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PEER-REVIEWED ARTICLE

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Prevalence of *Escherichia coli* and *Salmonella* in Runoff of Two Cattle Feedlots in North Dakota

ABSTRACT

Livestock waste is used extensively as manure in crop farming. Improperly managed manure can contaminate foods such as raw produce and can serve as a major source of zoonotic foodborne pathogens such as *Escherichia coli* and *Salmonella.* The objective of this study was to detect and estimate the prevalence of *E. coli* (O157 and non-O157) serotypes and *Salmonella* species in runoff samples collected from two cattle feedlots (A and B) in North Dakota. Runoff samples were collected using automatic samplers (ISCO 6712) and processed by following standard culture and confirmatory methods for the isolation of *E. coli* and *Salmonella.* Of 136 samples collected from feedlots A and B, 106 (78%) tested positive for at least one of the *E. coli* serotypes (O26, O45, O103, O111, O113, O121, O145 and O157), and 54 (40%) tested positive for *Salmonella.* One to seven *E. coli* serotypes were detected in each of the positive runoff samples, and a total of 237 *E. coli* serotypes were detected in the 106 positive runoff samples. Of the 237 serotypes, the most frequent was O45 (22%), followed by O103 (19%), O157 (18%), O121 (15%), O26 (9%), O111 (5%), O113 (5%), and O145 (5%). The number of serotypes recovered from feedlot A (169/237) was higher ($P < 0.001$) than the number recovered from B (68/237). These data provide evidence of the presence of *E. coli* serotypes and *Salmonella* in feedlot runoff, underscoring the need for pretreatment of feedlot runoff before it is disposed into the environment or used as fertilizer.

INTRODUCTION

Contamination of water and food with zoonotic pathogens such as *Escherichia coli* and *Salmonella* species can have severe public health consequences including, but not limited to, large disease outbreaks. According to the United States (U.S.) Centers for Disease Control and Prevention, *E. coli* O157 and non-O157 STEC cause an estimated 175,905 domestically acquired foodborne illnesses annually, and over 60% of these infections are caused by non-O157 STEC *(26)*.

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Nontyphoidal *Salmonella* spp. cause 11% of the estimated 9.4 million episodes of foodborne illness in the U.S. annually and are the leading cause of hospitalizations (35%) and deaths (28%) *(26)*.

Although the gastrointestinal tract of ruminants is a recognized reservoir for *E. coli* and *Salmonella* spp. *(18, 20)*, these pathogens can persist for up to 92 days and 210 days, respectively, in animal manure and contaminated soil *(21, 25)*, from which they can be transferred to watercourses through runoff during heavy rainfall or thawing of snow *(11, 17)*. Freshwater ecosystems are often at the interface between agriculture activities and environmental concern, and the contamination of drinking water, recreational surface waters and shellfish harvesting areas can play an important role in human exposure to *E. coli* O157, and non-O157 serotypes, to *Salmonella (22)*.

Feedlot manure can serve as a primary source of contamination of water sources and fresh produce with *E. coli* and *Salmonella*. Both *E. coli* O157 *(9)* and non-O157 *E. coli (24)* have been isolated from a range of habitats within the farm environment, including soil, manure, slurry, runoff, drinking troughs, irrigation water, vegetation and farm equipment *(1, 35)*, with persistence ranging from weeks to years. During and following periods of heavy rainfall, pathogens from all these habitats are readily transferred to watercourses through runoff *(34)*. *Salmonella* has been reported in U.S. cattle feedlots *(31)*, and fresh produce following soil contamination with irrigation water and compost manure *(28)*.

Although *E. coli* O157 and non-O157 serotypes as well as *Salmonella* have been detected in cattle feedlots in North Dakota *(18, 19)* and elsewhere *(24, 31, 32)*, no studies have investigated the occurrence of these pathogens in feedlot runoff in North Dakota. Knowledge of the presence of these pathogens and the potential public health risk posed by untreated feedlot manure and runoff is essential for justifying implementation of appropriate feedlot manure disposal and management strategies that promote environmental and public health safety.

The objective of this study was to detect and estimate the prevalence of *E. coli* O157, other *E. coli* serotypes (O26, O45, O103, O111, O113, O121, and O145), and *Salmonella* in runoff of two cattle feedlots located in two counties in North Dakota.

MATERIALS AND METHODS

Sampling sites

Runoff samples were collected from two cattle feedlots in two counties in North Dakota (hereafter referred to as feedlot A and feedlot B) during April and June 2012. Feedlot A became operational in 2011 on an area of 115 m by 50 m and had a capacity of 192 beef cattle housed in six pens, with approximately 32 steers per pen. The overall slope of the feedlot area was 5%. A 65 m by 115 m vegetative filter strip (VFS) was constructed immediately next to the feedlot pen surface *(Fig. 1, feedlot A) (23)*. The VFS was seeded with common cattails *Typha latifolia* grass and graded to a uniform slope of 2% on clay soil. A settling basin constructed at the end of the VFS collected runoff exiting the VFS *(Fig. 1, feedlot A)*.

