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Effects of Animal Diet, Manure Application Rate, and Tillage on Transport of Microorganisms from Manure-Amended Fields▽

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Manure from cattle fed distillers' grain or corn diets was applied to fields, and the fields were subjected to rainfall simulation tests. Manure was added at three rates on till and no-till plots. Correlations between microbial transport and runoff characteristics were identified. Results indicate that diet affects phage but not bacterial transport from manure-amended fields.

One goal of sustainable agricultural systems is to utilize nutrients in cattle manure while minimizing its adverse environmental impacts, including the transfer of bacterial pathogens or excess nutrients within watersheds (8). Manure from animals fed distillers' grains has different physical, nutrient, and microbial characteristics from manure from animals fed traditional corn finishing diets (2), including higher dissolved and total phosphorus levels (11, 13) and greater concentrations of some pathogenic bacteria (16, 17). Numerous factors affect how fecal microorganisms are transported in manure-amended fields. *Escherichia coli* bacteria from different animals have different transport characteristics (1). Significant transfer of fecal bacteria to watersheds was found to take place if rainfall occurred shortly after manure application (10). The amount of manure applied also impacts the total number of microorganisms present. Since manure nitrogen and phosphorus levels are affected by animal diet (i.e., corn or distillers' grains) and land application rates depend on the levels of these nutrients in manure, the total amount of manure applied will vary depending on individual manure nutrient characteristics (2). Also, manure can be applied to meet single- or multiple-year crop nutrient requirements. An additional factor impacting the transport of fecal microorganisms is whether the manure is surface applied or incorporated into the soil. Tillage of cropland after the application of manure affects both nutrient and microbial concentrations (7).

While the variations in physical, chemical, and microbiological characteristics of manures from animals fed different diets are well documented, little is known about how these differences impact the transport of manure-borne microorganisms once manure is land applied. We conducted experiments to measure bacterial and phage transport in runoff from fields amended with manure from animals fed either corn or 40% wet distillers' grain rations. Because the distillation process concentrates phosphorus in the by-product, we examined field plots using 1-, 2-, and 4-year phosphorus application requirements for corn under both till and no-till cropping conditions.

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Thirty-six 0.75- by 1.5-m plots were established on an Aksarben silty clay loam soil (fine, smectitic, mesic Typic Argiudoll) containing 11% sand, 54% silt, 35% clay, and 18.5 g kg^{-1} of organic C in the top 15 cm of the soil profile sample. Plots were delineated with sheet metal boarders, and runoff was collected in a trough located across the bottom of each plot. The field was cropped using a grain sorghum-soybean-winter wheat rotation under no-till management and was planted with sorghum the previous year. Experimental treatments included three replications each, on separate days, for the following factors: type of manure, manure application rate, and single-pass disk tillage in a randomized complete block design. Six control plots (three tilled and three no-till plots) to which no manure was applied were also established. Manure characteristics were measured to determine appropriate application amounts. Since manure was applied to meet corn phosphorus requirements, supplemental inorganic N fertilizer was added at rates required to meet annual crop growth requirements.

National Phosphorus Research Project rainfall simulation procedures were used to apply rainfall to paired plots as described previously (6, 12). Briefly, irrigation water was used to simulate rain using a portable rainfall simulator (12). Rainfall was applied for 30 min at an intensity of 70 mm h^{-1}. Runoff from each plot was pumped into a large storage container. At the completion of each run, the entire volume of water was thoroughly mixed and subsamples were taken for analysis. All microbial samples were placed immediately on ice and transported to the laboratory within 2 h for immediate analysis. Two additional rainfall simulation tests were conducted with the same duration and intensity at approximately 24-h intervals. Nutrient analyses were performed as described previously (4) and included measurements of dissolved phosphorus, total phosphorus, NO_3-N and NH_4-N, total nitrogen, pH, and electrical conductivity. Bacteriological analyses included enumeration of total coliforms, *E. coli* bacteria, and enterococci using the EPA-approved Quanti-Tray system (IDEXX Laboratories, Westbrook, ME), quantification of somatic phages (14), and isolation of naturally occurring Shiga-toxigenic *E. coli* O157, O111, and O26 strains (3) from runoff samples and the source manure.

