

Cattle Feedlot Soil Moisture and Manure Content: II. Impact on *Escherichia coli* O157

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ABSTRACT

The moisture and manure contents of soils at cattle feedlot surfaces vary spatiotemporally and likely are important factors in the persistence of *Escherichia coli* O157 in these soils. The impacts of water content (0.11–1.50 g H₂O g⁻¹ dry feedlot surface material [FSM]) and manure level (5, 25, and 75% dry manure in dry FSM) on *E. coli* O157:H7 in feedlot soils were evaluated. Generally, *E. coli* O157:H7 numbers either persisted or increased at all but the lowest moisture levels examined. Manure content modulated the effect of water on *E. coli* growth; for example, at water content of 0.43 g H₂O g⁻¹ dry FSM and 25% manure, *E. coli* O157:H7 increased by 2 log₁₀ colony forming units (CFU) g⁻¹ dry FSM in 3 d, while at 0.43 g H₂O g⁻¹ dry FSM and 75% manure, populations remained stable over 14 d. *Escherichia coli* and coliform populations responded similarly. In a second study, the impacts of cycling moisture levels and different drying rates on naturally occurring *E. coli* O157 in feedlot soils were examined. Low initial levels of *E. coli* O157 were reduced to below enumerable levels by 21 d, but indigenous *E. coli* populations persisted at >2.50 log₁₀ CFU g⁻¹ dry FSM up to 133 d. We conclude that *E. coli* O157 can persist and may even grow in feedlot soils, over a wide range of water and manure contents. Further investigations are needed to determine if these variables can be manipulated to reduce this pathogen in cattle and the feedlot environment.

FOODBORNE ILLNESS due to *Escherichia coli* O157:H7 has most often been associated with the consumption of undercooked beef and unpasteurized milk products, making the reduction of colonization of cattle by *E. coli* O157:H7 a high priority for the beef industry (Centers for Disease Control, 1993; Keene et al., 1997). In addition, *E. coli* O157:H7 disease has been linked to water and other foods likely contaminated with bovine manure from runoff or by use as soil amendment (Besser et al., 1993; Cieslak et al., 1993; Jackson et al., 1998; Licence et al., 2001). Contamination of the municipal water supply by runoff from land-applied livestock manure containing *E. coli* O157:H7 and *Campylobacter jejuni* was the probable cause of the large waterborne disease outbreak that occurred in Walkerton, Ontario, Canada, in 2000 (O'Connor, 2002). Thus, the application of effective preharvest control measures that reduce *E. coli* O157 incidence in the live animal should not only reduce the prevalence of this organism in beef and milk, but also reduce the incidence of environmental contamina-

tion by this organism via livestock waste, thereby reducing the risk of human foodborne and waterborne illness.

Preharvest control strategies that successfully reduce *E. coli* O157:H7 in cattle and the animal production environment will include measures that interrupt the cycling of pathogens in the production environment and subsequent reinfection of the herd. Horizontal transmission by direct or indirect fecal–oral exposure appears to be the primary means of dissemination of *E. coli* O157:H7 and other Shiga toxin–producing *E. coli* (STEC) in cattle. Faith et al. (1996) found that both transmission among cattle and contact with areas previously occupied by cattle shedding the pathogen were important factors in the dissemination of *E. coli* O157:H7 in a dairy herd. Similarly, Cobbold and Desmarchelier (2002) reported a higher prevalence of an inoculated STEC strain among calves housed as a group than among calves housed in separate pens. These researchers found that contamination of pen floors and hides were important environmental sources for STEC transmission to the animals (Cobbold and Desmarchelier, 2002). Several studies have also suggested that water troughs contaminated with feces serve as a reservoir for *E. coli* O157 for source cycling in cattle and the production environment (Faith et al., 1996; Hancock et al., 1998; LeJeune et al., 2001; Shere et al., 1998).

Although high prevalence of shedding of this pathogen is seasonal (Hancock et al., 1997; Mechie et al., 1997) and cattle appear to shed the pathogen intermittently (Besser et al., 1997; Rahn et al., 1997; Shere et al., 1998), *E. coli* O157:H7 can persist for long periods in bovine feces. When inoculated at an initial level of 10⁵ CFU g⁻¹ of feces, *E. coli* O157:H7 survived up to 70 d at 5°C (Wang et al., 1996). At the higher temperatures of 22 and 37°C, the organism survived in bovine feces for up to 56 and 49 d, respectively, in spite of low moisture content and water activity (Wang et al., 1996). Aerated bovine manure from cattle that were inoculated with *E. coli* O157:H7 remained culture-positive for 47 d (Kudva et al., 1998). Serotypes of STEC other than O157 can also survive in bovine feces for long periods (Fukushima et al., 1999). However, little or no information is available regarding the survival and growth potential of *E. coli* O157 in soils at the feedlot pen surface, the medium in which bovine feces is deposited in beef animal feeding operations and in which the fecal matter concentration increases during the finishing period. In addition, the effects of the various environmental factors that can influence the survival of this pathogen in feedlot soils are unknown. The objective of this work was to

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Abbreviations: CFU, colony forming units; FSM, feedlot surface material; SMAC, MacConkey sorbitol agar; STEC, Shiga toxin–producing *Escherichia coli*; TSB, Trypticase soy broth.