Feedlot B became operational in 2006 and had a capacity of 999 steers housed in five pens, with approximately 200 steers per pen. In this feedlot, a two-stage VFS system was constructed *(Fig. 1, feedlot B)*. At the initial stage, runoff from the feedlot ran through an area approximately 165 m long by 3 m wide, seeded with smooth brome grass (*Bromus inermis*) and western wheatgrass, which extended to a solids separator. At the second stage, runoff from the solids separator was channeled through a pipe and spread onto a vegetative filter strip 330 m long by 30 m wide. The VFS was established on fine sandy loam soil, with an overall slope of 2%. At the end, runoff exiting from the VFS was contained in a retaining pond and used for irrigating croplands.

Climate of sampling sites

Feedlot A was located in a county with a continental climate, characterized by cold winters (average temperature -14°C) and warm summers (average temperature 21.4°C). The county receives on average 538.23 mm of rainfall in a year *(12)*. Feedlot B was located in a county without a weather station. Data on weather conditions were made by inference from a neighboring county 28.324 km from the county where feedlot B was located. This neighboring county has a sub-humid climate characterized by long, cold winters, with about six days per month of temperatures above freezing. The average annual rainfall in that county was 508 mm, and average temperatures varied from -14.5°C in January to 21.5°C in July.

Runoff samples were collected during the months of April and June 2012 and were received at the laboratory on April 13, June 11, June 13, and June 20, 2012. The total precipitation for the month of April and June in the county where feedlot A was located was 28.96 mm (1.14 in) and 56.9 mm (2.24 in), respectively, while the total precipitation for the month of April and June in the county closest to feedlot B was 90.68 mm (3.57 in) and 41.66 mm (1.64 in), respectively. All precipitation data were obtained from the North Dakota Agricultural Weather Network (http://ndawn.ndsu.nodak.edu/).

Runoff sample collection

The two experimental sites were equipped with five automatic samplers (ISCO 6712, Teledyne ISCO Inc., Lincoln, NE), to collect runoff samples sequentially at one-hour intervals upon activation of the sampler through an actuator (model: 1640, sampler actuator, Teledyne ISCO Inc., Lincoln, NE). Samplers were powered by heavy-duty marine batteries, which were charged by a solar panel, as described previously *(23)*.

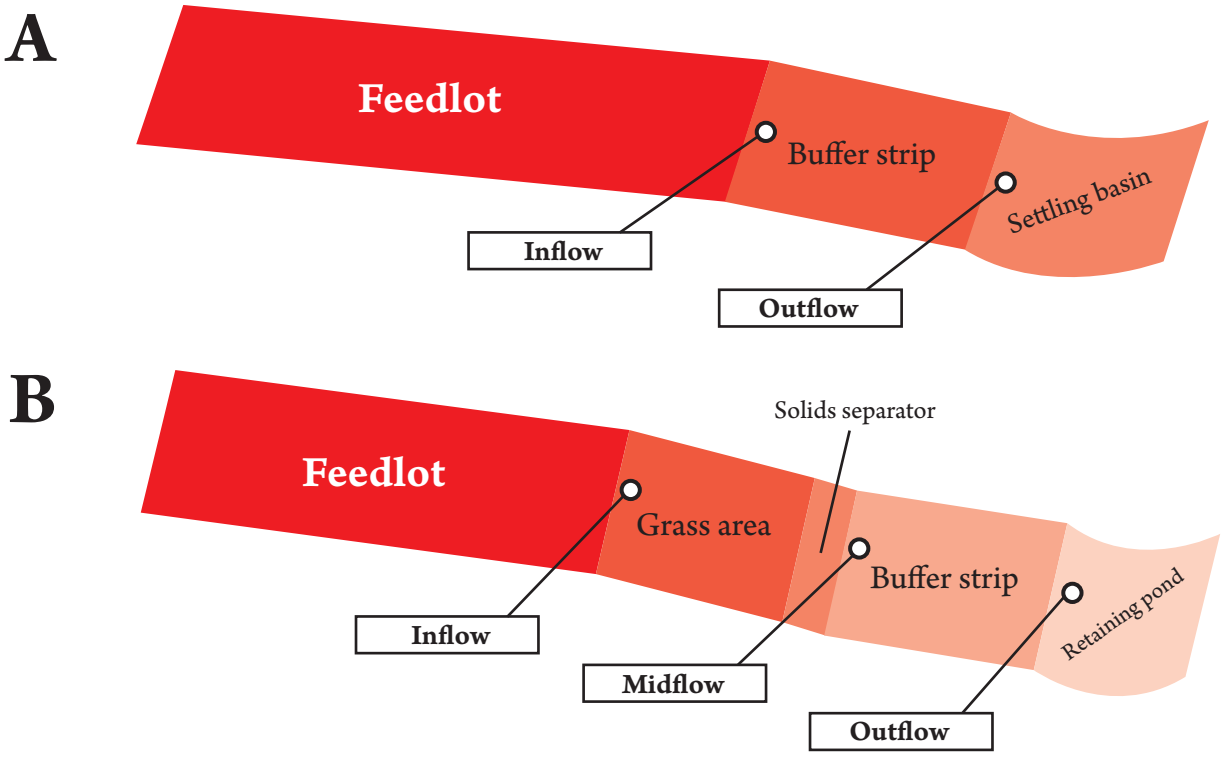

Figure 1. Layout of the two feedlots, vegetative filter strip (buffer strip), and water spreading area/ settling basin. Feedlot A has a settling basin and Feedlot B has a solid separator and retaining pond. Small circles represent sampling locations (inflow, midflow, and outflow). Figures are not to scale (23)

