Incidence and Persistence of Zoonotic Bacterial and Protozoan Pathogens in a Beef Cattle Feedlot Runoff Control–Vegetative Treatment System

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Determining the survival of zoonotic pathogens in livestock manure and runoff is critical for understanding the environmental and public health risks associated with these wastes. The occurrence and persistence of the bacterial pathogens *Escherichia coli* O157:H7 and *Campylobacter* spp. in a passive beef cattle feedlot runoff control–vegetative treatment system were examined over a 26-mo period. Incidence of the protozoans *Cryptosporidium* spp. and *Giardia* spp. was also assessed. The control system utilizes a shallow basin to collect liquid runoff and accumulate eroded solids from the pen surfaces; when an adequate liquid volume is attained, the liquid is discharged from the basin onto a 4.5-ha vegetative treatment area (VTA) of bromegrass which is harvested as hay. Basin discharge transported *E. coli* O157, *Campylobacter* spp., and generic *E. coli* into the VTA soil, but without additional discharge from the basin, the pathogen prevalences decreased over time. Similarly, the VTA soil concentrations of generic *E. coli* initially decreased rapidly, but lower residual populations persisted. Isolation of *Cryptosporidium* oocysts and *Giardia* cysts from VTA samples was infrequent, indicating differences in sedimentation and/or transport in comparison to bacteria. Isolation of generic *E. coli* from freshly cut hay from VTA regions that received basin discharge (12 of 30 vs. 1 of 30 control samples) provided evidence for the risk of contamination; however, neither *E. coli* O157 or *Campylobacter* spp. were recovered from the hay following baling. This work indicates that the runoff control system is effective for reducing environmental risk by containing and removing pathogens from feedlot runoff.

Increased environmental regulation and public scrutiny of concentrated animal feeding operations have fueled the need for research to develop and validate affordable, effective, and sustainable manure treatment practices for livestock producers. To minimize the impact of animal production systems on the environment, producers must contend with a number of challenges associated with manure accumulation and runoff, including the pollution of soils and water with nutrients and zoonotic pathogens. Runoff from livestock feeding operations, manure storage, or manure-amended fields can contaminate surface and groundwaters, increasing the risk for water- or foodborne illness (Jackson et al., 1998; O'Connor, 2002; Johnson et al., 2003; Olson et al., 2004). Furthermore, either runoff or manure that bears pathogens may contaminate food or feed crops (Besser et al., 1993; Beuchat and Ryu, 1997; Brackett, 1999). Treatment systems that effectively contain and/or reduce the zoonotic pathogens in manure and runoff will not only protect the environment, but should also reduce a source of pathogens in the immediate production environment for recontamination or recolonization of the livestock.

Vegetative treatment systems for management and treatment of feedlot runoff are often proposed as economical and effective alternatives to traditional pond storage and land application systems. While different designs exist, a typical vegetative treatment system consists of a settling basin for solids collection and a vegetative treatment area (VTA) for water and nutrient utilization. Types of VTAs include sloped VTAs, terraces, infiltration basins, and constructed wetlands, and vegetation may include aquatic plants (in the case of wetlands or basins), trees, and grasses, including grass hay (Henry et al., 2006). Various studies have examined the abilities of constructed wetlands to remove fecal indicator bacteria and pathogens from agricultural and domestic wastewater (Hill and Sobsey, 2001; Thurston et al., 2001).

Woodbury et al. (2002, 2003) have designed and investigated a passive beef feedlot runoff control–vegetative treatment system as an effective and sustainable alternative to long-term lagoon storage of runoff. Important system design considerations included elimination of long-term, high volume liquid storage, reduced construction costs, as well as minimization of both the time and the need for specialized

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Abbreviations: VTA, vegetative treatment area; CFU, colony forming units; IMS, immunomagnetic separation; PCR, polymerase chain reaction; PBS, phosphate-buffered saline.

equipment for operation and maintenance. With regard to reducing nutrient discharge and reducing the total volume of liquid storage, this system appears to be an improvement over traditional runoff systems (Woodbury et al., 2002, 2003); however, the occurrence and fate of zoonotic pathogens in this system had not been determined. Both *Escherichia coli* O157 and *Campylobacter* spp. are regularly isolated from cattle and the production environment, and human foodborne disease caused by these bacteria is commonly associated with the consumption of beef and raw milk (Centers for Disease Control and Prevention, 1993, 2002; Shere et al., 1998; Bae et al., 2005). Furthermore, a large waterborne illness outbreak caused by both *E. coli* O157 and *Campylobacter jejuni* was linked to municipal water supply contamination by runoff from land-applied bovine manure (O'Connor, 2002). The protozoan pathogens *Giardia* and *Cryptosporidium* are common causes of human waterborne gastrointestinal disease (Leclerc et al., 2002). In addition, both of these parasites are recognized as significant agents of diarrhea in cattle, especially in young calves (Olson et al., 2004). Cattle are frequently infected with these microorganisms and can shed *Giardia* cysts and *Cryptosporidium* oocysts in high numbers (Olson et al., 2004). The objectives of this work were to examine the incidence, transport, and survival of the bacterial pathogens *E. coli* O157 and *Campylobacter* spp. in the feedlot runoff control-vegetative treatment system. Generic *E. coli* populations also were determined, as a means of assessing how similar Gram-negative enteric pathogen populations would fare. In addition, the occurrence and levels of *Cryptosporidium* oocysts and *Giardia* cysts in selected system samples were assessed.

Materials and Methods

Beef Feedlot Runoff Control–Vegetative Treatment System

The design and operation of the passive beef cattle feedlot runoff control-vegetative treatment system have been described previously (Woodbury et al., 2002, 2003). Briefly, the system manages runoff from eight 30 by 90 m feedlot pens, which are typically stocked with 70 to 80 head of finishing cattle. Runoff from the pen surfaces is accumulated in a 300-m-long flat-bottom debris basin, which provides temporary liquid storage and accumulates settlable solids (Fig. 1). When an adequate volume is attained in the basin, the liquid fraction is discharged uniformly across a 4.5-ha VTA through 13 discharge pipes that are installed at the same elevation and spaced at 21-m intervals along the basin, through a terrace that separates the basin and VTA. The VTA is planted to bromegrass, which is harvested as hay. Runoff from each of two pairs of the easternmost pens and discharge from each of two pipes from the basin is directed through Parshall flumes at which ISCO samplers/flowmeters (ISCO Industries, Lincoln, NE) are installed to measure runoff and basin discharge events and take water samples for quality measurements (Fig. 1). Weather data, including rainfall volumes, air temperature, wind direction and speed, and relative humidity are monitored and recorded at 15-min intervals. Solids are removed annually, typically in the early fall. Natural runoff flow paths in the VTA were determined by electromagnetic induction mapping (Woodbury et al., 2002).

