Prevalence and risk factor investigation of *Campylobacter* species in beef cattle feces from seven large commercial feedlots in Alberta, Canada

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Abstract

This fecal prevalence study targeted cattle from 7 large (10 000 to > 40 000 head) commercial feedlots in Alberta as a means of establishing *Campylobacter* levels in cattle just prior to animals entering the food chain. Overall, 87% [95% confidence interval (CI) = 86–88] of 2776 fresh pen-floor fecal samples were culture positive for *Campylobacter* species, with prevalences ranging from 76% to 95% among the 7 feedlots. *Campylobacter* spp. prevalence was 88% (95% CI = 86–90) in the summer (n = 1376) and 86% (95% CI = 85–88) in the winter (n = 1400). In addition, 69% (95% CI = 66–71) of 1486 *Campylobacter* spp. positive samples were identified as *Campylobacter jejuni* using hippurate hydrolysis testing. Of those, 64% (95% CI = 58–70) of 277 and 70% (95% CI = 67–72) of 1209 *Campylobacter* isolates were identified as *C. jejuni* in winter and summer, respectively. After accounting for clustering within pen and feedlot, feedlot size and the number of days on feed were associated with *Campylobacter* spp. isolation rates. The high isolation rates of *Campylobacter* spp. and *C. jejuni* in feedlot cattle feces in this study suggest a potential role for feedlot cattle in the complex epidemiology of campylobacters in Alberta.

Résumé

Cette étude sur la prévalence fécale ciblait les bovins de 7 grands parcs d'engraissement commerciaux en Alberta (10 000 à > 40 000 têtes) afin d'obtenir des données sur les niveaux de Campylobacter *chez les bovins tout juste avant leur entrée dans la chaîne alimentaire. De manière globale, 87 % [intervalle de confiance 95 % (CI) = 86–88] des 2776 échantillons fécaux frais prélevés sur les planchers se sont avérés positifs pour la présence de* Campylobacter, *avec des prévalences variant de 76 % à 95 % entre les 7 parcs. La prévalence de* Campylobacter *spp. était de 88 % (95 % CI = 86–90) durant l'été (*n = *1376) et 86 % (95 % CI = 85–88) durant l'hiver (*n = *1400). De plus, 69 % (95 % CI = 66–71) des 1486 échantillons positifs pour* Campylobacter *spp. ont été identifiés comme étant* Campylobacter jejuni *à l'aide du test d'hydrolyse de l'hippurate. Parmi ceux-ci, 64 % (95 % CI = 58–70) de 277 et 70 % (95 % CI = 67–72) de 1209 isolats de* Campylobacter *ont été identifiés comme* C. jejuni, *respectivement en hiver et en été. Après avoir pris en considération l'agrégation à l'intérieur d'un enclos et d'un parc, la taille du parc d'engraissement et le nombre de jours en hébergement ont été associés avec les taux d'isolement de* Campylobacter *spp. Les taux d'isolement élevés de* Campylobacter *spp. et de* C. jejuni *dans les fèces des bovins d'embouche obtenus dans la présente étude suggèrent un rôle potentiel pour les bovins d'embouche dans l'épidémiologie complexe des campylobacters en Alberta.*

(Traduit par Docteur Serge Messier)

Introduction

Thermophilic *Campylobacter* species are important targets for veterinary and public health research because of their zoonotic potential, large range of reservoir hosts, and environmental persistence (survivability in water, for example). *Campylobacter jejuni (C. jejuni)* and *Campylobacter coli (C. coli)* cause most of the human cases of illness, of which *C. jejuni* is responsible for 80% to 85% (1). Transmission to people may be through the ingestion of contaminated food or undercooked meat, water, or raw milk (2). In Alberta, campylobacteriosis is the most common notifiable bacterial enteric disease with 36.1 cases per 100 000 people (2005 data) (3,4).

Thermophilic *Campylobacter* species are commonly isolated from poultry and livestock species such as cattle, swine, and sheep, with poultry generally recognized as the primary source of infection for people (2). Cattle have been identified as a source of infection for people and have been implicated in the environmental spread of campylobacters to water (5,6). In addition, *C. jejuni* clonal isolates have been found in cattle, wild birds, insects, and rodents on the same farm (7), and bovine strains have been found capable of colonizing poultry (8). The role of cattle in the epidemiology of campylobacters is not fully known and likely a complex web of transmission between people, poultry, cattle, other livestock species, wild reservoir hosts, and the environment exists.

In cattle, *C. jejuni* colonizes the proximal small intestine and may be found in digesta throughout the intestinal tract (9). In Alberta feedlots, fecal *Campylobacter* spp. prevalences in cattle range from 46% to 100% using culture or polymerase chain reaction (PCR)

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methodology (10–12), and 32% to 62% for *C. jejuni* using biochemical or molecular identification techniques (10–14).

