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Bacterial Transport from Agricultural Lands Fertilized with Animal Manure

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Abstract A plot scale study was conducted to determine bacterial transport in runoff from cropland treated with poultry litter and dairy manure applied at phosphorus (P) agronomic rates. Treatments included surface application of dairy manure, surface application of poultry litter, incorporation of dairy manure and control. A rainfall simulator was used to induce runoff 1 and 2 days after manure application. Runoff was analyzed to determine the concentration of indicator bacteria-fecal coliform, Escherichia coli, and Enterococcus. Observed edge-of-field bacterial concentrations were 10^2 to 10^5 times higher than Virginia's in-stream bacteria criteria for primary contact recreation waters. No significant treatment effects were observed on edge-of-field bacteria concentration or yield. Results suggest that the manure application based on agronomic P rates may yield significant bacterial loading to downstream waterbodies if rainfall occurs soon after manure application. This research underscores the need for BMPs that reduce runoff volumes and filter pollutants associated with animal manures.

Keywords Enterococcus · *Escherichia coli* · Fecal coliform · Indicator bacteria · Rainfall simulator

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1 Introduction

In the report to 105th congress, the Senate Agriculture, Nutrition and Forestry Committee identified the excessive or ill-timed land application of animal manure as a major source of water quality impairment (Harkin 1997). The land application of animal manure can pollute water sources by introducing pollutants such as nutrients, organic matter, sediment, pathogens, heavy metals, hormones, and antibiotics. The National Water Quality Inventory Report-2000 identified the runoff from agricultural lands as a leading source of water quality impairment in USA, and pathogens as the one of the most widespread pollutants (USEPA 2002). About 13.3% of the assessed river length (~150,362 km), and 3.3% of assessed lakes, reservoir and ponds (~2,347 km^2) in USA are impaired due to excessive pathogens (USEPA 2002). In Virginia, over 8,000 km of streams and rivers are impaired due to pathogens (VADEQ 2004).

Fecal material from warm blooded animals carries several microorganisms, some of which may be pathogenic. Since it is impractical to test water for individual pathogens, impairment by pathogens is often quantified based on indicator species such as total coliform, *Enterococus*, *Escherichia coli* (*E. coli*), fecal coliform, and fecal streptococcus, which are found in the intestine of warm blooded animals (Walker et al. 1990). Total coliform is not a reliable indicator of fecal contamination as this category includes several non-fecal sources (Rosen 2000).

Fecal coliform is a subgroup of total coliform and originates specifically from intestinal tract of warm blooded animals. Currently, fecal coliform is the predominant indicator used to assess bacterial pollution in watersheds (Rosen 2000). The other indicator species for pathogen impairment are *E. coli* and *Enterococcus*. *E. coli* is a member of the fecal coliform group and its presence correlates well with illnesses that result from exposure to fouled water. Fecal streptococcus is less dominant in animal feces compared to fecal coliform. Fecal streptococcus does not multiply in the environment and the die-off in the environment occurs rapidly. *Enterococcus* is a subgroup of fecal streptococcus and found in intestinal tract of humans and other warm blooded animals. *Enterococcus* correlates well with human illnesses that result from exposure to fouled water.

Several researchers have attempted to quantify the amount of indicator bacteria transported in runoff from pastures where animal waste has been applied (Doran and Linn 1979; Edwards et al. 2000; Kunkle 1970; Soupir et al. 2006) and from feedlots (Miner et al. 1966; Young et al. 1980). Moore et al. (1988) summarized several researches reporting indicator bacteria concentration in runoff from pasture, grazing systems, cropland and feedlot. However, relatively few experiments have been conducted to quantify the concentration of indicator bacteria present in surface runoff from cropland following manure application. Barker and Sewell (1973) studied the losses of indicator bacteria in runoff from cropland following irrigation with dairy manure slurries.