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evaluate the impacts of a range of different moisture contents and manure levels on the survival of *E. coli* O157:H7, generic *E. coli*, and coliforms in cattle feedlot soils. In a second study, the effects of repeated cycles of wetting and drying and of different drying rates of feedlot soils on these target bacterial populations in feedlot soils were determined.

MATERIALS AND METHODS

Inocula Preparation

A streptomycin-resistant strain of *Escherichia coli* serotype O157:H7 ATCC 43895 was used as the experimental inoculum in the first study examining the effects of manure and water content of feedlot soils (Riley et al., 1983; Wells et al., 1983). This streptomycin-resistant mutant was isolated by selective enrichment with increasing concentrations of antibiotic using a procedure similar to the gradient plate technique described by Eisenstadt et al. (1994), and is similar to the parental strain in growth rate, survival characteristics in manure, and acid resistance (data not shown). For each experiment, 100- μ L volumes of frozen glycerol stock cultures (-20°C) were inoculated into 50-mL volumes of Trypticase soy broth (TSB; BBL, Becton, Dickinson and Company, Sparks, MD) containing 250 $\mu\text{g mL}^{-1}$ streptomycin, which were statically incubated for 24 h at 37°C . Cells were collected by centrifugation ($950 \times g$ for 30 min) and resuspended in 50 mL of sterile phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 , 2 mM KH_2PO_4). Immediately before addition to the feedlot soils, the resuspended cells were diluted 10^{-1} in sterile deionized distilled H_2O . This diluted cell preparation contained 10^7 to 10^8 CFU mL^{-1} and was added to the feedlot soils along with additional deionized distilled H_2O at rates necessary to achieve an initial level of 10^5 CFU g^{-1} of dry FSM.

In the second study, the feedlot soils were prepared with bovine manure that was naturally contaminated with *E. coli* O157. Freshly shed bovine feces from cattle housed in the 6000 head capacity beef feedlot at the Roman L. Hruska U.S. Meat Animal Research Center (MARC) were screened to identify feces containing *E. coli* O157, using a modification of the nonselective enrichment and immunomagnetic separation (IMS) procedures described by Barkocy-Gallagher et al. (2002). Briefly, 10 g of each fecal sample was measured into a sterile filtered sample bag (Spiral Biotech, Norwood, MA), 90 mL of TSB was added, and the bag contents were mixed using a Stomacher Circulator (200 rpm, 1 min; Model 400; Seward Limited, London, UK). To approximate the concentrations of *E. coli* O157 in any positive samples, 1 mL of each initial feces-TSB preparation was further serially diluted in tubes containing 9 mL of TSB. Both the initial 10^{-1} enrichment preparations and the diluted samples were incubated at 37°C for 7 h. One-milliliter volumes of the enrichments were subjected to IMS using Dynabeads anti-*E. coli* O157 (Dynal Biotech ASA, Oslo, Norway) and 50- μ L volumes of the concentrated bead suspensions were spread-plated onto sorbitol MacConkey agar (SMAC) containing 0.05 $\mu\text{g mL}^{-1}$ cefixime and 2.5 $\mu\text{g mL}^{-1}$ potassium tellurite (CT-SMAC). The CT-SMAC plates were incubated at 37°C for 24 h before examination for *E. coli* O157. Sorbitol-negative colonies typical of *E. coli* O157 growth were tested for agglutination with *E. coli* O157 latex test reagents (Oxoid Limited, Basingstoke, England). These isolates were further confirmed as *E. coli* by the demonstration of lactose fermentation with gas production, indole production, inability to utilize citrate as a sole carbon source, negative Voges-Proskauer reaction, and positive methyl

red reaction (Hitchins et al., 1998), and as *E. coli* O157 by polymerase chain reaction screening for the presence of the enterohemorrhagic *Escherichia coli* (EHEC) and *E. coli* O157 genes *stx*₁, *stx*₂, *eaeA*, EHEC *hlyA*, and *rfbE*_{O157} (Paton and Paton, 1998). Immediately following their collection and initial sampling, the bovine fecal samples were frozen at -20°C . Before their use in the second study, the *E. coli* O157-positive fecal samples were thawed overnight at 4°C .

Effects of Manure Content and Moisture Level of Feedlot Soils

The feedlot soils were prepared and mixed as described by Miller and Berry (2005). Freshly deposited bovine feces were collected from cattle housed in the MARC feedlot. Hastings silt loam (fine, smectitic, mesic Udic Argiustolls) was collected from surface soil near the feedlot pens. The manure and soil were dried to final water contents of less than 0.1 g $\text{H}_2\text{O g}^{-1}$ dry matter. Three different manure contents (5, 25, and 75% manure, dry matter basis) were examined at each of six different moisture levels of 0.11, 0.25, 0.43, 0.67, 1.00, and 1.50 g $\text{H}_2\text{O g}^{-1}$ dry FSM. Powdered urea was mixed into the feedlot soils at a level of 1 g kg^{-1} dry FSM before the addition of H_2O . Urea is the primary form of nitrogen excreted in bovine urine and this level of urea is consistent with that which can be found in fresh manure. Each treatment combination was prepared in triplicate in plastic pans, with each pan receiving approximately 400 g of FSM with added H_2O . Moisture levels were maintained by daily weighing and addition of deionized distilled H_2O to the feedlot soils, which were held at room temperature. Room temperature was monitored and ranged from 18.2 to 22.3 $^{\circ}\text{C}$, with an average temperature of 19.0 $^{\circ}\text{C}$. To avoid any potential transport of the pathogen by flies or other vectors, the table bearing the experimental pans containing the soils was draped with mosquito bar netting (Cabela's, Sidney, NE). The feedlot soils were stirred thoroughly before removing samples at Days 0, 1, 2, 3, 7, and 14. The samples were analyzed as described below.

