The prevalence of verotoxins, *Escherichia coli* 0157:H7, and *Salmonella* in the feces and rumen of cattle at processing

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Abstract — Fecal samples collected from cattle at processing during a 1-year period were tested for verotoxins (VT1, VT2), *Escherichia coli* 0157:H7, and *Salmonella*. Verotoxins were detected in 42.6% (95% CI, 39.8% to 45.4%), *E. coli* 0157:H7 in 7.5% (95% CI, 6.1% to 9.1%), and *Salmonella* in 0.08% (95% CI, 0.004% to 0.5%) of the fecal samples. In yearling cattle, the median within-lot prevalence (percentage of positive samples within a lot) was 40% (range, 0% to 100%) for verotoxins and 0% for *E. coli* 0157:H7 (range, 0% to 100%) and *Salmonella* (range, 0% to 17%). One or more fecal samples were positive for verotoxins in 80.4% (95% CI, 72.8% to 86.4%) of the lots of yearling cattle, whereas *E. coli* 0157:H7 were detected in 33.6% (95% CI, 26.0% to 42.0%) of the lots. In cull cows, the median within-lot prevalence was 50% (range, 0% to 100%) for verotoxins and 0% (range, 0% to 100%) for *E. coli* 0157:H7 and *Salmonella* (range, 0% to 0%). Verotoxins were detected in one or more fecal samples from 78.0% (95% CI, 70.4% to 84.2%) of the lots of cull cows, whereas *E. coli* 0157:H7 were detected in only 6.0% (95% CI, 3.0% to 11.4%) of the lots of cull cows. The prevalence of verotoxins in fecal samples was lower in yearling cattle than in cull cows, whereas the prevalence of *E. coli* 0157:H7 in fecal samples was higher in yearling cattle than in cull cows. The prevalence of *E. coli* 0157:H7 in fecal samples was highest in the summer months. Rumen fill, body condition score, sex, type of cattle (dairy, beef), and distance travelled to the plant were not associated with the fecal prevalence of verotoxins or *E. coli* 0157:H7. The prevalence of verotoxins in fecal samples of cull cows was associated with the source of the cattle. It was highest in cows from the auction market (52%) and farm/ranch (47%) and lowest in cows from the feedlot (31%). In rumen samples, the prevalence of verotoxins was 6.4% (95% CI, 4.2% to 9.4%), and it was 0.8% (95% CI, 0.2% to 2.3%) for *E. coli* 0157:H7, and 0.3% (95% CI, 0.007% to 1.5%) for *Salmonella*.

Résumé — Prévalence des vérotoxines, d'*Escherichia coli* 0157:H7 et de *Salmonella* dans les fèces et le rumen de bovins à l'abattoir. Des échantillons fécaux prélevés chez des bovins à l'abattage sur une période d'un an ont été analysés afin d'identifier les vérotoxines (VT1, VT2), les *Escherichia coli* 0157:H7 et les *Salmonella*. Les vérotoxines ont été détectées dans 42,6 % (95 % IC, 39,8 à 45,4 %) des échantillons fécaux, les *E. coli* 0157:H7 dans 7,5 % (95 % IC, 6,1 % à 9,1 %) et les salmonelles dans 0,08 % (95 % IC, 0,004 % à 0,5 %). Chez les bovins d'un an, la prévalence médiane au niveau du groupe (pourcentage d'échantillons positifs au niveau du groupe) était de 40 % (intervalle, 0 % à 100 %) pour les vérotoxines et 0 % pour les *E. coli* 0157: H7 (intervalle, 0 % à 100 %) et les salmonelles (intervalle, 0 % à 17 %). Un ou plusieurs échantillons fécaux étaient positifs aux vérotoxines dans 80,4 % (95 % IC, 72,8 % à 86,4 %) des groupes de bovins d'un an alors que les *E. coli* 0157:H7 étaient détectés dans 33,6 % (95 % IC, 26 % à 42 %) des groupes. Chez les vaches de réforme la prévalence médiane au sein du groupe était de 50 % (intervalle de 0 % à 100 %) pour les vérotoxines et 0 % pour les *E. coli* 0157:H7 (intervalle, 0 % à 100 %) et les salmonelles (intervalle, 0 % à 0 %). Les vérotoxines ont été détectées dans un ou plusieurs échantillons fécaux de 78,0 % (95 % IC, 70,4 % à 84,2 %) des groupes de vaches de réforme alors que les *E. coli* 0157:H7 l'ont été dans seulement 6,0 % (95 % IC, 3 % à 11,4 %). La prévalence des vérotoxines dans les échantillons fécaux était plus faible chez les bovins d'un an que chez les vaches de réforme alors que celle des *E. coli* 0157:H7 était plus élevée chez les bovins d'un an que chez les vaches de réforme. La prévalence de *E. coli* 0157:H7 dans les échantillons fécaux était plus élevée dans les mois d'été. Le contenu du rumen, la cotation de la condition physique, le sexe, le type de bovin (laitier, de boucherie) et la distance franchie pour se rendre à l'usine n'étaient pas associés à la prévalence de vérotoxines ou de *E. coli* 0157: H7 au niveau fécal. La prévalence des vérotoxines dans les échantillons fécaux des vaches de réforme était associée à la provenance des bestiaux. Elle était plus élevée chez les vaches achetées au enchères (50 %) et chez celles provenant de fermes/ranches (47 %) et plus basses chez les vaches provenant des parcs d'engraissement (31 %). Dans les échantillons du rumen, la prévalence de vérotoxines était

