Microbial quality of runoff following land application of cattle manure and swine slurry

Jeanette A. Thurston-Enriquez, John E. Gilley and Bahman Eghball

ABSTRACT

Concentrations of human health-related microorganisms in runoff from agricultural plots (0.75 m × 2 m) treated with fresh and aged cattle manure, swine slurry and no manure (control) were determined. Three consecutive simulated rainfall events, producing 35 mm rainfall and separated by 24 h, were carried out for each plot. Fecal indicator (*Escherichia coli*, enterococci, *Clostridium perfringens* and coliphage) loads released in rainfall runoff from plots treated with fresh cattle manure, aged cattle manure and swine slurry treatments ranged from 5.52×10^5 to 4.36×10^9, 3.92×10^4 to 4.86×10^8, and 9.63×10^5 to 3.05×10^8, respectively. Plot runoff concentrations of protozoa (*Cryptosporidium* oocysts and *Giardia* cysts) ranged from 1.65×10^5 to 1.04×10^6, 2.93×10^3 to 2.75×10^5, and 9.12×10^4 to 3.58×10^6 for fresh cattle manure, aged cattle manure and swine slurry plot treatments, respectively. These results suggest that large microbial loads could be released via heavy precipitation events that produce runoff from livestock manure-applied agricultural fields, of even modest size, and could have a significant impact on water bodies within the watershed. Because of the lack of multiplication in the environment, highly elevated concentrations in manured land runoff, and correlation to protozoan parasite presence, *Clostridium* may be an alternative indicator for livestock manure contamination.

Key words | *Clostridium*, *Cryptosporidium*, *Escherichia coli*, indicators, *Giardia*, runoff

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INTRODUCTION

Approximately 1.36 billion tons of manure is generated by about 376,000 livestock operations in the US each year (EPA 2001). Compounding this issue is the increase in the number of livestock with a decrease in the number of animal feeding operations (AFOs) (EPA 2001). This intensification of the livestock industry generates large amounts of manure within small geographic areas (USDA 2003). Because of these large amounts of manure, animal feeding operations have emerged as a major potential source of water pollution with a primary focus on excessive nutrients, especially phosphorus and nitrogen. In addition to nutrients, pathogenic microorganisms may also occur in manure and are a concern to human and animal health.

To deal with large amounts of livestock manure, livestock producers store it in piles or lagoons, manage it to decrease nutrient and pathogen concentrations (i.e. composting), or spread or inject it into the soil to meet the nutrient requirements of crops. Feces are also directly deposited onto land within outdoor pens and pastures. Since animal feces can harbour human pathogenic microorganisms, pathogen transmission from livestock manure to water (ground, irrigation, surface and recreational waters) and food (contamination of food animals and crops) from direct deposition, water runoff events or other routes increases risks to human and animal health.

Waterborne outbreaks of disease in the United States have been associated with precipitation events (Curriero *et al.* 2001). Rainfall runoff events may carry human pathogens in water runoff from contaminated sites, such as manured land,

to water bodies serving as recreational, irrigation or drinking water sources. With increasing heavy rainfall events in the United States (Easterling *et al.* 2000*a*, *b*), rainfall runoff from manured land reaching water supplies is a growing concern. In fact, in May 2000 runoff from a field treated with cattle manure contaminated a groundwater supply with human pathogenic bacteria. *Escherichia coli* O157:H7 and *Campylobacter jejuni* were the aetiological agents identified in this waterborne outbreak that caused 2,300 illnesses and 7 deaths (McQuigge 2000; Hrudey *et al.* 2003). In addition to human pathogenic *E. coli* and *Campylobacter*, other manure-borne pathogens can be present and survive in livestock manure. For example, human pathogenic protozoa, *Giardia lamblia* and *Cryptosporidium parvum*, can also be fecally excreted by infected livestock and be disseminated by rain runoff events (Atherholt *et al.* 1998; Tate *et al.* 2000; Kistemann *et al.* 2002). Compounding this issue, these manure-borne protozoan parasites are infectious at very low doses (Smith 1993) and may be able to survive in manure and surface water for long periods (Deng and Cliver 1992; Fayer *et al.* 2000; Jenkins *et al.* 2002).

Runoff from livestock-agricultural areas has been reported as an important source of microbial contamination of water bodies. Studies involving fecal bacterial contamination in streams near dairy farms and cattle pastures (Gary *et al.* 1983; Niemi and Niemi 1991), surface runoff from grazed pastures (Doran and Linn 1979; Jawson *et al.* 1982), and subsurface runoff from manure-applied fields (Culley and Phillips 1982) demonstrated the ability of rain water runoff to horizontally transport fecally derived bacteria from manure-laden land to surface water supplies. Rain events can also flush manure-borne bacteria vertically, contaminating shallow groundwater (McMurry *et al.* 1998), and springs and wells within the hydrological catchments of pastures (Howell *et al.* 1996).

In addition to indicators of fecal pollution, manure-borne protozoan pathogens have been released into surface waters in close proximity to manure-applied fields. Since the majority of manure-borne protozoan parasites are transported in the aqueous phase of runoff (Mawdsley *et al.* 1996), high concentrations of infectious protozoa present on manure-applied cropland have potentially serious human health implications. Sischo *et al.* (2000) suggested that increased manure spreading frequency and the duration of rain events are risk factors for manure-borne protozoan parasite contamination of surface water (Sischo *et al.* 2000). A large proportion of *Cryptosporidium* oocysts that were inoculated into fecal pats were flushed during the first two rainfall events following manure application to rangeland plots (Tate *et al.* 2000). Limited information, however, is available regarding naturally occurring *Giardia* and *Cryptosporidium* concentrations transported in rainfall runoff from manure-applied cropland.