Effect of Fluctuating Moisture Levels of Feedlot Soils

The effects of repeated cycles of water addition followed by drying on the growth and survival of indigenous *E. coli* O157, generic *E. coli*, and coliforms were examined in cattle feedlot soils containing 25% manure and 75% soil, dry matter basis. Approximately 1.5 kg of the previously frozen *E. coli* O157-positive bovine feces were supplemented with additional fresh manure for a total mass of 5.5 kg manure. The manure was mixed with additional dried manure, soil, and urea as described above and by Miller and Berry (2005) to obtain feedlot soil of the targeted manure and soil levels. Prepared in this fashion, the initial moisture content of the feedlot soil and manure mixture was 0.78 g $\text{H}_2\text{O g}^{-1}$ dry FSM. The feedlot soils were distributed in triplicate to the pans in three different amounts, to also examine the effect of three different levels of moisture loss: the fast-drying, or high moisture flux, pans contained 360 g of feedlot soil; the intermediate moisture flux pans contained 900 g of feedlot soil; and the low moisture flux pans contained 1800 g of feedlot soil. The pans were held at room temperature (approximately 19 $^{\circ}\text{C}$) and covered with mosquito bar netting as described above. The pans were weighed daily to determine moisture loss and deionized distilled H_2O was added weekly to return the soil to the initial water content of 0.78 g $\text{H}_2\text{O g}^{-1}$ dry FSM. The feedlot soils were stirred thoroughly before removing samples

at 0, 2, 7, 14, 21, 28, 35, 49, 63, 77, 105, and 133 d. The samples were processed and analyzed as described below.

Sample Analyses

In the first study, populations of the inoculated *E. coli* O157:H7 streptomycin-resistant mutant were enumerated on SMAC containing 250 $\mu\text{g mL}^{-1}$ streptomycin (SMAC-Str). Five-gram samples of the feedlot soils were mixed with 45 mL of 2% buffered peptone water (BPW) in a filtered stomacher bag as described above. Following mixing, the filtered samples were serially diluted further as needed in BPW and spread-plated in duplicate onto SMAC-Str plates (0.1 mL per 100- \times 15-mm Petri plate). The SMAC-Str plates were incubated for 24 h at 37°C and typical *E. coli* O157 colonies were enumerated.

In the second study, *E. coli* O157 in the feedlot soils were enumerated using a most probable number (MPN)–immunomagnetic separation (IMS) technique. Five-gram samples of the feedlot soils were mixed with 45 mL of TSB in filtered stomacher bags as described above. These 10^{-1} dilutions were split and further diluted 10-fold in TSB as a three-replicate tube MPN. All MPN dilutions were incubated at 37°C for 7 h, after which 1-mL volumes were subjected to IMS and plating onto CT-SMAC as described above. *Escherichia coli* O157 positive tubes (confirmed as described above) were recorded and the MPN of each soil sample was calculated using the formula of Thomas (1942). After *E. coli* O157 numbers dropped below levels enumerable by the MPN (less than 1 cell in 3 g of feedlot soil), the entire volumes of the initial 10^{-1} dilutions were enriched by incubation for 7 h at 37°C and 1-mL volumes were subjected to IMS to determine if low levels of the pathogen were present.

Indigenous generic *E. coli* and coliforms in the feedlot soils were enumerated in both studies. The initial 10^{-1} soil sample dilutions in either BPW or TSB were serially diluted further if needed in BPW and 1-mL volumes were plated in duplicate onto Petrifilm *E. coli*/coliform count plates (3M Microbiology Products, St. Paul, MN). Following incubation at 37°C for 24 h, characteristic *E. coli* and coliform colonies were counted.

For both studies, water content of feedlot soils was determined by mass loss after drying overnight at 105°C. Additional analyses, including pH determination, organic matter content, and headspace gases composition, were as described in Miller and Berry (2005).

Statistical Analyses

Numbers of bacteria from duplicate plates were averaged and converted to \log_{10} CFU g^{-1} dry FSM. Least squares means of bacterial populations were analyzed using the general linear model procedure for repeated measurements (SAS Institute, 2001). The unit of observation was the pan. The model included effects of manure level, water content, day, pan (manure level \times water content), manure level \times water content, manure level \times day, water content \times day, and manure level \times water content \times day. The effects of manure level, water content, and manure level \times water content were tested using pan (manure level \times water content) as the error term. Differences in water loss per drying cycle data were analyzed by Bonferroni's *t* test (SAS).