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Funding provided by the Beef Industry Development Fund and National Tripartite Opt-Out Funds from the Canadian Cattlemen's Association, the Matching Investment Initiative from Agriculture and Agri-Food Canada, and in-house funding from the Beef and Dairy Group from Alberta Agriculture, Food and Rural Development.

de 6,4 % (95 % IC, 4,2 % à 9,4 %), celle des *E. coli* 0157:H7 de 0,8 % (95 % IC, 0,2 % à 2,3 %) et celle des salmonelles de 0,3 % (95 % IC, 0,007 % à 1,5 %).

(Traduit par docteur André Blouin)

Can Vet J 1999; 40: 332–338

Introduction

Food safety is one of the leading issues for the agricultural industry, for both livestock and produce. Food-borne pathogens, such as *Escherichia coli* 0157:H7 and *Salmonella,* can cause human disease, and beef is one vehicle for human infection (1–28). Surveys have been conducted in cattle on farms across the world to determine the prevalence of verotoxin-producing *E. coli* (VTEC), *E. coli* 0157:H7, and *Salmonella,* in an attempt to determine the sources of the bacteria and to identify management strategies that would help to reduce the risk of animal shedding and carcass contamination (1,6,9,10,14–16,18–22,26–28).

Verotoxin-producing *E. coli* are widespread in cattle and other ruminants (6,11,24,28,29). The prevalence of *E. coli* 0157 in beef and dairy cattle on farms has been reported to range from 0% to 68%, and the herd prevalence has been reported to range from 1.8% to 100%, with a wide distribution (1,7,28,29). The prevalence of VTEC and *E. coli* 0157:H7 in cattle at slaughter has been reported infrequently (2–5). In 3 recent studies conducted at slaughter, *E. coli* 0157 was isolated in 3.6% (4) and 13.4% of beef cattle (2), and 3.9% (5) and 16.1% (2) of dairy cattle.

On dairy farms, between 12% and 75% of the herds have been shown to be exposed to *Salmonella* (24,25). In a recent feedlot survey in the United States (18), *Salmonella* were recovered from 38% of the feedlots, 26% of the pens, and 5.5% of the fecal samples. *Salmonella* have also been isolated from the feces and rumen of cattle at slaughter in a limited number of studies, with prevalence estimates of 0% (23) and 27% (16) in the feces, and 36% (16) and 37% (26) in the rumen. Knowledge of the prevalence of these bacteria in the feces and rumen of cattle and the associated risk factors are important, because it may lead to intervention strategies before slaughter and during processing that will reduce the risk of bacterial contamination of the carcass. Current preslaughter management strategies that are being investigated include vaccination and competitive exclusion (1).

