Published January 12, 2017

Journal of Environmental Quality

TECHNICAL REPORTS

WASTE MANAGEMENT

Evaluation of Fecal Indicators and Pathogens in a Beef Cattle Feedlot Vegetative Treatment System

Lisa M. Durso,* Daniel N. Miller, Daniel D. Snow, Christopher G. Henry, Monica Santin, and Bryan L. Woodbury

Abstract

Runoff from open-lot animal feeding areas contains microorganisms that may adversely affect human and animal health if not properly managed. One alternative to full manure containment systems is a vegetative treatment system (VTS) that collects runoff in a sediment basin and then applies it to a perennial vegetation (grass) treatment area that is harvested for hay. Little is known regarding the efficacy of large-scale commercial VTSs for the removal of microbial contaminants. In this study, an active, pump-based VTS designed and built for a 1200-head beef cattle feedlot operation was examined to determine the effects of repeated feedlot runoff application on fecal indicator microorganisms and pathogens over short-term (2 wk) and long-term (3 yr) operations and whether fecal bacteria were infiltrating into deeper soils within the treatment area. In a short-term study, fecal bacteria and pathogen numbers declined over time in soil. Measurements of total coliforms and Enterococcus counts taken on control soils were not effective as fecal indicators. The repeated application of manure-impacted runoff as irrigation water did not enrich the pathogens or fecal indicators in the soil, and no evidence was seen to indicate that pathogens were moving into the deeper soil at this site. These results indicate that large-scale, active VTSs reduce the potential for environmental contamination by manure-associated bacteria. Also, this study has implications to full-containment systems that apply runoff water to land application areas (cropland) and the fate of pathogens in the soils of land application sites.

Core Ideas

- Repeated application of runoff did not enrich pathogens or indicators in the soil.
- No evidence of vertical migration of pathogens in the soil profile was found.
- Pathogens were reduced via infiltration and cell death over time in the soil.
- Total coliform and *Enterococcus* counts are not reliable for environmental samples.

J. Environ. Qual. 46:169–176 (2017)
doi:10.2134/jeq2016.05.0192

Supplemental material is available online for this article.
Received 24 May 2016.
Accepted 12 Sept. 2016.
*Corresponding author (lisa.durso@ars.usda.gov).

Feedlot runoff has been identified as a vehicle by which manure-borne microorganisms, including zoonotic pathogens, can contaminate waterways (Venglovsky et al., 2009), with the potential for both environmental and public health risks (Berry et al., 2007; Blaustein et al., 2015). Feedlots and other concentrated animal feeding operations (CAFOs) in the United States are required by the Clean Water Act to get a permit from the state or federal National Pollutant Discharge Elimination System if they meet the regulatory definition of CAFOs (Koelsch et al., 2006). The USEPA requires that CAFOs contain all the wastewater and runoff produced from a 25-yr, 24-h design storm, which means that they can hold the rain from a once-in-25-years storm as defined by the National Weather Service. For this location, that means 4.7 inches of rain. The design storm is determined from Miller (1964) for a geographic site, and this precipitation amount is used as criteria to determine the largest event that manure management systems are expected to manage. The probability of a storm exceeding a 25-yr, 24-h design storm event is 4% in any given year. Typically, CAFOs use full containment systems, such as holding ponds or runoff retention basins, to contain feedlot runoff. Although the focus of the National Pollutant Discharge Elimination System regulatory guidelines is predominantly on the nutrient components of animal manures, it is increasingly being recognized that sustainable manure management involves more than just a focus on nutrients and now includes assessment of zoonotic pathogens and veterinary pharmaceuticals in animal manures (Leytem et al., 2013). Additional regulations apply to recreational surface waters, which are monitored for fecal indicator bacteria, as a proxy for the presence of fecal pathogens, and there are health concerns when contaminated water is used to irrigate or process crops used for food production (Blaustein et al., 2015). The USEPA clarified the agricultural storm water exemption by stating that when there is a discharge of manure from land application areas, it is exempt if the manure was applied at agronomic

L.M. Durso and D.N. Miller, USDA–ARS, Agroecosystem Management Research Unit, 251 Filley Hall, UNL East Campus, Lincoln, NE 68583; D.D. Snow, School of Natural Resources, Univ. of Nebraska, 202 Water Sciences Laboratory, 1840 North 37th St., Lincoln, NE 68583; C.G. Henry, Univ. of Arkansas, Rice Research and Extension Center, Stuttgart, AR, 72160, previously Biological Systems Engineering, Univ. of Nebraska-Lincoln, Lincoln, NE 68583-0726; M. Santin, USDA–ARS, Environmental Microbial and Food Safety Laboratory, 10300 Baltimore Avenue, Building 173 BARC-East, Room 103, Beltsville, MD 20705-2350; B.L. Woodbury, USDA–ARS, U.S. Meat Animal Research Center, Nutrition and Environmental Management Research Unit, State Spur 18D, Clay Center, NE 68933. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. Assigned to Associate Editor Hailin Zhang.

Abbreviations: CAFO, concentrated animal feeding operation; VTA, vegetative treatment area; VTS, vegetative treatment system.

rates (Davis, 2003). Therefore, although most CAFOs do not allow manure to leave the manure management system, once the manure is land applied, it can become a nonpoint source of pathogens. Thus, understanding the fate of pathogens after land application events was the purpose of this study.

Feedlot runoff consists of ground-deposited cattle fecal material that is carried off of the immediate feedlot pen surface via rain water or snowmelt. The microbial components of the manure can be mobilized in the liquid portion of the runoff or be adsorbed to suspended or settled particulates (Blaustein et al., 2015). The rate and means by which pathogens are transported through the environment is a key component of risk assessments (Blaustein et al., 2015; USEPA, 2012). One strategy that has been evaluated for its ability to reduce the transport of manure-associated pathogens and fecal indicator bacteria from runoff into surface and ground waters is the use of vegetative filters and grass buffer strips. These are used to reduce the impact of runoff from manure-amended fields and have been shown to be effective at reducing the transport of nutrients such as phosphorus (Gilley et al., 2011). However, the transport dynamics of microbes differ from that of nutrients. The results examining the efficacy of vegetation for removal of microbes are mixed, with some researchers finding the vegetative strips to be effective at reducing microbe numbers (Mankin et al., 2006; Sullivan et al., 2007; Tate et al., 2006) and others concluding that the transport of manure-impacted runoff through vegetation either does not significantly reduce the concentration of the target bacteria (Durso et al., 2011) or may even increase the number of fecal indicator bacteria exiting the system (Beck et al., 2013; Entry et al., 2000a; Fajardo et al., 2001). The primary mechanism of removal is thought to be from the infiltrating effect and storage capacity of soils as opposed to sedimentation and tortuous flow that occurs when manures are applied across vegetation (Powers et al., 2010). Additional information about season-long water balance and nutrient performance of this VTS system is presented in Powers et al. (2010).