RESULTS AND DISCUSSION

Effect of Manure Content and Moisture Level of Feedlot Soils

In the first study, the impacts of a wide range of water contents of feedlot soils of three different soil–manure

combinations on *E. coli* O157:H7, generic *E. coli*, and coliforms were examined. The typical concentrations of *E. coli* O157:H7 that occur in feedlot soils are unknown. Because of the seasonality of shedding (Hancock et al., 1997) and the wide spatial variability of fecal deposition in feedlot pens (Woodbury et al., 2001), there is a potentially wide range of concentrations of the pathogen in the soils. We inoculated *E. coli* O157:H7 at an initial level of 10^5 CFU g^{-1} of dry feedlot soil to have an adequate concentration of cells to resolve either inactivation or growth. Recent studies reporting concentrations of *E. coli* O157:H7 in cattle feces indicate that the range of counts is wide and that an upper range of 10^5 CFU g^{-1} of feces is common (Fegan et al., 2004; Hutchinson et al., 2004; Omisakin et al., 2003). Although there were some differences, generally the effects of water and manure content on populations of indigenous *E. coli* and coliforms were similar to those seen with *E. coli* O157:H7 (Fig. 1). In both studies, the total coliform counts of the feedlot soils were composed primarily of generic *E. coli*, and therefore behaved similarly to generic *E. coli*. For all three groups of bacteria (*E. coli* O157:H7, generic *E. coli*, and coliforms), the manure level, water content, and manure level \times water content were significant ($P \leq 0.0001$).

For the most part, *E. coli* O157:H7 populations either increased or persisted during the two-week study period in all but the driest of the feedlot soils (Fig. 1). In feedlot soils containing 5% manure, there was a rapid loss of *E. coli* O157:H7 viability in the 0.11 g $\text{H}_2\text{O g}^{-1}$ dry FSM soils, and viable cells were below detectable levels by Day 2. At water content of 0.25 g $\text{H}_2\text{O g}^{-1}$ dry FSM, *E. coli* O157:H7 numbers declined slowly, with 4.04 \log_{10} CFU g^{-1} dry FSM remaining at Day 14. In soils of 5% manure of 0.43, 0.67, 1.00, and 1.50 g $\text{H}_2\text{O g}^{-1}$ dry FSM, *E. coli* O157:H7 populations increased slightly ($P \leq 0.01$) during the first day or two, then remained stable throughout the remainder of the 14-d study period.

Unlike the results seen in feedlot soils of 5% manure, *E. coli* O157:H7 populations increased in some of the water content treatments of the soils with 25 and 75% manure. In soil with 25% manure at 0.43 g $\text{H}_2\text{O g}^{-1}$ dry FSM, the *E. coli* O157:H7 numbers increased by 2 \log_{10} CFU g^{-1} by Day 3, and persisted at this high level for the remainder of the 14-d study. The reduction of *E. coli* O157:H7 in the 0.11 g $\text{H}_2\text{O g}^{-1}$ dry FSM treatment soils containing 25% manure was similar to that seen in 5% manure soils, although the rate was not as rapid. At Day 7, populations had dropped below detectable levels. The response seen at 0.25 g $\text{H}_2\text{O g}^{-1}$ dry FSM was somewhat between that at 0.11 and 0.43 g $\text{H}_2\text{O g}^{-1}$; following an initial decline, the *E. coli* O157:H7 numbers remained steadily high at approximately 5.0 \log_{10} CFU g^{-1} dry FSM through Day 14. At the higher water contents of 0.67, 1.00, and 1.50 g $\text{H}_2\text{O g}^{-1}$ dry FSM in the 25% manure soils, levels declined slowly over the course of the experiment to levels of 2.92, 3.21, and 3.62 \log_{10} CFU g^{-1} of dry FSM, respectively.

Increasing the manure level of the feedlot soils from 25% to 75% shifted the range of soil water content at which the pathogen could grow. In 25% manure soils