The following study was designed to determine the prevalence of verotoxins, *E. coli* 0157:H7, and *Salmonella* in the feces and rumen of yearling cattle and cull beef and dairy cows at slaughter. A second objective of the study was to determine risk factors (rumen fill, season, source, sex, body condition score, distance travelled to plant) associated with the prevalence of these bacteria. Previously, researchers have suggested that fasting cattle before slaughter will result in higher levels of *E. coli* 0157:H7 and *Salmonella* (14,16).

Materials and methods

Sampling

An abattoir in Alberta was visited every 2 to 3 wk from September 1995 to August 1996. The cattle at this plant originated from Alberta, Saskatchewan, and Manitoba. The number of fecal samples that were collected from cattle was based on the following calculations. Assuming a prevalence of 1.8% for *E. coli* 0157:H7 (10) and 5.5% for *Salmonella* (18), approximately 707 to 2079 individual fecal samples needed to be collected to estimate the individual fecal sample prevalence within 1% of the true value, with a 95% certainty (30).

By definition, a lot referred to a group of cattle from one owner that were processed and managed together at the plant, and the size of the lots ranged from 1 to 443 animals. In lots of yearling cattle and cows from feedlots or farms, the animals within the same lot came from the same feedlot or farm and generally represented specific groups of cattle (feeding pens). In lots of cattle from the auction market, the cattle may have originated from multiple herds when assembled at the auction market. To determine the sample size for prevalence estimates of lots of cattle at processing, herd prevalence estimates were used as a surrogate measure, because prevalence estimates for lots of cattle at processing have not been reported previously and process lots of cattle from feedlots and farms generally represented a herd. Assuming a herd prevalence of 63% for *E. coli* 0157:H7 (10) and 38% for *Salmonella* (18), between 259 and 262 lots of cattle needed to be sampled to estimate the lot prevalence within 6% of its true value (30). The maximum number of fecal samples that could be cultured for bacteriology on each sampling date was 50. In an attempt to achieve the total sample size required during the study to reliably estimate the prevalence, and taking into account the number of visits to the plant and the constraints of the packing environment, the study was designed so that 5 lots of yearling cattle were conveniently selected on each sampling date and, then, from each of these lots, 5 animals were randomly sampled on a systematic basis for a total of 25 fecal samples from yearling cattle (stratified random sampling); for example, if the lot size was 100 animals, every 20th animal on the process line within that lot was sampled. Cull cows were processed at the end of the day, and the number of cows and the size of the lots to be processed each day was not known with certainty. In an attempt to collect a minimum of 25 individual fecal samples from cull cows on each sampling date, an estimate was made of the total number of cows to be killed that day; then samples were randomly collected on an individual systematic basis (simple random sampling); for example, if 250 cows were estimated to be processed that afternoon, every 10th cow on the process line was sampled. On those dates when no cows were processed, 50 samples were collected from 10 lots of yearling cattle.

Fecal samples were collected from the bung (bagged off rectum) on the gut table; separate clean bags had been used to seal each rectum to reduce the risk of cross-contamination from one bung to another. Approximately 10 g of fecal material was collected

from the rectum by using a sterile spoon, and the feces was placed in 15 mL of enteric transport media (modified Cary-Blair, Dalynn, Calgary, Alberta). Technicians wore latex gloves and used a different spoon for each sample, changing gloves between each animal. Occasionally the number of cows in a lot was small and the line speed was too fast to change gloves between each animal sampled within a lot.

A total of 373 rumen samples was collected from yearling cattle (n = 223) and cull cows (n = 150) during the study. These samples were collected when time and help were available. The rumen samples were collected from the same lots and cattle that were sampled for fecal testing. Approximately 10 g of rumen contents were sampled on the gut table, by using a sterile scoop as the rumen was opened and emptied, and then placed in transport media, as described above. Latex gloves were worn and changed between each sample.