In feedlot A, one sampler was installed to collect runoff at the entry of the VFS (hereafter referred to as inflow), and another sampler was installed at the exit of the VFS to collect runoff leaving the VFS (hereafter referred to as outflow) *(Fig. 1)*. In feedlot B, the first sampler was installed at the grassed area immediately after the pens (hereafter referred to as inflow), another sampler was installed to collect runoff samples after the solids separator (midflow), and a third sampler collected runoff samples exiting the VFS (outflow) *(Fig. 1)*.

All samples were collected automatically by use of ISCO samplers. Each sampler contained 24 Teflon bottles of 1L capacity. In each sampling bottle, about 750 mL runoff sample was collected. The number of samples collected on each date in each feedlot varied depending on the runoff amount accumulated in the samplers. Information on number of runoff samples collected from each sampler on different dates is provided in *Table 1*. On certain dates *(see Table 1)*, no runoff occurred, and therefore no samples were collected. After collection, samples were placed in coolers and transported to the laboratory, where each sample was subdivided for analyses and kept refrigerated until analyses were performed. Laboratory isolation of *E. coli* and *Salmonella* was performed within 6 hours of sample collection.

Laboratory isolation of *E. coli* O157

Isolation of *E. coli* O157 was performed as previously described *(18, 29)*. Briefly, for each runoff sample, 90 ml of gram negative (GN) enrichment broth was added to a Whirl Pak bag (Nasco VWR, USA), into which 10 ml of runoff was added. To prevent growth of unwanted bacteria, the GN broth was supplemented with vancomycin (8.0 mg/l), cefixime-tellurite (0.05 mg/2.5mg/l) and cefsulodin (10.0 mg/L). Each bag of runoff and broth mixture was gently mixed and then incubated at 37°C for 18 to 24 hours. Following enrichment and incubation, immunomagnetic beads functionalized with antibodies against the *E. coli* O antigen were used to retrieve *E. coli* O157 from the enriched runoff broth, according to the manufacturer's protocol (Dynal Biotech ASA, Norway). One ml of runoff broth was transferred into pre-labeled microcentrifuge tubes containing 20 µl of the anti-*E. coli* O157 immunomagnetic beads and then mixed thoroughly by rotating, using a Dynal rotator mixer (Dynal Biotech ASA, Norway), for 30 minutes. The tubes were then inverted continuously for 3 minutes, using a magnetic particle concentrator to capture the beads, which were washed 3 times with phosphate-buffered saline plus tween 20 (PBS). The final product was reconstituted in 100

Table 1. Distribution and prevalence of *E. coli* positive feedlot runoff samples by feedlot and sampling site during 2012

Location	Sampling site	Date and number of samples collected				Samples tested	*E. coli* positive samples	
		04/13	06/11	06/13	06/20		n	%
Feedlot A	Inflow	18	18	10	27	73	64	88
	Outflow	-*	-	9	9	18	5	28
	Sub-total					91	69	76
Feedlot B	Inflow	-	-	-	27	27	21	78
	Outflow	-	-	-	12	12	10	83
	Midflow	-	-	-	6	6	6	100
	Sub-total					45	37	82
Total		18	18	19	81	136	106	78

*Dash indicates no runoff samples collected on those dates.

µl of PBS containing tween 20 and vortexed. An aliquot of 20 µl (Dynal immunomagnetic beads and captured bacterial cells) was spread onto sorbitol MacConkey agar (Difco, Becton Dickinson MD, USA) plates supplemented with cefixime (0.05 mg/ml) and potassium tellurite (2.5 mg/ml; CT–SMAC, Dynal Biotech ASA, Norway) and incubated at 37°C for 18–24 hours. Suspected colorless isolates (characteristic of *E. coli* O157) that did not ferment sorbitol in CT-SMAC agar were sub-cultured on MacConkey and Fluorocult agars (Difco, Becton Dickinson & Company, MD, USA). The latex agglutination test using the Remel Kit (Remel, Lenexa, KS, USA) was used to detect the presence of O antigen from *E. coli* O157 isolates that did not ferment sorbitol but that fermented lactose within 24 hours and tested negative for 4-methylumbelliferyl-β-D-glucuronide (MUG, no fluorescence was produced by the colonies).