Few risk factors for *Campylobacter* spp. shedding in beef cattle have been identified (15,16). In an Irish longitudinal study, shedding of *Campylobacter* spp. in feedlot cattle was associated with the pen involved, environmental contamination of pen dividing bars and water troughs, and the month of sampling (16). Distinct seasonal trends in *Campylobacter* spp. prevalence have been reported in human and poultry studies, although the exact timing of the peaks varies among countries (17,18). In cattle, the effect of season on *Campylobacter* spp. shedding has not been as definitive. A targeted United Kingdom study sampled beef cattle at slaughter and dairy herds over a 2-year period and found seasonal differences in *Campylobacter* spp. prevalence in dairy cattle but not beef cattle (19).

This paper reports the results of a fecal survey in cattle from 7 large commercial feedlots in Alberta. The goals of this project were 1) to obtain isolation rates of *Campylobacter* spp. and *C. jejuni* in cattle feces prior to the animals entering the food chain, 2) to obtain isolation rates of *Campylobacter* spp. and *C. jejuni* in cattle feces in summer and winter as indicators of seasonal fluctuation, 3) to conduct preliminary risk factor analyses based on *Campylobacter* spp. isolation rates in feces after adjusting for clustering at pen and feedlot levels, and 4) to collect *C. jejuni* isolates for molecular characterization (20).

Materials and methods

Sample size calculation

For a survey using simple random sampling, 22 samples would have been necessary to measure a 68% expected prevalence of *C. jejuni* (16) with 20% precision and 95% confidence (Epi-Info, version 3.01, 2003; Centers for Disease Control and Prevention, Atlanta, Georgia, USA). After applying an inflation factor formula (21) to account for clustering of the expected frequency of *Campylobacter* within pens, the survey required 7 feedlots, assuming an intraclass correlation coefficient (ICC) of 0.3, the unadjusted sample size of 22, collection from 40 pens per feedlot, and 1 sample per pen. An ICC for clustering in *C. jejuni* in cattle feces was not available from previous publications, and the choice of 0.3 was a slightly more conservative estimate than previously published ICCs for non-enteric cattle conditions (22). To assess within-pen variability, it was decided that 10 fecal pats per pen would be sampled for a total of 2800 fecal samples. Ethics approval for this project was received from both the University of Saskatchewan Biomedical Research and the University of Calgary Conjoint Health Research Ethics Boards.

Study animals and sampling protocol

The sampling target was feedlot cattle near the end of their feeding program. Animals sampled in this study were crossbred steers or heifers typical of the beef herds in western Canada (British Columbia, Alberta, Saskatchewan, and Manitoba). Geographically, feedlots were located within 4 Alberta regional health authorities: RHA1 (Chinook, 4 feedlots), RHA2 (Palliser, 1 feedlot), RHA3 (Calgary, 1 feedlot), and RHA5 (East Central, 1 feedlot). Seven commercial Alberta feedlots (a non-random sample) agreed to participate. Four feedlots had 1-time capacities of 10 000 to 19 999 cattle, 2 had capacities between 20 000 and 39 999 head, and 1 had a capacity of ≥ 40 000 head. Each feedlot was visited twice, once in winter (January 17 to February 1, 2005) and once in summer (August 22 to September 13, 2005). Cattle were kept in open-air, dirt floor pens with 20% porosity wood fences. The number of animals ranged from 14 to 538 head per pen, with 70% of pens containing 83 to 300 head.

Upon arrival to feedlots, cattle were processed as per the standard practices of the feedlot based on the age class, gender, weight, and health risk category of the animal. This may have included recording body weight, individual animal identification (such as, ear tagging), administration of ear implants, castration of bulls, induction of abortion in heifers, parasiticide treatment (topical avermectin, for example), and vaccination against agents such as infectious bovine rhinotracheitis virus, parainfluenza-3 virus, bovine viral diarrhea virus, bovine respiratory syncytial virus, *Mannheimia haemolytica, Clostridia* spp. or *Histophilus somni.* In addition, injectable metaphylactic antimicrobials may have been administered at processing depending on the assigned health risk category of each group of animals. Individual animal injectable antimicrobial use data were not collected for this study.

For each sampling date, feedlot personnel identified the 20 pens closest to slaughter based on expected shipping dates. Within the feedlot industry, "on feed" refers to cattle confined within feedlot pens and fed ad libitum rations formulated to maximize growth prior to slaughter. While rations varied between feedlots, the "finishing" diets fed to cattle near slaughter weight generally contained ~80% carbohydrate sources (barley or other cereal grains), ~18% roughage sources (cereal or corn silage), and ~2% vitamin and mineral supplements (all as-fed). Ionophores (monensin) and antimicrobials (oxytetracycline, chlortetracycline, tylosin) fed for ≥ 7 d during the feeding period were documented by pen, and the average number of days pens of cattle were on feed was 150 (range: 38 to 462 d).

In this study, 85% of pens were sampled within 47 d of slaughter (range: 22 to 120 d). Pen weight estimates were supplied by feedlots and pen sizes (area) were supplied by feedlots or approximated at the time of sampling for calculation of pen density (# animals/m^2).