A VTS is an alternative to the traditional full containment system and should not be confused with vegetative filters. A VTS is an engineered manure management system that collects open-lot runoff in sediment or settling basins for temporary storage after a precipitation event. The runoff is then applied to a vegetated area when the soil has the capacity to infiltrate the collected runoff, using a dedicated irrigation system. Vegetative treatment areas (VTAs) are only used for the runoff from the feedlot area, and no additional commercial fertilizers or other manure nutrients are applied to VTAs. Conceptually, a VTS replaces the need for a long-term runoff retention pond. The primary mechanism of operation is infiltration into land planted with perennial grasses that utilize the nutrients in the runoff (Koelsch et al., 2006). Thus, VTSs are used as part of long-term manure management systems based on their ability to recycle nutrients into plant matter that can be fed back to livestock.

There are a variety of VTS designs, all meant to replace the need for large, lined, long-term runoff retention ponds. The VTS for open lot control is comprised of a sediment basin, an outlet structure or pump, and an area of perennial vegetation (i.e., a VTA). The VTS couples livestock and crop systems by quickly applying feedlot runoff to fields and is designed to comply with USEPA National Pollutant Discharge Elimination System requirements for CAFOs (Bond et al., 2011; Koelsch et al., 2006). Federal guidelines allow for the use and evaluation of VTS as an alternative to runoff retention ponds (Bond et al., 2010; Khanijo et al., 2007; Moody et al., 2006; Ostrem et al., 2010). Passive, gravity-based systems have been proposed as a cost-effective alternative for larger feedlots (Woodbury et al., 2003). Active systems have also been proposed and evaluated that include valves, pumps, or sprinkler systems to manage and apply runoff to the vegetated areas (Bond et al., 2010; Gross and Henry, 2007, 2010; Koelsch et al., 2006; Melvin and Lorimor, 2007; Moody et al., 2006; Ostrem et al., 2010; Powers et al., 2010) Berry et al. (2007) evaluated a passive, gravity-fed brome grass VTS and found that bacterial and protozoal pathogen numbers generally declined over time, although some manure-borne bacteria were able to survive in the soil for extended periods. Andersen et al. (2013) evaluated the performance of six commercial VTS in Iowa for nutrient parameters; however, microbial data were not collected in that study. In general, the efficacy of VTS for fecal coliform removal is not clear, and few studies have been conducted to study large-scale VTS performance (Koelsch et al., 2006).

The objectives of this study were (i) to evaluate the fate and transport of fecal indicators and microbial pathogens over time in the soil of these active VTS systems and (ii) to determine if the microbial dynamics in these active systems mirror the results found by Berry et al. (2007) for the passive VTS, particularly because there was more frequent application of runoff in the active system. We examined the dynamics of particular pathogens and fecal indicators in the soil over the course of 2 wk after a single application event, evaluated the impact of precipitation on the mobility of bacteria after a feedlot runoff application to the vegetative area, and collected annual soil cores to determine vertical transport of fecal bacteria in VTS soil during a 3-yr period.

Materials and Methods

Vegetative Treatment System

The VTS was installed next to a 1200-head beef cattle CAFO in central Nebraska (Fig. 1). Beef cattle are typically weaned between 7 and 8 mo of age and raised by a backgrounder or stocker until they are sent to a feedlot, typically at 12 to 16 mo of age. There were no young cattle at this feedlot. The estimated weight for animals present during spring and summer rain events is 225 to 500 kg. This site was located on silty loam/sandy loam soil that had been graded and planted to cool-season grasses 3 yr before the beginning of this investigation. Feedlot pen runoff accumulated in unlined settling basins on the downslope end of each pen, where solids were allowed to settle; runoff was then pumped using a vertical turbine pump to one of eight 244 m by 20 m treatment cells. Collected feedlot pen runoff

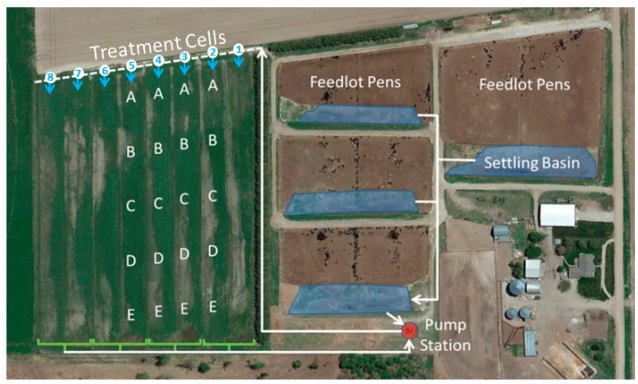

Fig. 1. Satellite photograph of the feedlot and vegetative treatment system with specific elements of the feedlot runoff management system highlighted. Sites A through E in the treatment cells indicate the location where soil samples were taken.

was retained in the basins for several hours to several days depending on the precipitation event, so that the VTA had time to dry and infiltrate the applied runoff. An intake structure screened large particles, but most solids that would settle did so within a few hours. Coarse screening prevented larger particles from entering the pump. Each pen had its own settling basin, and the basins were interconnected to a single pump station that transferred the open lot runoff water to the vegetative area by way of underground pipe. The producer could apply the runoff water to one of eight treatment cells at a time. Runoff water was applied to the uppermost end of each treatment cell. Within each treatment cell, the water was uniformly distributed across the treatment cell using a gated pipe, and the vegetative area was border irrigated so each cell was separated by a small berm. Each treatment cell had been land leveled for uniform cross slope and 1% down slope, with each treatment cell being 250 m by 20 m, for a total of 4.5 ha. Vegetation from the VTA is harvested as hay by the feedlot owner. Any remaining applied runoff or tail water from a treatment cell was returned to the pump station by way of an intake structure and underground pipe (Andersen et al., 2013; Powers et al., 2010). This VTS was permitted under the National Pollutant Discharge Elimination System permitting program (Gustafson et al., 2006; USEPA, 2008) using an alternative performance criteria specified in Section 40, part 412 of the Code of Federal Regulations documenting that it was functionally equivalent for regulatory purposes to conventional runoff retention ponds (Bond et al., 2011). The system was unique in that it was instrumented to measure the volume of runoff water applied to the treatment areas with a propeller-style flow meter (Mccrometer). The return volume was measured with an area-velocity sensor (ISCO-teledyne) so that total volume of applied runoff water and tail water could be measured from each application event and from precipitation events.

