Article

Occurrence of foodborne bacteria in Alberta feedlots

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Abstract — The occurrence of generic *Escherichia coli, E. coli* O157, *Salmonella*, and *Campylobacter* in cattle manure, beef carcasses, catch basin water, and soils receiving manure application was determined in 21 Alberta feedlots. In cattle manure, generic *E. coli* (98%, 2069/2100) and *Campylobacter* (76%, 1590/2100) were frequently detected; *E. coli* O157 (7%, 143/2100) and *Salmonella* (1%, 20/2100) were less frequently detected. Samples from beef carcasses in the cooler following Hazard Analysis Critical Control Point interventions yielded only 1 isolate each of generic *E. coli* and *Campylobacter* (1/1653) and no *Salmonella* (0/1653). Catch basin water specimens were positive for generic *E. coli* in both the spring (62%, 13/21) and the fall (52%, 11/21). Other bacteria were detected only in the spring water specimens, including *E. coli* O157 (29%, 6/21), *Salmonella* (5%, 1/21), and *Campylobacter* (52%, 11/21). Generic *E. coli* was frequently isolated from soil specimens (30%, 27/88), but *E. coli* O157 was not found in soil samples obtained in the spring and was only occasionally detected in the fall samples (9%, 3/32). *Salmonella* were occasionally found in the soil specimens collected in the spring (3%, 2/56), but not in the fall season (0/32). *Campylobacter jejuni* was frequent in cattle manure (66%, 1070/1623), but rare in carcass and environmental samples. *E. coli* O157 and *Salmonella* were rarely detected in cattle or the environment. Generic *E. coli* and *Salmonella* were rarely detected on carcasses.

Résumé — Présence de bactéries d'origine alimentaire dans des parcs d'engraissement de l'Alberta. La présence de *E. coli* générique, de *E. coli* O157, de *Salmonella* et de *Campylobacter* dans le fumier de bovins, les carcasses de bœuf, les bassins de réception des eaux et les sols enrichis de fumier a été déterminée dans 21 parcs d'engraissement de l'Alberta. Dans le fumier de bovins, *E. coli* générique (98 %, 2069/2100) et *Campylobacter* (76 %, 1590/2100) ont été fréquemment détectés; *E. coli* O157 (7 %, 143/2100) et *Salmonella* (1 %, 20/2100) ont été moins fréquemment détectés. Les échantillons prélevés sur les carcasses ont fourni un isolat de *E. coli* générique et un de *Campylobacter* (1/1653), mais aucun de *Salmonella* (0/1653). Les spécimens provenant des bassins de réception des eaux étaient positifs pour *E. coli* générique au printemps (62 %, 13/21) et à l'automne (52 %, 11/21). Les autres bactéries ont été détectées uniquement dans les spécimens provenant des eaux de printemps, dont *E. coli* O157 (29 %, 6/21), *Salmonella* (5 %, 1/21) et *Campylobacter* (52 %, 11/21). Le *E. coli* générique était fréquemment isolé à partir d'échantillons de sol (30 %, 27/88, alors que *E. coli* O157 n'était pas retrouvé dans les échantillons de sol du printemps mais uniquement à l'occasion, dans le sol d'automne (9 %, 3/32). *Salmonella* a été occasionnellement retrouvé dans les échantillons de sol recueillis au printemps (3 %, 2/56) mais pas à l'automne (0/32). *Campylobacter jejuni* était fréquent dans le fumier de bovins (66 %, 1070/1623) mais rare dans les carcasses et les échantillons environnementaux. *E. coli* O157 et *Salmonella* étaient rarement détectés chez les bovins ou dans l'environnement. *E. coli* générique et *Salmonella* étaient rarement détectés sur les carcasses.

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Introduction

Food and environmental safety is a high priority for feedlot producers. There has been much speculation on the risk to humans of zoonotic pathogens from feedlot cattle, their carcasses, and the environment. Studies have been conducted to determine the occurrence of *Escherichia coli* O157 in live cattle and carcasses (1–3), but few have evaluated its occurrence in the Alberta feedlot environment (4,5). Few studies have measured the occurrence of *Salmonella* and *Campylobacter* in feedlot cattle (6–8). Microbial data are needed to conduct a quantitative analysis of the risk associated with these bacteria from cattle on food and environmental safety. Risk assessments are used as a tool to identify risk management strategies to control unacceptable levels of risk.

This study was designed to determine the occurrence of generic *E. coli, E. coli* O157, *Salmonella,* and *Campylobacter* in manure, the feedlot environment, and cattle carcasses from Alberta feedlots.