Sample Collection

The microbiological investigation of the runoff control-VTA system was initiated in August 2003 and weather permitting, samples were collected every 2 to 3 wk through October 2005. Inclement winter weather during January or February in some cases extended the sampling interval to 4 wk. Samples collected on this schedule included both liquids and solids from the basin (when available), and soils collected from the basin approach and the VTA; the numbers of samples collected at each time are indicated in Table 1. At each sampling, basin liquid and solids samples were collected at different points along the length of the basin. The basin liquids were collected into sterile 50-mL tubes by immersion of the tube below the surface of the basin slurry. The basin solids were collected into sterile 50-mL tubes by immersion of the tube into the bottom sediment layer. Clean latex gloves were donned for the collection of each sample. Typically, five soil samples were collected from the approach to the basin and ten soil samples were collected from the VTA. Soils were sampled to a depth of 5 cm using a small spade, which was wiped clean, sanitized with 70% ethanol, and wiped dry between uses. The VTA soil samples were collected from regions of the VTA that received basin discharge; in addition to the VTA soils, two control soil samples were collected from the berms, which do not receive basin discharge (Fig. 1).

As a means of determining if and when the cattle were shedding the target pathogens, manure from the drained pens was sampled approximately every 4 wk. Manure samples were three-sample composites of three separate freshly-shed fecal pats from each of the eight pens. Clean latex gloves were donned for the collection of each sample, which was placed into a sterile sample bag.

Bromegrass hay was harvested from the VTA once annually during the study, in June. Mowing and windrowing were initiated at the north end of the VTA and proceeded to the south end, and were oriented perpendicular (east-west) to the discharge pipes (Fig. 1). Hay samples were collected immediately following cutting and periodically after baling into large round bales, up to 104 d after cutting. Following each cutting, fifteen loose hay samples were collected at evenly spaced intervals across the regions of the VTA that had received basin discharge during the study period (treatment hay), which was approximately the south half of the VTA. Similarly, control hay samples were fifteen loose hay samples collected from across the north half of the VTA, which did not receive basin discharge during the study. Following baling, the bales were segregated according to the VTA region from which the hay was cut. Loose hay was collected and cut into approximately 12-cm-long pieces using clean, ethanol-sanitized scissors before placement into a sterile sample bag. Baled hay samples were taken using a bale corer, which was sanitized with 70% ethanol and wiped dry between uses. Coring was done at the bale ends, with the corer oriented at a 45° angle from the horizontal (up). Two samples were collected from each bale, placed in sterile sample bags, and examined separately.

Following the annual removal of basin solids in September 2004, nine soil cores were taken from the basin. Taking care to avoid cross-contamination with the outside portion of the cores, subsamples from the inside of the soil cores were

Table 1. Rainfall and generic *E. coli*, *E. coli* O157, and *Campylobacter* spp. in vegetative treatment area (VTA) soil, basin solids, and basin liquid samples during the study period.†‡