The rumen fill was assessed on the gut table by palpation, and a scoring system of 0 to 4 was used, with 0 being empty and 4 being full. The body condition score was measured on a 5-point scale after the hide had been removed; a score of 1 was very thin and a score of 5 was very fat. One technician scored body condition scores throughout the study, and at most, 3 technicians scored rumen fill, in an attempt to reduce the inherent variability in these subjective measures. Data were collected from the livestock manifests and plant's driver schedules on the size of the lots, age of cattle (yearling, cow), sex of cattle, type of cattle (beef, dairy), source of cattle (feedlot, auction, farm/ranch), and distance cattle travelled to the plant.

Bacteriology

The samples in transport medium were transferred to the laboratory in a cooler containing ice and stored overnight at 4°C. Portions of these fecal and rumen content suspensions were taken for each of the 3 microbiological procedures outlined below.

For detection of verotoxins, fecal suspensions were inoculated into modified trypticase soy broth (mTSB) and incubated for 18 h to 24 h at 42°C. Culture supernatants were prepared and tested in the Vero cell cytotoxicity assay (VCA), incorporating verotoxin neutralization (31). The cytotoxicity was termed positive if it was neutralized by a prepared combination of anti-VT1 and anti-VT2 polyclonal antisera.

For detection of *E. coli* 0157:H7, fecal and rumen content suspensions were 1) plated directly on cefixime tellurite sorbitol MacConkey's agar (CT-SMAC (32)), consisting of sorbitol MacConkey's agar containing 2.5 mg/L of potassium tellurite and 0.05 mg/L of cefixime, and 2) placed in mTSB and incubated at 42°C for 6 h. Immunomagnetic separation (IMS) was performed on these 6-hour broth cultures by using magnetic polystyrene beads coated with 0157-antibody (Dynabeads anti-*E. coli* 0157, Dynal, Oslo, Norway), according to the manufacturer's instructions. Following IMS, a 100-µL aliquot of the bead suspension was plated onto CT-SMAC agar and incubated at 37°C overnight. Five sorbitol-negative colonies were selected at random and tested in the slide agglutination assay using anti-0157 antisera (Difco, Detroit, Michigan, USA). Agglutination-positive isolates were tested in the VCA, and toxin-positive isolates were confirmed as *E. coli* 0157:H7 by biochemical testing and standard serotyping procedures at the Reference Laboratory of the Health of Animals Laboratory, Health Canada, Guelph, Ontario.

Salmonella were isolated by using the following procedures. Feces and ruminal content samples were 1) directly inoculated into modified semisolid Rappaport Vassiliadis (MSRV) medium (33), and 2) inoculated into tetrathionate broth. Inoculated MSRV medium plates were incubated at 42°C for 24 h. Tetrathionate cultures were incubated at 42°C for 48 h and then subcultured to Rappaport Vassiliadis broth, which was incubated at 37°C for 24 h. The Rappaport Vassiliadis broth culture was then subcultured onto brilliant green sulfa agar and xylose-lysine-tergitol agar (34). The latter plating media were incubated at 37°C for 24 h. Suspect *Salmonella* were biochemically tested and serotyped at the Health of Animals Laboratory.

Statistical methods

Data were entered into a database in a statistical analysis program (Statistix for Windows, 1996, Analytical Software, Tallahassee, Florida, USA). Prevalence was determined on an individual basis (percent positive fecal samples) and on a lot basis (percent positive samples within a lot). Confidence intervals (CI) of a proportion (prevalence) were estimated using the Fleiss quadratic approximation method, because this approximate CI is valid even when P is near 0 or unity (Epi Info, Version 6, 1995, Centers for Disease Control and Prevention, Atlanta, Georgia, USA). No adjustments were made for clustering, since no widely accepted means were identified to adjust asymmetrical CI for cluster effects. A lot was considered positive when bacteria were identified in at least 1 fecal sample from the lot (% positive lots). A variable for season was created using the following sampling dates: 1) fall: September, October, November; 2) winter: December, January, February; 3) spring: March, April, May; and 4) summer: June, July and August. Individual and lot level associations between the prevalence of bacteria in the feces and factors, such as age (yearling cattle, cull cow), rumen fill, body condition score, source (feedlot, farm/ranch, auction), type (dairy, beef), sex, season, and distance travelled to the plant were assessed by using the chi-square test, Fisher's exact test, and Krushkal-Wallis ANOVA test. Since the fecal prevalence of bacteria differed between yearling cattle and cull cows, the associations between fecal prevalence and the other factors described above were assessed separately for each age group. The P value for significance of all associations was set at 0.05.