Materials and methods

Feedlots

Twenty-one Alberta feedlots with greater than 5000-head capacity were randomly selected within size strata. The cattle marketing agency in Canada (CANFAX) provided randomly ordered lists of feedlots within 4 feeding capacity ranges. There were 46 feedlots with a 5000 to 10 000 head capacity, 14 with a 10 001 to 15 000 head capacity, 11 with a 15 001 to 20 000 head capacity, and 11 with an over 20 000 head capacity. Starting from the top of each of the 4 random lists of feedlots, feedlot producers were contacted to request their participation in the study until the required sample size per stratum was achieved. If a feedlot producer was not willing to participate, then the next feedlot on the random list within that feeding capacity stratum was contacted. Eleven feedlots from the 5001 to 10 000 head capacity, 4 from the 10 001 to 15 000 head capacity, 3 from the 15 001 to 20 000 head capacity, and 3 from the over 20 000 head capacity were included in the study.

Feedlots were visited once in the spring (March–July) and once in the fall (September–December). At each visit, 1 pen that was the shortest on feed and 1 pen within 2 wk of slaughter (preslaughter) were sampled at each feedlot. Twenty-five fresh manure samples (25 to 50 g) were collected from the floor of each feedlot pen and sent to various laboratories for analysis. During each visit, a water sample was collected from each of the 4 corners of 1 catch basin in each feedlot and then pooled into 1 water sample per feedlot for analysis.

Arrangements were made with 3 federally inspected beef processors in Alberta to follow the processing pens of cattle from which manure samples had been collected to collect carcass samples in the cooler following Hazard Analysis Critical Control Point (HACCP) intervention processes. For each group of cattle that originated from a pen that had been sampled, the processing plant personnel conveniently pulled 60 carcasses onto a cooler line where each half was sampled for a total of 120 samples. These samples were randomly split into 3 sets of 40 samples, which were then sent to each of 3 laboratories for testing. The Food Safety and Inspection Service (FSIS) procedures devloped by the United States Department of Agriculture (USDA) were followed for carcass sample collection (9).

In the spring or fall, 30 soil samples (0.5 kg) were collected from 1 field using a whole field composite sampling method (10) to determine the presence of generic *E. coli, E. coli* O157, *Salmonella,* and *Campylobacter* at 2 depths of soil (0 to 15 cm and 15 to 60 cm). Samples were taken from the field at various times before and after manure application (48 h, 1 wk, 1 mo). The manure that was applied to the land was sourced from multiple pens within the feedlot; these pens may or may not have included the specific pens in which manure samples were collected from the feedlot floor. The number of fields sampled in the spring or fall was based on the feedlot's pen cleaning practices and was restricted because of budget limitations.

Samples sent by courier overnight to the laboratories were cultured for generic *E. coli, E. coli* O157, *Salmonella,* and *Campylobacter.*

Isolation of generic *E. coli* from manure, soil, and water samples

Sterile cotton swabs were used to transfer manure to Violet Red Bile agar plates containing 50 mg/L of 4-methylumbelliferyl-β-D-glucuronide (VRB-MUG). Sterile loops were then used to streak each inoculated plate for isolation and the plates were incubated overnight at 37°C. Colonies that had purple fluorescence under UV illumination (312 nm) were picked as presumptive *E. coli* and were streaked onto a Columbia Blood Agar (CBA) plate and incubated overnight at 37°C. Isolated colonies from the CBA plate were used for a cytochrome oxidase test. Oxidase negative colonies were inoculated into Triple Sugar Iron (TSI) slants and 3 mL Luria Broth (LB, Hardy Diagnostics, Santa Maria, California, USA) and incubated overnight at 37°C. The LB was tested for tryptophanase (indole) and indole positive isolates with appropriate TSI reactions (acid/acid) were transferred to 1 mL Brain Heart Infusion (BHI) agar plugs, incubated overnight at 37°C, and then stored at room temperature.

For isolation of generic *E. coli* from soil samples, the shaken buffered peptone water (BPW) pre-enrichment broth, used in the *Salmonella* isolation method for soil samples (description follows), was plated on VRB-MUG (1-mL and 0.1-mL aliquots onto separate plates) and incubated overnight at 37°C. The number of coliforms (purple colonies on VRB-MUG) and putative *Escherichia coli* (fluorescent under UV illumination) were noted. *Escherichia coli* isolates were confirmed by oxidase, indole, and TSI tests and stored on BHI agar plugs as described previously.

For isolation of generic *E. coli* from catch basin water samples, 1 mL, 0.1 mL, and 0.01 mL of water were spread onto separate VRB-MUG agar plates and incubated overnight at 37°C. The purple, fluorescent colonies on VRB-MUG were counted as generic *E. coli* and 3 colonies per sample were confirmed as *E. coli* by oxidase, indole, and TSI tests and stored on BHI agar plugs as described previously.

Isolation of generic *E. coli* from beef carcass samples

Beef carcass samples were collected with sterile pre-moistened sponges from 3 sites (flank, brisket, and rump) on the carcass

using Beef/Swine Carcass Sampling Kits (Solar Biologicals, Ogdensburg, New York, USA). One mL of sample suspension was plated onto 3M Petrifilm *E. coli* Coliform Count Plates (3M Microbiology, St. Paul, Minnesota, USA). After incubation at 37°C for 48 h, the colonies on the plates were recorded and blue colonies surrounded by a gas bubble were presumptive *E. coli* (11–13). At least 4 representative colonies on the Petrifilm plates (3M Microbiology) were picked and streaked onto MacConkey (MAC) agar plates to test for lactose fermentation. Suspect colonies were verified with TSI, oxidase and indole tests as described previously. The *E. coli* isolates were confirmed with API 20E tests (bioMerieux Canada, Montreal, Quebec) or BBL Crystal tests (BD Biomedicals, Franklin Lakes, New Jersey, USA). *Escherichia coli* (ATCC 25922 and ATCC 35218) and *Pseudomonas aeruginosa* (ATCC 27853) were used as positive and negative controls, respectively.