The feedlot operator had a mix of spring and fall calves, and thus there was a mix of lightweight (500 lbs) to near fat cattle (1000–1200 lbs) in the feedlot at all times during the study. Generally, there were younger cattle in the feedlot in the autumn sample events than during the spring and summer events. Thus, there would have been a range of diets based on the growth stage of cattle, generally more roughage to grain in younger cattle diets than in older animal diets. The rations were corn and distillers based.

Sample Types

Four types of samples were collected as part of this study: VTA rain runoff, applied feedlot runoff, tailwater, and soil (Table 1). Vegetative treatment area rain runoff consisted of rain water that fell on the treatment cells, ran off, and was collected at the lower end of the vegetative area. This sample was used to evaluate whether fecal indicators and pathogens were mobilized from the vegetative area by the rain. The VTA rain runoff was pumped from the bottom of the cells and applied to the treatment cells before wastewater application. Applied feedlot runoff was cattle feedlot pen runoff that had been screened to remove solids, was pumped to the top of the treatment cells, and distributed via irrigation pipe directly onto the treatment cell. Samples were collected at the up-slope end of the cells because the runoff was applied from the gated pipe to the treatment cells. Tailwater was the applied feedlot runoff that did not infiltrate into the treatment cell soil and was collected from the bottom of the cells.

Under normal operating conditions, the feedlot operator would wait a few hours or days until the soil in the VTA dried on the surface and could accept applied feedlot runoff. Thus, during high-precipitation events the soil may be near field capacity at the surface as water percolates through the soil profile. Application consists of monitoring the advance of the wetting front until it is near the end and then moving to another treatment cell. Some application events would result in small tailwater volumes; other event would result in no tailwater. In extreme cases, a large tailwater volume may be experienced, but this would be expected under a chronic wet period or events near the design storm and when less than 50% of the available water-holding capacity was available.

For the purpose of these experiments, dates were chosen where large volumes of feedlot runoff were available (large storm events had taken place) and the antecedent moisture conditions of the VTA would have been high. Unlike normal operation, the research conditions were designed to simulate the worst-case conditions that may be experienced by VTAs during large storm events by applying runoff until large tailwater volumes were generated. Although some feedlot owners might subject their systems to large tailwater volumes, it is counterproductive for them to do so because it takes more labor and energy to apply the Applied Feedlot Runoff. Here, the overapplication of large tailwater volumes simulated the conditions where more runoff is applied than the VTA can assimilate. The applied runoff could be seen as a front of water moving down the length of the cells. Under normal operating conditions, when the front reached the bottom third of the

Table 1. Descriptions of experimental sample types and sample collection parameters.

Sample type	Description	Timing of collection	*n*
VTA† rain runoff	Rain water that fell on the treatment cells and did not infiltrate. Collected at the bottom of the paired cells (i.e., Cells 1 and 2 had common runoff collection, as did Cells 3 and 4, 5 and 6, and 7 and 8).	Runoff samples were collected on arrival at the site, before wastewater application.	37
Applied feedlot runoff	Feedlot pen runoff accumulated in unlined basins at the bottom of the pens. Most solids settled out, and coarse screens in the settling basin prevented larger particles from being included. This liquid was applied to the tops of treatment cells.	For each treatment cell, wastewater samples were taken at 10, 20, and 30 min during application.	121
Tail water	Liquid that was collected near the bottom of the VTS‡ cells directly after application of wastewater. Used to measure the filtering capacity of the vegetation.	Collected when the wastewater front traveled at least 4/5 of the treatment cell length (times ranged from 45–90 min per cell).	62
Soil	Soil cores collected along transects in four cells: Sites A through E in treatment cells 2 through 5, (Fig. 1).	Short-term soil study: Days 0, 1, 3, 7, and 14. Annual 50-cm-deep cores in the fall of each year. Sectioned into 5- and 10-cm increments.	80 short-term; 60 composites; 420 total sections

† Vegetative treatment area.

‡ Vegetative treatment system.

cell, the producer would close the valve for that cell and start applying the runoff to a new cell.

Soil samples consisted of composites (three cores) of either surface cores (0–6 cm) collected with a hand probe (short-term soil study) or 50-cm-deep cores collected with a truck-mounted hydraulic probe (2010, 2011). A pneumatic probe was used to collect 50-cm-deep cores in 2012 because the surface soil was too hard for the hydraulic probe to work.

Sample Collection: Liquid Samples

Liquid samples (VTA rain runoff, applied feedlot runoff, and tailwater) were collected in the spring and summer for 3 yr. Because there was insufficient rainfall in autumn to produce runoff, no liquid samples were collected. Within 48 h of a rain event, samples were collected, stored on ice, and processed within 12 h. All samples were collected in 500-mL Nalgene screw-topped plastic bottles. Upon arrival at the site, VTA rain runoff was collected first from standing water at the bottom of the cells. Each VTA rain runoff sample was a composite of rainwater runoff from two cells due to the configuration of berms and gates. Next, three applied feedlot runoff samples were collected directly from the outflow pipes 10, 20, and 30 min after beginning application to a particular cell. Typically, feedlot runoff was applied for 30 to 45 min to a particular cell. Finally, excess tail water, which had been intentionally overapplied (45–60 min of application), was collected from the liquid front near the bottom of the cells. On average, this wetting front reached the bottom of the cell 50 min after beginning application. Flow data were downloaded from an automated sampler and an area-velocity sensor located at the tailwater return pipe, and the applied runoff volume was measured by reading the totalizer values on the flow meter between treatment cell changes. The valves were then switched between treatment cells, and feedlot runoff was applied to the next cell, with sample collection representing the concentration and volume of what was entering and exiting treatment cells as described above.

Sample Collection: Soil Samples

The short-term soil study examined changes in the surface soil over 2 wk after a summer wastewater application event (2011). Four replicate cells were chosen for sampling, and samples were taken at five locations in each cell along a transect (Fig. 1) on Days 0, 1, 3, 7, and 14. For the fall deep soil study, three 50-cm-deep cores were taken from the same five locations in four cells described in the short-term soil study, subdivided into six different depths (0–5, 5–10, 10–20, 20–30, 30–40, and 40–50 cm), and then pooled by depth. For the soil survival study (Event 8), soil samples were also collected from the berms between the vegetative cells. The berm did not receive runoff, and berm samples were used to provide information on background levels of the measured parameters. Cores were cut and pooled in the field, stored in coolers with ice, and processed within 12 h of collection.