Laboratory isolation of other *E. coli* serotypes

For the isolation of other *E. coli* serotypes, runoff samples were prepared using a protocol for the isolation of *E. coli* O157, with slight modifications. A sterile 15 ml centrifuge tube was loaded with 1 ml of runoff sample and 9 ml of buffered peptone water (BPW) (Difco, Becton Dickinson & Company, MD, USA) and incubated overnight at 37°C. Immunomagnetic beads (Dynal Biotech ASA, Norway) functionalized with antibodies against O antigens for different *E. coli* strains (O26, O45, O103, O111, O113, O121, and O145) were used to retrieve the respective strains from the enriched runoff samples and plated on Violet Red Bile (VRB) MUG agar. The runoff and beads were mixed thoroughly by rotating, using a Dynal rotator mixer (Dynal Biotech ASA, Norway). Suspected *E. coli* colonies were sub-cultured onto Eosin Methylene Blue (EMB) agar plates and incubated overnight at 37°C. Colonies with typical *E. coli* characteristics (dark purple flat colonies or colonies with a blue-green sheen) were subjected to biochemical testing by stabbing in 7 ml Triple Sugar Iron (TSI) agar slants (Becton Dickinson, Franklin Lakes, NJ, USA), and the results read after 24 hours following incubation overnight at 37°C. Positive isolates (by production of acid and gas) were further confirmed by use of the indole test. TSI positive isolates were inoculated into test tubes containing 3 ml of tryptic soy broth and incubated at 37°C for 24 hours. Thereafter, 3 to 5 drops of Kovac's reagent were added to the positive isolate to test for indole production. Indole positive samples (a red-violet color at the surface of the broth) were stored at –80°C until further analyses.

Genomic DNA extraction

Bacterial DNA used for the multiplex polymerase chain reaction was prepared from the *E. coli* isolates using established protocols *(13, 33)*, following lysis with single cell lysing buffer (SCLB). A single isolated freshly cultured bacteria colony from a tryptic soy agar plate was suspended in 40 µl of SCLB in a 0.2 ml microcentrifuge tube. The SCLB master mix consisted of 10 µl of 20 mg/ml proteinase K (Amresco) and 990 µl of 10 mM Tris HCL and 1mM EDTA (TE) buffer solution (Amresco). The entire mixture was placed in a thermocycler (Eppendorf) and run under the following conditions: 80°C for 10 minutes, cooled at 55°C for 10 min, and held at 4°C. Following lysis, 80 µl of sterile double distilled water was added to the suspension, which was then centrifuged for 30 sec at 4500 × g. The samples were stored at -20°C until further analyses.

Multiplex polymerase chain reaction (mPCR)

The primer sets specific for the O gene of each serotype and PCR conditions used for the amplification of *E. coli* O157:H7 and non-O157 target genes are shown in *Table 2 (7)*. The *E. coli* isolates were tested in an eight primer multiplex PCR assay for detection and amplification of the *E. coli* serogroups for *E. coli* O26, O45, O103, O111, O113, O121, O145 and O157. Each reaction consisted of a 25.5 µl reaction mixture containing 2 µl of template DNA, 12.5 µl of multiplex buffer, 0.5 µl of primer (composite) set and 10.5 µl of double distilled water. Each PCR assay had a positive and a negative control. Amplification of target genes was performed following the Qiagen's multiplex kit instructions (Qiagen, Valencia, CA, USA) and included initial denaturation at 95°C for 15 min, followed by 30 cycles of denaturation at 94°C for 30 seconds; primer annealing at 57.5°C for 60 sec, and a final extension for 10 min at 72°C and then cooling to 4°C. The DNA amplicon was electrophoresed in a 1.5% agarose gel for 45 minutes at 170V, stained with ethidium bromide for 15 minutes, destained with water for 15 minutes, and observed under ultraviolet light using an Ultra Violet AutoChemi System (Tiles Scientific, USA). Gel images were captured electronically and amplicon size determined using DNA molecular size standards (PCR marker, Promega, Madison, WI, USA).

Laboratory isolation of *Salmonella*

Runoff samples were cultured in the laboratory by use of a protocol for the detection of *Salmonella* as previously described *(19)*. A sterile 15 ml centrifuge tube was loaded with 1 ml of runoff sample and 9 ml of buffered peptone water (BPW) (Difco, Becton Dickinson & Company, MD, USA) and incubated overnight at 37°C. This was followed by immunomagnetic beads separation as already described, but with immunobeads (Dynabeads anti-*Salmonella*, Dynal Biotech, Inc., Lake Success, NY, USA) specific for *Salmonella* species. After the final wash, the beads were transferred to 10 ml of Rappaport Vassiliadis R10 (RV) broth for resuscitation of potential *Salmonella* bacteria (Becton Dickinson, Sparks, MD, USA) and incubated (with constant gentle shaking) at 42°C for 24 h. Following incubation, the RV cultures were streaked on modified brilliant green agar (mBGA, Becton Dickinson, Sparks, MD, USA) and mannitol lysine crystal violet brilliant green agar (MLCB, Oxoid LTD, Basingstoke, UK) and incubated for 18 to 24 h. Characteristic *Salmonella* colonies were stabbed in 7 mL TSI agar slants (Becton Dickinson, Sparks, MD, USA), and the results were read after 24 hours of incubation. Tubes with characteristic black pigments due to hydrogen sulfide gas production by *Salmonella* spp. were considered positive. Positive *Salmonella* isolates were