Indicator bacteria loads in surface runoff vary according to the type of animal whose manure is applied to the land surface, how the manure was stored, age of the manure, time interval between manure applications and the rainfall, rainfall intensity and other soil and environmental factors affecting bacteria survival (e.g., ambient temperature, soil pH, soil type, moisture availability). Robbins et al. (1971) concluded that on an annual basis, 3 to 23% of fecal coliform deposited on the soil surface through manure application, or directly by animals is lost in runoff. Kunkle (1979) reported a decline in population of fecal coliform in runoff with time. Barker and Sewell (1973) observed that in irrigated production systems a majority of bacteria were lost in the first sprinkler irrigation event following manure application. Other than the increased time interval between manure application and the first rainfall or irrigation event, incorporation of animal manure in soil has been suggested as the method to reduce bacterial loading in surface runoff (Walker et al. 1990).

A better understanding of bacterial movement from land applied animal manures is needed to improve the design of best management practices and water quality modeling associated with the development and implementation of Total Maximum Daily Loads (TMDLs) and other similar watershed management processes. This study compares bacterial transport in runoff from conventionally tilled cropland plots receiving different types of animal manure, resulting due to rainfall occurring shortly after manure application. The specific objective of this study was to determine the indicator bacteria losses in the runoff from conventionally tilled plots, treated with dairy manure and poultry litter applied at P-based agronomic rates.

2 Materials and Methods

Bacteria transport plots were constructed at the Virginia Tech Prices Fork Research Center, Blacksburg, VA. The soil is a Groseclose silt loam (clayey, mixed, mesic Typic Hapludult). Five treatments were evaluated; dairy manure surface applied (DS), dairy manure incorporated (DI), poultry litter surface applied (PL), inorganic fertilizer incorporated (IF), and no fertilizer or control (CO). The IF treatment was used to study and compare the transport of nutrient in runoff, as described in (Mishra et al. 2006). Three replications of each treatment produced a 15 plot Randomized Complete Block Design (RCBD).

2.1 Plot Construction

An area approximately, 25×50 m, was tilled twice, each time to a depth of 0.15 m. Following the second tillage operation, 2,4-dichlorophenoxyacetic acid (at the rate of 0.57 kg active ingredient per ha) was applied to kill any emergent vegetation. Following the tillage operations, 15 18.3×3.3 m plots were constructed, each adjacent to the others with the plot longitudinal direction perpendicular to the contour. The average slope of the study area is 2%. The plots were separated by 0.30 m tall plywood borders, buried to a depth of 0.15 m. The borders served to isolate each plot hydrologically. At the downslope end of each plot a "V" shaped outlet concentrated the overland flow into a 0.15 m rectangular H-flume fitted with a Belfort model FW-1 stage recorder (Belfort Instrument, Baltimore, MD, USA) to continuously measure runoff rate. Runoff rate and volume from the plots were also recorded manually using a stop watch and a 5-l bucket.

2.2 Manure Characteristics and Application Rates

Soil samples were collected following the second tillage operation and analyzed for residual nutrient content to determine the appropriate fertilizer rate. Recommended N and P (as P_2O_5) application rates were 170 and 90 kg ha^{-1}, respectively (VADCR 1995). Dairy manure was obtained 17 days prior to the experiment from an anaerobic lagoon located at the Virginia Tech dairy, Blacksburg, VA and stored in plastic tanks. The dairy manure was collected early to accommodate the dairy's lagoon pump-out schedule. Lagoon contents were well mixed during pump-out. Dairy manure samples were sent to Agriculture Service Laboratory, Clemson University, Clemson, SC, on the day of manure collection for nutrient analysis (Table 1). Poultry litter was obtained from a broiler operation one day before it was applied. The availability of nutrients in poultry litter was estimated from the nutrient analysis result of previous year for poultry litter (same farm and same management system; Table 1).

The amount of dairy manure and poultry litter applied to the plots (Table 2) was calculated based on nutrient availability of the manure and the litter and the P-based (as P_2O_5) agronomic requirements of "Early Sunglow Sweet" corn (*Zea Mays*) seeded at a rate of 10.8 kg ha^{-1}. The availability of P in animal manure and litter is comparable to N; however, when applied at a P-based rate, the manure and litter do not supply sufficient N for proper crop growth. In this research the N deficiency was alleviated by applying ammonium nitrate (34–0–0; Table 2).

Dairy manure was applied on each plot manually by transferring the stored manure to buckets that were used to uniformly spread the manure. To resuspend the solids, the dairy manure was constantly stirred while being transferred to the buckets. Poultry litter was uniformly broadcast by hand. For the incorporated treatment (DI), dairy manure was incorporated by tilling the soil to a depth of 0.15 m after application. Plots were planted with "Early Sunglow Sweet" corn (*Zea Mays*) at a rate of 10.8 kg ha^{-1} following manure application on May 13, 2003.