An enrichment method was also included during the first sampling period, given the low positive rate for *E. coli* using the standard method on initial samples. A 0.1-mL volume of sample suspension was added to 2 mL of EC medium with MUG broth and incubated at 42°C for 48 h with shaking. Bacterial growth was monitored by plating 0.1 mL of broth onto MAC agar plates. Colonies on the MAC plates were subjected to TSI, oxidase, indole, API, or BBL Crystal tests as described previously.

Isolation of *Salmonella* spp. from bovine feces and beef carcasses

Salmonella spp. were cultured as described in the USDA/FSIS Microbiology Laboratory Guidebook (14) with minor modifications. Difco brand media (Difco Laboratories, Becton Dickinson Microbiology Systems, Sparks, Maryland, USA) were used unless otherwise stated. For manure samples, approximately 10 g of thoroughly mixed manure was added to 90 mL of sterile BPW and mixed well. For carcass sponges, 40 mL of BPW was added to the sponge using a Dilumat Dispenser (AES Laboratories, Combourg, France) to bring the total volume to 60 to 65 mL and mixed by vigorous shaking, ensuring that the sponge was immersed in the BPW. The manure and carcass sponge samples were incubated at 35°C for 24 h. One and 0.1 mL of BPW pre-enrichment culture was transferred to 10 mL of tetrathionate broth (TT) and 10 mL of Rappaport Vassiliadis broth (RV; EM Science, Merck, Darmstadt, Germany), respectively. The TT and RV enrichment cultures were incubated at 42°C for 24 h. Each enrichment culture was streaked onto Xylose-Lysine-Tergitol 4 agar (XLT4), Brilliant Green Sulfa agar (BGS) and Rambach agar (RAM; Merck, Darmstadt, Germany) plates and incubated at 35°C. Plates were examined after 22 to 24 h of incubation and candidate colonies (black or red colonies with or without black centers on XLT4; pink, smooth, and opaque colonies on BGS; and red, pink, peach, or magenta colonies on RAM) were selected for biochemical testing. Plates were incubated for an additional 24 h and re-examined for additional candidate colonies. Candidate colonies were streaked on Blood agar (BAP) and MAC plates and inoculated into urea agar, TSI agar and Lysine Iron agar (LIA) slants. Plates were examined for purity and reactions of the slants and MAC plates were noted. Isolates that were presumptive *Salmonella* based on typical color reactions were confirmed by agglutination in polyvalent O and O1 antisera (Denka Seiken Co., Tokyo, Japan). One isolate per sample confirmed as positive for *Salmonella* spp. was stored in sheep blood at -70°C for subsequent antimicrobial susceptibility testing and serotyping. Control samples were analyzed at the same time and included *Salmonella* Typhimurium ATCC 14028 as the positive control and uninoculated broths as the sterility control.

Isolation of *Salmonella* from catch basin water samples

For isolation of *Salmonella* from water samples, 225 mL of water was added to 225 mL 2 × BPW (Hardy Diagnostics) and incubated overnight at 37°C. Enrichment culture (100 µL) was added to 10 mL freshly activated TT (Hardy Diagnostics) and incubated for 48 h at 37°C. Tetrathionate broth (100 µL) was then added to 9 mL of R-10 medium (Hardy Diagnostics) and both the TT and R-10 media were incubated for 24 h at 37°C. The TT and R-10 media enrichment cultures were plated on selective XLT4 agar medium (Hardy Diagnostics) and incubated for 48 h at 37°C. Black colonies on XLT4 were transferred to LIA (Hardy Diagnostics) slants and incubated for 24 h at 37°C. Presumptive *Salmonella* cultures from LIA slants were transferred to TSI slants and urea slants (Hardy Diagnostics) and incubated overnight at 37°C. Isolates that had alkaline/acid, H_2S positive reactions on TSI and were urease negative were serogrouped (Salmonella O Grouping kit, BD Diagnostics, Franklin Lakes, New Jersey, USA).

Isolation of *Salmonella* from soil samples

For isolation of *Salmonella* from soil samples, 4 sub-samples (25 g each) of each specimen were separately pre-enriched in 225 mL BPW for 24 h at 37°C. The pre-enriched flasks were mixed (15 min shaking, room temperature), sub-samples were removed for *E. coli* culture as described previously, and the BPW was incubated overnight at 37°C. Subsequent isolation steps were as described for water samples.