The benefits of no-tillage, a soil conservation practice, include erosion control, greater water infiltration and decreased evaporation (Unger 2003). However, the impact that no-till, manure-applied cropland has on the transport of manure-borne fecal indicator and protozoa in runoff has not been investigated previously. Therefore, the objectives of this study were to determine: 1) the load of manure-borne microorganisms, important in microbial water quality assessments (fecal indicator microorganisms) and human health (protozoan parasites), in runoff from cattle manure and swine slurry treated no-tilled cropland; 2) the effect that fresh and aged cattle manure have on microbial concentrations in cropland runoff; 3) the ability of traditional (*E. coli* and enterococci) and alternative (*Clostridium perfringens* and coliphage) fecal microorganisms to serve as indicators of protozoa and livestock manure contamination; and 4) the effect that corn plant residue has on microbial concentrations in runoff.

METHODS

Study site

This field study was conducted from May to August 2001 at the University of Nebraska Rogers Memorial Farm located about 18 km east of Lincoln, Nebraska, in Lancaster County. The Sharpsburg silty clay loam soil (fine, smectitic, mesic Typic Argiudoll) at the site contained 11% sand, 54% silt, and 35% clay with 18.5 g kg^{-1} organic C in the top 15 cm of soil. The pH of the soil was 6.6 and 5.3 for 0–5 cm and 5–15 cm depth, respectively. The soil formed from loess under prairie vegetation and had a mean slope of 7%. The site had been cropped using a grain sorghum (*Sorghum bicolor* (L.) Moench), soybean (*Glycine max* (L.) Merr.), winter wheat (*Triticum aestivum* L. cv. Pastiche) rotation

under a no-till management system, and was left undisturbed following soybean harvest in autumn 2000. Herbicide was applied immediately before and midway through the study to prevent weed growth.

Manure and residue

Aged beef cattle manure was collected from animal pens in May 2001 from a private confined livestock operation near Waterloo, Nebraska. At this cattle feeding operation, the yearling to 2-year-old cattle were fed a diet of ground alfalfa, cracked corn and liquid protein. The uncovered animal pens contained 100–120 cattle per pen. Pens were scraped to remove manure build-up 1–2 times per year, therefore the 'aged manure' used for this research contained a mixture of fresh and aged cattle feces. In order to achieve a homogeneous application to each experimental plot, aged cattle manure was sieved through a screen with 12 mm openings and then stored at 4°C until application. Fresh beef cattle manure and swine manure was obtained from the University of Nebraska Agricultural Research and Development Center near Ithaca, Nebraska. Fresh beef cattle fecal pats were collected directly from animal pens. These cattle pens housed 16-month-old steers that were fed 48% moist corn, 40% wet corn gluten, 7% alfalfa hay and 5% mineral supplements. The swine production unit contained 100 swine weighing 36 to 45 kg that were fed a corn/soybean diet. Fresh cattle and swine manure was mixed prior to land application. Sieving of these manure types was not necessary since their liquid composition enabled a more homogeneous application to experimental plots compared with aged beef cattle manure. Corn residue materials, containing mainly stalks, were collected at the Rogers Memorial Farm in May 2001 and dried in an oven at 60°C prior to plot application.

Experimental design

A randomized complete block design was used with three replications. Each block included: 1) corn (*Zea mays* L.) residue at a rate of 4 Mg ha^{-1} and manure (three plot replications); 2) no corn residue but with manure (three plot replications); and 3) no corn residue or manure (three plot replications). The types of livestock manure included aged beef cattle, fresh beef cattle and swine. Cattle and swine manure were applied at rates of 32.3 Mg ha[1] and 66.5 Mg ha^{-1}, respectively, which were the amounts that would have been required to meet corn N requirements. Application rates were determined using 40% N availability for beef cattle manure (Eghball and Power 1999) and 70% N availability for swine manure (Eghball *et al.* 2002). The applied corn residue mass of 4 Mg ha^{-1} provided approximately 37% surface cover (Gilley *et al.* 1986). Ground water, used for irrigation of cropland at the research site, was used for rainfall simulation tests.

The dimension of each experimental plot was 0.75 m wide by 2 m long. Plot borders consisted of a sheet metal lip that emptied into a collection trough. The trough extended across the bottom of each plot and diverted runoff into aluminium washtubs. Two rain gauges were placed along the outer edge of each respective plot, and one rain gauge was located between the paired plots. Soybean material (from the previous year) was removed from each experimental plot prior to the initial rainfall event. To provide more uniform antecedent soil water conditions between treatments, water was applied to the plots with a hose until runoff began. Following prewetting, crop residue followed by manure or only manure was added uniformly by hand immediately before the initial rainfall event. To represent an extreme rainfall event, simulated rainfall was applied for 30 min at an intensity of 70 mm h^{-1} using a portable rainfall simulator based on a previous design (Humphry *et al.* 2002). Each plot, therefore, received 52.5 l of water from each rainfall event. Runoff from each rainfall simulation event was collected in each plot's collection trough. Before a sample was collected, runoff water was agitated to suspend solids. Less than 60 min was taken to saturate the plot soil, apply manure or manure and corn residue, perform rainfall simulation, and collect runoff water from each experimental plot. Water samples were collected in 1-l sterile plastic bottles and kept on ice until arrival at the laboratory where they were stored at 4°C until analysis.

At the end of the day, each plot was covered with a canvas tarp to limit input from natural rainfall and wildlife. Two additional rainfall simulation events were conducted at approximately 24-h intervals for every experimental plot treatment. During each of the three rain runoff experiments,

plots were exposed to sunlight from approximately 9 am to 3 pm each day.

Physical and chemical water analyses

Turbidity of each sample was determined using a Hach 2100P turbidimeter (Hach Co., Loveland, Colorado). Relative humidity and average air temperature were recorded by a weather station located within 7 miles of the study site. Runoff water sediment (percentage solids) was determined by weighing sediment after drying overnight at 105°C.