2.3 Rainfall Simulation

The Virginia Tech Biological Systems Engineering rainfall simulator (Dillaha et al. 1988) was set up following corn planting to conduct a series of rainfall simulations. The source water used for simulation was obtained from an on-site pond. Rainfall was applied at a rate of 50 mm h^{-1}. The first rainfall simulation (S1) was conducted on May 14, 2003, within 24 h of manure application, to simulate a scenario where a rainfall event occurs shortly after manure application. The S1 event lasted 60 min and represented a precipitation event occurring on dry soil conditions.

Table 1 Concentration and availability of nutrients in manure and fertilizer used in the experiment as reported by the Agriculture Service Laboratory, Clemson University, Clemson, SC

Manure	Available nitrogen	Phosphorus as P_2O_5
Dairy manure (4/26/03)[a]	9.74×10^{-4} (kg l^{-1}) – surface applied[b] 1.2×10^{-3} (kg l^{-1}) – incorporated[c]	7.97×10^{-4} (kg l^{-1})
Poultry litter (previous year record, same farm and management system)	2.25%[d]	2.46%
Ammonium nitrate	34.00%	–

[a] Dates refer to the day when manure was shipped for nutrient analysis.

[b] Surface applied available nitrogen includes 50% of ammonium-N, 60% of organic-N, and 100% of nitrate-N, in dairy manure.

[c] Incorporated available nitrogen includes 80% of ammonium-N, 60% of organic-N, and 100% of nitrate-N, in dairy manure.

[d] All percentages are reported by weight.

Table 2 Amount of manure and fertilizer applied to each treatment

Treatment	Manure/fertilizer applied for P_2O_5	N deficiency alleviated by ammonium nitrate (kg ha^{-1})	Ammonium nitrate applied (kg ha^{-1})
DS (surface applied dairy manure)	112,923 (l ha^{-1})	60.0	176.0
DI (incorporated dairy manure)	112,923 (l ha^{-1})	34.5	101.5
PL (surface applied poultry litter)	3,660 (kg ha^{-1})	88.0	258.0
CO (control)	–	–	–

The second rainfall simulation (S2) was conducted 24 h later (May 15, 2003), and represented precipitation event under wet soil conditions. The duration for the S2 event was 48 min. The duration of simulated rainfall events was a function of the time required for the runoff from the plots to reach steady-state conditions. The coefficient of uniformity for the two events was greater than 90%.

2.4 Sampling and Data Analysis

Runoff grab samples from each plot were collected at the outfall of the flumes every three minutes on the rising limb of the hydrograph. This interval was increased to 6 min after the runoff hydrograph reached steady-state. A simulated rainfall event continued until the hydrograph from all plots reached steady state. Between 8 and 14 water samples were collected from each plot during each rainfall event. One flow-weighted composite sample for each plot and each rainfall event was prepared, following the event, and analyzed. The flow weighted samples were analyzed for fecal coliform, *E. coli*, and *Enterococcus* (Clesceri et al. 1998).

2.5 Statistical Analysis

Analysis of variance (ANOVA) was performed on runoff volumes (mm), bacteria concentration (cfu/100 ml), and yield (cfu/ha) to determine treatment and simulation effects (Ott and Longnecker 2001). An adjusted Tukey's pairwise comparison (Ott and Longnecker 2001) was performed among the treatments and rainfall simulation events. Significance was determined at the $P<0.05$ level. The null hypotheses were that there is no difference in indicator bacteria concentrations and yield among treatments within each simulated event and across the simulated rainfall events.

3 Results and Discussion

3.1 Runoff

The effect of antecedent soil moisture conditions was evident on the peak runoff rate and runoff volume for the two simulated events (Table 3). The event mean peak runoff rate and volume were significantly greater for the S2 event, some 25 and 53% greater, respectively when compared to S1. Before the S1 event, the soil was dry and freshly tilled, the plot surface was disturbed, and the infiltration capacity was greater in comparison to the second rainfall simulation event. The S1 event settled the tilled soil smoothing the surface. Additionally, raindrop impact redistributed soil particles producing a surface sealing crust. The surface crust and the additional soil moisture from the S1 rainfall reduced infiltration capacity for the S2 event. With respect to treatment effects, the DS treatment produced the lowest peak runoff rate for S1 and lowest runoff volume for both S1 and S2. The mulching effect of the solids in the surface applied dairy manure was likely a contributing factor. A similar mulching effect was observed by Bruggeman and Mostaghimi (1993) for freshly applied sludge.