Isolation of *E. coli* O157 from manure, soil, and catch basin water samples

For isolation of *E. coli* O157 from manure samples, 10 g of manure were added to 90 mL of Tryptic Soy Broth (TSB; Hardy Diagnostics) and incubated overnight at 42°C. Immunomagnetic separation (IMS) for the O157 antigen was then performed on 2 × 500 µL aliquots of the enriched culture as per the manufacturer's instructions (Dynal Biotech, Brown Deer, Wisconsin, USA), plated onto Sorbitol MAC plates containing cefixime (50 µg/L) and potassium tellurite (2.5 mg/L) (SMAC-CT, Hardy Diagnostics) and incubated overnight at 37°C. Small white colonies on SMAC-CT were transferred to MAC agar and incubated overnight at 37°C. Red (lactose-fermenting) colonies were transferred to EC agar containing 50 mg/L MUG (EC-MUG, Hardy Diagnostics) and incubated overnight at 37°C. The EC-MUG plates were observed under UV light and MUG-negative (nonfluorescent) colonies were tested for O157 antigen expression by latex agglutination (*E. coli* O157 Test Kit;

Table 1. Percent of feedlot manure samples positive for specified bacteria by season (spring, fall) and pen type (shortest on feed, preslaughter)

Season — Pen type	*E. coli*	*E. coli* O157	*Salmonella*	*Campylobacter*
Spring — shortest-on-feed				
% individual	98[a] (514/525)	15[a] (81/525)	0[a] (0/525)	69[a] (362/525)
(95% CI)	(96.3 to 98.9)	(12.4 to 18.8)	NA	(64.8 to 72.9)
% positive pens	100[a] (21/21)	57[a] (12/21)	0[a] (0/21)	100[a] (21/21)
(95% CI)	(84 to 100)	(34.0 to 78.2)	NA	(84 to 100)
Spring — preslaughter				
% individual	99[a] (518/525)	4[a] (22/525)	1[a] (6/525)	86[b] (450/525)
(95% CI)	(97.3 to 99.5)	(2.6 to 6.3)	(0.4 to 2.5)	(82.4 to 88.6)
% positive pens	100[a] (21/21)	38[a] (8/21)	9[a] (2/21)	100[a] (21/21)
(95% CI)	(84 to 100)	(18.1 to 61.6)	(1.2 to 30.4)	(84 to 100)
Fall — shortest-on-feed				
% individual	98[a] (517/525)	5[a] (24/525)	1[a] (6/525)	58[a] (307/525)
(95% CI)	(97.0 to 99.3)	(2.9 to 6.7)	(0.4 to 2.5)	(54.4 to 62.8)
% positive pens	100[a] (21/21)	38[a] (8/21)	19%[a] (4/21)	100[a] (21/21)
(95% CI)	(84 to 100)	(18.1 to 61.6)	(5.4 to 41.9)	(84 to 100)
Fall — preslaughter				
% individual	99[a] (520/525)	3[a] (16/525)	1[a] (8/525)	90[b] (471/525)
(95% CI)	(97.8 to 99.7)	(1.8 to 4.9)	(0.7 to 3.0)	(86.8 to 92.2)
% positive pens	100[a] (21/21)	42[a] (9/21)	28[a] (6/21)	100[a] (21/21)
(95% CI)	(84 to 100)	(21.8 to 66.0)	(11.3 to 52.2)	(84 to 100)

[a,b] $P < 0.05$ between shortest on feed and preslaughter pens within season and type of analysis (individual, pen) significant differences in percent positive samples.
NA — Not available; could not be calculated.
95% CI — 95% confidence interval.

Oxoid). Isolates positive for the O157 antigen were transferred to 1 mL BHI and incubated overnight at 37°C. Buffered glycerol (15% v/v) was added to the enriched BHI and the tubes were stored at -80°C.

For isolation of *E. coli* O157 from soil samples, 4 sub-samples (25 g each) from each specimen were added to separate 225 mL aliquots of TSB and then incubated overnight at 42°C. Two 500 μL aliquots of each TSB enrichment sample were subjected to colony isolation with the aid of IMS and were characterized as described previously for manure samples.

For isolation of *E. coli* O157 from water, 225 mL samples were added to an equal volume of 2 × TSB and incubated overnight at 42°C. Two 500 μL aliquots of each enriched TSB culture were subjected to IMS and O157 colonies that were isolated were characterized as described previously for manure specimens.