Microbial Analysis

Coliforms, *Escherichia coli*, and Enterococcus counts were performed using IDEXX Quanti-tray (IDEXX Laboratories, Inc.), which is based on the Standard Methods Most Probable Number model. These are standard methods approved by the USEPA and documented in the US Federal Register (40 CFR Parts 136 and 503, Vol 72, No. 157, March 26, 2007). Shiga-toxigenic *E. coli* O157 was detected using previously published methods (Durso, 2013). Briefly, 10 g of sample were combined with 90 mL of 1.5′ Brilliant Green Bile Broth, incubated for 6 h at 37°C, and plated on CHROMagar O157 (CHROMagar). Mauve colonies were confirmed using a multiplex PCR procedure to detect *stx1*, *stx2*, rfb_{O157}, *eae*, *hly*, and $fliC_{H7}$ as previously described (Hu et al., 1999; Paton and Paton, 1998). For *Salmonella* detection, 10 g of sample was mixed with 90 mL of Difco Trypticase soy broth (Becton Dickinson) and processed as previously described (Brichta-Harhay et al., 2011), including confirmation via PCR of the *invA* gene. A subset of the samples was evaluated for the presence of parasites *Cryptosporidium* (Santín and Zarlenga, 2009), Giardia (Santín et al., 2009), and *Enterocytozoon bieneusi* (Santín and Fayer, 2009), as previously described. Each sample was sieved, and parasite forms were concentrated by CsCl density centrifugation followed by DNA extraction using a modification of the DNeasy Tissue Kit (Qiagen, Valencia, CA) as previously described (Santín et al., 2004). The PCR protocols for amplifying gene fragments from *Cryptosporidium* (SSU rRNA), *G. duodenalis* (SSU rRNA), and *E. bieneusi* (SSU rRNA, ITS, and LSU rRNA) have been described (Buckholt et al., 2002; Hopkins et al., 1997; Xiao et al., 1999). The PCR products were analyzed on 1% agarose gel and visualized by ethidium bromide staining. All PCR products were purified with Exonuclease I/Shrimp Alkaline Phosphatase (Exo-SAP-IT) (USB Corp.) and sequenced in both directions using Big Dye chemistries and an ABI3130 sequencer analyzer (Applied Biosystems). All sequences were subjected to BLAST searches in the GenBank database to confirm their identity.

Statistics

Microbial abundance data were log transformed before statistical analysis using the repeated-measures ANOVA available in the SAS statistical package (version 9.2, SAS Inst. Inc.). Multiple covariance structures were evaluated to select the model with the lowest Akaike information criterion.

Results

A total of 720 liquid and soil samples were evaluated for fecal indicators and zoonotic foodborne pathogens over 3 yr, including enumeration of total coliforms, *E. coli*, and Enterococcus and detection of STEC O157 and *Salmonella*. The feedlot runoff source material was rich in fecal-associated bacteria (*E. coli*, coliforms, and Enterococcus) and included specific zoonotic pathogens *E. coli* O157:H7 and *Salmonella* (Supplemental Table S1). The tailwater was also rich in all three fecal indicators; however, their numbers were reduced by 2 to 4 logs in rainwater runoff. *Escherichia coli* O157:H7 was detected at all eight runoff events in the applied feedlot runoff and in excess tailwater from the treatment cells in five of the six events where treatment cells were purposely overapplied with feedlot runoff. *Salmonella* was detected only in the first three events, which were all in 2010. For two of those 2010 events, feedlot runoff was purposely overapplied, and *Salmonella* was detected in the tailwater for those particular events. Protozoan pathogens were also detected in applied feedlot runoff and tailwater in all events during 2011 and 2012 (Table 2). These included *Cryptosporidium*, occasionally Giardia, and Microsporidia (*E. bieneusi*). "Clean" VTA rain

runoff was negative for *Cryptosporidium* and Giardia but positive in one case for Microsporidia during spring of 2012.

The 2-wk soil-survival studies conducted concurrently with Event 8 (summer of 2011) showed that the microbes initially remain viable on the surface of the VTS cells at concentrations of 10^4 total coliforms, 10^3 *E. coli*, and 10^3 Enterococcus per gram of soil (Table 3). Pathogens were undetectable by the third day, and *E. coli* numbers in soil dropped by an order of magnitude over the 14-d study period. *Salmonella* were not detected in feedlot runoff or soil samples.

In soil cores collected during the fall for three consecutive years, fecal bacteria were found predominantly in the surface soil. There did not appear to be substantial downward movement of fecal indicators or pathogens at this location during the 3-yr time frame (Table 4).

Specific zoonotic pathogens were only rarely detected in the soil core samples. Out of 420 soil samples collected at the end of each year, only a single sample was positive for *E. coli* O157:H7, and only two samples were positive for *Salmonella*. All three detections occurred during the 2010 sample coring, with no detections in 2011 or 2012. During the Event 8 study where soils were sampled immediately after wastewater application and periodically for 2 wk, only two of the samples were positive for *E. coli* O157:H7; one soil sample immediately after wastewater application and one soil sample on the following day. All other soils collected on the following days were negative for *E. coli* O157:H7. *Salmonella* was not detected in any wastewater or soil samples (Fig. 2).

Discussion

In this study we evaluated the performance of a pump-based VTS designed and built to serve a 1200-head beef cattle operation. Immediate reductions in total bacterial numbers, as the runoff moved down the vegetated cell, were due to infiltration by the microbes into and onto the soil, effectively immobilizing them in the short-term. In addition to having been immobilized, short-term reductions likely resulted from loss of cell viability and bacterial cell death. Bacterial numbers were highest for all three fecal indicators in the top 5 cm of the soil. These VTS results were similar to results collected from riparian filter strips, which also saw the highest coliform numbers in the top 0 to 5 cm of soil (Entry et al., 2000b). Under normal operating conditions, the wastewater front would not have been allowed to reach the bottom of the individual cells, and thus the microbes would have been contained within the VTS. In this VTS design, runoff that accumulated at the bottom of treatment cell was subsequently reapplied and resulted in 100% containment of these pathogens within the treatment cells.

It is likely that the soil within the vegetative cells was saturated immediately after heavy rainfall. Over 24 to 48 h, evapotraspiration would move water out of the soil. Runoff application likely brought

Table 2. Protozoal pathogens detected in applied feedlot runoff and tailwater in 2011 and 2012. Detection of specific zoonotic parasites in vegetative treatment area (VTA) rain runoff, applied feedlot runoff, and tailwater within the vegetative treatment system.

Event (date)	Sample type	Samples (*n*)	*Cryptosporidium* (Y/N)	*Giardia* (Y/N)	*Microsporidium* (Y/N)
Event 6 (18 Apr. 2011)	VTA rain runoff	1	N	N	N
	applied feedlot runoff	4	Y (100%)	N	Y (100%)
	tailwater	1	Y	N	Y
Event 8 (6 June 2011)	manure	10	Y (80%)	Y (70%)	Y (30%)
	VTA rain runoff	–†	–	–	–
	applied feedlot runoff	3	Y (100%)	N	Y (100%)
	tailwater	3	Y (100%)	Y (33%)	Y (100%)
Event 10 (17 Apr. 2012)	VTA rain runoff	2	N	N	Y (50%)
	applied feedlot runoff	4	Y (100%)	N	Y (75%)
	tailwater	1	Y	Y	Y
Event 11 (29 May 2012)	VTA rain runoff	–	–	–	–
	applied feedlot runoff	4	Y (100%)	Y (25%)	Y (100%)
	tailwater	1	Y	N	Y

† No samples tested.