Table 2. Specific primers used for multiplex PCR to detect *E. coli* serotypes

E. coli serotype		Primer sequence	Reference
O26	F	CAATGGGCGGAAATTTTAGA	7
	R	ATAATTTTCTCTGCCGTCGC	
O45	F	TGCAGTAACCTGCACGGGCG	7
	R	AGCAGGCACAACAGCCACTACT	
O103	F	TTGGAGCGTTAACTGGACCT	7
	R	GCTCCCGAGCACGTATAAAG	
O111	F	TGTTTCTTCGATGTTGCGAG	7
	R	GCAAGGGACATAAGAAGCCA	
O113	F	TGCCATAATTCAGAGGGTGAC	7
	R	AACAAAGCTAATTGTGGCCG	
O121	F	TCCAACAATTGGTCGTGAAA	7
	R	AGAAAGTGTGAAATGCCCGT	
O145	F	TTCATTGTTTTGCTTGCTCG	7
	R	GGCAAGCTTTGGAAATGAAA	
O157	F	TCGAGGTACCTGAATCTTTCCTTCTGT	7
	R	ACCAGTCTTGGTGCTCTGACA	

sub-cultured in tryptic soy broth supplemented with 5% glycerol and stored at −80°C until further analyses.

Data analysis

Following testing for *E. coli* and *Salmonella*, binary data (positive or negative) for each runoff sample were recorded in an Excel spread sheet and analyzed using XLSTAT software (XLSTAT 2014, Addinsoft SARL, Paris, France). The number of runoff samples that tested positive for *E. coli* serotypes (O26, O45, O103, O111, O113, O121, O145 and O157) and *Salmonella* were determined and proportions of positive samples computed for each feedlot.

The Chi-square test and Fisher's exact test were used to compare the number of runoff samples that tested positive for *E. coli* and *Salmonella* between (i) the two feedlots (feedlot A versus B), and (ii) sampling locations within each feedlot (feedlot A: inflow versus outflow; and feedlot B: inflow versus outflow, inflow versus midflow, and midflow versus outflow).

For runoff samples that tested positive for *E. coli* and for which more than one *E. coli* serotype (O26, O45, O103, O111, O113, O121, O145 and O157) was recovered from a single positive sample, the number of *E. coli* serotype isolates recovered from each sample was compared for feedlots A and B. In all analyses, statistical significance was set at $P < 0.05$.

RESULTS

Prevalence of *E. coli* in feedlot runoff

Of 136 samples tested, 106 (78%) samples from both feedlots tested positive for at least one *E. coli* serotype; feedlot A had a prevalence of 76% (69/91) while feedlot B had a prevalence of 82% (37/45), and comparison of the two prevalence estimates revealed no statistically significant difference ($P = 0.69$) *(Table 1)*.

In feedlot A, the proportion of *E. coli* positive samples collected from inflow sites (64/73) was significantly different ($P = < 0.001$) from the proportion for outflow

