the estimated crop uptake, or applications should be restricted to every other or every third year so that eventually the P concentrations in the soil would reduce. Manure was applied to the plots at the rate of 56 kg P₂O₅ per hectare (50 lbs P₂O₅ per acre), a minimum application rate that allows for even distribution of manure by farm equipment (Dean Gall, personal communication, Blacksburg, Virginia, 27 September 2002). Information on the manure characteristics and land application rates is presented in Table I.

The dried turkey litter, comprised of pine shavings and manure, was collected from a pile stacked under a covered shed for three weeks and then uniformly broadcast onto the plots. The liquid dairy manure was obtained from the Virginia Tech Dairy manure storage pond. The storage pond contents were agitated, pumped into a tank, and uniformly applied to the plots. "Standard" cowpies were constructed from fresh dairy cow deposits scraped from dairy stalls. The standard cowpies were

formed by taking fresh manure and mixing it in a cement mixer for approximately 15 min. The manure was then placed in a mold with a diameter of 20.3 cm (8 in) and a depth of 2.54 cm (1 in) until a weight of 0.9 kg (2.0 lbs) was reached (Thelin and Gifford, 1983). A total of 360 cowpies were randomly positioned in the two transport plots. Such high concentrations of cowpies are typically seen near water troughs, gates, fence lines, and shady areas or bedding areas, where cattle tend to congregate.

2.4. RAINFALL SIMULATION

The Biological Systems Engineering Department's rainfall simulator (Dillaha *et al.*, 1987) generated storm events to produce runoff from the transport plots. Rainfall was applied at a uniform rate (44.5 mm/h) to all pasture plots. Two rainfall simulations were conducted within 24 h after manure application. The first simulation (S1) lasted approximately 3 h because of initial dry soil conditions. The rainfall was applied until a steady state runoff resulted. The S1 simulation represented the bacteria transport during dry field conditions. Before the second simulation (S2) began (22 h after the end of S1), soils were saturated due to the long simulated rainfall event during S1 and an overnight natural rainfall event which did not produce additional runoff. Simulation S2, which lasted one hour, represented the bacteria transport characteristics under very wet soil conditions with very high potential for producing runoff. Rainfall amounts were measured using volumetric rain gages installed at two locations in the plots. The rainfall application uniformity coefficients (Schwab *et al.*, 1993) for S1 and S2 were 93% and 96%, respectively.

2.5. SAMPLE AND DATA ANALYSIS

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 runoff samples during S1 and 68 samples during S2 were collected from all eight plots. FW-1 stage recorders tracked runoff hydrographs and recorded the sampling time. Each sample was analyzed for total suspended solids, *E. coli*, FC, and *Enterococcus* concentrations so that temporal distributions during runoff events could be examined. A magnesium chloride and potassium dihydrogen phosphate buffer solution (Hach, 2003) was added to the water and manure samples before testing for bacteria. The samples were analyzed using the Spread Plate (Clesceri *et al.*, 1998) and membrane filtration methods (Clesceri *et al.*, 1998).

The commercial Biolog System (Biolog, 2003) identified the species of *Enterococcus* present in the source manure and those species that were in the runoff samples. The procedures for the Biolog assay, validation with control cultures, and confirmation of species identifications were performed as described by Hagedorn *et al.* (2003). Twenty isolates each were taken from the plated source manure and from the onset, peak, and cessation of runoff during both rainfall simulations.

The Repeated Measures Design and Tukey's pairwise comparison (Ott and Longnecker, 2001) tested for significance among the transport plot treatments. Significance was determined at the $p < 0.05$ level. The null hypothesis was that there would be no difference in the concentrations of the suspended solids or bacteria in surface runoff among the treatments.

3. Results and Discussion

3.1. RUNOFF AND TOTAL SUSPENDED SOLIDS

Runoff increased during the second simulation (S2), due to the saturated soil conditions compared with the initial simulation (S1). The runoff varied among the plots due to differing soil moisture conditions, manure treatments prior to the rainfall simulation, and different runoff start times. The liquid dairy manure increased the soil moisture content of the plots prior to the rainfall simulation due to the nature of the waste, accounting for the slight increased runoff from the liquid dairy plots during S1. No statistically significant differences in runoff were detected among the different treatments or between the S1 and S2 simulations at a p -value of 0.05. Thus, the differences in the bacterial concentrations reported here were mainly due to the different manure treatments, and not necessarily due to the differences in runoff volumes or other factors affecting runoff. Flow-weighted concentrations (Novotny and Olem, 1994) were calculated for the TSS in runoff from each of the transport plots. There were also no statistical differences in the TSS concentrations between the two simulations or among any of the different treatments (Table II).