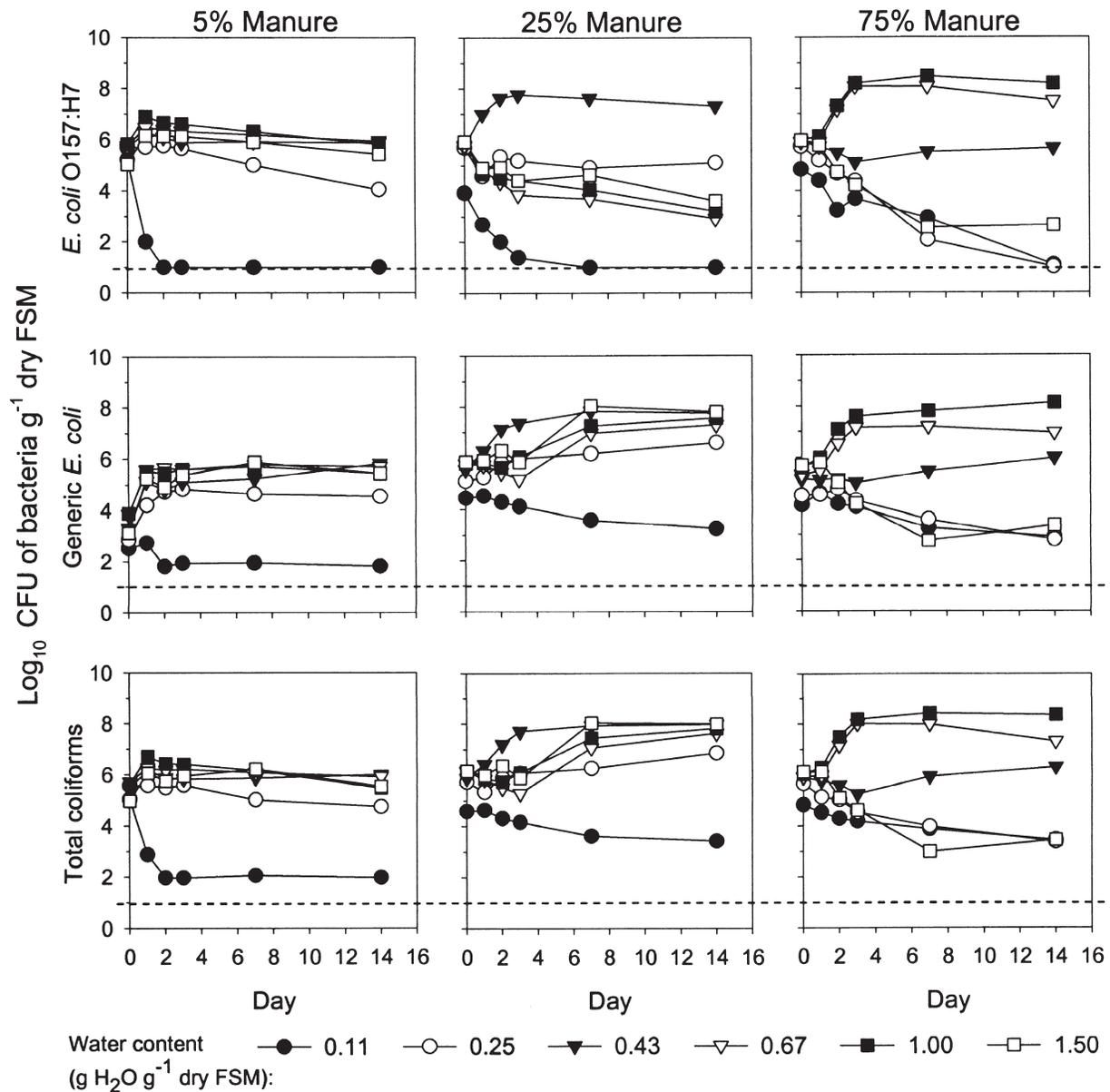


Fig. 1. Effects of water content and manure level on populations of *Escherichia coli* O157:H7 ATCC 43895, and indigenous generic *E. coli* and total coliforms in feedlot soils during storage at room temperature. Water content was maintained by daily addition of deionized distilled H₂O. The minimum detection level is marked by dashed lines and was 1.00 log₁₀ colony forming units (CFU) g⁻¹ of dry feedlot surface material (FSM). The standard errors of the least squares means were 0.15, 0.20, and 0.18 for *E. coli* O157:H7, generic *E. coli*, and total coliforms, respectively.

with water contents of 0.67 and 1.00 g H₂O g⁻¹ dry FSM, *E. coli* O157:H7 populations dropped; however, in feedlot soils of 75% manure and of 0.67 and 1.00 g H₂O g⁻¹, the initial populations of 5.9 to 6.0 log₁₀ CFU g⁻¹ dry FSM increased to levels greater than 8.0 log₁₀ CFU g⁻¹ dry FSM by Day 3. And, while the pathogen grew at water content of 0.43 g H₂O g⁻¹ in the 25% manure soils, at water content of 0.43 g H₂O g⁻¹ in the 75% manure soils, the growth of the organism was inhibited. At 1.50 g H₂O g⁻¹ dry FSM and 75% manure, *E. coli* O157:H7 numbers dropped slowly to 2.65 log₁₀ CFU g⁻¹ dry FSM by Day 14. In 75% manure soils of 0.11 and 0.25 g H₂O g⁻¹ dry FSM, *E. coli* O157:H7 populations were below detectable levels by Day 14.

As noted above, the generic *E. coli* and coliform populations generally behaved like the *E. coli* O157:H7 populations in response to the manure levels or water contents of the feedlot soils. The main two divergences in response between *E. coli* O157 and generic *E. coli* or coliforms are revealed by visual comparison of the growth and survival curves of the three groupings of bacteria shown in Fig. 1. First, in feedlot soils of all the manure levels examined at water contents of 0.11 g H₂O g⁻¹ dry FSM, *E. coli* O157:H7 populations dropped below detectable levels (<1.00 log₁₀ CFU g⁻¹ of dry FSM) during the course of the study. Populations of generic *E. coli* and coliforms, on the other hand, remained at detectable levels even though they were reduced sub-

Table 1. Prevailing soil environmental conditions in feedlot soils prepared with varying manure levels and water contents, based on microbial metabolic activity. Appropriate volumes of deionized distilled H₂O were added daily to maintain the water content of the feedlot soils. Table reproduced with modifications from Miller and Berry (2005).