Statistical analysis was performed using the analysis of variance (ANOVA) procedure in SAS software (SAS Institute,

TABLE 1. Effects of animal diet, manure application rate, and tillage on microbial transport

Factor	Value for:			
	Phages (log PFU ha^{-1})	Total coliforms (log CFU ha^{-1})	*E. coli* (log CFU ha^{-1})	Enterococci (log CFU ha^{-1})
Animal diet				
Corn	10.20	12.96	12.66	11.88
Distillers' grains	11.35	12.85	12.60	11.73
$LSD_{0.05}$[a]	0.38			
Manure application rate[b]				
1-yr P requirement	10.44	12.68	12.32	11.54
2-yr P requirement	10.70	12.87	12.62	11.87
4-yr P requirement	11.18	13.16	12.95	12.00
$LSD_{0.05}$	0.47	0.19	0.20	0.36
Tillage				
Till	10.86	13.01	12.53	12.10
No till	10.69	12.80	12.73	11.51
$LSD_{0.05}$				
ANOVA value for[c]:				
Diet	0.01	0.23	0.51	0.30
Application rate	0.02	0.01	0.01	0.04
Tillage	0.81	0.24	0.27	0.13
Diet × application rate	0.50	0.91	0.87	0.74
Diet × tillage	0.83	0.51	0.17	0.59
Tillage × application rate	0.43	0.19	0.77	0.51
Diet × tillage × application rate	0.26	0.72	0.77	0.74

[a] The LSD test was used to identify significant differences among experimental treatments. A probability level of <0.05 was considered significant. The $LSD_{0.05}$ is the LSD at the probability level of <0.05.
[b] Beef cattle manure was applied to meet 1-, 2-, or 4-year P requirements for crop growth.
[c] ANOVA values are displayed as *P* values.

2003), and the least-significant-difference (LSD) test was used to identify significant differences among experimental treatments. A probability level of <0.05 was considered significant.

The manure application rate impacted total counts for all four measured microbial parameters (phages, total coliforms, *E. coli* bacteria, and enterococci) (Table 1). Correlation coefficients for microbial constituents and runoff characteristics are presented in Table 2. All four measured microbial parameters were found to be significantly correlated to NH_4-N. Total coliform and *E. coli* numbers were also correlated to dissolved, particulate, and total phosphorus levels. Juhna et al. (9) reported that phosphorus concentrations affect the survival of *E. coli* in drinking water, but it is unlikely that the same mechanism is applicable in this system.

Our field-based results from manure-amended cropland show no significant differences in the numbers of total coliforms, *E. coli* bacteria, and enterococci in runoff from agricultural fields amended with manure from animals fed either distillers' grain or corn diets (Table 1). The mean total coliform, *E. coli*, and enterococcus concentrations in runoff from all treatments were 9.47×10^6, 5.35×10^6, and 1.16×10^6 CFU hectare $(ha)^{-1}$, respectively. The numbers for total coliforms in this investigation are similar to results reported by Mishra et al. (10) for fecal coliforms in dairy and poultry manure-amended plots, and both *E. coli* and enterococcus numbers were higher in the study conducted by Mishra et al. (10). Even though the three rainfall events occurred on three consecutive days after manure application, the majority of the manure-borne bacteria remained on the plots. *E. coli* O157:H7, O26:H11, and O111:H8 bacteria were isolated in runoff from 17, 4, and 0 of 36 plots, respectively.

Based on the counts of bacteria in the source manure at the time of application, there was a relatively low percentage of bacteria transported in the runoff (Fig. 1). It has been shown previously that the physical and microbial parameters of cattle manure can be influenced by diet (2, 11, 13, 16, 17) and that *E. coli* bacteria from different kinds of manure can have different transport characteristics in the laboratory (1). Although the differences were not statistically significant in this study, it was interesting that the transport percentages for fecal coliform, *E. coli*, and enterococcus bacteria in runoff were higher for plots receiving manure from corn-fed animals than from those receiving manure from distillers' grain-fed animals.

In contrast, there were statistically significant differences in phage counts between plots that were amended with manure from corn- versus distillers' grain-fed animals, with higher phage recovery from runoff from plots amended with manure from distillers' grain-fed animals (Table 1). At the time of application, the pHs of the manures from animals fed the

TABLE 2. Correlation coefficients for microbial constituents and runoff characteristics