			VTA soil			Basin solids			Basin liquid		
Basin discharge event	Sample dates	Precipitation§	Generic *E. coli*	*E. coli* O157	*Campylobacter* spp.	Generic *E. coli*	*E. coli* O157	*Campylobacter* spp.	Generic *E. coli*	*E. coli* O157	*Campylobacter* spp.
		cm	$\log_{10}$ CFU kg^{-1} soil	–positive samples/total samples–		$\log_{10}$ CFU kg^{-1} solids	–positive samples/total samples–		$\log_{10}$ CFU L^{-1} liquid	–positive samples/total samples–	
	03 Aug. 11	2.13	4.61a¶	0/4	0/4	5.58a	0/4	0/4	4.43a	0/4	0/4
	03 Sept. 8	4.75	4.50a	0/4	0/4	6.53b	3/4	0/4	5.24a	1/4	0/4
1	03 Sept. 12†	5.72	9.15a	2/4	0/4	8.47a	1/4	1/4	9.38a	1/4	0/4
	03 Sept. 22	0.94	7.50b	0/4	0/4	7.88ab	1/4	0/4	NA	NA	NA
	03 Oct. 6	0.91	6.87bc	3/6	0/6	NA	NA	NA	NA	NA	NA
	03 Oct. 20	2.59	6.48bcd	2/3	0/3	8.36a	4/4	0/4	8.41ab	0/4	0/4
	03 Nov. 4	1.65	6.15cd	0/3	0/3	7.54ab	2/4	0/4	7.89b	1/4	1/4
	03 Nov. 17	0.00	6.29cd	0/10	0/10	7.21b	3/4	0/4	NA	NA	NA
	03 Dec. 1	0.00	6.15cd	3/10	0/10	NA	NA	NA	NA	NA	NA
	03 Dec. 15	0.00	6.05d	2/10	0/10	NA	NA	NA	NA	NA	NA
	04 Jan. 12	0.00	5.84de	2/10	0/10	NA	NA	NA	NA	NA	NA
	04 Feb. 17	12.07	5.33e	1/10	0/10	NA	NA	NA	NA	NA	NA
	04 Mar. 1	2.74	5.95de	3/10	0/10	NA	NA	NA	NA	NA	NA
2	04 Mar. 9†	2.00	6.49a	1/10	6/10	6.97ab	1/5	1/5	6.85a	1/5	2/5
	04 Mar. 15	0.00	5.95ab	0/10	4/10	7.44a	0/5	4/5	6.71a	1/5	1/5
	04 Mar. 29	2.50	5.35bc	0/10	2/10	6.63bd	0/5	4/5	5.74b	0/5	3/5
	04 Apr. 19	0.25	4.50c	0/10	0/10	5.61c	0/5	2/5	4.36c	1/5	0/5
	04 May 3	2.84	4.61c	0/10	0/10	6.03cd	3/5	0/5	5.40b	0/5	0/5
3	04 May 17†	6.30	5.90ab	5/10	3/10	7.76a	3/5	2/5	6.13a	3/5	1/5
	04 June 1†	7.01	6.56a	4/10	0/10	8.21a	5/5	1/5	5.47ab	2/5	0/5
	04 June 14	3.25	5.35b	0/10	0/10	6.51b	1/5	0/5	4.83bc	1/5	1/5
	04 June 28	4.52	4.96b	0/10	0/10	5.90b	0/5	0/5	4.38c	1/5	0/5
4	04 July 6†	14.40	7.51ab	4/10	1/10	7.84ade	4/5	0/5	6.87ac	1/5	0/5
	04 July 19	3.68	7.63a	0/10	0/10	6.82ab	1/5	0/5	5.51bc	0/5	0/5
	04 Aug. 2	1.07	6.26bc	2/10	0/10	6.21bc	0/5	0/5	4.79b	0/5	0/5
	04 Aug. 16	0.46	5.73cde	0/10	0/10	5.29c	0/5	0/5	4.82b	0/5	0/5
	04 Aug. 30	2.79	5.97cd	1/10	0/10	NA	NA	NA	NA	NA	NA
	04 Sept. 14	0.33	5.38cdef	0/10	0/10	NA	NA	NA	NA	NA	NA
	04 Sept. 27	7.62	5.33cdef	0/10	0/10	8.71d	4/5	0/5	6.37cd	5/5	1/5
	04 Oct. 18	3.76	5.51cde	0/10	0/10	7.76ade	5/5	0/5	6.19cd	5/5	0/5
	04 Nov. 8	2.46	4.93def	0/10	0/10	7.81ad	3/5	0/5	5.90cde	0/5	0/5
	04 Nov. 22	1.68	5.37cdef	0/10	0/10	6.73be	3/5	2/5	6.93ad	3/5	1/5
	04 Dec. 7	0.00	4.74def	0/10	0/10	6.76abe	3/5	1/5	6.20acd	2/5	1/5
	04 Dec. 20	0.00	4.64ef	0/10	0/10	NA	NA	NA	NA	NA	NA
	05 Jan. 24	3.81	4.97def	0/10	0/10	NA	NA	NA	NA	NA	NA
	05 Feb. 15	0.46	4.67ef	0/10	0/10	5.64c	2/5	3/5	5.91acde	3/5	3/5
	05 Mar. 7	0.05	4.47ef	0/10	0/10	6.17bc	0/5	3/5	4.98be	3/5	5/5
5	05 Mar. 28†	3.81	4.89ab	4/10	1/10	6.73ab	5/5	1/5	6.15a	5/5	3/5
	05 Apr. 12†	5.44	5.22ab	5/10	7/10	7.43a	4/5	1/5	7.58b	5/5	2/5
	05 Apr. 25	3.28	5.28ab	0/10	0/10	6.12bc	2/5	0/5	5.99a	0/5	4/5
	05 May 9	0.00	4.72ab	0/10	0/10	5.56c	0/5	2/5	5.37ac	0/5	2/5
	05 May 24	0.13	5.49a	1/10	0/10	7.39a	2/5	0/5	6.18a	0/5	0/5
	05 June 6	2.59	4.98ab	0/10	0/10	6.63ac	1/5	0/5	5.41ac	0/5	0/5
	05 June 27	6.30	4.47ab	0/10	0/10	5.92bc	0/5	0/5	5.42ac	0/5	0/5
	05 July 11	1.70	4.15b	0/10	0/10	5.86bc	0/5	0/5	4.94c	2/5	0/5
	05 Aug. 1	5.03	4.03b	0/10	0/10	5.64c	1/5	0/5	5.42ac	1/5	0/5
6	05 Aug. 22†	5.33	4.95a	4/10	0/10	7.13a	5/5	1/5	6.58a	1/5	1/5
	05 Sept. 12†	0.74	5.98b	0/10	0/10	7.56a	0/5	1/5	6.71a	0/5	0/5
	05 Oct. 3	0.69	5.53ab	0/10	1/10	NA	NA	NA	NA	NA	NA

† Sample dates that were preceded by basin discharges.

‡ NA, no sample available.

§ Total precipitation since the previous sampling date.

¶ Within a basin discharge event, generic *E. coli* population means in each column followed by the same letter are not significantly different ($P > 0.05$).

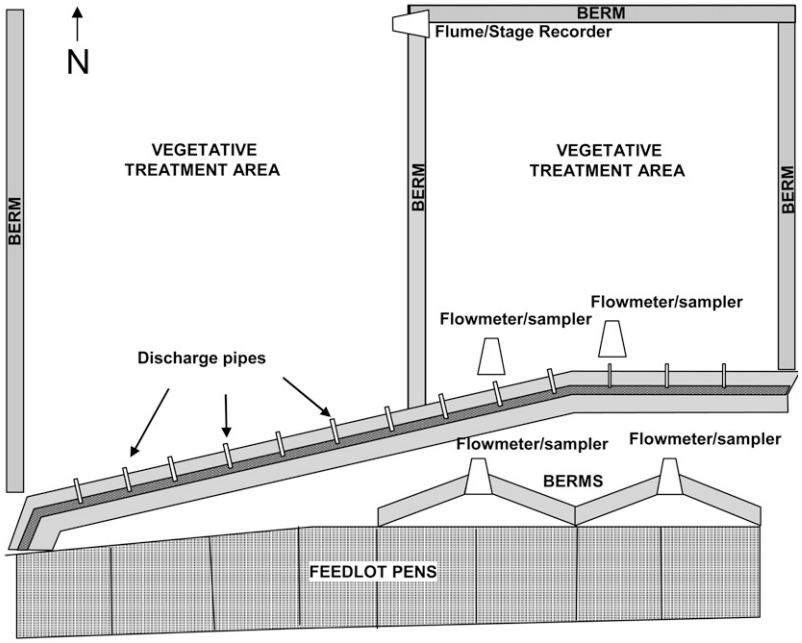

Fig. 1. Diagram of the feedlot runoff control and vegetative treatment system, reproduced with modifications from Woodbury et al. (2003).

recovered at depths of up to approximately 0.91 m for use in microbial analyses.