3.2 Fecal Coliform (FC)

The fecal coliform (FC) concentration (cfu/100 ml) in runoff from all the treatments ranged from 8.0×10^2 to 1.0×10^6 (Table 4). An ANOVA analysis was performed using the bacteria concentration data transformed to log base 10. This transformation permitted analysis using mean comparison tests (Gilbert 1987). No significant treatment effect or rainfall event effects were observed for FC concentration or yield, save the CO treatment which yielded a significantly lower FC concentration during the S2 event. The high variabil-

Table 3 Runoff response from tested treatments for all simulated events

Treatments	Simulated event 1 (S1)		Simulated event 2 (S2)	
Mean treatment peak runoff rate (mm/h)				
DI (incorporated dairy manure)	32.1a[a]	(10.9)[b]	39.6a	(7.8)
PL (surface applied poultry litter)	28.6a	(4.8)	37.2a	(1.9)
DS (surface applied dairy manure)	22.7a	(4.3)	38.4a	(4.7)
CO (control)	28.6a	(6.2)	33.0a	(4.8)
Event mean	28.0A[c]		37.1B	
Mean treatment runoff volume (mm)				
DI (incorporated dairy manure)	6.5ab	(2.1)	11.9ab	(1.7)
PL (surface applied poultry litter)	7.9ab	(4.1)	12.0ab	(0.6)
DS (surface applied dairy manure)	4.1a	(1.5)	8.9a	(0.7)
CO (control)	6.3ab	(1.9)	9.5ab	(2.3)
Event mean	6.2A		10.6B	

[a] Similar lowercase letters indicate no statistically significant difference among the treatments for a simulated event at the 0.05 level.

[b] Numbers in parenthesis are standard deviations.

[c] Similar uppercase letters indicate no significant difference between simulated rainfall events at the 0.05 level

ity of the observed bacteria concentration data was the primary reason why no significant response was found. No significant treatment effect may suggest that manure incorporation may not help in reducing bacteria transport in surface runoff when rainfall occurs closely following the manure application. This result is in contrast with the general recommendation: that manure incorporation is an effective method for reducing pathogens in runoff (Crane et al. 1980).

The relatively high FC concentration in the CO treatment, even though 6 to 40 times less than the concentrations measured for other treatments was unexpected, since no manure was applied to the CO plots. The reason for the high FC concentration from

Table 4 Mean concentration and yield of fecal coliform in runoff

Treatments	Simulated event 1 (S1)		Simulated event 2 (S2)	
Mean fecal coliform concentration (cfu/100 ml)				
DI (incorporated dairy manure)	3.7 E5a[a]	(1.9 E4)[b]	3.7 E5a	(1.4 E2)
PL (surface applied poultry litter)	1.0 E6a	(5.5 E5)	1.4 E5a	(2.7 E5)
DS (surface applied dairy manure)	1.7 E5a	(2.2 E5)	2.9 E5a	(4.8 E4)
CO (control)	2.6 E4a	(9.8 E5)	8.0 E2 b	(7.1 E4)
Event mean	4.0 E5 A[c]		2.0 E5 A	
Mean fecal coliform yield (cfu/ha)				
DI (incorporated dairy manure)	3.2 E12a	(5.0 E12)[a]	4.5 E12a	(3.0 E12)
PL (surface applied poultry litter)	1.2 E13a	(1.3 E13)	1.6 E12a	(7.7 E11)
DS (surface applied dairy manure)	1.0 E12a	(1.5 E12)	2.5 E12a	(7.3 E11)
CO (control)	1.9 E11a	(1.9 E11)	8.2 E9 a	(1.8 E8)
Event mean	3.5 E12A		2.2 E12A	

[a] Similar lowercase letters indicate no statistically significant difference among the treatments for a simulated event at the 0.05 level.