Microbiological analyses

Bacterial analysis was conducted within 6 hours of sample collection. *E. coli*, *Clostridium perfringens* and enterococci were assayed from runoff water samples using membrane filtration (*Standard Methods* 1998). Selective and differential microbiological media for *E. coli*, *Clostridium perfringens* and enterococci included mI (EPA 2000*a*), mCP (Armon and Payment 1988) and mEnterococcus agars, respectively. Coliphage analysis was performed within 24 hours of sample collection. Somatic coliphage was assayed using the double layer agar method (EPA 2000*b*).

Giardia cysts and *Cryptosporidium* oocysts were concentrated from runoff water samples by centrifugation (520 × g, 20 min) and the resulting pellet was preserved in formalin (1:1) within 96 hours of sample collection. Protozoan parasite purification and immunofluorescent microscopy was carried out using the ICR method (EPA 1995).

Data analysis

The objectives of this study were to report and compare microbial loads released in runoff from manured land. Thus, the microbial concentration released by each 0.75 m wide by 2 m long experimental plot was determined. The microbial concentration released in runoff from each plot was calculated by multiplying the microbial concentration per litre by the total litres of runoff collected after each rainfall simulation event. These microbial plot runoff concentrations were log-transformed prior to statistical analysis. Statistical calculations, analysis of variance (ANOVA), Pearson's correlations and regression, were performed using GraphPad Prism version 4.00 for Windows (San Diego, California). The level of significance P(α) of <0.100 was considered statistically significant.

Percentage increase calculations were determined for the first rainfall simulation event using the formula:

$$\%\,\text{Increase} = [(T - C)/C] \times 100; \quad (1)$$

where T and C are the geometric average microbial concentration determined for runoff water collected after the first rain event from manure-treated and control experimental plots, respectively.

Percentage release calculations were carried out for each rainfall simulation event using the formula:

$$\%\,\text{Release} = [(T - C)/M] \times 100; \quad (2)$$

where T is the geometric average microbial runoff concentration per manure-treated plot, M is the geometric average microbial manure concentration applied to each plot, and C is the geometric average microbial runoff concentration from control plots.

RESULTS

Concentrations of fecal indicator microorganisms in manure were determined prior to field application (Table 1). Manure concentrations of *Giardia* cysts and *Cryptosporidium* oocysts, however, were not measured. Geometric average runoff concentrations of fecal indicator microorganisms and protozoa for each experimental treatment are listed in Table 2.

Percentage increase values; control compared with manure-applied plot runoff concentrations, for each fecal indicator microorganism are presented in Table 3. Percentage increase was not determined for *Giardia* cysts and *Cryptosporidium* oocysts because they were not detected in control plot runoff. Only microbial concentrations recorded for the first runoff event were used for percentage increase calculations since little to no growth of fecal indicator microorganisms would have occurred during the 60 minutes taken to apply manure onto plots, carry out the rainfall simulation and collect runoff. Table 4 lists the

Table 1 | Geometric mean concentrations of fecal indicator microorganisms per gram manure and the total microbial load in manure applied per 1.5 m^2 plot

Manure type	*E. coli* (CFU g^{-1}; CFU per 1.5 m^2)	Enterococci (CFU g^{-1}; CFU per 1.5 m^2)	Clostridium (CFU g^{-1}; CFU per 1.5 m^2)	Coliphage (PFU g^{-1}; PFU per 1.5 m^2)
Fresh cattle[a]	3.33×10^6	5.93×10^5	1.27×10^4	1.03×10^6
	1.61×10^{11}	5.79×10^{10}	4.36×10^8	4.96×10^{10}
Aged cattle[a]	2.33×10^4	2.51×10^5	8.01×10^2	1.51×10^4
	4.29×10^9	1.04×10^{10}	1.18×10^8	1.42×10^9
Swine[b]	6.01×10^5	2.23×10^5	1.16×10^5	9.25×10^4
	4.36×10^9	7.97×10^9	7.21×10^8	4.55×10^9

[a]32.3 Mg ha^{-1} of cow manure applied to each experimental plot.
[b]66.51 Mg ha^{-1} swine slurry applied to each experimental plot.

percentage of study microorganisms released from manure-applied experimental plots. Figure 1 illustrates trends in fecal indicator concentrations, average of three replicates, released per plot for each rainfall simulation event (over the 3-day experimental period).

Fecal indicator microorganisms

Escherichia coli and enterococci

Control plots containing no manure or residue, had significantly ($P < 0.050$) lower average concentrations of *E. coli* and enterococci in runoff water compared with aged cattle, fresh cattle and swine manure-applied experimental plots. The numbers of *E. coli* recovered in runoff were highest for swine and fresh cattle manure followed by the aged cattle manure treatment. For enterococci, however, the highest concentrations were observed in fresh and aged cattle compared with swine manure-applied plots. Corn residue did not have an effect on *E. coli* and enterococci concentrations in runoff since the levels of these bacteria were not significantly different in runoff water collected from plots containing manure alone and manure with corn residue ($P > 0.100$).

Regression analysis of *E. coli* and enterococci concentrations in runoff water for the first, second and third rainfall simulation events, each separated by 24 h, revealed statistically significant trends (Figure 1). *E. coli* concentrations in runoff increased over the 3-day study period from plots containing fresh cattle manure, with ($P = 0.054$) and without corn residue ($P = 0.002$). A significant decline in runoff concentrations of *E. coli* in plots treated with swine manure ($P = 0.020$) was observed. For enterococci concentrations in runoff, significant declines occurred over the three rainfall events for aged cattle ($P = 0.025$), aged cattle with corn residue ($P = 0.020$) and swine ($P = 0.036$) manure-applied experimental plots. There were no significant trends in *E. coli* concentrations in runoff from plots treated with swine slurry and corn residue ($P = 0.835$). Enterococci runoff concentrations increased over the 3-day study period for plots containing fresh cattle manure ($P = 0.012$) and fresh cattle manure with corn residue ($P = 0.043$). For control plots, no significant trends were observed for *E. coli* ($P = 0.728$) and enterococci ($P = 0.160$) runoff concentrations over the three rainfall events.