Results

Twelve hundred and forty-seven fecal samples were collected from 293 lots of cattle from 194 owners at processing during 12 consecutive months (Tables 1 and 2). Verotoxins were detected in 42.6% (95% CI, 39.8% to 45.4%) of the fecal samples. The prevalence of *E. coli* 0157:H7 was lower on direct culture than on IMS-enrichment culture. On direct culture, *E. coli* 0157:H7 were detected in 2.6% (95% CI, 1.9% to 3.7%) of the

Table 1. Description of cattle from which fecal samples were collected

Descriptor	Yearling cattle	Cull cows
Number sampled	654	593
Sex		
Male	62%	0%
Female	38%	100%
Type		
Beef	100%	88%
Dairy	0%	12%
Source		
Feedlot	91%	17%
Farm/ranch	7%	27%
Auction	2%	56%
Body condition score		
1	0.6%	25.9%
2	2.3%	17.0%
3	31.7%	25.1%
4	51.0%	23.4%
5	14.4%	8.6%
Rumen fill		
0	0.9%	15.0%
1	14.2%	29.2%
2	51.0%	34.6%
3	25.8%	17.2%
4	8.1%	4.0%

fecal samples, and on IMS-enrichment culture, *E. coli* 0157:H7 were detected in 7.5% (95% CI, 6.1% to 9.1%) of the samples. Verotoxin-positive fecal samples were not indicative of *E. coli* 0157:H7 positive samples. Only 11% of the samples that were test positive for verotoxin were also test positive for *E. coli* 0157:H7. There was only 1 isolation (0.08%) of *Salmonella* from the feces (95% CI, 0.004% to 0.5%). It was found in the feces of a yearling animal during the summer, and it was typed as *Salmonella typhimurium* phage type 104.

The median within lot prevalence was 40% for verotoxins and 0% for *E. coli* 0157:H7 and *Salmonella*. Seventy-nine percent of the lots of cattle had at least 1 positive verotoxin culture, and 19.5% of the lots had at least 1 positive *E. coli* 0157:H7 culture.

The prevalence of verotoxins was higher in cull cows than in yearling cattle, yet the within-lot prevalence and percentage of positive lots were similar. The individual prevalence and percentage of positive lots of *E. coli* 0157:H7 were higher in yearling cattle than in cull cows, but the within-lot prevalence was similar. There was no difference in the prevalence of *Salmonella* between yearling cattle and cull cows.

In yearling cattle, verotoxins were not associated with rumen fill, season, source, sex (steer, heifer), body condition score, or distance travelled. In cull cows, the prevalence of verotoxins was associated with the source of the cattle. The prevalence of verotoxins in fecal samples was higher in cows from the auction market (52%) and farm or ranch (47%) and lower in cows from the feedlot (31%).

The prevalence of *E. coli* 0157:H7 was significantly higher in the summer than in the other 3 seasons (Figure 1). The prevalence of *E. coli* 0157:H7 in fecal samples

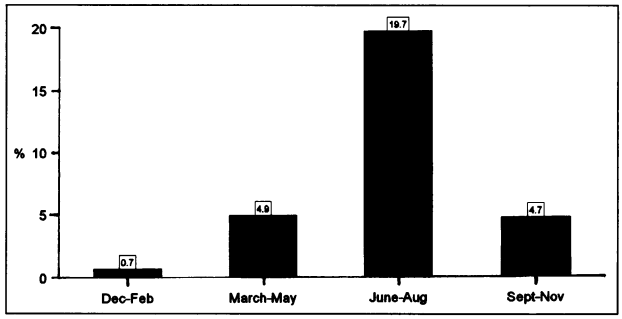

Figure 1. Prevalence of *Escherichia coli* 0157:H7 in fecal samples from yearling cattle and cull cows at slaughter in Alberta by season.