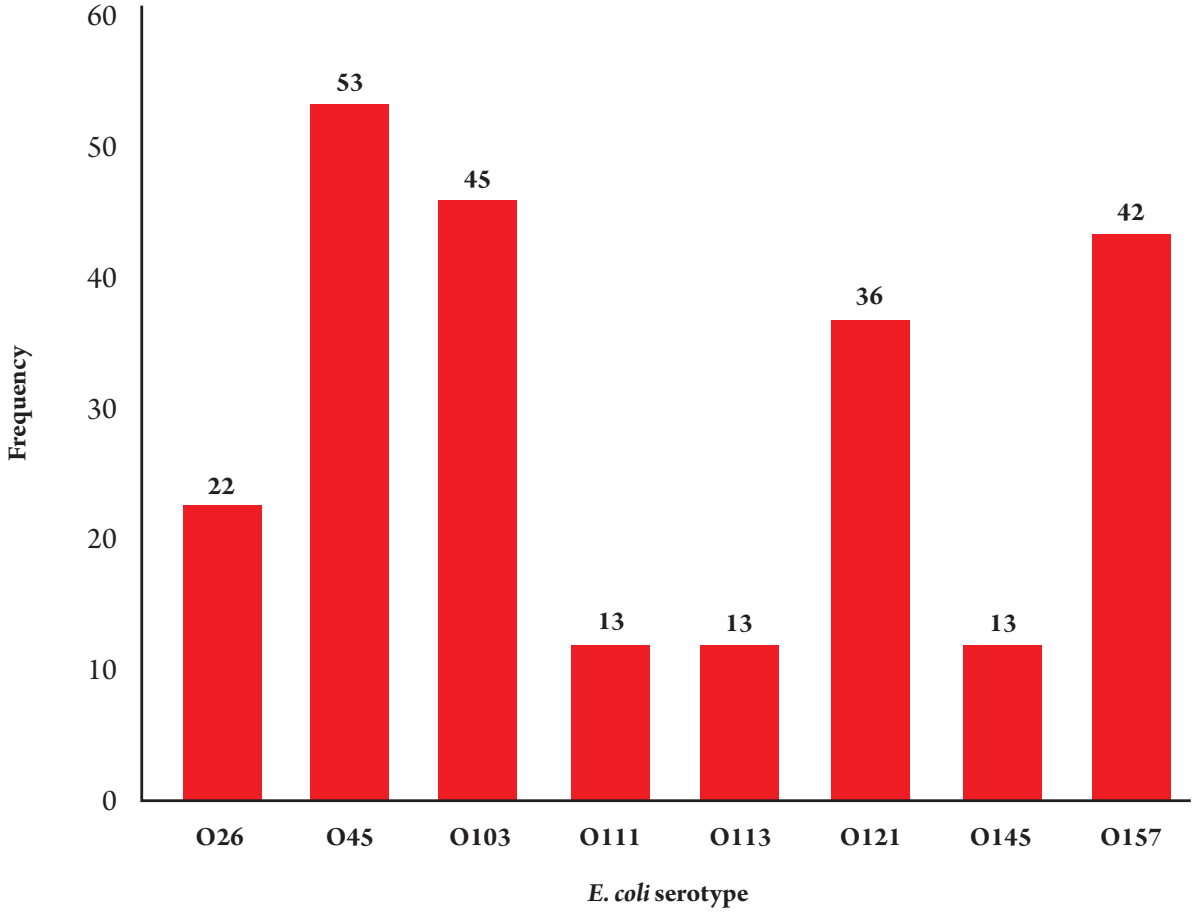

Figure 2. Frequency distribution of 237 E. coli *serotype isolates recovered from 106 positive runoff samples collected from feedlots A and B*

sites (5/18) *(Table 1)*. In feedlot B, the proportion of *E. coli* positive samples collected from inflow sites (21/27) was not significantly different from the proportion for outflow sites (10/12) ($P = 0.92$), or midflow sites (6/6) ($P = 0.62$) *(Table 1)*. The proportion of positive samples from midflow sites (6/6) was not significantly different from the proportion for outflow sites (10/12) ($P = 0.52$).

One to seven *E. coli* serotypes were detected by multiplex polymerase chain reaction in each of the 106 positive runoff samples: one serotype from 42 (40%) samples, two serotypes from 27 (25%), three serotypes from 20 (19%), four serotypes from 9 (8%), five serotypes from 4 (4%), six serotypes from 3 (3%), and seven serotypes from 1 (1%) sample.

A total of 237 *E. coli* serotypes were detected in the 106 *E. coli* positive samples. Of the 237 serotypes, the most frequent serotype was O45 (22%) followed by O103 (19%), O157 (18%), O121 (15%), O26 (9%), O111 (5%), O113 (5%), and O145 (5%) *(Fig. 2)*.

The number of *E. coli* serotypes detected was higher ($P < 0.001$) for feedlot A (169/237) than for feedlot B (68/237). In feedlot A, the most frequent of the 169 serotypes was O103 (19%), followed by O45 (18%), O121 (18%), O157 (16%), O26 (9%), O111 (8%), O113 (7%), and O145 (6%) *(Fig. 3)*. In feedlot B, the most frequent of the 68 serotypes was O45 (34%), followed by O157 (22%), O103 (19%), O26 (9%), O121 (9%), O145 (4%), O113 (3%), and O111 (0).

Prevalence of *Salmonella* in feedlot runoff

Of the 136 samples tested, 54 (40%) tested positive for *Salmonella*, and prevalence was higher ($P < 0.001$) for feedlot A (47/91; 52%) than for feedlot B (7/45; 16%) *(Table 3)*.

In feedlot A, the proportion of *Salmonella* positive samples collected from inflow sites (38/73) was not significantly different ($P = 0.88$) from the proportion for outflow sites (9/18) *(Table 3)*. In feedlot B, the proportion of *E. coli* positive samples collected from inflow sites (3/27) was not