Table 3. Survival of fecal indicators and pathogens in soil over a 2-wk time course. Fecal indicators initially remain viable on the surface of the vegetative treatment system cells and slowly declined over the 14-d study period. *Salmonella* were not detected in feedlot runoff or soil samples; *Escherichia coli* O157:H7 were undetectable by the third day.

Day	Source	*Escherichia coli*		Total coliform		Enterococcus		*E. coli* O157:H7 (Y/N)	*Salmonella* (Y/N)
		Log cfu†	SD	Log cfu	SD	Log cfu	SD		
0	manure	6.64	0.50	6.67	0.46	4.25	0.67	Y	N
0	runoff	3.04	0.36	3.45	0.20	3.35	0.19	Y	N
0	soil	3.17	0.93	4.69	0.65	3.05	0.23	Y	N
1	soil	3.20	0.50	4.18	0.55	3.20	0.41	Y	N
3	soil	3.22	0.46	4.50	0.55	3.34	0.33	N	N
7	soil	2.88	0.81	4.25	0.74	3.18	0.54	N	N
14	soil	2.18	0.56	4.52	0.61	3.31	0.54	N	N
3	berm	−0.40	1.34	3.17	0.77	2.83	0.48	N	N
3	hay	0.25	1.72	5.34	0.94	0.86	1.77	N	N

† Values for manure, soil, berm, and hay are per gram. Values for runoff are per milliliter.

Table 4. Detection of fecal indicators and pathogens at multiple soil depths, 2010–2012. There did not appear to be substantial downward movement of fecal indicators or pathogens at this location during the 3-yr time frame. Dashed line indicates no sample collected.

	Escherichia coli		Total coliform		Enterococcus		*E. coli* 157:H7 (Y/N)		*Salmonella* (Y/N)	
	Treatment	Berm	Treatment	Berm	Treatment	Berm	Treatment	Berm	Treatment	Berm
	log cfu g^{-1} soil									
Event 5 (2010)										
0–5 cm	0.59	–	4.21	–	4.16	–	Y	–	N	–
5–10 cm	−0.16	–	3.38	–	3.91	–	N	–	N	–
10–20 cm	−0.51	–	3.01	–	3.72	–	N	–	N	–
20–30 cm	−0.85	–	2.36	–	3.76	–	N	–	Y	–
30–40 cm	−0.58	–	1.75	–	3.86	–	N	–	Y	–
40–50 cm	−0.88	–	1.40	–	3.58	–	N	–	N	–
Event 9 (2011)										
0–5 cm	−0.21	−0.24	2.97	3.42	2.44	2.29	N	N	N	N
5–10 cm	0.37	0.12	2.49	2.12	2.20	2.53	N	N	N	N
10–20 cm	0.01	−0.15	2.52	1.73	2.11	2.26	N	N	N	N
20–30 cm	−0.45	−0.80	2.24	1.00	2.06	1.66	N	N	N	N
30–40 cm	−0.41	−1.00	2.08	0.71	2.12	2.36	N	N	N	N
40–50 cm	−0.78	−0.80	1.98	0.64	2.18	2.91	N	N	N	N
Event 12 (2012)										
0–5 cm	−0.93	−0.64	1.80	2.84	2.53	2.73	N	N	N	N
5–10 cm	−1.00	−1.00	0.55	2.24	2.43	3.01	N	N	N	N
10–20 cm	−1.00	−0.74	0.42	1.79	2.39	2.65	N	N	N	N
20–30 cm	−1.00	−0.59	−0.27	1.22	2.44	2.95	N	N	N	N
30–40 cm	−0.95	−1.00	0.11	2.10	2.40	3.17	N	N	N	N
40–50 cm	−0.95	−1.00	0.28	2.57	2.47	3.22	N	N	N	N

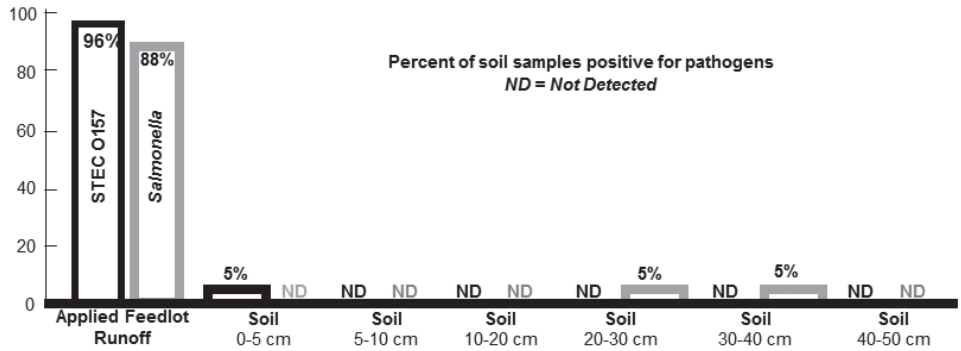

Fig. 2. Human pathogens in soil at selected depth intervals. Percent of samples that were culture positive for Shiga toxigenic *Escherichia coli* O157:H7 and *Salmonella*.

soils back up to saturation because the soils would have absorbed water as the runoff front moved down the vegetative cell. Excess water would move off the vegetative cell and collect in the basin at the bottom. Because of the sloped design of the treatment cells, runoff did not collect or pool on the surface of the vegetative cells.

When postapplication rain events were examined and enough rain fell on the VTS cells to yield runoff from the treatment cells, fecal-associated bacteria remained predominantly in the soil because the abundance of fecal bacteria in the VTA rain runoff was only a small fraction of the fecal microbes that had been loaded onto the treatment cell during previous events. The total number of bacteria in tailwater was 2 to 4 logs lower in concentration than the applied feedlot runoff numbers. More importantly, when total abundance or mass of fecal-associate bacteria in the VTA rain runoff was compared with total abundance or mass previously applied to the treatment cell, then the overall efficiency of removal was 3 to 5 logs (up to 99.999% removal). This was an important finding because these results showed that if the soil had time to assimilate feedlot runoff after an application event, then very little potential existed for off-site transport of the measured fecal-associated bacteria. It should be noted that the scenario with the VTS was a worst-case because the treatment cells received more frequent application events than would be expected from typical land application events with conventional full containment systems.