Adequate rainfall rate and volume accompanied by high runoff flow rates are required to trip the sample collectors located at the Parshall flumes (Woodbury et al., 2002). When available, runoff entering and/or exiting the basin (pen runoff and basin discharge, respectively) that was collected at the flumes was subjected to microbial analyses.

Microbiological Analyses

For isolation of *E. coli* O157, samples were subjected to nonselective enrichment and immunomagnetic separation (IMS) procedures as described by Berry and Miller (2005). Briefly, 10-g samples of soils, basin solids, manure, hay, basin liquid (10 mL), or runoff (10 mL) and 90 mL of tryptic soy broth (TSB; Becton, Dickinson and Company, Sparks, MD) were measured into a sterile filtered sample bag and the bag contents were blended with a Stomacher 400 Circulator (200 rpm, 30 sec; Seward Limited, London, UK). A 1.2-mL volume was removed to a sterile tube for use in generic *E. coli* enumeration as described below. The remaining sample mixture was incubated at 37°C for 7 h. Following this incubation, a 1-mL volume of the sample enrichment was subjected to IMS using Dynabeads anti-*E. coli* O157 (Dynal Biotech ASA, Oslo, Norway) and plating onto sorbitol MacConkey agar plates containing 0.05 mg L^{-1} cefixime and 2.5 mg L^{-1} potassium tellurite. The plates were incubated at 37°C for 22 to 24 h and examined for suspect colorless, sorbitol negative colonies of 1 to 2 mm in diameter, which were tested with *E. coli* O157 latex agglutination reagents (Oxoid Limited, Basingstoke, England). Identities of presumptive isolates were confirmed using standard biochemical procedures (Hitchins et al., 1998) and by PCR screening for genes specific for enterohemorrhagic *E. coli* and *E. coli* O157 (Paton and Paton, 1998).

Procedures similar to those described by Berry et al. (2006) were used for isolation of *Campylobacter* spp. from runoff system samples. One-g samples of soils, basin solids, or manure, or 1-mL samples of basin liquid or runoff were added to 13.5 mL of Bolton selective enrichment broth (BEB; Oxoid) in a sterile 15-mL conical tube. For hay samples, 1 mL of the initial 10^{-1} TSB dilutions of the hay (see above; before incubation) were placed into the BEB tubes. The BEB tubes were incubated at 37°C for 4 h, followed by incubation at 42°C for 20 h, after which 20 μL were streaked onto Campy-Cefex agar plates (Stern et al., 1992) and incubated microaerobically at 42°C for 48 h in anaerobic jars containing CampyGen (Oxoid). Suspect colonies were tested using PANBIO-Campy (jcl) latex agglutination reagents (PANBIO, Inc., Columbia, MD) and further confirmed by PCR screening for genes specific for the thermophilic *Campylobacter* spp. (Eyers et al., 1993; Fermér and Olsson Engvall, 1999).

For determination of generic *E. coli* concentrations, the retained volumes of the initial 10^{-1} TSB dilutions of soils, basin solids and liquid, and hay were serially diluted further if necessary in 2% buffered peptone water and 1 mL volumes were plated onto Petrifilm *E. coli*/coliform count plates (3M Microbiology Products, St. Paul, MN), which were incubated at 37°C for 24 h before counting characteristic *E. coli* colonies (Berry and Miller, 2005).

From September 2003 to January 2004, the collected manure samples were examined for the presence of *Cryptosporidium* oocysts and *Giardia* cysts. Analyses of the runoff system samples for these protozoan pathogens were conducted from May 2004 through April 2005. For these analyses, 10 g of each of the five basin solids samples were pooled and mixed. In addition, 200 mL of each of the five basin liquid samples were combined into a 1-L pooled sample. Equivalent amounts of five each of the 10 VTA soil samples were combined and mixed to make two separate pooled VTA soil samples. A modification of USEPA Method 1623, utilizing concentration, IMS, and immunofluorescent staining, was used for determining *Cryptosporidium* oocyst and *Giardia* cyst concentrations in basin liquid, pen runoff, and basin discharge samples (USEPA, 2001). Filtration was not performed due to the turbid nature of these samples. Instead, 1 L of basin wastewater was concentrated by centrifugation (1500 × *g* for 10 min) and the pellet resuspended 1:1 in formalin. For manure samples, 5 g was resuspended in 40 mL phosphate-buffered saline (PBS) and shaken for 10 min. The sample was concentrated by centrifugation (1500 × *g* for 15 min) and the pellet resuspended 1:1 in formalin. For recovery of protozoa in soil, a modification of the method described by Kuczynska and Shelton (1999) was performed. After extraction and sieving soil samples, the resulting solution was centrifuged at 1500 × *g* for 10 min. The supernatant was discarded and the pellet resuspended 1:1 in

formalin. All pellets (basin, manure, and soil) were stored at 4°C until IMS and immunofluorescent antibody staining (IFA) could be conducted. Immunomagnetic separation (Aureon Biosystems, Vienna, Austria) and IFA (Waterborne, New Orleans, LA) were performed for all pellets according to manufacturers' instructions.

Statistical Analysis

Generic *E. coli* populations were converted to $\log_{10}$ CFU per kg or L values for statistical analyses. Analysis of variance and the Tukey-Kramer multiple comparisons test were performed on the converted bacterial population data using InStat (version 3.00, GraphPad Software, San Diego, CA); the model included the fixed effect of day of year. Differences in the isolation of generic *E. coli*, *E. coli* O157, and *Campylobacter* spp. from basin discharge hay and control hay were assessed by using the two-tailed Fisher exact test (Uitenbroek, 1997). This test was also used to compare differences in the numbers of pathogen-positive runoff system samples during different seasons. For all analyses, differences were considered significant when P values were less than 0.05.