Isolation of *Campylobacter* from manure, catch basin water, carcass, and soil samples

For isolation of *Campylobacter* from manure samples, sterile swabs were used to transfer approximately 0.1 g of feces into 12 mL Bolton Broth (Oxoid) containing laked horse blood (50 ml/L, Oxoid) and Bolton Broth selective supplement (2 vials/L, Oxoid), and incubated aerobically for 24 h at 37°C. Enriched samples were subcultured by sterile swab to Cefoperazone-Vancomycin-Amphotericin B agar plates (CVA; Hardy Diagnostics) and incubated at 42°C under microaerophilic conditions (CampyGen; Oxoid). The CVA plates were examined for candidate *Campylobacter* colonies based on colony morphology after 48 h and 96 h incubation. Enriched broths that exhibited contaminant overgrowth on CVA plates were filtered (0.45 μm, Millipore) and incubated microaerophilically on blood agar plates for 48 h at 42°C. Three candidate *Campylobacter* colonies per sample were stained and screened for typical curved rod *Campylobacter* spp. morphology (Victoria Blue 4R stain; Pfeltz & Bauer, Waterbury, Colorado, USA) by light microscopy. Isolates were stored at -80°C after placing a heavy suspension of 24 h growth into 1% proteose peptone with 10% glycerol (Hardy Diagnostics).

For isolation of *Campylobacter* from water samples, 225 mL of water were combined with an equal volume of 2 × Bolton Broth plus laked horse blood and incubated aerobically at 37°C for 24 h. After incubation, 0.45 μm filtrates were plated on blood agar and 300-μL unfiltered aliquots were spread plated and streaked for isolation on CVA plates. Inoculated plates were incubated under microaerophilic conditions for 48 h at 37°C. After addition of Bolton Broth selective supplement (5 mL), each sample was re-incubated under microaerophilic conditions for 24 h at 37°C and then subcultured to blood agar and CVA. Candidate *Campylobacter* colonies were isolated and stored as described for candidate isolates from manure samples.

For isolation of *Campylobacter* from carcass swabs, the swabs and excess diluent were transferred to sterile specimen cups containing 100 mL of Bolton Broth with laked horse blood and incubated aerobically for 24 h at 37°C. After addition of Bolton Broth selective supplement, the specimen cups were re-incubated aerobically for 24 h at 42°C. One hundred microlitres of enriched samples were spread plated to CVA and incubated under microaerophilic conditions for 48 h at 37°C. Candidate *Campylobacter* were isolated and stored as described for manure samples.

For isolation of *Campylobacter* from soil samples, 4 soil subsamples (25 g each) were inoculated into separate 225 mL aliquots of Bolton Broth containing laked horse blood and

Table 2. Percent of feedlot beef carcass samples positive for specified bacteria by season

Season — Level of analysis	*E. coli*	*Salmonella*	*Campylobacter*
Spring			
% individual (95% CI)	0.1 (1/835) (0.003 to 0.7)	0 (0/835) NA	0.1 (1/835) (0.003 to 0.7)
% positive pens (95% CI)	5 (1/21) (0.1 to 23.8)	0 (0/21) NA	5 (1/21) (0.1 to 23.8)
Fall			
% individual (95% CI)	0 (0/818) NA	0 (0/818) NA	0 (0/818) NA
% positive pens (95% CI)	0 (0/21) NA	0 (0/21) NA	0 (0/21) NA

NA — Not available; could not be calculated. 95% CI — 95% confidence interval.

Bolton Broth selective supplement and incubated aerobically for 24 h at 37°C. Enriched samples were mixed by inversion and re-incubated aerobically for 24 h at 42°C. Filtrates (0.45 μm) of the Bolton Broth enrichments were then streaked for isolation onto blood agar plates and incubated under microaerophilic conditions for 48 h at 37°C. Candidate *Campylobacter* were isolated and stored as described for manure samples.

Candidate thermophilic *Campylobacter* isolates were identified using 1) colony blot hybridization with a *hipO* gene probe to identify *C. jejuni* (15) and for 2) *hipO*-negative isolates, a *lpxA* multiplex PCR (16). Isolates negative for *hipO* hybridization and for *C. jejuni* and *C. coli* multiplex PCR products were designated "other thermophilic *Campylobacter.*"

Statistical analysis

Data were entered into Excel spreadsheets (Microsoft, Bellevue, Washington, USA) and then imported into Statistix 8 (1985–2003) (Analytical Software, Tallahassee, Florida, USA) where simple statistics (frequency, chi-squared test, Fishers exact test) were performed to determine the proportion of positive samples and compare differences in the proportion positive between season and type of pen (shortest on feed, preslaughter). Fisher's exact binomial 95% confidence limits for proportions were calculated. Pens were considered positive if any one of the 25 manure samples within that pen was positive. Soil samples were considered positive if any one of the 4 sub-samples cultured was positive. Chi-squared statistics were adjusted for clustering within feedlots using analysis of variance (ANOVA) to calculate the correction factor, as previously described (17). A statistical level of significance was set at $P \leq 0.05$.

Results

Twenty-one feedlots in Alberta were visited and sampled between March and December, 2004. Fifteen feedlots in the 5001 to 10 000 head capacity did not participate because they did not have enough cattle going to be processed at the time periods during which the study was conducted. Three feedlots in the 15 001 to 20 000 head capacity, and 1 feedlot in the > 20 000 head capacity did not participate because of time constraints or lack of interest in the study. The 42 remaining feedlots from the original list were not contacted.