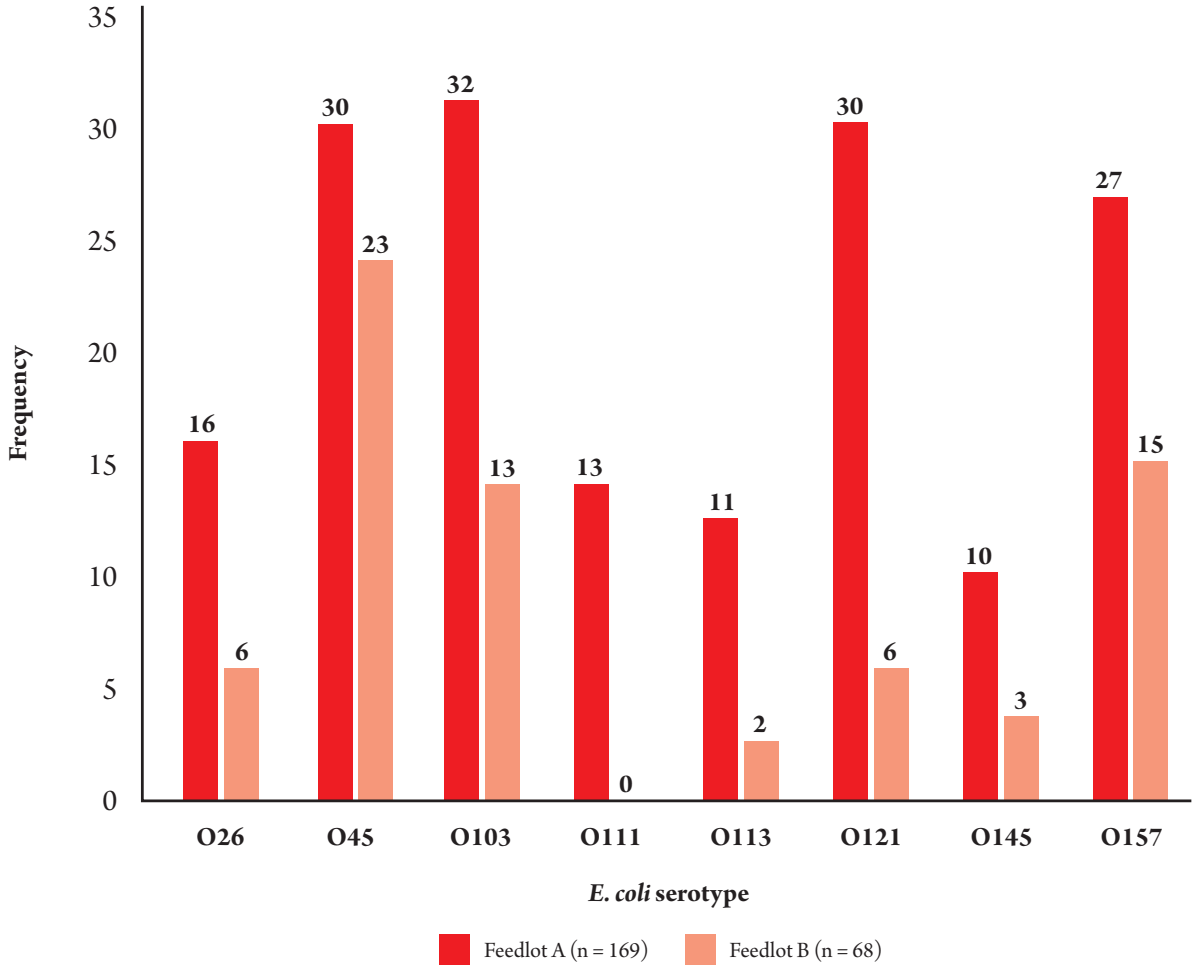

Figure 3. Frequency distribution of E. coli *serotype isolates recovered from positive runoff samples collected from feedlots A and B*

Table 3. Distribution and prevalence of *Salmonella* positive feedlot runoff samples by feedlot and sampling site

Location	Sampling site	Date and number of samples collected				Samples tested	*Salmonella* positive samples	
		04/13	06/11	06/13	06/20		n	%
Feedlot A	Inflow	18	18	10	27	73	38	52
	Outflow	-*	-	9	9	18	9	50
	Sub-total					91	47	52
Feedlot B	Inflow	-	-	-	27	27	3	11
	Outflow	-	-	-	12	12	2	17
	Midflow	-	-	-	6	6	2	33
	Sub-total					45	7	16
Total		18	18	19	81	136	54	40

*Dash indicates no runoff samples collected on those dates.

significantly different from the proportion for outflow sites (2/12) ($P = 0.87$) or for midflow sites (2/6) ($P = 0.34$) *(Table 3)*. The proportion of positive samples from midflow sites (2/6) was not significantly different from that from outflow sites (2/12) ($P = 0.56$).

DISCUSSION

The objective of this study was to detect and estimate the prevalence of *E. coli* O157, other *E. coli* serotypes (O26, O45, O103, O111, O113, O121, and O145), and *Salmonella* in runoff of two cattle feedlots. Findings revealed an estimated prevalence of *E. coli* of 78% in runoff samples collected from both feedlots. One to seven *E. coli* serotypes were isolated from each positive runoff sample, the most frequent serotype detected in both feedlots being O45 (22%), followed by O103 (19%), O157 (18%), O121 (15%), O26 (9%), O111 (5%), O113 (5%), and O145 (5%). The detection of *E. coli* O157 and other *E. coli* serotypes O26, O45, O103, O111, O121, and O145 in runoff indicates a potential risk to food safety and environmental health. In the absence of proper cattle manure and runoff management, *E. coli*-containing runoff from feedlots may contaminate the surrounding environment, particularly following heavy rainfall or thawing of snow. Of particular concern are feedlots that are close to pastures, water sources for agricultural or drinking or recreational use, and crop/farmland used for cultivation of leafy green vegetables and other produce. Runoff may adulterate fresh produce, or contaminate drinking and recreational water sources, resulting in infections in humans who consume the food or utilize the water sources. Studies conducted elsewhere detected *E. coli* O157 and non-O157 in farm environments, including water, sediment, irrigation water, soil adjacent to produce, and produce *(1, 4, 5, 35)*.