[b] Numbers in parenthesis are standard deviations.

[c] Similar uppercase letters indicate no significant difference between simulated rainfall events at the 0.05 level.

the CO treatment could be the cross-contamination from other treatments, or the influence of wildlife. The sources of cross contamination could be graduate students walking through adjacent plots while planting corn, or setting up the rainfall simulator, or wind may have spread some poultry manure to adjacent plots during application. Similar effects of cross contamination were observed for other indicator bacteria species (*E. coli* and *Enterococcus*) as well.

The concentration of FC from dairy manure treatments (DI and DS) increased slightly during the S2 event compared to S1, although runoff volume and peak runoff rate increased from the S1 and S2 event and a dilution effect was expected. The increase in FC concentration and runoff volume resulted in increased FC yields from the dairy manure treatments. The increase in concentration coupled with increased runoff volume and peak runoff rate have been observed in previous studies (Dudley and Karr 1979; Kunkle 1970; Robbins et al. 1971). These results are particularly significant as they show that the release of bacteria from dairy manure is gradual, and that the bacterial contamination from animal manure treatments can extend over a period of time, unlike nutrient contamination where significant losses of nutrients were observed during the first simulated rainfall event (Mishra et al. 2006).

Except for the CO treatment, the FC concentrations observed in this study were at least three orders of magnitude higher than values previously reported (Barker and Sewell 1973; Janzen et al. 1974; Robbins et al. 1971) and the state water quality criteria for primary contact (SWCB 2006). Although these values are edge of field concentrations and some dilution, filtration and die-off is expected before the bacteria in the runoff would reach a receiving waterbody, the high observed concentrations have the potential to increase the FC concentration in the receiving waterbody well above the applicable water quality criteria resulting in a water quality impairment.

3.3 *Escherichia coli* (EC)

The *E. coli* (EC) concentration (cfu/100 ml) in runoff from all the treatments ranged from 1.9×10^3 to 2.8×10^4 (Table 5). As with the FC indicator, no significant treatment effect nor rainfall event effects were observed. For the S2 event, an increase in EC concentration compared to S1 was observed for the DI and PL treatments. An increase in yield was also observed for all the treatments except CO. Although not significant, this increase in EC concentration and yield is consistent with observations made in previous studies (Dudley and Karr 1979; Kunkle 1970;

Table 5 Mean concentration and yield of *E. coli* in runoff

Treatments	Simulated event 1 (S1)		Simulated event 2 (S2)	
Mean *E. coli* concentration (cfu/100 ml)				
DI (incorporated dairy manure)	6.5 E3a[a]	(5.7 E3)[b]	1.0 E4a	(1.1 E4)
PL (surface applied poultry litter)	1.9 E3a	(2.0 E3)	2.8 E4a	(3.6 E4)
DS (surface applied dairy manure)	8.6 E3a	(2.3 E3)	7.8 E3a	(5.1 E3)
CO (control)	1.9 E3a	(2.2 E3)	3.6 E3a	(5.3 E3)
Event mean	4.7 E3A[c]		1.2 E4A	
Mean *E. coli* yield (cfu/ha)				
DI (incorporated dairy manure)	4.0 E10a	(2.9 E10)	7.6 E10a	(1.1 E11)
PL (surface applied poultry litter)	2.2 E10a	(2.6 E10)	3.2 E11a	(4.0 E11)
DS (surface applied dairy manure)	3.3 E10a	(1.6 E10)	4.2 E10a	(4.1 E10)
CO (control)	8.8 E9a	(8.5 E9)	5.6 E9a	(6.2 E9)
Event mean	3.0 E10A		1.0 E11A	

[a] Similar lowercase letters indicate no statistically significant difference among the treatments for a simulated event at the 0.05 level.

[b] Numbers in parenthesis are standard deviations.

[c] Similar uppercase letters indicate no significant difference between simulated rainfall events at the 0.05 level.

Robbins et al. 1971). The presence of bacteria in the runoff from the CO treatment is again attributed to the cross-contamination from other treatments.

For both simulated rainfall events, the EC concentration from all treatments was 10 to 100 times higher than the EC water quality criterion of 126 cfu/100ml (SWCB 2006). Again, these are edge of field values, and some dilution, filtration and die-off is expected as runoff travels overland and flows downstream.