Results and Discussion

Bovine manure was sampled and analyzed approximately every 4 wk as a means of assessing cattle shedding status of the target bacteria over the course of the study, and thereby demonstrating a source of bacteria to the runoff system in all seasons. The proportion of *E. coli* O157-positive manure composites was slightly higher during the fall (22.6%) than during the winter (9.7%), spring (15.5%), and summer (12.8%). These observations are consistent with the higher fecal prevalence of this organism in cattle during the late summer and early fall that has been reported previously (USDA-APHIS, 2001). The percentage of manure samples positive for *Campylobacter* spp. was 14.9% in the summer, 9.4% in the fall, 9.7% in the winter, and 2.2% in the spring. Thus, both pathogens were sporadically shed by the cattle during the study. This was reflected in the frequent isolation of both *E. coli* O157 and *Campylobacter* spp. from samples collected in the runoff control-VTA system.

During the 26-mo study, there were four separate runoff events, occurring in September 2003, March 2004, May/June 2004, and June/July 2004, where rainfall volume in combination with snowmelt and/or liquid volume in the basin were adequate to result in measurable basin discharge to the VTA (Table 1). In addition to these four events, rains occurring in late March/early April 2005 and mid-August/early September 2005 also resulted in minor basin discharges before sampling on 28 March, 12 April, 22 August, and 12 September of that year. Basin discharge introduced generic *E. coli*, *E. coli* O157, and *Campylobacter* spp. to the VTA, as indicated by their detection in collected basin discharge liquid (data not shown) and in VTA soils following discharge (Table 1). Before the first basin discharge in September 2003, there had been no discharge into the VTA for over a year. This was reflected in the low concentrations of generic *E. coli* recovered from the VTA soils before this event. Generic *E. coli* levels in the control soil, that does not receive basin discharge, were typically below detectable levels (the detection limit was 1.00 $\log_{10}$ CFU g^{-1}; data not shown).

Following discharge to the VTA, generic *E. coli* levels in the VTA soils increased ($P < 0.05$), and then steadily decreased ($P < 0.05$) over time (Table 1). However, lower residual populations persisted for long periods. This was especially evident for generic *E. coli* populations following the first and fourth basin discharge events (occurring in September 2003 and June/July 2004, respectively). Following the first basin discharge, average generic *E. coli* populations in VTA soils were 9.15 $\log_{10}$ colony forming units (CFU) kg^{-1} of soil. Over a period of 53 d (from 12 Sept. 2003 to 4 Nov. 2003), the average populations declined ($P < 0.001$) to 6.15 $\log_{10}$ CFU kg^{-1} soil, and then remained approximately constant ($P > 0.05$) at about 6.00 $\log_{10}$ CFU kg^{-1} soil for an additional 118 d through the winter until the second discharge event which occurred in March 2004. Initial generic *E. coli* populations in VTA soils following the second and third basin discharges in March 2004 and May/June 2004 were not as high ($P < 0.05$) as those occurring in September 2003, at 6.49 and 6.56 $\log_{10}$ CFU kg^{-1} of soil, respectively, and the lengths of time monitored before the next discharge events were shorter. However, the VTA soil concentrations of generic *E. coli* that remained before the subsequent discharges were also lower ($P < 0.05$), at 4.61 and 4.96 $\log_{10}$ CFU kg^{-1} soil. Average generic *E. coli* populations in VTA soils following the fourth basin discharge occurring in late June/July 2004 were 7.51 $\log_{10}$ CFU kg^{-1} soil. Similar to VTA soil populations observed following the first basin discharge event, the initial generic *E. coli* concentrations declined ($P < 0.001$) about 3 $\log_{10}$ CFU kg^{-1} soil (from 7.51 to 4.74 $\log_{10}$ CFU kg^{-1} soil from 6 July 2004 to 7 Dec. 2004), then remained constant ($P > 0.05$) at a lower level of about 4.50 to 4.70 $\log_{10}$ CFU kg^{-1} soil through the winter until 7 Mar. 2005, the sampling date before the next, minor basin discharge.

These data suggest that temperature or other environmental or biological factors associated with the fall and/or winter seasons may enhance persistence of *E. coli* in soil. Log reductions per day were calculated from generic *E. coli* population data from the first samples collected following a basin discharge and the final samples collected before the next discharge event. Log_{10} CFU reductions per day in VTA soils for the first and fourth basin discharges, which encompassed the fall and winter months, were lower (0.019 and 0.013 $\log_{10}$ CFU d^{-1}, respectively) than for the second and third basin discharge events (0.034 and 0.022 $\log_{10}$ CFU d^{-1}, respectively), which were restricted to the spring and summer months. Within the first basin discharge, $\log_{10}$ reduction of generic *E. coli* in VTA soil from 12 Sept. 2003 to 4 Nov. 2003 was 0.057 $\log_{10}$ CFU d^{-1}, and from 4 Nov. 2003 to 1 Mar. 2004 was much lower at 0.002 $\log_{10}$ CFU d^{-1}. Similarly, within the fourth basin discharge event, $\log_{10}$ reduction of generic *E. coli* in VTA soil from 6 July 2004 to 8 Nov. 2004 was 0.021 $\log_{10}$ CFU d^{-1}, and from 8 Nov. 2004 to 7 Mar. 2005 was 0.004 $\log_{10}$ CFU d^{-1}.

This apparent seasonal effect was also evident in the incidence of *E. coli* O157 in VTA soils following the first basin discharge event. At the initial sampling and on 3 Oct. and 20 Oct. 2003, 50, 50, and 67% of VTA soil samples were positive for *E. coli* O157, respectively. *E. coli* O157 was recovered from VTA soil samples on all sample dates from 1 Dec. 2003 to 1 Mar. 2004, with percentages of positive samples ranging from 10 to 30%.

In contrast, *E. coli* O157 introduced to the VTA by subsequent basin discharges in the late spring and summer, including the minor discharges occurring in 2005, appeared to decrease more rapidly to levels below the detection limit (Table 1). For example, on the first sampling following the fourth basin discharge in late June/July 2004, 4 of 10 samples (40%) were *E. coli* O157-positive. Upon continued sampling on 19 July 2004 and 2, 16, and 30 Aug. 2004, 0, 20, 0, and 10% of VTA soil samples were positive for *E. coli* O157, respectively. Thereafter, from 14 Sept. 2004 until the minor discharge occurring in late March 2005, all VTA soil samples were negative for this pathogen.