was 19.7% (95% CI, 15.3% to 24.3%) in the summer, 4.7% (95% CI, 2.6% to 7.6%) in the fall, 0.7% (95% CI, 0.09% to 2.6%) in the winter, and 4.9% (95% CI, 2.9% to 7.7%) in the spring. The percent positive lots was 39.4% (95% CI, 28.0% to 51.7%) in the summer, 17.1% (95% CI, 9.4% to 27.5%) in the fall, 3.3% (95% CI, 0.4% to 11.3%) in the winter, and 16.5% (9.3% to 26.1%) in the spring.

The individual and lot prevalences of *E. coli* 0157:H7 were not associated ($P > 0.05$) with rumen fill, body condition score, source, sex, type (beef, dairy), or distance travelled.

During the study, a total of 373 rumen samples were collected from 223 yearling cattle and 150 cull cows. The prevalence in the rumen of verotoxins was 6.4% (95% CI, 4.2% to 9.4%), *E. coli* 0157:H7 was 0.8% (95% CI, 0.2% to 2.3%), and *Salmonella* was 0.3% (95% CI, 0.007% to 1.5%). There was one isolate of *Salmonella*, and it was typed as *Salmonella thompson*. The rumen prevalence rates were similar in yearling cattle and cull cows (data not presented).

Discussion

Verotoxins were frequently found in the feces of yearling cattle and cull cows at processing, suggesting, but not proving, that VTEC was also frequently identified in these cattle. In this study, Vero cell cytotoxicity assays incorporating verotoxin neutralization were used as indicators for the detection of verotoxin *E. coli* (VTEC), but were not provers that VTEC were present. Samples were not subsequently cultured and characterized to confirm the isolation of VTEC. Verotoxins were frequently detected in these cattle, supporting the findings of previous studies on VTEC (6,11,24,28,29). While verotoxins were common, they were a poor indicator of positive *E. coli* 0157:H7 fecal samples, since only 11% of the verotoxin-positive fecal samples were also test positive for *E. coli* 0157:H7.

The prevalence of *E. coli* 0157:H7 in slaughter cattle reported here (Table 2) falls near or within the 95% CI of prevalence estimates from 3 recent studies conducted in cattle at slaughter. Four percent (95% CI, 1.1% to 9.6%) of the fecal samples from cull dairy cattle in herds in the states of Idaho, Oregon, and Washington tested positive for *E. coli* 0157 in the feces at slaughter (5). A 1-year study in England found *E. coli* 0157 in the feces of 13.4% (95% CI, 11.8% to 15.0%) of prime

Table 2. Prevalence of verotoxins, *Escherichia coli* 0157:H7 by direct culture and IMS-enrichment, and *Salmonella* in the feces of cattle at processing in western Canada from September 1995 to August 1996