Water content	Manure level		
	5%	25%	75%
g H ₂ O g ⁻¹ dry FSM†			
0.11	low activity, aerobic‡	no activity§	no activity§
0.25	aerobic¶	aerobic¶,#	no activity§
0.43	aerobic¶	aerobic¶,#	aerobic¶,#
0.67	fermentative¶,††	fermentative¶,††	aerobic¶,#
1.00	fermentative¶,††	fermentative¶,††	fermentative¶,††
1.50	fermentative¶,††	fermentative¶,††	fermentative¶,††

† Feedlot surface material.

‡ Slow reduction of glucose from the soil.

§ Persistence of glucose in soil.

¶ Lactate accumulation in soil.

High oxygen consumption by soil microorganisms

†† Decline in soil pH and/or CH₄ emission.

stantially at this low water content. Second, generic *E. coli* and coliforms were capable of growth in some of the feedlot soils in which *E. coli* O157:H7 did not grow. These results were particularly apparent in the feedlot soils containing 25% manure (Fig. 1). Populations of *E. coli* O157:H7, generic *E. coli*, and coliforms all grew readily in the 25% manure soils with water content of 0.43 g H₂O g⁻¹ dry FSM. *Escherichia coli* O157:H7 levels were also stably persistent in the soils with 0.25 g H₂O g⁻¹ dry FSM, but declined gradually in the soils with 0.67, 1.00, and 1.50 g H₂O g⁻¹ dry FSM. Conversely, generic *E. coli* and coliforms multiplied in the 25% manure soils of water contents of 0.25 and 0.67 to 1.50 g H₂O g⁻¹ dry FSM, although the lag times were extended. In both situations, these differences in responses between the three groupings of bacteria are likely due to variations in the abilities of individual strains of *E. coli* and/or species of coliform bacteria to grow in the feedlot soils under different conditions of manure or moisture content. Furthermore, these results call attention to the importance of using several strains of the same species or of a closely related group (e.g., generic *E. coli* or coliforms, respectively) and/or using multiple strains of a specific type (e.g., a mixed cocktail of different isolates of *E. coli* O157) in such studies.

Miller and Berry (2005) found that three different general environmental conditions occur in feedlot soils based on their water and manure contents and the microbial metabolic activities taking place in the soils (Table 1). By and large, the growth and/or survival of *E. coli* O157:H7 in the feedlot soils examined in the first study were reflective of these three general environments. At the lowest water contents, in which microbial activity was not detectable or very low, *E. coli* O157:H7 viability generally was rapidly lost. The feedlot soils of intermediate water contents typically were an aerobic environment; *E. coli* O157:H7 populations tended to persist at high levels in these soils, and if moisture was adequate, the pathogen could multiply in these soils. At higher water contents, anaerobic, fermentative conditions prevailed. There was an accumulation of lactic acid in these soils and in some cases, soil pH declined. As a rule, the growth of *E. coli* O157:H7 was inhibited in these soils and populations slowly declined.

As previously noted, changes in the manure contents of the feedlot soils shifted the range of soil water contents at which *E. coli* O157:H7 and the other target bacteria could or could not multiply (Fig. 1). Fundamentally, as the manure level increased, more water was needed to generate the same environmental conditions that were produced at a lower manure level, as was also observed by Miller and Berry (2005) with regard to numerous microbial processes occurring in the soil, including glucose consumption, fermentation, lactate and other acid production, and gas fluxes (Table 1). The observed effects suggest that increases in manure content may reduce the water activity and/or water potential of feedlot soils. It is generally acknowledged that these parameters are more accurate measures of the potential for microbial activity than is water content; water content indicates how much water is present, but water activity and water potential indicate how much water is available for microbial growth (Parr et al., 1981). Further work is planned to confirm if indeed the observed effects of manure levels are due to reduced soil water potential, and if so, to determine if these are parameters that can be influenced to control *E. coli* O157:H7 persistence in feedlot soils.

Effect of Fluctuating Moisture Levels of Feedlot Soils

In the first study, relatively constant soil moisture levels were maintained by replenishing water daily. Such constant conditions of hydration would be the exception rather than the rule in nature and in the open feedlot environment. Therefore, the second study was designed to ascertain the effects of repeated cycles of water addition and drying, and therefore the effects of the resultant cycles in soil environment (fermentative, aerobic, and/or dry), on the target bacterial populations. Because water was replenished weekly over the course of the 133-d experiment, there were 19 one-week-long drying cycles. Adding different masses of feedlot soils to the pans was useful for obtaining treatment units with different drying rates ($P > 0.05$). Mean values of H₂O loss per cycle for the high, intermediate, and low moisture flux pans were 0.87, 0.41, and 0.20 g H₂O g⁻¹ dry FSM, re-

spectively. Because samples were removed from the pans weekly, the final soil water contents tended to decline over the course of the experiment. However, these water contents for feedlot soils before adding back H₂O in the high, intermediate, and low moisture flux pans were generally less than 0.11 g H₂O g⁻¹, between 0.25 and 0.43 g H₂O g⁻¹, and between 0.43 and 0.67 g H₂O g⁻¹ of dry FSM, respectively.