The association of cooler soil temperatures and the longer environmental survival of *E. coli* in soils has been described previously. Environmental *E. coli* isolates survived longer in soil when held at 4, 15, and 25°C, than at 30 or 37°C (Ishii et al., 2006a). Topp et al. (2003) reported that initial *E. coli* soil populations of 6.00 $\log_{10}$ CFU g^{-1} soil persisted at levels greater than 5.00 $\log_{10}$ CFU g^{-1} soil at 4°C after 30 d, while declining to about 3.00 $\log_{10}$ CFU g^{-1} soil at 30°C during the same timeframe. Greater persistence of *E. coli* and/or *E. coli* O157:H7 at cooler temperatures in stream water (Terzieva and McFeters, 1991) and in livestock manure (Wang et al., 1996; Kudva et al., 1998) has also been reported. In contrast, Mukherjee et al. (2006) reported a more rapid reduction rate of *E. coli* O157:H7 populations in manure-amended soil at 4°C than at ambient temperatures of about 12 to 15°C. Natvig et al. (2002) found that a 1 March manure application in Wisconsin reduced soil survival of *E. coli* and *Salmonella* as compared to 1 June manure application. They concluded that these bacteria may have survived better in the manure-fertilized soil at warmer temperatures and/or that freeze-thaw cycling of the soil in the early spring may have been unfavorable to bacterial survival. In vitro experiments demonstrated that while freeze-thaw cycling did not eliminate *E. coli* or *Salmonella* in manure-amended soils, it may cause reductions in viable cells (Natvig et al., 2002).

The survival in soil of fecal indicator bacteria and pathogens has been a current research topic because of the risks for food crop and water contamination when livestock manure is used as a soil amendment. These studies have also reported that these microorganisms can often survive for long periods following manure application to land, and have also shown they can multiply in these soils. When bovine manure was incorporated into soil under temperatures typical of late spring or early summer in Wisconsin, initial *E. coli* levels of about 5.00 $\log_{10}$ g^{-1} soil at first increased by 1 to 2 logs, and then persisted for up to 19 wks (Lau and Ingham, 2001). Nicholson et al. (2005) applied beef manure slurry by both incorporation into arable soil and by surface application to grassland soil, and found that *E. coli* O157, *Campylobacter*, and *Salmonella* at initial levels of about 2 to 3.5 $\log_{10}$ g^{-1} soil survived in the soils for up to a month. *E. coli* survived up to 162 d in pasture soils following natural deposition by cattle or swine (Avery et al., 2004). Factors affecting *E. coli* (and *E. coli* O157) persistence in manure-amended soils may include, but are not limited to: soil type, amount of manure, application (incorporation vs. surface-applied), temperature, moisture, competition for limited nutrients, and protozoan predators (England et al., 1993; Fenlon et al., 2000; Lau and Ingham, 2001; Gagliardi and Karns, 2002; Avery et al., 2004; Byappanahalli and Fujioka, 2004; Hutchison et al., 2004; Berry and Miller, 2005). Several studies have observed that the presence of clay in soils may improve the survival of *E. coli* (Fenlon et al., 2000; Lau and Ingham, 2001; Gagliardi and Karns, 2002). The Hastings silt-loam soil of the VTA has an appreciable clay content (fine, smectitic, mesic Udic Argiustolls) which may have played a role in *E. coli* persistence in the VTA soil following basin discharge.

Most studies of *E. coli* survival in soil have focused in manure-amended soils. Because of the high nutrient levels present in the liquid basin discharge (Woodbury et al., 2003), the VTA soils receiving this discharge may in many ways resemble these manure-fertilized soils. However, several recent works have demonstrated that *E. coli* can adapt to life outside of the mammalian gastrointestinal tract, becoming "naturalized" to (Ishii et al., 2006a), and existing and growing in a variety of natural soil and sediment environments (Byappanahalli and Fujioka, 1998, 2004; Desmarais et al., 2002; Byappanahalli et al., 2006; Ishii et al., 2006a). Furthermore, these *E. coli* are not confined to soils in tropical or subtropical environments, but have also been found to persist and grow in soils in temperate environments that experience a wider range of extreme temperatures and weather conditions (Byappanahalli et al., 2006; Ishii et al., 2006a). The existence of natural *E. coli* in soils and sediments, and its potential introduction to waterways, confounds its use as an indicator of fecal contamination.

Campylobacter were not recovered from VTA soils following the first basin discharge event occurring in September 2003 (Table 1), although cattle in the pens were shedding *Campylobacter* spp. during this time. Following discharge occurring in March 2004, 60, 40, and 20% of VTA soil samples were positive on 9 Mar., 15 Mar., and 29 Mar. 2004, respectively, and then were negative again until the next discharge. Immediately following the third basin discharge in May/June 2004, 3 of 10 VTA soils on 17 May 2004 were positive for *Campylobacter* spp. However, subsequent VTA samples were again negative until after the fourth basin discharge. On 6 July 2004, *Campylobacter* was recovered from 1 of 10 VTA soil samples. All VTA soil samples collected from this time until after the minor basin discharge events occurring in March and April 2005 were negative for this pathogen. On 28 Mar. 2005 and 12 Apr. 2005, 10% and then 70% of VTA soil samples were *Campylobacter*-positive. Thereafter, VTA soils were negative for the pathogen until after the next minor discharge events that occurred in mid-August/early September 2005. Thus, while basin discharge clearly introduced *Campylobacter* spp. into the VTA, the results indicate that *Campylobacter* do not survive in soils as well as *E. coli* O157. When present, the higher numbers of *Campylobacter*-positive VTA soils following discharge corresponded to greater numbers of positive samples in basin solids and liquids. The apparent greater prevalence of *Campylobacter* spp. in runoff system samples collected during the spring is discussed below.