Clostridium

Control plots had significantly ($P < 0.050$) lower *Clostridium* concentrations in water runoff compared with plots containing manure. The numbers of *Clostridium* transported in runoff were highest for swine followed by the fresh and aged cattle manure-applied plots. Significantly ($P = 0.007$) higher concentrations of *Clostridium* were observed in runoff from swine manure plots without corn residue

Table 2 | Concentration of study microorganisms released in simulated rainfall runoff from 0.75 m wide by 2 m long experimental plots lacking (control) or containing manure (no corn residue)

Microorganism plot treatment	Runoff event 1	Runoff event 2	Runoff event 3
E. coli[a] (CFU[b] per plot)			
Control	2.43×10^5	4.54×10^5	1.36×10^5
Aged cattle	1.10×10^8	1.23×10^8	2.68×10^7
Fresh cattle	9.92×10^6	1.44×10^9	9.56×10^8
Swine	3.05×10^8	2.24×10^8	1.25×10^7
Enterococci[a] (CFU per plot)			
Control	1.90×10^6	2.99×10^6	3.80×10^6
Aged cattle	4.86×10^8	9.64×10^7	9.64×10^7
Fresh cattle	4.46×10^7	2.26×10^8	3.39×10^8
Swine	4.36×10^7	2.02×10^7	5.39×10^6
Clostridium[a] (CFU per plot)			
Control	1.64×10^2	4.02×10^3	7.04×10^2
Aged cattle	1.47×10^5	3.92×10^4	8.38×10^4
Fresh cattle	1.64×10^6	6.18×10^5	5.52×10^5
Swine	2.16×10^7	1.46×10^6	9.63×10^5
Coliphage[a] (PFU[c] per plot)			
Control	6.36×10^5	7.11×10^5	3.04×10^5
Aged cattle	3.41×10^7	1.68×10^7	1.85×10^7
Fresh cattle	3.43×10^7	4.36×10^9	3.33×10^9
Swine	2.96×10^7	1.15×10^6	1.00×10^6
Giardia (cysts per plot[d])			
Control	DL[e]	DL	DL
Aged cattle	1.72×10^4 [f]	2.93×10^3 [f]	1.54×10^4 [f]
Fresh cattle	3.38×10^5	DL	NA[g]
Swine	3.58×10^6	9.12×10^4	NA

Table 2 | (*continued*)

Microorganism plot treatment	Runoff event 1	Runoff event 2	Runoff event 3
Cryptosporidium (oocysts per plot[d])			
Control	DL	DL	DL
Aged cattle	2.75×10^5 [f]	3.62×10^3	1.08×10^5 [f]
Fresh cattle	1.04×10^6	1.65×10^5	NA
Swine	2.20×10^6	1.18×10^5	NA

[a]Geometric average of microbial concentration per plot (three experimental replicates) where the microbial concentration per plot = runoff volume (l) × microbial concentration per l.
[b]CFU = colony forming units.
[c]PFU = plaque forming units.
[d]Arithmetic mean of protozoa concentration in runoff of two to three plot replicates, where the protozoa concentration = number of protozoa per l × runoff volume (l).
[e]DL = protozoa not detected. Detection limit ranged from $<3.6 \times 10^3$ to $<5.3 \times 10^4$ for cysts and oocysts in runoff water (average control plot runoff volume was 39.8 l per rainfall event).
[f]Not an average concentration, only one runoff sample assayed.
[g]NA = not analysed.

compared with those with residue. No residue effects, however, were observed for fresh or aged cattle manure-applied plots ($P > 0.10$).

Runoff concentrations of *Clostridium* significantly ($P < 0.002$) decreased from the first to the third runoff event for swine slurry treatments. Over the 3-day study period, plots containing fresh cattle manure ($P = 0.093$) and fresh cattle manure with corn residue ($P = 0.086$) showed a significant decline in *Clostridium* runoff concentrations (Figure 1). No significant trends were observed for control plots ($P = 0.862$) and aged cattle manure treatments ($P = 0.553$) (Figure 1).

Table 3 | Percentage increase in fecal indicator microorganisms in runoff from plots receiving livestock manure compared with control plots for the first rainfall runoff simulation event

Microbe	Percentage increase[a] Aged cattle	Fresh cattle	Swine
E. coli	4.52×10^4	4.08×10^5	1.25×10^5
Enterococci	2.55×10^4	2,247	129
Clostridium	8.95×10^4	1.0×10^6	1.32×10^7
Coliphage	5,262	5,293	4,554

[a]Percentage increase formula: $\%\,\text{increase} = [(T - C)/C] \times 100$; where T and C are the geometric average microbial runoff concentration per plot collected after the first rain event from manure-treated (T) and control (C) experimental plots, respectively.

Coliphage

Runoff water coliphage concentrations were significantly ($P < 0.050$) lower from control plots compared with manure-treated plots. The highest to lowest concentrations of coliphage were recovered from fresh cattle, aged cattle and swine manure-applied plots. Coliphage concentrations were greater ($P = 0.027$) in runoff from plots treated with fresh swine slurry and 4 Mg ha^{-1} corn residue compared with plots on which only swine slurry was applied. There was no significant difference between runoff coliphage concentrations collected from plots containing only manure and manure with corn residue for fresh ($P = 0.542$) and aged ($P = 0.206$) cattle manure treatments.

Regression analysis revealed significant trends in coliphage concentration in runoff during the three rainfall events (Figure 1). On the plots containing fresh cattle manure and corn residue, an increase in coliphage numbers over the three runoff events was significant ($P = 0.086$). For experimental treatments containing swine manure ($P = 0.012$) and swine manure with corn residue ($P = 0.004$), the number of coliphage declined over the 3-day experimental period.