Comparing surface soils collected in Event 8 where feedlot runoff was applied and monitored over 2 wk with surface soils collected annually in the fall, the concentrations of *E. coli* and total coliforms per gram of soil were reduced by 2 to 3 logs from June to September 2011, indicating that the fecal-associated microorganisms naturally declined over time. Interestingly, in 2012, fecal-associated microbes in the soil cores were reduced even further (3–5 logs) when compared with the previous year, which was likely due to the harsh conditions of extreme drought from that year on these particular microorganisms.

An important set of control samples collected from the berm area between treatment cells indicated that selected groups of bacteria that were measured in the soil were not necessarily related to feedlot runoff because the abundances of total coliforms were similar between berm and treatment cells (and even sometimes more

numerous in berm samples). For the total coliform group, this may have been due to the presence of plant-associated coliform bacteria in the berm samples (Leclerc et al., 2001). Enterococcus numbers remained stable at 10^3 g^{-1} when the two soils were compared (fresh runoff applied to surface soil vs. end-of-season soil samples) and when compared across the years. These data suggest it was likely that native populations of Enterococcus were present in these soils. Although the general premise was that the fecal indicator bacteria were associated exclusively with feces, there are instances where these bacteria have been isolated or enumerated from soil with no or limited fecal inputs. For example, in Illinois, a field experiment found high numbers of fecal coliforms and *E. coli* in control soils neighboring the treatment plots (Beck et al., 2013), and a study of ungrazed native Nebraskan prairies found mean levels of total coliforms and Enterococcus occurred naturally in soil at 1.60E+04 and 3.39E+03 cfu g^{-1} dry soil, respectively (Durso et al., 2016). Data from the current study reinforce these previous results and highlight the utility of taking background or control samples when measuring microbial parameters in manure-impacted soils. Additionally, these data support criticisms of the use of these indicator organisms as definitive markers of the microbiological safety of water due to the common appearance of coliforms in the environment (Leclerc et al., 2001). Also of note is that this was an open field site, accessible to birds, rodents, and other wildlife that may have contributed fecal inputs. Although these inputs are not expected to influence the coliform numbers of the applied feedlot runoff and tailwater, there is the possibility that they contribute to the coliform counts in the rainfall runoff and berm control samples.

The fecal-indicator bacteria and specific zoonotic pathogens measured over the 3 yr of the study remained suspended in the feedlot runoff as it was applied to the treatment areas. The VTS grasses did not physically remove these microbes or prevent them from traveling further along the treatment cell (i.e., "straining"). These results support conclusions from laboratory-scale experiments demonstrating no reduction in *E. coli* concentrations in the vegetative filter strips (Fox et al., 2010). In plot-based studies of grass filter strips, the flow rate was reported to influence entrapment of bacteria. Collins et al. (2004) reported 0 to 5% entrapment of *E. coli* under high flow rates, compared with 95% entrapment at the slowest flow rate. Of note is the difference in interpretation of microbial numbers by concentration, typical of microbiological reports, or by mass (concentration times volume), typical of engineering reports. One point of view evaluates concentration of microbial numbers per unit of volume, whereas some interpret results by mass reductions. Thus, researchers and end users should consider which method is being reported or used to evaluate performance or efficacy.

The specific transport of the different indicator bacteria groups is thought to differ due to underlying physical and chemical parameters of the species measured (Blaustein et al., 2015). *Escherichia coli* is thought to be more frequently associated with the liquid portions of runoff compared with enterococci, and enterococci are thought to be more likely to attach to particulate matter compared with *E. coli* (Blaustein et al., 2015; Guber et al., 2007; Muirhead et al., 2006; Soupir et al., 2010). In the VTS measured for this study, we did not observe any reduction in Enterococcus concentration when comparing the feedlot runoff that was initially applied to the tailwater that was collected at the bottom of the VTS cells after transport through 800 feet of vegetation. If there was preferential attachment of Enterococcus to particulate matter, it was not to the larger particles that settled out during the passage of the wastewater from the top to the bottom of the VTS cell. Due to infiltration of the applied feedlot runoff, the total number or mass of Enterococcus cells in the tailwater was reduced by 99% when compared with the total number or mass of Enterococcus cells in the applied feedlot runoff.

In comparing the microbial performance of the active, pump-based VTS here with the passive, gravity-fed system evaluated by Berry et al. (2007), we observed the same general trends for bacterial and protozoan numbers to decline over time. The numbers of *E. coli* in the passive system ranged between 10^4 and 10^6 cfu per gram of soil or milliliter of liquid, whereas the *E. coli* in the active system were generally less concentrated and displayed a larger range of values. Immediately after application of the applied feedlot runoff in the active system, there were only 10^3 cfu *E. coli* g^{-1} dry soil, declining to 3.8 cfu *E. coli* g^{-1} dry soil at the end of the season. In the liquid samples, the highest *E. coli* numbers were associated with the applied feedlot runoff (10^5), declining to 10^1 in the VTA rain runoff. When comparing these numbers, the current analysis was based on gram dry weight of soil, whereas Berry et al. (2007) used wet weight of soil without drying. Per gram, the Berry et al. (2007) samples contained proportionately less soil, and their reported *E. coli* concentrations would likely be higher had they used dry weight. Despite these differences in starting concentrations, both systems resulted in a decline of target organisms during field operations.

Applied feedlot runoff and tailwater that was purposely over-applied contained high concentrations of fecal-associated bacteria, and pathogens such as *E. coli* O157:H7, *Cryptosporidium*, and Microsporidium were routinely detected. Fecal bacteria and pathogen numbers declined over time in the soil. Short-term (2-wk) reductions were observed with many fecal-associated bacteria, and *E. coli* O157:H7 in the soil was reduced within a few days to undetectable levels. No evidence was seen to indicate that pathogens were moving into the deeper soil at this site. Total coliform and *Enterococcus* counts on control berm samples reinforce previous work suggesting that these two fecal indicators are not reliable for environmental samples. Results from this study suggest that VTSs for CAFOs can be effectively used with minimal risk of soil and water contamination.

Acknowledgments

The authors thank Crystal Powers, Jennifer McGhee, Jaime LaBrie, Amy Mantz, Alisha Craft, Angel Iverson, Katie Herman, Paul Koerner, and Ryan McGhee for technical and field assistance in collection and processing of samples; K and N Farms for access to the VTS; and Kevin Gustafson, Jason Gross, and Owen Kvitten for design and operation of the VTS. This work was supported by USDA–ARS and National Program 214, the USEPA Section 319 non-point source pollution program, the Nebraska Environmental Trust Fund, and the Natural Resource Conservation Service Conservation Innovation Grant Program and the Natural Resource Conservation Service Environmental Quality Incentives Program.