	Verotoxins	*E. coli* 0157:H7 (direct culture)	*E. coli* 0157:H7 (IMS-enrichment)	*Salmonella*
All cattle				
— individual (%)	531/1247 (42.6%)	33/1247 (2.6%)	93/1247 (7.5%)	1/1247 (0.08%)
(95% CI)	(39.8% to 45.4%)	(1.9% to 3.7%)	(6.1% to 9.1%)	(0.004% to 0.5%)
— % positive lots[a]	232/293 (79.2%)	23/293 (7.8%)	57/293 (19.5%)	1/293 (0.3%)
(95% CI)	(74.0% to 83.6%)	(5.1% to 11.7%)	(15.2% to 24.6%)	(0.02% to 2.2%)
— within-lot[b]	40% (0%, 100%)	0% (0%, 80%)	0% (0%, 100%)	0% (0%, 17%)
Yearling cattle				
— individual (%)	250/654 (38.2%)	32/654 (4.9%)	81/654 (12.4%)	1/654 (0.2%)
(95% CI)	(34.5% to 42.1%)	(3.4% to 6.9%)	(10.0% to 15.2%)	(0.008% to 1.0%)
— % positive lots[a]	115/143 (80.4%)	22/143 (15.4%)	48/143 (33.6%)	1/143 (0.7%)
(95% CI)	(72.8% to 86.4%)	(10.1% to 22.6%)	(26.0% to 42.0%)	(0.04% to 4.4%)
— within-lot[b]	40% (0%, 100%)	0% (0%, 80%)	0% (0%, 100%)	0% (0%, 17%)
Cull cows				
— individual (%)	281/593 (47.4%)	1/593 (0.2%)	12/593 (2.0%)	0/593 (0%)
(95% CI)	(43.3% to 51.5%)	(0.009% to 1.1%)	(1.1% to 3.6%)	(0% to 0.8%)
— % positive lots[a]	117/150 (78.0%)	1/150 (0.7%)	9/150 (6.0%)	0/150 (0%)
(95% CI)	(70.4% to 84.2%)	(0.03% to 4.2%)	(3.0% to 11.4%)	(0% to 3.1%)
— within-lot[b]	50% (0%, 100%)	0% (0%, 50%)	0% (0%, 100%)	0% (0%, 0%)

[a]at least 1 positive fecal culture in a lot
[b]median prevalence within lots of processed cattle (range)

beef animals and 16.1% (95% CI 14.4% to 17.9%) of cull dairy animals at slaughter (7). In cattle arriving for slaughter in Italy, 3.6% (95% CI, 2.0% to 5.8%) of the feedlot cattle were test positive for *E. coli* 0157 in the feces (4).

Salmonella were rarely found in our study, suggesting that they were uncommon fecal bacteria in these cattle. A recent study in cull dairy cows at slaughter in New York found *Salmonella* in 0% of the fecal samples, 13.8% of the hide-surface sponge samples, and 1.2% of the carcass samples (23). The one fecal isolate in our study was *S. typhimurium* phage type 104; this serotype has been reported with increasing frequency around the world (35).

Caution must be exercised when making direct comparisons among prevalence estimates of bacteria from various studies. There are differences in culture techniques, including a large variability in the sensitivity of tests (36). Direct culture is not as sensitive as IMS-enrichment culture, as shown in Table 1. Additionally, the number, frequency, and timing of sampling (on-farm, slaughter); the handling, transport and storage of samples; the type and age of cattle; the type of sample (fecal pat, fecal swab, weight of fresh feces); the season of sampling; the unit of analysis (individual, herd, process lot); and the serotype of bacteria may affect prevalence estimates.

The highest occurrence of these bacteria in the feces during the summer months (Figure 1) supports results from previous research (1,2,12), suggesting that ecological and management factors may influence their shedding. *Escherichia coli* 0157:H7 has been cultured or been shown to grow in water, feed, and soil (1,15,20,21), and it may survive in feces for prolonged periods, depending on temperature and moisture conditions (13). Thus, environmental factors may partly explain the seasonal fluctuations.

Verotoxin-positive samples were not associated with season, rumen fill, body condition score, distance travelled, and sex and type of cattle, suggesting widespread distribution in all cattle. In cull cows, an association was found between the prevalence of verotoxins and the source of the cows. The prevalence of verotoxins was higher in cows that came from the auction market and farm or ranch compared with cows that came from the feedlot. This association might be explained by differences in management, environment, and diet. Alternatively, this association may have been due to chance, because of the multiple comparisons made between the prevalence estimates and potential explanatory factors.

The higher prevalence of *E. coli* 0157:H7 in the feces of yearling cattle compared with cull cows supports findings of previous studies that showed a higher prevalence in younger animals (1,12). Differences in the prevalence of *E. coli* 0157:H7 in these 2 age groups of cattle may also reflect differences in management and nutrition (1). It is not known if immunity is a factor, since limited studies have found a poor association among serological titers, shedding, and reinfection (8,37). Management practices that have been tentatively, but not consistently, associated with the fecal prevalence of *E. coli* 0157 include herd size, grouping, weaning, manure management, equipment sanitation (including water troughs), feed composition, feed additives (by products, ionophores), and parenteral antibiotics (1,9,10,15,19–22,27).