Table 4 | Percentage release of manure-borne microorganisms (excluding background concentrations measured in control plots) in runoff from plots receiving manure

	Percentage release[a]		
Microbe	**Fresh cattle**	**Aged cattle**	**Swine**
E. coli			
1st runoff event	0.01	2.56	6.99
1st, 2nd & 3rd runoff event	ND[b]	6.04	12.40
Enterococci			
1st runoff event	0.07	4.65	0.52
1st, 2nd & 3rd runoff event	ND	6.44	0.76
Clostridium			
1st runoff event	0.38	0.12	3.00
1st, 2nd & 3rd runoff event	0.64	0.22	3.33
Coliphage			
1st runoff event	0.07	2.36	0.64
1st, 2nd & 3rd runoff event	ND	4.77	0.66

[a]Percentage release formula: $\%\,\text{release} = [(T - C)/M] \times 100$; where M is the geometric average microbial manure concentration applied per plot, and T and C are the geometric average microbial runoff concentration per plot after the indicated rainfall event (s) from manure-treated (T) and control (C) experimental plots, respectively.
[b]Percentage release not determined (ND) due to increase in fecal bacteria and phage 24 h after manure application.

Protozoan parasites

Due to the high cost of the detection assay for *Giardia* cysts and *Cryptosporidium* oocysts, only a selected number of samples were analysed. Concentrations of these protozoa were not determined in manure prior to land application. Runoff water collected from control and livestock manure-applied plots, however, was quantified (Tables 3 and 4). Detection limits are listed for treatment plots where protozoa were not detected in the volume of sample assayed. *Giardia* cysts and *Cryptosporidium* oocysts were not detected in control plots. For control plots, the detection limit range for cysts and oocysts was $<3{,}634$ to $<53{,}726$ per 34.6 l (arithmetic average) of runoff water.

Owing to the limited number of samples analysed, statistical calculations were carried out combining data from both residue and no residue treatments. Table 2, however, lists arithmetic means of cysts and oocysts in plots treated with manure and without residue. *Giardia* cysts were found in significantly ($P = 0.004$) higher concentrations in runoff water from swine slurry compared with fresh or aged cattle manure-applied plots. There was no significant difference ($P = 0.895$) in *Giardia* cyst runoff concentrations between aged and fresh cattle manure treatments. Higher concentrations of *Cryptosporidium* oocysts were detected in runoff from swine slurry ($P = 0.010$) and fresh cattle manure ($P = 0.016$) compared with aged cattle manure treatments. There was no significant difference ($P = 0.200$) in runoff concentrations of *Cryptosporidium* oocysts between swine slurry and fresh cattle manure treatments. Overall, concentrations of *Giardia* cysts and *Cryptosporidium* oocysts were not significantly different in runoff from swine slurry ($P = 0.600$) or aged cattle ($P = 0.987$) manure treatments; however higher concentrations of oocysts were detected in runoff from fresh cattle manure plots ($P = 0.011$).

Runoff concentrations of *Giardia* cysts and *Cryptosporidium* oocysts were positively correlated ($P < 0.001$) to one another for all manure treatments. No significant relationships were found between protozoan parasites and indicator microbial concentrations in runoff water collected from plots containing aged and fresh cattle manure. For swine manure, runoff concentrations of *Clostridium* correlated to decreasing *Giardia* cyst ($P = 0.002$) and *Cryptosporidium* oocyst ($P = 0.025$) runoff concentrations over the 3-day experimental rainfall runoff simulation. Also, declining runoff concentrations, over the 3-day experimental period, were observed between coliphage and *Giardia* cyst ($P = 0.0515$) and *Cryptosporidium* oocyst ($P = 0.0693$) from swine slurry treatments. Cysts ($P = 0.006$) and oocysts ($P = 0.020$) were positively correlated with declining runoff volume from swine slurry treatments. Turbidity levels were positively correlated with declining cyst ($P = 0.017$) and oocyst ($P = 0.006$) levels in runoff from swine slurry treatments. For fresh cattle manure-applied plot runoff, there were positive correlations between turbidity and the decline in oocysts ($P < 0.050$), and runoff water volume and the decrease in cysts ($P = 0.063$) over the 3-day experimental period. No significant relationships ($P > 0.100$) were

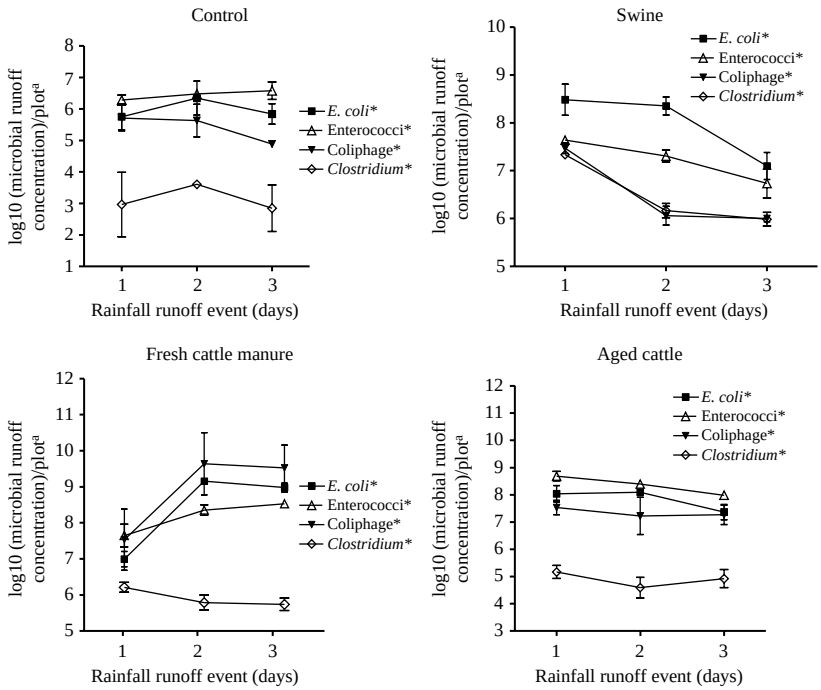

Figure 1 | Rainfall runoff concentration trends for fecal indicator bacteria over the 3-day period for plot treatments without corn residue (asterisks denote significant increasing or decreasing trend). [a] Average log transformed microbial runoff concentrations (three replicate plots) released per 0.75 m wide by 2 m long experimental plot.

observed between protozoan parasite concentrations and runoff volume, turbidity or sediment amount in runoff from plots treated with aged cattle manure.