Microbial constituent	Value[a] for:							
	DP[b]	PP[c]	TP[d]	NH_4-N[e]	TN[f]	NO_3-N[g]	EC[h]	pH
Phages	0.23 (0.02)	−0.10 (*)	0.14 (*)	0.22 (0.02)	−0.23 (0.02)	−0.11 (*)	−0.14 (*)	0.09 (*)
Fecal coliforms	0.28 (0.01)	0.29 (0.01)	0.31 (0.01)	0.29 (0.01)	0.05 (*)	0.07 (*)	0.13 (*)	−0.21 (0.03)
E. coli bacteria	0.49 (0.01)	0.42 (0.01)	0.52 (0.01)	0.36 (0.01)	0.17 (*)	−0.36 (0.01)	0.33 (0.01)	−0.21 (0.03)
Enterococci	0.18 (*)	0.14 (*)	0.19 (*)	0.25 (0.01)	−0.01 (*)	0.11 (*)	0.01 (*)	−0.37 (0.01)

[a] A correlation coefficient is significant at the 95% level if |correlation| is >0.19 for *n* of 108. The value in parentheses represents the following: $Pr > |r|$, which is a two-tailed *P* value testing that the correlation is zero. Asterisks indicate values of >0.05.
[b] DP, dissolved phosphorus.
[c] PP, particulate phosphorus.
[d] TP, total phosphorus.
[e] NH_4-N, ammonium nitrogen.
[f] TN, total nitrogen.
[g] NO_3-N, nitrate nitrogen.
[h] EC, electrical conductivity.

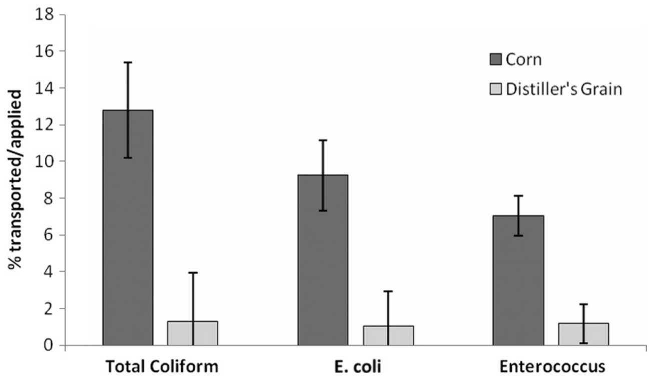

FIG. 1. Transport percentages for fecal coliform, *E. coli*, and enterococcus bacteria in runoff as affected by diet. Microbial transport values were averaged across manure application rates and tillage conditions. Vertical bars represent standard errors.

distillers' grain and corn diets were 7.7 and 8.3, respectively. It has been reported previously that pH can alter the electrochemical properties of soils and microorganisms (15). Therefore, the observed differences in runoff may be attributed, in part, to variations in pH (5). Phage populations increased rapidly following manure application. The transport percentage for phages was highest for the no-till plots receiving manure from corn-fed animals (Fig. 2), although the differences were not statistically significant. In some instances, phage numbers in runoff were found to be higher than those in the source manure. Since bacteriophage replication involves the lysing of bacteria, it is possible that the higher phage concentrations are responsible for lower numbers of fecal indicator bacteria; however, this specific issue was not investigated here. Comparisons of transport percentages for somatic phages in runoff as affected by diet and tillage suggest that multiple factors influence phage transport in runoff.

In conclusion, animal diet significantly affected the transport of bacteriophages in runoff. However, diet did not significantly

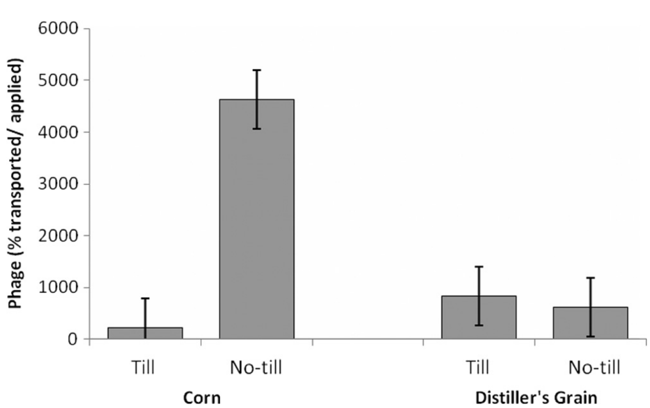

FIG. 2. Transport percentages for phages in runoff as affected by diet and tillage. Microbial transport values were averaged across manure application rates. Vertical bars represent standard errors.

influence the transport of fecal indicator bacteria. Microbial transport increased significantly with the manure application rate. An interesting spike in phage transport was observed for the no-till plots amended with manure from corn-fed animals, but these data were not statistically significant. Tillage conditions did not significantly affect the transport of fecal indicator bacteria.

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