Previously, researchers have suggested that fasting cattle for 24 h to 120 h will result in higher levels of fecal shedding of *E. coli* 0157:H7 and *Salmonella* (14,16). Failure to find an association between *E. coli* 0157:H7 and rumen fill may be due to the inaccuracy of rumen fill as a measure of fasting. Rumen fill could not be substantiated in this study as a surrogate measure of fasting

by the distance travelled to the plant, because the distance travelled to the plant was not a measure of the time from the farm to slaughter, and we did not record holding time at the plant to determine the time from the farm to slaughter. Lack of variability in rumen fill would explain a null association; however, there was variability in rumen fill (Table 1). A recent study conducted in sheep (15) failed to find increased shedding of *E. coli* 0157:H7 with a 24-hour fast. The average time from shipment to slaughter in cattle at this plant was approximately 7 h, ranging from 2 h to 23 h (unpublished observations). Under most commercial situations in western Canada, cattle are shipped and slaughtered within a 24-hour time frame; thus, fasting may not be a risk factor of practical significance.

The prevalence of verotoxins, *E. coli* 0157:H7 and *Salmonella* in the rumen were low, suggesting the rumen content may not be a major source of carcass contamination of these bacteria. However, other studies have found a higher prevalence of *Salmonella* in the rumen content, at rates of 36% (16) and 37% (26). Differences in prevalence estimates among studies may be due to those factors previously described, and may also be affected by the year in which the study was conducted and the geographical location of the study.

One limitation of this study is its small sample size, which affects the reliability of prevalence estimates. When data were stratified by age, this effectively reduced the sample size and, therefore, the precision of the prevalence estimates, as illustrated by the wide 95% CI (Table 2). In yearling cattle, samples were not collected by a simple random method, but stratified by lot and then randomly sampled within lot. Failure to adjust CI in yearling cattle for clustering may result in wider CI, but this is partially offset by the stratification, which tends to narrow the CI (variance).

Another potential limitation of this study is bias. The prevalence estimates may have been slightly biased, because lots of yearling cattle were chosen conveniently, rather than randomly. In yearling cattle, a fixed number of samples per lot were collected and the number sampled per lot was small and not weighted on lot size. This may have reduced the reliability of the estimate and biased downward the within lot prevalence, and it may have caused misclassification bias by reducing the detection of positive lots (lot sensitivity). However, the size of the lot was not statistically associated with the within-lot prevalence or the percentage of positive lots. Bias may also have resulted from failure to change latex gloves between each sample collected in a cull cow lot. Occasionally, the number of cows in a lot was small and the line speed was too fast to change gloves between each animal sampled within a lot. This could have inflated the lot prevalence, if cross contamination occurred. However, sterile spoons were used between each animal, so the risk of cross-contamination was most likely low.

Although these factors may have slightly affected the validity and reliability of the prevalence estimates reported here, the results of this study do support those of previous research, namely, that both the feces and rumen content of cattle at slaughter are sources of verotoxins, *E. coli* 0157:H7, and *Salmonella*. Thus, processing procedures must continue to reduce both fecal and rumen content contamination of carcasses, and other methods should also be pursued to minimize the presence of these bacteria at various stages of production and processing. This study suggests that fasting may not be an important risk factor for increased fecal shedding of *E. coli* 0157:H7 in slaughter cattle under existing commercial transport and processing conditions in western Canada.

Acknowledgments

We thank Suneeta D'Souza, Darcy Goncii, Robin King and Craig Pienkowski from the Animal Diseases Research Institute in Lethbridge for their help in sample collection and bacteriology. Roger Johnson, Cornelius Poppe, Kris Rahn, Susan Read, and Pascal Michel of the Health of Animals Laboratory in Guelph are acknowledged for their assistance in bacteriology methods, serotyping of isolates, and manuscript review. The Management Committee of the Canadian Cattlemen — Quality Starts Here Program is gratefully acknowledged for supporting this on-farm food safety research. CVJ

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