Physical and chemical parameters

Groundwater used for the rainfall simulation tests had a mean EC value of 0.73 $dS\,m^{-1}$ and a pH of 7.62. Table 5 lists the ranges in physical and chemical parameters measured for all experimental plot treatments. The amount of sediment in runoff from plots treated with swine and fresh cattle manure was not determined.

The volume of water applied by each rainfall simulation event was 52.5 l (35 mm), thus the total amount of water applied to each plot over the 3-day study period equalled 157.5 l (104 mm). The average volume of runoff collected over the 3-day rainfall simulation experiments for control,

Table 5 | Ranges of physical and chemical parameters measured from three replicate plots containing no manure, fresh cattle manure, aged cattle manure and swine slurry

Plot treatment	% Relative humidity	Air temperature (°C)	Runoff water pH	Sediment in runoff water ($Mg\,ha^{-1}$)	Runoff water volume (l)[a]	Turbidity (NTU)
No manure	58–90	13–31	6.7–8.4	0.11–1.41	32–45	67–1420
Fresh cattle	58–86	21–28	7.4–8.1	ND[b]	30–48	313–1,880
Aged cattle	51–90	13–29	7.7–8.6	0.06–0.79	9–41	104–798
Swine	56–64	21–31	7.4–8.1	ND	31–45	360–5230

[a]For each rainfall simulation event, 52.5 l water was applied per plot.
[b]Not determined.

fresh cattle manure, swine slurry and aged cattle manure plots was 119.4 l, 116.4 l, 116.9 l and 78.8 l, respectively. There were no significant differences ($P > 0.100$) in average runoff volumes observed for each rainfall experiment between control, fresh cattle manure and swine slurry plots. Significantly lower volumes, however, were measured for plots treated with aged cattle manure ($P < 0.050$). The presence of corn residue did not significantly ($P > 0.500$) affect runoff volume for swine slurry, aged cattle manure or fresh cattle manure-applied plots.

Declining levels of *Clostridium* were positively correlated ($P < 0.015$) with turbidity levels in rainwater runoff over the 3-day study period for every plot treatment. Furthermore, the amount of sediment measured from control and aged cattle manure runoff was also significantly ($P < 0.010$) correlated to declining *Clostridium* concentrations (sediment was not analysed for fresh cattle manure or swine slurry).

DISCUSSION

Since pathogenic microorganisms are found in feces, the presence of fecally derived indicator microorganisms has been used to assess microbial water quality of recreational and drinking water sources. By definition, fecal indicator microorganisms should: 1) be useful for all water types; 2) be a member of the intestinal micro flora of mammals; 3) occur in the same or greater concentration than the associated pathogen(s) and the density should have a direct relationship to the degree of fecal contamination; 4) have a reasonably longer survival time under environmental conditions than the associated pathogen(s); 5) not multiply in the environment; and 6) have an easy, inexpensive, rapid and uncomplicated assay that allows detection and enumeration (Gerba 2000). Since one microorganism cannot serve as a suitable surrogate for every manure-borne pathogen, detection of a group of indicators may be more appropriate for estimating microbial water quality effects by livestock wastewater runoff. In this study, multiple microorganisms were surveyed to determine if widely used or alternative fecal indicator microorganisms are adequate fecal contamination indicators of different manure sources. All of the studied indicator microorganisms were detected in aged and fresh cattle manure and swine slurry used for plot application. Furthermore, the studied fecal indicators were also detected in runoff samples collected from aged cattle manure, fresh cattle manure and swine slurry treated plots.

Concentrations of fecal indicator microorganisms were significantly higher in runoff collected from manure treatments than control (no manure) plots. Experimental plots were covered during a portion of the daylight hours and overnight; therefore any effects caused by sunlight or desiccation to microorganisms were reduced. While higher reductions in microorganisms may have occurred if the plots remained uncovered between rainfall runoff events, manure-borne microorganisms are likely to be associated with organic or other particles in manure. These manure particles can, therefore, serve to protect health-related microorganisms (pathogens and indicators) from the effects of sunlight, desiccation and even predation by other microbes. The amount of rainfall applied in this study, 35 mm each day for 3 days (104 mm rainfall total), is not representative of an average daily rainfall event, but rather an extreme event. The number of days each year in the US that exceed 50.8 mm precipitation, however, is increasing (Easterling *et al.* 2000*a*).

Under this study's experimental conditions, concentrations of fecal indicator microorganisms released in runoff water from agricultural plots lacking corn residue ranged from 5.52×10^5 to 4.36×10^9, 3.92×10^4 to 4.86×10^8, and from 9.63×10^5 to 3.05×10^8 for fresh cattle manure, aged cattle manure and swine slurry, respectively (Table 2). Fecal indicator microbial levels in runoff from control plots were significantly lower, from 1.64×10^2 to 3.80×10^6 (Table 2). Since fecal indicator microorganisms are used to assess the microbial quality of surface water, large fecal indicator microbial loads from manured land could cause a water body to exceed state water quality standards. These results suggest that large microbial loads could be released from heavy precipitation events that produce runoff from livestock manure-applied agricultural fields, of even modest size, and could significantly affect water bodies within the watershed.

High levels of fecal indicator microorganisms (*E. coli*, enterococci, *Clostridium* and coliphage) were detected in aged cattle manure, fresh cattle manure and swine slurry before application to experimental plots (Table 1).