Table 3. Percent of feedlot catch basin water samples positive for specified bacteria by season

Season	*E. coli*	*E. coli* O157	*Salmonella*	*Campylobacter*
Spring (95% CI)	62%[a] (13/21) (38.4 to 81.9)	29%[a] (6/21) (11.3 to 52.2)	5%[a] (1/21) (0.1 to 23.8)	52%[a] (11/21) (29.8 to 74.3)
Fall (95% CI)	52%[a] (11/21) (29.8 to 74.3)	0%[b] (0/21) NA	0%[a] (0/21) NA	0%[b] (0/21) NA

[a,b] $P < 0.05$ between seasons ([a]spring; [b]fall).
NA — Not available; could not be calculated. 95% CI — 95% confidence interval.

The median days-on-feed for the pens shortest on feed was 14 d and ranged from 1 to 152 d. For preslaughter pens, the median days-on-feed was 184 d and ranged from 55 to 394 d.

The percent of culture-positive samples for feedlot manure is shown in Table 1, beef carcasses in Table 2, catch basin water in Table 3, and soil in Table 4.

Discussion

As expected, generic *E. coli* were commonly found in feedlot manure (98%, 2069/2100) regardless of season. *Escherichia coli* O157 was occasionally isolated from feedlot manure samples (7%, 143/2100) (Table 1), similar to levels that have been previously reported in Alberta feedlots (4). *Escherichia coli* O157 were more common in manure collected in the spring (10%, 103/1050) than in the fall (4%, 40/1050), which supports previous research (18,19) where these bacteria were most commonly isolated in the summer. Additionally, *E. coli* O157 were more common in shortest-on-feed pens (4%, 38/1050) than in preslaughter pens (1%, 105/1050), which has also been previously reported (18).

Salmonella was rarely isolated from feedlot manure (1%, 20/2100), as has been previously reported in Alberta feedlots (7). Serotypes identified among these isolates included *S.* Rubislaw (40%, 8/20), *S.* Saintpaul (25%, 5/20), *S.* Mbandaka (15%, 3/20), *S.* Enteritidis (10%, 2/20), *S.* Typhimurium (5%, 1/20), and *Salmonella* 4,5,12:i:-. (5%, 1/20). Many different serotypes have been isolated in manure samples from cattle in Canadian feedlots (7), with similar serotypes being found in humans that were ill (20).

Campylobacter was found in 76% (1590/2100) of the feedlot manure samples (Table 1). Since some manure samples yielded more than one type of *Campylobacter,* a total of 1623 isolates were characterized including *C. jejuni* (66%, 1070/1623), *C. coli* (1%, 17/1623), and other thermophilic *Campylobacter* spp. (33%, 536/1623). The occurrence of *Campylobacter* spp. in feedlot manure was similar between seasons; however, it was higher in preslaughter pens (88%, 921/1050) than in shortest-on-feed pens (64%, 669/1050); similar to that previously reported (6,8).

Generic *E. coli* was isolated only once from beef carcasses (1/1653) (Table 2), suggesting that HACCP systems in the processing plants and intervention processes were highly effective in minimizing the effects of manure contamination on beef carcasses. Testing for *E. coli* O157 was not permitted in the processing plants; therefore, the occurrence of *E. coli* O157 on carcasses remains unknown. Given the low animal-level occurrence of *E. coli* O157 compared with the very high animal-level

Table 4. Percent of field soil samples positive for specified bacteria by season, soil depth, and time to manure application

Season (soil depth)	*E. coli*	*E. coli* O157	*Salmonella*	*Campylobacter*
Spring				
Before manure application				
(shallow)	14% (1/7)	0% (0/7)	14% (1/7)	14% (1/7)
(95% CI)	(0.4 to 57.9)	NA	(0.4 to 57.9)	(0.4 to 57.9)
(deep)	0% (0/7)	0% (0/7)	0% (0/7)	0% (0/7)
(95% CI)	NA	NA	NA	NA
48 h after manure application				
(shallow)	57% (4/7)	0% (0/7)	0% (0/7)	71% (5/7)
(95% CI)	(18.4 to 90.1)	NA	NA	(29.0 to 96.3)
(deep)	43% (3/7)	0% (0/7)	14% (1/7)	43% (3/7)
(95% CI)	(9.9 to 81.6)	NA	(0.4 to 57.9)	(9.9 to 81.6)
1 wk after manure application				
(shallow)	29% (2/7)	0% (0/7)	0% (0/7)	57% (4/7)
(95% CI)	(13.7 to 71.0)	NA	NA	(18.4 to 90.1)
(deep)	14% (1/7)	0% (0/7)	0% (0/7)	29% (2/7)
(95% CI)	(0.4 to 57.9)	NA	NA	(13.7 to 71.0)
1 mo after manure application				
(shallow)	14% (1/7)	0% (0/7)	0% (0/7)	29% (2/7)
(95% CI)	(0.4 to 57.9)	NA	NA	(13.7 to 71.0)
(deep)	14% (1/7)	0% (0/7)	0% (0/7)	14% (1/7)
(95% CI)	(0.4 to 57.9)	NA	NA	(0.4 to 57.9)
Fall				
Before manure application				
(shallow)	0% (0/4)	25% (1/4)	0% (0/4)	0% (0/4)
(95% CI)	NA	(0.6 to 80.6)	NA	NA
(deep)	25% (1/4)	0% (0/4)	0% (0/4)	0% (0/4)
(95% CI)	(0.6 to 80.6)	NA	NA	NA
48 h after manure application				
(shallow)	75% (3/4)	25% (1/4)	0% (0/4)	25% (1/4)
(95% CI)	(19.4 to 99.4)	(0.6 to 80.6)	NA	(0.6 to 80.6)
(deep)	50% (2/4)	0% (0/4)	0% (0/4)	0% (0/4)
(95% CI)	(16.8 to 93.2)	NA	NA	NA
1 wk after manure application				
(shallow)	75% (3/4)	0% (0/4)	0% (0/4)	0% (0/4)
(95% CI)	(19.4 to 99.4)	NA	NA	NA
(deep)	50% (2/4)	0% (0/4)	0% (0/4)	0% (0/4)
(95% CI)	(16.8 to 93.2)	NA	NA	NA
1 mo after manure application				
(shallow)	50% (2/4)	25% (1/4)	0% (0/4)	0% (0/4)
(95% CI)	(16.8 to 93.2)	(0.6 to 80.6)	NA	NA
(deep)	25% (1/4)	0% (0/4)	0% (0/4)	0% (0/4)
(95% CI)	(0.6 to 80.6)	NA	NA	NA