TABLE II
Runoff and flow-weighted total suspended solid concentrations

	Rainfall simulation	Liquid dairy	Cowpie	Turkey	Control
Runoff (mm)	S1 ^a	6.9a*	4.7a	3.8a	2.8a
	S2 ^b	9.8a	12.9a	9.2a	10.4a
Total suspended solids – FWC ^c (mg/L)	S1	59.3	177.2	37.3	85.1
	S2	83.6	54.8	22.5	29.0
	Average ^d	71.5a	116.0 a	29.9 a	57.1 a

* Average values followed by the same letter in each row do not differ at the 5% level of significance according to Tukey's pairwise comparison.

^aSimulation 1.

^bSimulation 2.

^cFlow-weighted Concentration.

^dAverage of S1 and S2.

TABLE III

Flow-weighted bacterial concentrations in runoff from the transport plots for rainfall simulations S1 and S2

	Rainfall simulation	Liquid dairy	Cowpie	Turkey	Control
<i>E. coli</i> (cfu/100 ml)	S1 ^a	3.13×10^4	2.00×10^5	9.27×10^3	16
	S2 ^b	5.52×10^3	7.33×10^4	1.65×10^4	11
	Average ^c	1.84×10^4 ab*	1.37×10^5 a	1.29×10^4 b	14 b
Fecal coliform (cfu/100 ml)	S1	7.42×10^4	2.34×10^5	1.67×10^4	51
	S2	6.81×10^3	9.61×10^4	1.90×10^4	36
	Average	4.05×10^4 b	1.65×10^5 a	1.79×10^4 b	44 b
<i>Enterococcus</i> (cfu/100 ml)	S1	9.34×10^3	1.87×10^5	6.75×10^3	6
	S2	3.18×10^3	5.04×10^4	6.52×10^3	2
	Average	6.26×10^3 a	1.19×10^5 a	6.64×10^3 a	4 a

*Average values followed by the same letter in each row do not differ at the 5% level of significance according to Tukey's pairwise comparison.

^aSimulation 1.

^bSimulation 2.

^cAverage of S1 and S2.

3.2. FECAL BACTERIA

Flow-weighted concentrations were calculated for the *E. coli*, FC, and *Enterococcus* in runoff from each of the transport plots (Table III). A large number of samples were processed at once, making it impossible to always estimate the correct dilution level within the allotted time. The highest bacterial counts achieved were averaged for all three treatments.

The results from the transport plots indicated that for a runoff event occurring soon after land application of waste; the cowpie treatment had the highest average fecal bacteria indicator flow-weighted concentrations, followed by the liquid dairy manure and turkey litter treatments. The differences among the treatments were primarily due to the different initial bacterial concentrations in the source manure applied to the plots (Table I). The fresh cowpies protected the bacteria from environmental factors that could increase die-off, but were easily broken apart by the impact of the raindrops. Based on observations of the disintegrating cowpies, bacteria were more readily released for transport off of the plots during the runoff event. Following application of the liquid dairy manure, the bacteria either infiltrated into the soil prior to the rainfall event or remained on the surface and died off due to the overnight environmental exposure.

The turkey litter treatment had the lowest concentrations of bacteria in the source manure (Table I); however, the concentrations in runoff were much higher than expected. The average enterococcal concentrations exceeded the concentrations from

the plots treated with the liquid dairy manure even though 223 times more bacteria were applied to the liquid dairy manure plots; thus, the turkey litter treatment released a greater percentage of the bacteria present in the source manure. The bacterial concentrations in runoff from the control plots were very low, indicating that background contributions were minimal.

The bacterial concentrations from the liquid dairy and cowpie treatments decreased during S2, compared with the S1 values. The bacteria present on the soil surface appear to have been washed off the plots during S1 or died off due to the additional overnight environmental exposure. The FC and *E. coli* concentrations in runoff from the plots treated with turkey litter increased during S2, when the litter remaining on the plots was saturated. The turkey litter had lower moisture content and might require a more significant runoff event to release the bacteria for transport. The bacteria could also be more easily released when the moisture content of the litter increases as was the case prior to S2.

3.3. TEMPORAL DISTRIBUTION OF BACTERIAL CONCENTRATIONS

The distribution of the bacterial concentrations along the runoff hydrograph was investigated to determine if a relationship exists between flow rates and bacterial indicator concentrations. Two trends were most commonly observed in the runoff intensity/bacterial indicator relationship (Figure 1). Most frequently, the bacterial concentrations increased as flow rates increased, peaking simultaneously (trend 1). At other times, the first sample, taken immediately after the initiation of runoff, had the highest indicator bacterial concentration which then decreased with subsequent samples (trend 2). On occasion, the bacterial concentration peaked for the last sample, taken three minutes after the rain stopped. This pattern indicated that substantial dilution reduced bacterial concentrations during high runoff rate periods which occurred during the rising limb of the hydrograph.