The estimated percentage release of manure-borne indicator microorganisms after land application and the first rainfall event ranged from 0.01 to 6.99%. Thus, 93.01% to 99.99% of the fecal indicators originating in the different manure types were not carried by runoff following the first 30 min (35 mm) rainfall event. It is likely that these microorganisms were either adsorbed to particles too large to be transported by runoff water, died-off, preyed on by other microorganisms, or infiltrated into the soil soon after land application. In swine slurry and aged cattle manure, the estimated release of manure-borne indicator microorganisms in runoff over the course of the three rain runoff events, separated by 24 h periods, was estimated to be from 0.66 to 12.40% and 0.22 to 6.44%, respectively. Higher percentage release values for aged cattle manure plots were probably because runoff (78.7 l) from these plots was more concentrated than runoff collected from swine slurry (116.9 l) and fresh cattle manure (116.4 l) treatments. The release of microorganisms from fresh cattle manure-applied plots after the second and third rainfall events, however, was not estimated since it appears that these microorganisms were able to multiply over the course of the 3-day experimental period.

Protozoan parasites were undetectable in runoff from control plots, but ranged from 9.12×10^4 to 3.58×10^6 and 2.93×10^3 to 1.04×10^6 in runoff from plots lacking corn residue and treated with swine slurry and cattle manure (fresh and aged), respectively (Table 2). High concentrations of protozoa may be attributed to wild animal contributions during the study period, variable method detection efficiency, or naturally high concentrations in manure. Large sediment pellets resulted from the concentration of 600 ml of runoff, so the amount of sample analysed for protozoan parasites was very small; therefore high numbers of protozoa were reported for each sample where cysts or oocysts were detected.

In a survey currently being carried out, numbers of *Cryptosporidium* and *Giardia* range from < 1.0 to 112 oocysts per gram feces and 2 to 1,506 cysts per gram feces in beef cattle fecal pats collected from yearling cattle (16 fecal samples analysed to date) (Thurston-Enriquez, unpublished data). Furthermore, concentrations of *Cryptosporidium* oocysts and *Giardia* cysts have been observed to vary from 20 to 90 oocysts and 500 to 1,075 cysts per gram swine lagoon wastewater, respectively (five swine slurry samples analysed to date) (Thurston-Enriquez, unpublished data). The species of *Cryptosporidium* oocysts and *Giardia* cysts detected in runoff water samples could not be determined by the methods used for their detection. Thus, whether protozoa observed in runoff samples were infectious or were species not considered pathogenic to humans is unknown. Nevertheless, information pertaining to the transport of manure-borne protozoa in rainfall runoff has not been previously reported and is useful for understanding the movement of these organisms from manured land.

Although the viability of *Giardia* cysts and *Cryptosporidium* oocysts could not be determined by immunofluorescence microscopy methods, previous research suggests that these protozoan pathogens are capable of surviving long periods in soil (Jenkins *et al.* 2002) and several weeks in surface water (DeRegnier *et al.* 1989; Robertson *et al.* 1992; Fayer *et al.* 2000). Since the number of these protozoa that need to be ingested for infection is low and both can survive in soil and surface water for long periods, an increased risk of human infection can occur if contaminated runoff from manure-treated fields is within the hydrological catchments of recreational, irrigation or drinking water sources. *Giardia* and *Cryptosporidium* are also economically important livestock pathogens (Esteban and Anderson 1995; Olsen *et al.* 1995; Olsen *et al.* 1997); therefore another concern is protozoan parasite transmission from manured land to forage crops or water consumed or in contact with susceptible livestock.

The age (fresh or aged) and source (cattle or swine) of manure had significant effects on runoff water microbial concentrations from manure-treated land. The amounts of recently excreted and aged manure applied to land can vary. For example, swine feces, urine and other waste is commonly collected in a pit directly below swine pens and cattle feedlot pens are scraped from every few weeks to 1–2 times per year. Consequently, when swine and cattle feedlot manure is applied as a crop fertilizer, they contain a mixture of recently excreted and aged feces. In the current study, higher concentrations of *E. coli*, enterococci, *Clostridium* and coliphage were observed per gram of fresh cattle manure compared with swine slurry and aged cattle manure (not reflected in Table 1 values since different amounts of swine and cattle manure were applied to plots).

In runoff water, however, concentrations of indicator microorganisms did not always follow these same trends. Percentage release calculations demonstrate that aged cattle manure released the highest concentrations of enterococci and coliphage and that swine slurry released the highest concentrations of *E. coli* and *Clostridium* into runoff water from manure-applied plots. While the volume of runoff released from swine slurry plots (116.9 l) was not significantly different from the control (119.4 l) or fresh cattle manure (116.4 l) plots, aged cattle manure plots had significantly lower runoff volumes (78.7 l). Thus, the higher concentrations observed in runoff from aged cattle manure plots might be because the runoff is more concentrated. For swine slurry plots, however, these results indicate that the liquid state of swine manure allowed manure-borne microorganisms to be more readily transported in runoff compared with fresh cattle manure. Unlike swine slurry, fresh cattle manure is likely to be slowly eluted by rainfall and serve as a long-term microbial source, as demonstrated by Bradford and Schijven (2002). These investigators observed that components, including *Cryptosporidium* oocysts, in the surface layer of fresh cattle feces are slowly eluted by water (Bradford and Schijven 2002). This study's results further demonstrate that fresh cattle manure acts as a long-term source for manure-borne bacteria and coliphage not only because of their slow release from fresh manure, but also because of their apparent ability to multiply in fresh cattle manure.

Fresh cattle manure applied to soil may provide a more hospitable environment for fecal bacteria and coliphage survival and growth compared with aged cattle manure and swine slurry. Increases in *E. coli*, enterococci and coliphage concentrations were observed in runoff following fresh cattle manure application and each rainfall runoff event. These increases indicate that either the studied microorganisms were able to grow under the studied conditions or that an increasing number of microorganisms were released from manure particles with each rainfall event. The latter is not likely since *Clostridium* (anaerobic spore-former) and *Cryptosporidium* (cannot multiply outside of mammalian host) levels did not significantly increase in runoff from any of the manure treatments. Moreover, fecal indicator concentrations did not significantly increase in runoff from controls, suggesting that the fresh cattle manure plots provided a niche for fecal indicator growth.