Because of long periods of little rain, loss of liquid to evaporation, and/or frozen conditions, basin solids and liquids often were not available for sample analyses (Table 1). In general, any precipitation that was adequate to cause runoff from the pens served to replenish the basin with both generic *E. coli* and the target pathogens. This was indicated by changes in population levels of *E. coli* in basin solids, basin liquids, and approach soil

samples between basin discharge events, as well as by the sporadic isolations of both *E. coli* O157 and *Campylobacter* from these samples between discharges (approach soil data not shown). However, peak numbers of generic *E. coli* levels and pathogen isolation frequencies in basin samples tended to be highest following large rains that were adequate to fill the basin and result in discharge. The abilities of *E. coli* O157 or *Campylobacter* spp. to persist in basin liquids or solids were difficult to ascertain because of the continual addition of runoff to the basin. In addition, further work would be needed to compare survival of these fecal bacteria in shallow basins to their survival in deep anaerobic lagoons which are typically used for feedlot runoff collection and storage. All soil cores taken from below the basin in September of 2004 were negative for both *E. coli* O157 and *Campylobacter* spp. at all depths examined (Table 2). Enumerable generic *E. coli* were found in 5 of 9 soil samples at depths between 0 and 0.15 m, and in 1 of 9 samples at depths between 0.31 to 0.61 m. These results indicate that there was minimal leaching and transport of the target bacteria through the soils below the basin.

The percentage of approach soil, basin solids, and basin liquid samples that were positive for *E. coli* O157 was highest ($P < 0.05$) in the fall at 35.5%, and was similar ($P > 0.05$) in the winter, spring, and summer seasons at 21.7, 22.7, and 20.3%, respectively. (We excluded the VTA soils from this calculation because not all runoff events resulted in discharge; therefore, occurrences in the VTA might not be reflective of seasonal differences.) This is consistent with our observation of a higher proportion (22.6%) of *E. coli* O157-positive bovine manure samples in the fall as compared to the other seasons. As noted above, we saw higher ($P < 0.05$) numbers of *Campylobacter*-positive samples in the spring. The percentages of runoff system samples positive for *Campylobacter* spp. were considerably higher ($P < 0.05$) during the winter and spring (27.3 and 27.0%, respectively) than during the summer (2.0%) or fall (6.5%). The high percentage observed during the winter was primarily due to the numbers of *Campylobacter*-positive samples collected in late February 2005 (Table 1). Among bovine manure samples collected from the pens, 9.7 and 2.2% were positive for *Campylobacter* during the winter and spring seasons, respectively, with the highest percentage (14.9%) seen during the summer. Stanley et al. (1998) described peaks in the prevalence of *Campylobacter* shedding by cattle occurring both in the spring and fall. Interestingly, human *Campylobacter* infection has been shown to peak in the spring, and may be linked to environmental factors rather than food sources (Kovats et al., 2005; Louis et al., 2005). Furthermore, *Campylobacter* spp. have frequently been isolated from surface waters at low temperature and during the winter (Jones, 2001). Clearly, there are unknown factors involved in the environmental persistence and ecology of this pathogen. Recent work by Ishii et al. (2006b) found that *Campylobacter* and other pathogens (including Shiga toxin-producing *E. coli* and *Salmonella*) can be harbored in the macrophytic alga *Cladophora* along shoreline waters of Lake Michigan. In addition, wild animals or birds may have been sources of *Campylobacter* spp., as well as *E. coli* O157, in the runoff control–VTA system. Genotypic analyses by pulsed field gel electrophoresis and further characterization of the *E. coli* O157 and *Campylobacter* spp. isolates will be described in a separate report.

Table 2. Generic *E. coli*, *E. coli* O157, and *Campylobacter* spp. in soil core samples from cores taken from below the basin in September 2004.†

Depth of soil core sample	Generic *E. coli* (range of cell counts)‡	*E. coli* O157	*Campylobacter* spp.
0–0.15 m	5/9 (4.47 to 5.81 $\log_{10}$ CFU kg^{-1} soil)	0/9	0/9
0.15–0.31 m	0/9	0/9	0/9
0.31–0.61 m	1/9 (5.08 $\log_{10}$ CFU kg^{-1} soil)	0/9	0/9
0.61–0.91 m	0/9	0/9	0/9

† Number of positive samples/total number of samples.

‡ Range of cell counts in soil cores in which generic *E. coli* were detected, expressed as $\log_{10}$ CFU kg^{-1} soil. The detection limit for enumeration was 1.00 $\log_{10}$ CFU g^{-1} soil.

Utilization of basin discharge liquid for hay production raises the question of the risks for contaminating the hay and potentially reintroducing pathogens into the herd via feeding the hay. Rainfall and subsequent basin discharge events occurring on 13, 18, and 22 May 2004, before cutting the hay on 1 June 2004, provided an opportunity to assess these risks. On 17 May, 50 and 30% of VTA soil samples were positive for *E. coli* O157 and *Campylobacter* spp., respectively. On 1 June, the same day that the hay was cut, 40% of VTA soil samples were positive for *E. coli* O157. Among a total of 30 samples of loose hay collected from VTA regions that had received basin discharge (15 on 1 June and 15 on 3 June right before baling), one sample was positive for *E. coli* O157 and no samples were positive for *Campylobacter* spp. (Table 3). Neither pathogen was isolated from baled hay samples at 20, 41, or 69 d after cutting. The differences in generic *E. coli* isolation were not significant ($P > 0.05$) between hay from VTA regions that had received runoff as compared to control hay. Similar results were seen in the second season for hay cut on 16 June 2005. Neither pathogen was recovered from either loose hay or baled hay from basin discharge hay or control hay. Generic *E. coli* was recovered more frequently from basin discharge hay than control hay immediately following cutting; however, generic *E. coli* recovery from the basin discharge hay declined during storage of the bales. While these data indicate that there is some risk for hay contamination, the risk appears to be low.