The relationship between flow rate and bacterial concentration appeared to be dependent upon the indicator species and the animal waste treatment (Table IV). The concentrations in runoff from the plots treated with cowpies followed trend 1 during S1. However, during the S2 simulation, they followed trend 2 with a peak occurring at the last sampling time for all indicator species. These results would have implications for modeling the temporal concentrations of bacteria indicator species and the types of BMPs that need to be implemented to alleviate the peak concentrations. Different management practices are needed to reduce bacterial transport from the two patterns illustrated in Figure 1. In some cases the peak bacterial concentration may occur simultaneously with peak flow (trend 1) and BMPs that reduce peak runoff rates may be most suitable to reduce bacterial loading into waterways. Vegetative filter strips are often implemented to reduce peak flows. However, their effectiveness in removing fecal bacteria in runoff has produced mixed results (Chaubey *et al.*, 1994; Coyne *et al.*, 1995, 1998; Lim *et al.*, 1998), and they might not be a suitable management practice. Coyne *et al.* (1995)

TABLE IV
Temporal distributions of fecal bacteria depended upon manure treatment and indicator

Treatment	Rainfall simulation S1			Rainfall simulation S2		
	Liquid dairy	Cowpie	Turkey	Liquid dairy	Cowpie	Turkey
	Follows trend 1 or 2					
<i>E. coli</i>	2	1	1	2	2	1
Fecal coliform	1	1	1	2	2	1
<i>Enterococcus</i>	2	1	1	2	2	1

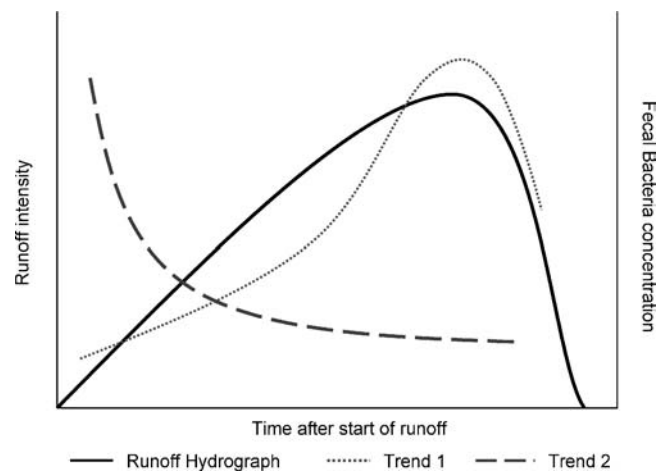


Figure 1. Temporal distribution of fecal bacteria concentrations in relation to runoff intensity.

concluded that grass filter strips (9 m long) were unable to reduce FC concentrations in runoff to meet primary water contact standards of 200 cfu/100 ml. The grass filter strips effectively removed 99% of the sediment in the surface runoff, but FC removal rates ranged from 43% to 74%. The peak bacteria concentration could also occur immediately after runoff begins (trend 2), which would indicate that BMPs that reduce the first flush effects, such as detention basins, could more effectively reduce bacterial transport.

3.4. ENTEROCOCCAL SPECIES IDENTIFICATION

Because of the large number of pastured cattle in Virginia, the cowpie treatment was selected for a species identification study. Even though the same species of *Enterococcus* can be found in several different host species, major variation in the distribution of the different enterococcal species among hosts have been found (Aarestrup *et al.*, 2002). The number of enterococcal isolates selected for species

TABLE V

Enterococcal species identified in dairy cowpies (source manure) and in runoff during S1 and S2 using the Biolog identification system

Species in dairy cowpies	Number identified	Percent identified	Species in runoff	Number identified	Percent identified
<i>Enterococcus mundtii</i>	11	55%	<i>Enterococcus faecalis</i>	44	36.7%
<i>Enterococcus gallinarum</i>	4	20%	<i>Enterococcus mundtii</i>	25	20.8%
<i>Enterococcus faecium</i>	2	10%	<i>Enterococcus gallinarum</i>	13	10.8%
<i>Enterococcus faecalis</i>	2	10%	<i>Enterococcus faecium</i>	13	10.8%
<i>Enterococcus solitarius</i>	1	5%	<i>Enterococcus casseliflavus</i>	8	6.7%
			Other	17	14.2%

identification was similar to those reported from other studies (Pourcher *et al.*, 1991). Table V presents the enterococcal species present in the cowpies and runoff.