NA — Not available; could not be calculated. 95% CI — 95% confidence interval.

occurrence of generic *E. coli* and the rare isolation of generic *E. coli* on carcasses in this study; we expect that the occurrence of *E. coli* O157 on carcasses would be very low. *Salmonella* (0/1653) were not isolated from beef carcasses, and they are infrequently isolated from Canadian beef (7). *Campylobacter* were only found once on a beef carcass and the isolate was typed as *C. jejuni.* These results indicate a very low occurrence of zoonotic *Salmonella* and *Campylobacter* on beef carcasses from Alberta feedlots.

As expected, generic *E. coli* were commonly found in the catch basin water (57%, 24/42), which is the runoff water from feedlot pens (Table 3). *Escherichia coli* O157 was only found in the spring catch basin water samples (29%, 6/21). *Salmonella* was rarely isolated from catch basin water (2%, 1/42). The single catch basin water isolate was *S.* Saintpaul. *Campylobacter* were only found in catch basin water in the spring (52%, 11/21), suggesting environmental factors may affect bacterial survival. The *Campylobacter* species isolated from catch basin water were not *C. jejuni* or *C. coli.* Catch basin water either evaporates or is irrigated onto feedlot silage crop. It is unknown whether the use of catch basin water to irrigate crops may risk infection of cattle consuming the silage after harvest.

Generic *E. coli* were isolated from soil samples before and after manure application (Table 4). The proportion of positive samples did not differ over time for all bacteria tested, which may be due to the small sample size. *Escherichia coli* O157 were

rarely isolated from soil samples (3%, 3/88) and the isolates found were each from different feedlots and not related to the timing of manure application on the land. Salmonellae were occasionally isolated from the soil (3%, 2/56) in the spring but not in the fall (0/32). One of the soil isolates was *S.* Typhimurium; the other could not be resuscitated, and therefore the serotype could not be determined. This study indicates a very low occurrence of *Salmonella* in cattle manure, on beef carcasses, and in feedlot environmental samples from Alberta feedlots.

Campylobacter were found in soil samples in the spring (32%, 18/56). However, only 1 shallow soil sample was positive (3%, 1/32) in the fall 48 h after manure application; suggesting environmental factors may play a role in bacterial survival during different seasons. Only 1 of the 134 *Campylobacter* isolates from the soil was *C. jejuni.* This study indicates a very low number of human pathogenic isolates of *Campylobacter* on beef carcasses and no human pathogenic isolates in environmental samples (soil, catch basin water) from Alberta feedlots.

In summary, while generic *E. coli* and *Campylobacter* spp. were commonly found in the manure of feedlot cattle in Alberta feedlots, *E. coli* O157, *Salmonella, C. jejuni,* and *C. coli* were infrequently isolated from beef carcasses or in feedlot catch basin water or soil samples. A quantitative risk assessment would need to be conducted to determine the level of risk to human safety from these sources. This study was representative of Alberta feedlots; although the days-on-feed by type of pen (shortest-on-feed, preslaughter) was atypical due to bovine spongiform encephalopathy (BSE) marketing challenges. The closure of international borders to Canadian live cattle and beef resulted in cattle being kept on feed much longer than normal. Some of the shortest-on-feed pens were on feed for some time after arrival and they did not represent cattle typical of newly arrived pens of cattle. This misclassification bias may have obscured finding differences in the proportion of bacteria by type of pen. A low sample size for pens, catch basins, and soil samples may have affected our ability to reliably estimate the occurrence of bacteria and detect differences amongst proportion estimates. Our laboratory culture methods were standard but sampling procedures and less than 100% culture sensitivity and specificity can result in apparent farm- and animal-level estimates that differ from the true estimate. Farm- and pen-level prevalence estimates might tend to be low due to the influences of reduced sample size and imperfect culture sensitivity, both of which would reduce farm- and pen-level sensitivity and ultimately, prevalence estimates. Additionally, the relatively high specificity of culture techniques would indicate less of an issue with farm- or pen-level specificity such that the prevalence estimates we provide would not have an upward bias. Consequently, we believe that the apparent prevalence estimates would tend to be lower than the true prevalence.