3.4 *Enterococcus* (ENT*)*

The *Enterococcus* (ENT) concentration (cfu/100 ml) in runoff from all the treatments ranged from 3.3×10^3 to 7.9×10^5 (Table 6). No significant treatment effect or rainfall event effects were observed except for the CO treatment which produced a significantly lower concentration and yield compared to other treatments for the S2 event. This result may suggest that manure application is a source of ENT yield in runoff. A decrease in concentration of ENT in runoff from S1 to S2 event was observed in DS and PL treatments, while the ENT concentration from the DI treatment was unchanged. These trends were different than that observed for the other bacterial indicators.

In general, the ENT concentration and yield were less than the FC and more than the EC yields. The ENT concentration in runoff observed from the two events was three to four orders of magnitude greater than the federal standard for primary contact (33 cfu/100 ml). Again, these are edge of field concentrations and these levels could decrease before entering and when in receiving waters.

4 Conclusions

A plot-scale study was conducted at Prices Fork Research Farm, Virginia Tech, to determine the indicator bacteria loss in runoff from conventionally tilled plots treated with dairy manure, and poultry litter. Manure and litter applications were based on agronomic P requirements. Runoff was induced by an artificial rainfall simulator with an intensity of 50 mm h^{-1} 1 and 2 days following manure application. The fecal coliform, *E. coli* and *Enterococcus* concentration in runoff from the two simulated events were one to four orders of magnitude higher than state in-stream water quality criteria that were developed to protect human health. Bacteria concentrations this high are in part due to the fact that the first simulated event was conducted in less than 24 h after manure application. We surmise that the moisture from first simulated event aided bacterial survival, which led to higher bacterial concentration during second simulated

Table 6 Mean concentration and yield of *Enterococccus* in runoff

Treatments	Simulated event 1 (S1)		Simulated event 2 (S2)	
Mean *Enterococcus* concentration (cfu/100 ml)				
DI (incorporated dairy manure)	4.2 E4a[a]	(2.3 E4)[b]	4.3 E4a	(2.6 E4)
PL (surface applied poultry litter)	2.8 E5a	(1.2 E4)	8.6 E4a	(1.1 E4)
DS (surface applied dairy manure)	7.9 E5a	(1.4 E6)	8.3 E4a	(7.2 E4)
CO (control)	3.3 E3a	(3.7 E3)	8.6 E3b	(4.2 E3)
Event mean	2.8 E5A[c]		5.5 E4A	
Mean *Enterococcus* yield (cfu/ha)				
DI (incorporated dairy manure)	2.9 E11a	(2.1 E11)	5.4 E11a	(4.0 E11)
PL (surface applied poultry litter)	2.7 E12a	(7.2 E11)	1.0 E12a	(1.3 E11)
DS (surface applied dairy manure)	5.2 E12a	(9.0 E12)	7.0 E12a	(6.3 E11)
CO (control)	2.2 E10a	(1.4 E10)	7.2 E10b	(4.8 E10)
Event mean	2.1 E12A		5.9 E11A	

[a] Similar lowercase letters indicate no statistically significant difference among the treatments for a simulated event at the 0.05 level.

[b] Numbers in parenthesis are standard deviations.

[c] Similar uppercase letters indicate no significant difference between simulated rainfall events at the 0.05 level.

event. The increase in runoff volume, and peak runoff rate helped increase the bacteria concentration, and consequently yield during the second simulated event. No significant treatment effects were observed on the bacterial loading in surface runoff. This is particularly important as previous studies have suggested that incorporation is a viable BMP to reduce bacterial losses from animal manure treated fields.

Although, the concentration and yield of bacteria reported in this study are edge of the field values and some filtering, dilution, and die-off of bacteria would occur prior to reaching a receiving waterbody, the magnitude of bacteria concentration and yield reported in this study underscores the need for BMPs to control bacterial loading when precipitation occurs soon after manure application. This study suggests that manure application on cropland based on agronomic P requirements can be a significant source of bacteria loading, if rainfall occurs shortly after manure application. The manure application should be planned in ways that there is sufficient time for bacteria die-off before the rainfall. In areas of high rainfall, or if the manure is applied in rainy season, sufficient BMPs should be installed to control bacteria losses and transport.

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