The source manure was dominated by *Enterococcus mundtii*, a species that is commonly related to cattle manure. The presence of *Enterococcus faecalis* in cattle source manure is somewhat controversial. The results from this study agree with findings from Rutkowski and Sjogren (1987) where *Enterococcus faecalis* was found to be present in cattle manure, but disagree with studies conducted by Pourcher *et al.* (1991) and Wheeler *et al.* (2002). As part of a phenotypic methods comparison study (Harwood *et al.*, 2003), 50 isolates were tested from cow manure in California with the Biolog method. Their study found *Enterococcus faecalis* to be 2% of the total isolates identified. A study by Hagedorn (C. Hagedorn, unpublished data) identified 53 isolates from cow manure in Delaware and found only 2% of the isolates tested were *Enterococcus faecalis*, using the Biolog method. Although only a small percentage of the available enterococcal isolates were identified, the results appear to be similar to previous results obtained from other studies that used the Biolog System (Harwood *et al.*, 2003).

In addition to evaluating the source manure to determine the existing species, it is also important to consider the transport capabilities of the different species. Even if an enterococcal species is present in the source manure, it might not be transportable in surface runoff. Twenty enterococcal isolates were taken from the beginning of the runoff event, the peak runoff point, and the end of the runoff event during S1 and S2. Table V also presents the percentage of each species collected in runoff samples.

These results indicate that *Enterococcus faecalis* may be the dominant species present in runoff from grazed pasturelands. *Enterococcus faecalis* was not present in high numbers in the source sample, but was consistently found in the runoff, indicating that it may be a more transportable species of *Enterococcus*. *Enterococcus mundtii* is one species of *Enterococcus* frequently found in runoff, but was present in runoff at much lower percentages. This species might die-off more quickly than other species and might not be present in runoff if additional rainfall events had

been performed at a later time after the land application of the waste. The enterococcal counts from the control plots were very low, so the likelihood that the isolates identified as *Enterococcus faecalis* were from some other source, such as birds, is highly unlikely, and since the manure for the cowpies was collected from fresh material in the dairy barns, it also minimized the likelihood of contamination from other sources.

The identification of predominant species of *Enterococcus* that are associated with specific sources of fecal pollution could greatly simplify identification of NPS pollution. The presence of a certain species of *Enterococcus* would indicate the source of the fecal pollution and proper measures could be taken to reduce loading without the use of computer simulation models or expensive bacterial source tracking (BST) analysis. In order to accomplish this task, the most transportable species (and die-off rates) related to fecal pollution from different sources should be identified. This research provides preliminary results for dairy cattle, but much more research is needed.

4. Summary and Conclusions

A field study was conducted to evaluate the transport of bacteria from livestock manure applied to pasturelands. Rainfall was simulated and runoff samples were collected to determine concentrations of TSS, *E. coli*, FC, and *Enterococcus* present in runoff and temporal trends of *E. coli*, FC, and *Enterococcus* as related to the runoff hydrograph. The commercial Biolog System was used to evaluate the species of *Enterococcus* present in the source manure and in runoff from plots treated with cowpies.

The average bacteria flow-weighted concentrations were highest in runoff samples from the plots treated with cowpies (*E. coli* concentrations of 137,000 cfu/100 ml, FC concentrations of 165,000 cfu/100 ml, and *Enterococcus* concentrations of 119,000 cfu/100 ml). Runoff from pasture treated with cowpies and liquid dairy manure had higher fecal bacteria concentrations during the initial rainfall event (S1), but the turkey litter treatment concentrations increased during S2. Results of this comparative study clearly indicate that cowpies have a greater potential to contribute high fecal bacteria concentrations into streams than the land application of liquid dairy manure or turkey litter, although bacteria concentrations in runoff from all treatments exceeded Federal standards for primary contact in the United States. The relationship between runoff rates and concentrations of the indicator species was dependent upon the animal waste application, the indicator species and antecedent soil moisture conditions.

The Biolog System identified the different species of *Enterococcus* present both in the cowpie source manure and in the runoff collected from the transport plots treated with cowpies. The source manure was dominated by *Enterococcus mundtii* (55%) and *Enterococcus gallinarum* (20%), while *Enterococcus*

faecalis (37%) was the dominant species in runoff. The identification of predominant species of *Enterococcus* that are associated with specific sources of fecal pollution could greatly simplify the identification of the origins of non-point source pollution.

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