Other fecally derived microorganisms have also been reported to multiply in manure and manure-treated soil (Guan & Holley 2003). Gagliardi & Karns (2000) observed the multiplication of pathogenic *E. coli* O157:H7 in undisturbed soil cores collected from no-till land treated with cattle manure. These authors reported that *E. coli* was able to multiply in manure-treated soil and that manure enhanced *E. coli* survival (Gagliardi & Karns 2000). In the current study, fresh cattle manure may have provided nutrients and protection from antagonistic environmental conditions thereby significantly influencing fecal indicator microbial concentrations in runoff water. Furthermore, while indicator microorganisms are not supposed to multiply in the environment, manure-borne pathogenic bacteria such as *E. coli* O157:H7 have been reported to multiply in manure, water and soil (Guan & Holley 2003). Thus, a microorganism that is able to replicate under conditions supportive of manure-borne pathogenic bacteria multiplication should be considered as a potential indicator of conditions conducive to the survival and growth of pathogenic bacteria.

In the current study, microbial concentrations significantly increased in runoff from fresh cattle manure-treated soil but declined significantly in runoff from plots receiving swine slurry and aged cattle manure (over the 3-day study period). The swine slurry and aged cattle manure used for plot application would have undergone increased microbial degradation compared with fresh cattle manure. These aged manures would have higher concentrations of ammonia and other degradation products, such as volatile fatty acids. These degradation products may have had a negative impact on the survival and growth of the studied microorganisms. Other researchers have reported increased microbial reduction in swine slurry and other manures (Deng and Cliver 1992; Pesaro *et al.* 1995) and that microbial reductions are influenced by ammonia levels in manure (Jenkins *et al.* 1998; Araki *et al.* 2001).

Clostridium perfringens has been suggested to be a reliable indicator for the presence of pathogens of fecal origin in surface water (Payment & Franco 1993), animal manure (Conboy & Goss 2001) and sewage contamination (Hill *et al.* 1996). It has been suggested that *Clostridium*, owing to its ability to form environmentally resistant spores,

is a poor indicator of recent contamination events. Therefore, *Clostridium* has been recommended as a conservative indicator of past fecal contamination (Gerba 2000). Large increases over background concentrations of these bacteria, however, can be used to identify recent livestock fecal pollution. In the current study, percentage increases of *Clostridium* concentrations in runoff from manured plots were higher than any of the other assessed indicators, ranging from 2X to $> 1.0 \times 10^5$X. Moreover, *Clostridium* may also be useful as a surrogate for protozoan parasite occurrence (Payment & Franco 1993). *Clostridium* concentrations, unlike the other fecal bacterial indicators assessed, correlated with *Giardia* cyst and *Cryptosporidium* oocyst concentrations in runoff from swine slurry-treated plots. Other positive correlations between *Giardia* cyst, *Cryptosporidium* oocyst and *Clostridium* concentrations with turbidity and sediment levels in runoff water were observed suggesting that these microorganisms are transported with particulates in runoff. Finally, *Clostridium* did not appear to grow within the manured plots; instead, *Clostridium* levels declined over the 3-day experimental period. Thus, the observations made by this study further demonstrate that *Clostridium* is a useful indicator of livestock fecal contamination.

Corn residue was applied to plots (4 Mg ha^{-1}) before manure application to determine its effect on microbial transport by runoff. For cattle manure experiments, residue did not affect microbial transport to runoff water. Levels of *Clostridium* were higher and coliphage levels were lower for swine manure treatments without corn residue. Further studies will be necessary to determine what factors affected runoff concentrations of *Clostridium* and coliphage in plots containing residue.

CONCLUSIONS

This study determined the load of microorganisms, important in microbial water quality assessments and human health, transported in runoff following land application of fresh cattle, aged cattle and swine manure. Only 0.01% to 6.99% of the fecal indicator microorganisms *(E. coli*, enterococci, *Clostridium* and coliphage) originating in manure were transported in runoff after a 30 min simulated rainfall event from 0.75 m wide by 2 m long experimental plots. However, fecal indicator runoff concentrations ranged from 5.52×10^5 to 4.36×10^9, 3.92×10^4 to 4.86×10^8, and from 9.63×10^5 to 3.05×10^8 for fresh cattle manure, aged cattle manure and swine slurry, respectively. Furthermore, protozoan parasite runoff concentrations ranged from 9.12×10^4 to 3.58×10^6 and 2.93×10^3 to 1.04×10^6 in swine slurry and cattle manure treatments, respectively.

E. coli, enterococci and coliphage levels in fresh cattle manure-applied plot runoff increased, suggesting that these microorganisms were able to multiply. Conversely, fecal indicator growth was not observed for swine slurry or aged cattle manure treatments. Over a 3-day rainfall runoff period, higher runoff concentrations of fecal indicators and protozoa were released from swine slurry and fresh cattle compared with aged cattle manure treatments. *Clostridium* appears to be a suitable indicator of livestock manure contamination. Moreover, *Clostridium* and coliphage runoff concentrations correlated to protozoan parasite numbers in plots treated with swine slurry. The presence of corn residue did not significantly affect runoff levels of manure-borne microorganisms from fresh or aged cattle manured plots. The effects that corn residue has on manure-borne microorganism transport in runoff from swine slurry-applied plots require further study.

ACKNOWLEDGEMENTS

The authors would like to thank Amy Kahler and Crystal Powers for their hard work in the laboratory and Brad Krajewski, Jeffrey Nicolaisen, Bill Sabatka and Mark Strand for their help with field activities.

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