References

Andersen, D.S., R.T. Burns, L.B. Moody, M.J. Helmers, B. Bond, I. Khanijo, and C. Pederson. 2013. Impact of system management on vegetative treatment system effluent concentrations. J. Environ. Manage. 125:55–67. doi:10.1016/j.jenvman.2013.03.046

Beck, W.J., J.E. Schoonover, K.W.J. Williard, and J.J. Zaczek. 2013. Attenuation and persistence of fecal indicator bacteria in vegetated buffer strips in Southern Illinois, USA. Agrofor. Syst. 87:1405–1419. doi:10.1007/s10457-013-9647-4

Berry, E.D., B.L. Woodbury, J.A. Nienabler, R.A. Eigenberg, J.A. Thurston, and J.E. Wells. 2007. Incidence and persistence of zoonotic bacterial and protozoan pathogens in a beef cattle feedlot runoff control-vegetative treatment system. J. Environ. Qual. 36:1873–1882. doi:10.2134/jeq2007.0100

Blaustein, R.A., Y.A. Pachepsky, D.R. Shelton, and R.L. Hill. 2015. Release and removal of microorganisms from land-deposited animal waste and animal manures: A review of data and models. J. Environ. Qual. 44:1338–1354. doi:10.2134/jeq2015.02.0077

Bond, B.J., R.T. Burns, C.G. Henry, T.P. Trooien, S.H. Pohl, L.B. Moody, M.J. Helmers, and J.D. Lawrence. 2010. Evaluating the Performance of Vegetative Treatment Systems on Open Beef Feedlots in the Midwestern United States. Proceeding of the XVIIth CIGR World Congress, Québec City, Canada. 13–17 June 2010.

Bond, B.J., R.T. Burns, C.G. Henry, T.P. Trooien, S.H. Pohl, L.B. Moody, M.J. Helmers, and J.D. Lawrence. 2011. Comparison of construction costs for vegetative treatment systems in the Midwestern United States. Appl. Eng. Agric. 27(3):413–423. doi:10.13031/2013.37067

Brichta-Harhay, D.M., T.M. Arthur, J.M. Bosilevac, N. Kalchayanand, S.D. Shackelford, T.L. Wheeler, and M. Koohmaraie. 2011. Diversity of multidrug-resistant *Salmonella enterica* strains associated with cattle at harvest in the United States. Appl. Environ. Microbiol. 77:1783–1796. doi:10.1128/AEM.01885-10

Buckholt, M.A., J.H. Lee, and S. Tzipori. 2002. Prevalence of *Enterocytozoon bieneusi* in swine: An 18-month survey at a slaughterhouse in Massachusetts. Appl. Environ. Microbiol. 68:2595–2599. doi:10.1128/AEM.68.5.2595-2599.2002

Collins, R., A. Donnison, C. Ross, and M. McLeod. 2004. Attenuation of effluent-derived faecal microbes in grass buffer strips. N. Z. J. Agric. Res. 47:565–574. doi:10.1080/00288233.2004.9513621

Davis, J. 2003. Factsheet #25: Making decisions about application rates. CAFO factsheet series. Midwest Plan Service, Iowa State Univ., Ames.

Durso, L.M. 2013. Primary isolation of Shiga toxigenic *Escherichia coli* from environmental sources. J. Environ. Qual. 42(5):1295–1307. doi:10.2134/jeq2013.02.0035

Durso, L.A., J.E. Gilley, R.A. Eigenberg, D.B. Marx, and B.L. Woodbury. 2011. Effects of diet, manure application rate, and tillage on transport of micro-organisms from manure-amended fields. Appl. Environ. Microbiol. 77(18):6715–6717. doi:10.1128/AEM.02995-10

Durso, L.M., D. Wedin, J.E. Gilley, D.N. Miller, and D.B. Marx. 2016. Assessment of selected antibiotic resistances in ungrazed native Nebraska prairie soils. J. Environ. Qual. 45:454–462. doi:10.2134/jeq2015.06.0280

Entry, J.A., R.K. Hubbard, J.E. Theis, and J.J. Fuhrmann. 2000a. The influence of riparian filterstrips on coliform bacteria: I. Movement and survival in water. J. Environ. Qual. 29:1206–1214.

Entry, J.A., R.I.K. Hubbard, J.E. Theis, and J.J. Fuhrmann. 2000b. The influence of vegetation in riparian filterstrips on coliform bacteria: II. Survival in soils. J. Environ. Qual. 29:1215–1224. doi:10.2134/jeq2000.00472425002900040027x

Fajardo, J.J., J.W. Bauder, and S.D. Cash. 2001. Managing nitrate and bacteria in runoff from livestock confinement areas with vegetative filter strips. J. Soil Water Conserv. 56:185–191.

Fox, G.A., E.M. Matlock, J.A. Guzman, D. Sahoo, and K.B. Stunkel. 2010. *Escherichia coli* load reduction from runoff by vegetative filter strips: A laboratory-scale study. J. Environ. Qual. 40:980–988. doi:10.2134/jeq2010.0391

Gilley, J.E., L.M. Durso, R.A. Eigenberg, D.B. Marx, and B.L. Woodbury. 2011. Narrow grass hedge control of nutrient loads following variable manure applications. Trans. ASABE 54(3):847–855. doi:10.13031/2013.37110

Gross, J., and C.G. Henry. 2007. A "sprinkler" vegetative treatment system "VTS." In: Proceedings of the International Symposium on Air Quality and Waste Management for Agriculture, Broomfield, CO. 16–19 Sept. 2007. doi:10.13031/2013.23875

Gross, J., and C.G. Henry. 2010. Advancements in sprinkler vegetative treatment systems for small and medium animal feeding operations. In: Proceedings of the International Symposium on Air Quality and Waste Management for Agriculture, Dallas, TX. 13–16 Sept. 2010. ASABE, St. Joseph, MI. doi:10.13031/2013.32672

Guber, A.K., J.S. Karns, Y.A. Pachepsky, A.M. Sadeghi, J.S. van Kessel, and T.H. Dao. 2007. Comparison of release and transport of manure- borne *Escherichia coli* and enterococci under grass buffer conditions. Lett. Appl. Microbiol. 44:161–167. doi:10.1111/j.1472-765X.2006.02065.x

Gustafson, K., O. Kvittem, and C. Henry. 2006. K and N mac farms vegetative treatment system design and permit documents. USDA–NRCS, Washington, DC.

Hopkins, R.M., B.P. Meloni, D.M. Groth, J.D. Wetherall, J.A. Reynoldson, and R.C. Thompson. 1997. Ribosomal RNA sequencing reveals difference between the genes of Giardia isolates recovered from humans and dogs living in the same locality. J. Parasitol. 83:44–51. doi:10.2307/3284315

Hu, Y., Q. Zhang, and J.C. Meitzler. 1999. Rapid and sensitive detection of *Escherichia coli* O157:H7 in bovine faeces by a multiplex PCR. J. Appl. Microbiol. 87:867–876. doi:10.1046/j.1365-2672.1999.00938.x

Khanijo, I.K., R.T. Burns, L.B. Moody, M. Helmers, J. Lawrence, C. Pederson, and D. Anderson. 2007. Vegetative treatment system models: Modeled vs. measured performance. In: Proceedings of the International Symposium on Air Quality and Waste Management for Agriculture, Broomfield, CO. 16–19 Sept. 2007. ASABE, St. Joseph, MI.