The pen manure composites examined from September 2003 to January 2004 frequently contained *Giardia* cysts (36/36, 100%) and *Cryptosporidium* oocysts (21/36, 58%), indicating a fairly high prevalence of shedding among the cattle (Table 4). Concentrations of *Cryptosporidium* ranged from 5.00×10^2 to 1.12×10^5 oocysts kg^{-1} manure, with an average count of 1.40×10^4 oocysts kg^{-1} manure. Concentrations of *Giardia* ranged from 1.33×10^3 to 1.51×10^6 cysts kg^{-1} manure, with an average count of 1.32×10^5 cysts kg^{-1} manure. We analyzed selected runoff system samples for these protozoa from May 2004 through April 2005. Despite the previously observed high frequency of shedding, we did not find high numbers of either *Cryptosporidium* oocysts or *Giardia* cysts in runoff system samples. Three of eight basin liquid samples were positive for *Cryptosporidium* oocysts, and four of eight basin liquid samples were positive for *Giardia* cysts. Notably, we did not recover either *Cryptosporidium* oocysts or *Giardia* cysts from basin solids. Three of 18 VTA soils samples contained small numbers of *Cryptosporidium* oocysts and

Table 3. Generic *E. coli*, *E. coli* O157, and *Campylobacter* spp. in vegetative treatment area (VTA) bromegrass hay.†

Date of harvest	Sample type (days from cutting)	Bromegrass hay treatment‡	Generic *E. coli*	*E. coli* O157	*Campylobacter* spp.
1 June 2004 cutting	Fresh-cut (d 0)	Control	0/15a§	0/15a	0/15a
		Basin discharge	3/15a	0/15a	0/15a
	Before baling (d 2)	Control	0/15a	0/15a	0/15a
		Basin discharge	3/15a	1/15a	0/15a
	Baled hay (d 41)	Control	1/26a	0/26a	0/26a
	(d 20)	Basin discharge	5/26a	0/26a	0/26a
	Baled hay (d 69)	Control	0/26a	0/26a	0/26a
		Basin discharge	1/26a	0/26a	0/26a
16 June 2005 cutting	Fresh-cut (d 0)	Control	1/15a	0/15a	0/15a
		Basin discharge	9/15b	0/15a	0/15a
	Baled hay (d 25)	Control	2/6a	0/6a	0/6a
		Basin discharge	1/8a	0/8a	0/8a
	Baled hay (d 67)	Control	0/6a	0/6a	0/6a
		Basin discharge	2/8a	0/8a	0/8a
	Baled hay (d 104)	Control	0/6a	0/6a	0/6a
		Basin discharge	1/8a	0/8a	0/8a

† Number of positive samples/total number of samples.

‡ Control hay was cut from regions of the VTA that did not receive basin discharge, while basin discharge hay was cut from VTA regions that had received basin discharge, during the study period from August 2003 through October 2005.

§ Within a sample type and day, the number of positive samples/total number of samples in each column followed by the same letter are not significantly different ($P > 0.05$).

Giardia cysts. These results likely are reflective of differences in transport and sedimentation of these protozoan oocysts and cysts in comparison to the target bacteria examined.

In clear water, *Cryptosporidium* oocysts and *Giardia* cysts settle out of suspension very slowly, and can thereby be transported long distances in water (Dai and Boll, 2003, 2006). However, *Cryptosporidium* oocysts tend to associate with sediments and suspended particles in water, which speeds their removal from suspension and affects their transport in water (Searcy et al., 2005). Furthermore, the presence of dilute manure, which would be the situation in the current work, can enhance the attachment of *Cryptosporidium parvum* oocysts to soil particles (Kuczynska et al., 2005). Low settling velocities may explain why we did not find *Cryptosporidium* oocysts or *Giardia* cysts in the basin solids. However, because of the large amounts of sediment and particulate matter that are present in runoff from feedlot pens, it is more likely that the protozoa were associated with sediments and suspended particles, thereby increasing their removal from overland flow and reducing their transport into the basin. In addition, it is possible that the basin approach grass served as a vegetative filter by trapping and retaining *Cryptosporidium* oocysts and *Giardia* cysts in the pen runoff. There is a 4% slope from the feed bunks to the basin terrace, and the approach from the pens to this terrace is planted to bromegrass (Fig. 1; Woodbury et al., 2002). Several studies have demonstrated that vegetative/vegetated filter or buffer strips are effective at reducing concentrations of *Cryptosporidium* oocysts in runoff from livestock production (Atwill et al., 2002; Tate et al., 2004). As discussed by Tate et al. (2004), vegetative buffer strips may more efficiently remove and retain *Cryptosporidium* oocysts and *Giardia* cysts from runoff because of their larger size, as compared to the removal of the smaller enteric bacteria like *E. coli*.

Conclusions

Basin discharge can transport generic *E. coli* and the pathogens *E. coli* O157 and *Campylobacter* spp. to the VTA. Following their introduction to the VTA, populations of generic *E. coli* declined over time, as did the isolation frequencies of *E. coli* O157 and *Campylobacter* spp., though residual populations could persist for long periods. Temperature or other environmental factors associated with fall and winter may favor persistence of *E. coli* in soils. Recovery of generic *E. coli* from VTA hay grown in the regions that had received basin discharge decreased following storage of

Table 4. *Cryptosporidium* oocysts and *Giardia* cysts in beef feedlot runoff control-VTA system samples.†

Sample type	*Cryptosporidium* oocysts (range of oocyst counts)‡	*Giardia* cysts (range of cyst counts)‡
Samples collected from September 2003 to January 2004		
Bovine manure	21/36 (5.00×10^2 to 1.12×10^5 oocysts kg^{-1})	36/36 (1.33×10^3 to 1.51×10^6 cysts kg^{-1})
Samples collected from May 2004 to April 2005		
Basin liquid	3/8 (7 to 5.56×10^2 oocysts L^{-1})	4/8 (7 to 1.85×10^2 cysts L^{-1})
Basin solids	0/7	0/7
VTA soils	3/18 (2.00×10^3 to 4.00×10^3 oocysts kg^{-1})	3/18 (1.00×10^3 to 3.00×10^3 cysts kg^{-1})
Control soils	0/8	0/8

† Number of positive samples/total number of samples.

‡ Range of (oo)cyst counts in samples in which *Cryptosporidium* or *Giardia* were detected, expressed as $\log_{10}$ (oo)cysts kg^{-1} soil. The detection limit ranged from <1.33 to <4 g^{-1} manure, <22 to <89 L^{-1} basin liquid, <1 to <8 g^{-1} basin solids, and <1 to <13 g^{-1} soils.

the baled hay. While isolation of *E. coli* from hay does indicate that there is some risk for pathogen contamination, this risk appears to be low. *E. coli* O157 was isolated from only one of a total of 60 freshly-cut hay samples harvested from VTA areas that had received basin runoff, before baling. Neither pathogen was recovered from hay following baling and storage. In summary, the feedlot runoff control–vegetative treatment system was effective for managing both bacterial and protozoan pathogens from feedlot runoff.

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