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References

1. Flint HJ, Stewart CS. Faecal shedding and rumen proliferation of *Escherichia coli* O157:H7 in calves: An experimental model. In: *Escherichia coli* O157 in Farm Animals, New York: CABI Publ, 1999: 59–69.
2. Sargeant JM, Sanderson MW, Smith RA, Griffin DD. *Escherichia coli* O157 in feedlot cattle feces and water in four major feeder cattle states in the USA. Prev Vet Med 2003;61:127–135.
3. Renter DG, Smith DR, King R, et al. Detection and determinants of *Escherichia coli* O157:H7 in Alberta feedlot pens immediately prior to slaughter. Can J Vet Res 2008;72:217–227.
4. Van Donkersgoed J, Berg J, Potter A, et al. Environmental sources and transmission of *E. coli* O157:H7 in feedlot cattle. Can Vet J 2001; 42:714–720.
5. Berry ED, Woodbury BL, Nienaber JA, Eigenberg RA, Thurston JA, Wells JE. Incidence and persistence of zoonotic bacterial and protozoan pathogens in a beef cattle feedlot runoff control vegetative treatment system. J Environ Qual 2007;36:1873–1882.
6. Inglis GD, Morck DW, McAllister JA, et al. Temporal prevalence of antimicrobial resistance in *Campylobacter* spp. from beef cattle in Alberta feedlots. App Environ Microbiol 2006;72:4088–4095.
7. Sorensen O, Van Donkersgoed J, McFall M, Manninen K, Gensler G, Ollis G. The incidence of *Salmonella* spp. shedding by Alberta beef cattle and the incidence of *Salmonella* spp. in ground beef. J Food Prot 2002;65:484–491.
8. Besser TE, Lejeune JT, Rice DH, et al. Increasing prevalence of *Campylobacter jejuni* in feedlot cattle through the feeding period. Appl Environ Microbiol 2005;71:5752–5758.
9. United States Department of Agriculture (USDA). FSIS Pathogen Reduction and HACCP Systems: Final Rule. Federal Register 1996;61:38917–38938.
10. Nutrient Management Planning Guide 2001, Alberta Agriculture, Food & Rural Development.
11. Townsend DE, Irving RL, Naqui A. Comparison of the SimPlate coliform and *Escherichia coli* test with Petrifilm, Three-Tube MPN, and VRSA + MUG methods for enumerating coliforms and *E. coli* in food. J Food Prot 1998;61:444–449.
12. Vail JH, Morgan R, Merino CR, Gonzales F, Miller R, Ram JL. Enumeration of waterborne *Escherichia coli* with petrifilm plates: Comparison to standard methods. J Environ Qual 2003;32:368–73.
13. Van Donkersgoed J, Manninen K, Potter A, et al. Antimicrobial susceptibility of hazard analysis critical control point *Escherichia coli* isolates from federally inspected beef processing plates in Alberta, Saskatchewan, and Ontario. Can Vet J 2003;44:723–728.
14. Rose BE. 1998. Isolation and Identification of Salmonella from Meat, Poultry, and Egg Products, Microbiology Laboratory Guidebook. US Department of Agriculture, Food Safety and Inspection Service, Washington, DC.
15. Bae W, Kaya KN, Hancock DD, Call DR, Park YH, Besser TE. Prevalence and antimicrobial resistance of thermophilic *Campylobacter* spp. from cattle farms in Washington State. Appl Environ Microbiol. 2005;71:169–174.
16. Werno AM, Klena JD, Shaw GM, Murdock DR. Fatal case of *Campylobacter lari* prosthetic joint infection and bacteremia in an immunocompetent patient. J Clin Microbiol 2002;40:1053–1055.
17. Donald A, Donner A. The analysis of variance adjustment to chi-square tests when there is litter or herd correlation in surveys or field trials. Acta Vet Scand Suppl 1988;84:490–492.
18. Hancock D, Besser T, Lejeune J, Davis M, Rice D. The control of VTEC in the animal reservoir. Int J Food Microbiol 2001;66:71–78.
19. Van Donkersgoed J, Graham T, Gannon V. The prevalence of verotoxins, *Escherichia coli* O157:H7, and Salmonella in the feces and rumen of cattle at processing. Can Vet J 1999;40:332–328.
20. Laboratory Surveillance Data for Enteric Pathogens in Canada. Annual Summary 2005. Public Health Agency of Canada. ISBN: 978-0-662-36864-6.