Koelsch, R.K., J.C. Lorimor, and K.R. Mankin. 2006. Vegetative treatment systems for management of open lot runoff: Review of literature. Appl. Eng. Agric. 22:141–153. doi:10.13031/2013.20190

Leclerc, H., D.A. Mossel, S.C. Edberg, and C.B. Struijk. 2001. Advances in the bacteriology of the coliform group: Their suitability as markers of microbial water safety. Annu. Rev. Microbiol. 55:201–234. doi:10.1146/annurev.micro.55.1.201

Leytem, A.B., R.S. Dungan, and P.J.A. Kleinman. 2013. Sustainable manure management. In: E. Kebraeab, editor, Sustainable animal agriculture. CAB International, Oxfordshire, UK. p. 83–112. doi:10.1079/9781780640426.0083

Mankin, K.R., P.L. Barnes, J.P. Harner, P.K. Kalita, and J.E. Boyer. 2006. Field evaluation of vegetative filter effectiveness and runoff quality from unstocked feedlots. J. Soil Water Conserv. 61:209–217.

Melvin, S.W., and J.C. Lorimor. 2007. Alternative technology systems for open feedlot runoff control. Presented at the ASABE International Meeting, Minneapolis MN. 17–20 June 2007. ASABE, St. Joseph, MI. doi:10.13031/2013.23101

Miller, J.F. 1964. Two to ten-day precipitation for return periods of 2 to 100 years in the contiguous United States. US Dep. of Commerce, Washington, DC.

Moody, L.B., C. Pederson, R.T. Burns, and I. Khanijo. 2006. Vegetative treatment systems for management of open lot runoff: Project design and monitoring methods for five commercial beef feedlots. Presented at the ASABE Annual International Meeting, Portland OR. 9–12 July 2006. ASABE, St. Joseph, MI. doi:10.13031/2013.21143

Muirhead, R.W., R.P. Collins, and P.J. Bremer. 2006. Numbers and transported state of *Escherichia coli* in runoff direct from fresh cowpats under simulated rainfall. Lett. Appl. Microbiol. 42:83–87. doi:10.1111/j.1472-765X.2005.01823.x

Ostrem, T.P. Trooin, S.H. Pohl, D.P. Todley, and N.O. Michael. 2010. Monitoring the performance of vegetative treatment systems across the subhumid and semiarid rainfall zones. In: Proceedings of the International Symposium on Air Quality and Manure Management for Agriculture, Dallas, TX. 13–16 Sept. 2010. ASABE, St. Joseph, MI. doi:10.13031/2013.3267

Paton, A., and J. Paton. 1998. Detection and characterization of toxigenix *Escherichia coli* by using multiplex PCR assays for *stx1, stx2, eaeA,* enterohemorrhagic *E. coli hlyA, rfb0111* and *rfbO157*. J. Clin. Microbiol. 36(2):598–602.

Powers, C.A., C.G. Henry, R. Walkowiak, J. Gross, J.A. George, K.D. Gustafson, R.T. Burns, B.L. Woodbury, and R.A. Eigenberg. 2010. Vegetated treatment system design and monitoring at two large CAFO beef feedlots in Nebraska. In: International Symposium on Air Quality and Manure Management for Agriculture, Dallas, TX. 13–16 Sept. 2010. ASABE, St. Joseph, MI. doi:10.13031/2013.32671

Santín, M., J.M. Trout, L. Xiao, L. Zhou, E. Greiner, and R. Fayer. 2004. Prevalence and age-related variation of Cryptosporidium species and genotypes in dairy calves. Vet. Parasitol. 122:103–117. doi:10.1016/j.vetpar.2004.03.020

Santín, M., and R. Fayer. 2009. *Enterocytozoon bieneusi* genotypes nomenclature based on the ITS sequence: A consensus. J. Eukaryot. Microbiol. 56(1):34–38.

Santín, M., J.M. Trout, and R. Fayer. 2009. A longitudinal study of *Giardia duodenalis* genotypes in dairy cows from birth to two years of age. Veterinary Parasitol. 162(1-2):40–45.

Santín, M., and D.S. Zarlenga, 2009. A multiplex PCR to simultaneously distinguish Cryptosporidium species of veterinary and public health concern in cattle. Veterinary Parasitol. 166(2009):32–37.

Soupir, M.L., S. Mostaghimi, and T. Dillaha. 2010. Attachment of *Escherichia coli* and enterococci to particles in runoff. J. Environ. Qual. 39:1019–1027. doi:10.2134/jeq2009.0296

Sullivan, T.J., J.A. Moore, D.R. Thomas, E. Mallery, K.U. Snyder, M. Wustenberg, J. Wustenberg, S.D. Mackey, and D.L. Moore. 2007. Efficacy of vegetated buffers in preventing transport of fecal coliform bacteria from pasturelands. Environ. Manage. 40:958–965. doi:10.1007/s00267-007-9012-3

Tate, K.W., E.R. Atwill, J.W. Bartolome, and G. Nader. 2006. Significant *Escherichia coli* attenuation by vegetative buffers on annual grasslands. J. Environ. Qual. 35:795–805.

USEPA. 2008. Federal Register. National pollutant discharge elimination system permit regulation and effluent limitation guidelines and standards for concentrated animal feeding operations (CAFO's). USEPA, Washington, DC.

USEPA. 2012. Microbial risk assessment guideline: Pathogenic microorganisms with focus on food and water. EPA/100/J-12/001. USEPA, Washington, DC.

Venglovsky, J., N. Sasáková, and I. Placha. 2009. Pathogens and antibiotic residues in animal manures and hygienic and ecological risks related to subsequent land application. Bioresour. Technol. 100:5386–5391. doi:10.1016/j.biortech.2009.03.068

Woodbury, B.L., J.A. Nienaber, and R.A. Eigenberg. 2003. Performance of a passive feedlot runoff control and treatment system. Trans. ASAE 46:1525–1530. doi:10.13031/2013.15641

Xiao, L., L. Escalante, C. Yang, I. Sulaiman, A.A. Escalante, R.J. Montali, R. Fayer, and A.A. Lal. 1999. Phylogenetic analysis of Cryptosporidium parasites based on the small-subunit rRNA gene locus. Appl. Environ. Microbiol. 65:1578–1583.