A higher proportion of *E. coli* serotypes were detected in feedlot A (71%) than in B (29%). The reason for the difference in number of serotypes detected in the two feedlots is unknown. However, the number of runoff samples tested from feedlot A (n = 91) and feedlot B (n = 45) may have affected the number of serotypes detected in the two feedlots. The number of *E. coli* positive samples and respective percentages in inflow and outflow for feedlot A and B were different. In feedlot A, the percentage was higher in inflow (88%) than in outflow (28%), while in feedlot B, the opposite was true; the percentage in outflow (83%) was higher than in inflow (73%), and midflow was 100%. The reason for this is unknown; differences in buffer design, soil types, and runoff collection for the two feedlots may explain some of this variation.

Although the present study targeted the *E. coli* serotypes O26, O45, O103, O111, O113, O121, O145 and O157, the presence of all these serotypes in runoff samples suggests these bacteria may be persistent in the feedlot environment. The diversity in serotypes among the non-O157 recovered from runoff samples in this study was also notable, and may suggest potential variability in virulence. Additional studies are required to investigate and confirm the virulence of the other *E. coli* serotypes recovered from feedlot runoff. Although virulence of the other *E. coli* serotypes was not determined in the present study, detection of those serotypes still has important implications for food safety, such as in situations where risk of cattle infection or re-infection exists, as occurs following runoff contamination of pastures. In wake of the ruling by the US Department of Agriculture, Food

Safety and Inspection Service *(30)* to regulate the presence of non-O157 belonging to serotypes O26, O45, O103, O111, O121, and O145 (referred to as the Big Six) in non-intact beef products (77 FR 31975) *(8)*, information on prevalence of these serotypes is important in developing on-farm prevention and control strategies.

The prevalence of *Salmonella* in feedlot runoff collected from both feedlots was 40%, and prevalence in feedlot A (52%) was higher than in feedlot B (16%). Within each feedlot, the proportion of *Salmonella*-positive samples collected from the inflow site was higher than from the outflow site in feedlot A, but not in B. Similar to the findings for *E. coli* O157 and non-O157, the detection of *Salmonella* in feedlot runoff may indicate a potential risk to public health safety.

In general, the detection of *E. coli* O157 and non-O157 and of *Salmonella* in runoff is likely linked to cattle housed in the feedlots. Cattle are known to harbor *E. coli* O157 and non-O157 *(14, 16, 18)* and *Salmonella (2, 6, 10, 14)*. In addition, *E. coli* O157:H7 *(27, 28)*, non-O157 *(3)*, and *Salmonella (15)* can survive for extended periods of time in manure and slurry. The presence of all of these organisms in both inflow and outflow samples, and in both feedlots, underscores the need to properly manage and control runoff from feedlots, barnyards and other livestock facilities. Proper manure and runoff management can minimize the risk of spread to, and contamination of, surrounding environments such as pastures, watersheds, irrigation water, and farmland used for produce, as well as rivers, streams and lakes. Reducing the pathogen load in manure and feedlot runoff is crucial to minimize the risk of environmental contamination and thus to protect public health.

The present study had a number of limitations. First, it was limited to two feedlot facilities in North Dakota that were selected on the basis of willingness to participate in the study; therefore, any extrapolations of findings should be done cautiously. Second, prevalence estimates for *E. coli* O157, non-O157, and *Salmonella* in cattle housed in the two feedlots were not known, because this was beyond the scope of the study. Further studies that link recovered isolates to feedlot cattle and that assess how long the pathogens thrived in the feedlot environment are therefore warranted. Third, virulence traits of the recovered *E. coli* O157 and non-O157 isolates were not known, because no additional testing for virulence genes was performed, and antimicrobial resistance and serotype data for the *Salmonella* isolates were not determined.

Last, it is noteworthy that feedlot buffer zones/grass areas in the present study were designed with the goal of reducing runoff of solids and nutrients. Typically, buffer zone design depends on the soil and hydrological conditions, as well as on feedlot sizes. Typical slope range varies from 3 to 5%, and in the present study, slopes were between 2 and 5%. The buffer zone is seeded with locally available grass species, and in the present study, cattail grass was the dominant species. The buffer zones were not designed to stop runoff of pathogens. Therefore, it is important that feedlot runoff be treated before it is discharged into the environment or used as a nutrient source, a recommendation that was made to management of the two feedlots involved in the present study.

In conclusion, this study reported the presence of *E. coli* O157, other *E. coli* serotypes (O26, O45, O103, O111, O113, O121, and O145), and *Salmonella* in runoff from cattle feedlots. The findings suggest that these pathogens may be present in both cattle manure and the feedlot environment, and may predispose the surrounding local environment to livestock fecal pathogen contamination. The findings indicate a potential risk to public health and safety and underscore the importance of proper manure management and pretreatment of feedlot runoff before disposal into the environment or use as organic manure.

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