An additional objective of this second study was to determine the effects of these moisture level cycles on *E. coli* O157 naturally occurring in feedlot soils. Therefore, fresh bovine feces collected from beef cattle that were shedding this pathogen were used to prepare the feedlot soil samples. The prevalence of fecal shedding of *E. coli* O157:H7 by cattle typically is higher during the summer and early fall (Hancock et al., 1997; Mechie et al., 1997), so the experiment was scheduled in September to improve our chances of obtaining adequate volumes of O157-positive feces. A total of 26 fecal samples were collected and analyzed on two different days, and seven of these samples were positive. Approximate concentrations of *E. coli* O157 in the fecal samples ranged from 10² to 10³ CFU g⁻¹ of fresh feces.

The initial concentrations of the naturally contaminating *E. coli* O157 in the feedlot soils immediately following their preparation were 2.4 to 2.5 log₁₀ MPN g⁻¹ of dry FSM (Fig. 2). These low levels of the pathogen declined in soils of all three moisture flux treatments examined. In all flux treatments, *E. coli* O157 populations were detectable at Day 14, but at Day 21, all were below detectable levels. On Day 35, two of three high moisture flux samples and one of three intermediate moisture flux samples were positive for *E. coli* O157 by enrichment. Thus, under conditions of moisture cycling and drying, these low initial populations of *E. coli* O157 were reduced rapidly in the feedlot soils, though their presence could be detected at Day 35.

As with the first study, *E. coli* made up the preponderance of the total coliform counts. During the first five to seven weeks of the study, both the generic *E. coli* and coliform populations either remained steady or increased slightly, despite the weekly cycles of water addition followed by drying. After these first few weeks, levels of both of these bacteria groups declined slowly throughout the remainder of the study, which was ended after 133 d. At this time, numbers of both generic *E. coli* and coliform bacteria still remained at enumerable levels. As seen with *E. coli* O157, there was no difference in the impact of the different moisture flux treatments on the populations of generic *E. coli* and coliforms ($P > 0.28$).

Depending on the particular study and the combination of feedlot soil water and/or manure content, all three groups of the target bacteria were observed to be capable of persistence in the feedlot soils. To our knowledge, feedlot soils have not been specifically examined; however, these observations are consistent with survival and persistence reported for *E. coli* and related bacteria in manure-amended soils. Depending on the date of manure application, Natvig et al. (2002) found that indigenous generic *E. coli* and *Salmonella enterica*

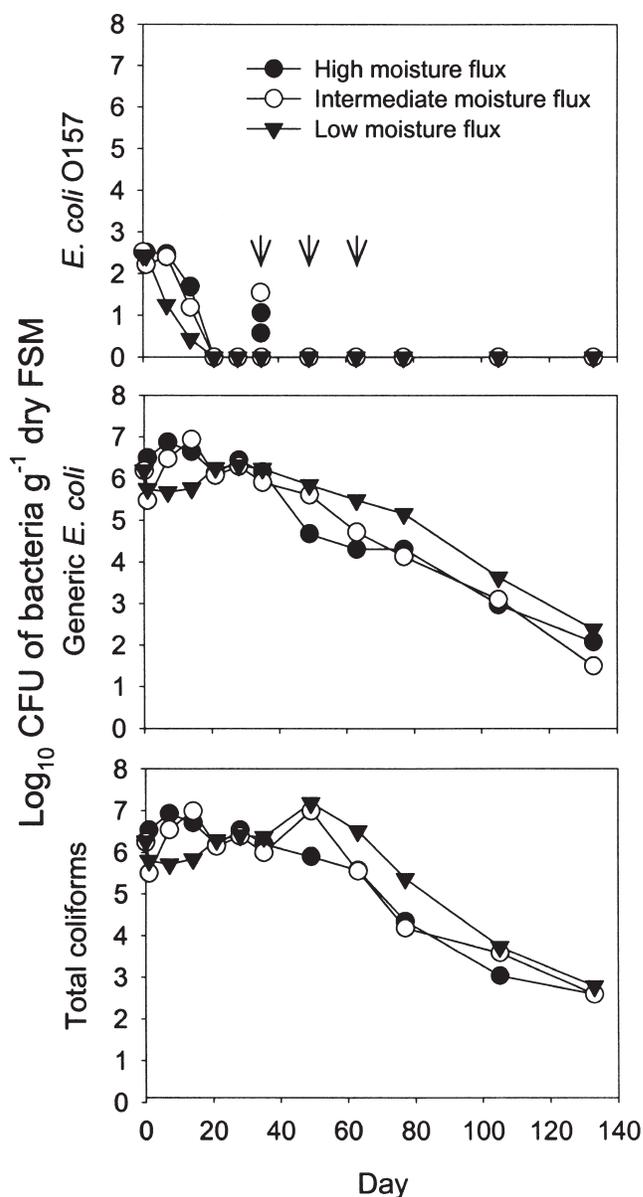


Fig. 2. Effect of fluctuating water contents on populations of naturally occurring *Escherichia coli* O157, generic *E. coli*, and total coliforms in feedlot soils containing 25% manure. The pans were filled with three different masses of soils to attain high, intermediate, and low moisture flux ranges. Appropriate volumes of deionized distilled H₂O were added weekly to return the soils to the initial water content of 0.78 g H₂O g⁻¹ of dry feedlot surface material (FSM). Where indicated by arrows, feedlot soil samples were enriched to determine the presence or absence of *E. coli* O157 on Days 35, 49, and 63. The three symbols at Day 35 indicate that two high moisture flux samples and one intermediate moisture flux sample were positive for *E. coli* O157 by enrichment. The standard errors of the least squares means were 0.21 and 0.20 for generic *E. coli* and total coliforms, respectively.

serovar Typhimurium (at initial levels of 4.5–5.0 log₁₀ CFU g⁻¹ of soil) could be detected and enumerated in manure-fertilized soil by direct plating at 17 weeks after the manure application. In a similar study, Lau and Ingham (2001) reported growth followed by persistence of indigenous *E. coli* in bovine manure incorporated into either loamy sand or silty clay loam soils (17 g ma-

nure per 400 g soil), although soil type did affect survival. Other studies have reported the growth of *E. coli* O157 in soils amended with bovine manure (Gagliardi and Karns, 2000; Jiang et al., 2002). Gagliardi and Karns (2002) reported that the presence of manure did not appear to affect the persistence of *E. coli* O157:H7 in soils when 3 g of manure was applied to 500-g soil microcosms. However, they also found that survival of *E. coli* O157:H7 in soils was improved by the presence of some of the cover crops tested and also by the presence of clay in the soils. Thus, the potential impact of different soil types should be a consideration for the design of similar experiments. The silt-loam soil used in the current work has a high clay content relative to its textural class (B.L. Woodbury, personal communication, 2004), which may have played a role in the high persistence of the pathogen that was observed in the first study. The ability of *E. coli* O157 to survive for extended periods in soils amended with manure has important implications for the microbial safety of food crops grown in the soils. Recent works have shown that vegetables can be contaminated with this organism during their cultivation in soils amended with manure or compost containing the pathogens, or by irrigation with contaminated water (Islam et al., 2004; Solomon et al., 2002). Furthermore, persistence of *E. coli* O157 in soils increases the probability of water supply contamination in cases of runoff from fields treated with manures.

The ability of this pathogen to survive for long periods and even grow in feedlot soils suggests that the feedlot pen floor may serve a source of transmission of *E. coli* O157:H7 to cattle. In their examination of *E. coli* O157:H7 prevalence in more than 3000 cattle from five Midwestern U.S. feedlots, Smith et al. (2001) found that higher percentages of cattle in muddy pens shed the pathogen than cattle from pens in a normal condition, and they reasoned that the muddy soil environment might facilitate the fecal-oral transmission of pathogens among cattle. A recent work by LeJeune et al. (2004) indicates that the production environment as a reservoir may play a larger role as a source for transmission of the pathogen than do incoming cattle. More work will be needed to determine the role for feedlot soil as a source of *E. coli* O157:H7 for cattle infection. However, taken together, this information suggests that the soils of the feedlot pen surface may be a potential target for preharvest control measures to reduce *E. coli* O157:H7 in cattle and the feedlot environment, as well as to reduce the risk of pathogen contamination of water supplies from feedlot runoff. Additional studies are planned to examine other variables, including temperature, that can affect the survival and activities of bacteria in feedlot soils. Future work will also identify specific treatments for the reduction of pathogens in feedlot soils.

CONCLUSIONS

Our work indicates that *E. coli* O157:H7 and other coliform bacteria not only can persist, but multiply in feedlot soils if appropriate conditions exist. Combinations of soil, manure, and moisture that resulted in aero-

bic environments either allowed growth or improved the survival of the pathogen. Similar results were seen for the indigenous generic *E. coli* and coliforms. Additional work will be necessary to determine if the variables of manure level or water content, or other variables, can be manipulated to inhibit *E. coli* O157:H7 growth and persistence in feedlot soils. Furthermore, this additional work should include the development of knowledge regarding how these and other variables might interact to control not only pathogens, but to control other negative environmental emissions associated with beef cattle feedlots such as odor, dust, ammonia, and greenhouse gases. For example, the combinations of soil, manure, and moisture that produced the low pH, fermentative soil conditions that limited *E. coli* O157:H7 growth are the same conditions that produce the most offensive odors, while the dry combinations that produced the low or no activity conditions that reduced viable *E. coli* O157:H7 numbers are the same that produced more dust (Miller and Berry, 2005). At 25 and 75% manure levels, increases in manure level of the soils changed the effect of moisture on *E. coli* O157 growth and survival by increasing the volume of water needed to produce the same effect on the pathogen's growth or survival. This suggests that higher manure levels may reduce soil water potential; further experiments are planned to examine this effect and determine if it can be manipulated to control *E. coli* O157:H7 in feedlot soils. Finally, the ability of high numbers of *E. coli* O157:H7 to persist in feedlot soils over a wide range of water and manure contents suggests that the pen surface may be an appropriate target for interventions to reduce this pathogen from cattle and the feedlot environment.

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