The first 10 fresh pen-floor fecal pats (steaming or observed defecation) were sampled in each pen using Starswab II (Starplex Scientific, Etobicoke, Ontario) charcoal transport media swabs. Efforts were made to avoid sampling multiple pats from the same animal. Each swab was inserted into 5 different locations within each fecal pat with care to avoid gross environmental contamination, and re-inserted into the charcoal transport media. Ten swabs from each pen were placed into a medium Ziploc bag (SC Johnson, Racine, Wisconsin, USA). The bags were placed into an insulated foil bag (KeepCool-GenericKC9, Winnipeg, Manitoba) with 4 frozen gel packs (Ice-Pak/Hot-Pak, Montreal, Quebec) with care to avoid placing the ice packs onto the swab tips. The insulated bag was then packaged into a cardboard box and shipped via courier (labelled as per International Air Transport Association regulations) to the Vaccine and Infectious Disease Organization (VIDO) in Saskatoon, Saskatchewan. A Hobo H08 Pro temperature monitor (Onset Computer Corporation, Pocasset, Massachusetts, USA) was included in each of the 14 shipments. Swabs were processed within

approximately 24 h of collection, with the exception of 1 shipment, which was processed at 48 h due to a weather related transport delay. Transport temperatures were monitored from 2 h after closure to 2 h before the shipment was opened.

Culture methodology

Each charcoal swab was streaked onto Karmali selective agar (CM935 with supplement SR0167E, Oxoid, Nepean, Ontario) and incubated microaerobically (85% N_2, 10% CO_2, 5% O_2) at 42°C for 48 h. Each incubation batch included a laboratory strain *C. jejuni* plate as a positive control. One colony considered positive for *Campylobacter* spp. from each sample (based on growth, color, and morphology of the colony, and color of the cell mass) was streaked onto a Karmali agar plate and incubated at 42°C (85% N_2,10% CO_2, 5% O_2) for a further 48 h.

Hippurate hydrolysis testing

Hippurate hydrolysis testing was used to identify *Campylobacter* colonies as *C. jejuni*. For the winter collection, 277 *Campylobacter* spp. positive isolates from 140 pens (approximately 2 samples per pen) and for the summer collection 1209 of 1210 *Campylobacter* positive isolates from 140 pens were tested for hippurate hydrolysis. A loopful of bacterial cells was emulsified in 100 μL of 1% aqueous sodium hippurate (Sigma-Aldrich H529, Oakville, Ontario) in a single well of a 96-well titer plate. After 2 h incubation at room temperature, 50 μL of ninhydrin solution (3.5% ninhydrin in a 1:1 mixture of acetone and butanol) was slowly added to each well and incubated at 37°C for 20 to 30 min. Purple color change indicated a positive reaction, whereas clear-to-yellow indicated a negative sample. Positive isolates were tentatively identified as *C. jejuni* and were frozen at -70°C in 25% glycerol for later molecular characterization.

Polymerase chain reaction testing

A subsample of study *Campylobacter* genomic DNA (104 isolates) was examined using multiplex PCR to assess the accuracy of culture and hippurate techniques. Isolates were randomly selected using a computer program (Microsoft Office Excel 2003; Microsoft Corporation, Redmond, Washington, USA) from all hippurate positive isolates after stratification by feedlot and season. Confirmation of isolates as *C. jejuni* was required for future molecular genotyping (20). Published PCR primers for *Campylobacter* spp. (23S rRNA), *C. jejuni (hipO)*, and *C. coli (glyA)* were used, with initial denaturation at 95°C for 30 s, annealing at 59°C for 30 s, with 2 extension steps at 30 s and 7 min at 72°C (23). Positive *C. jejuni* and *C. coli* controls were included in each PCR.

Data analysis

Of the 2800 fecal samples collected, 22 of the culture plates were overgrown with mold/bacteria and could not be read, and data were missing for 2 samples. The missing/unreadable data were distributed across 14 pens, and were excluded from analyses. All prevalence 95% confidence intervals (CI) were calculated using the binomial exact specification (Intercooled STATA 9.2; StataCorp LP, College Station, Texas, USA).

Factors affecting whether or not a fecal sample was positive for *Campylobacter* spp. using culture methodology were examined using mixed models with a binomial distribution and logit link function. Models were specified using restricted iterative generalized least square estimation and using second-order penalized quasi-likelihood estimates (MLwiN version 2.0; Centre for Multilevel Modeling, Institute of Education, London, England) (21). The strength of the association between outcome and exposure was reported as an odds ratio with 95% CI.

In the 1st stage of analysis, the absolute difference in prevalence among feedlots was investigated in a model with feedlot identifier as the only fixed effect. The potential lack of independence or clustering of samples within pens was accounted for using a pen random effect.

In the 2nd stage of the analysis, the importance of a series of feedlot- and pen-level risk factors for the occurrence of *Campylobacter* spp. was assessed after accounting for potential clustering of observations using random effects for both pen and feedlot (21). Continuous variables, including number of days on feed ($\leq$ 149, 150–299, or $\geq$ 300 d) for each pen, number of head per pen ($\leq$ 99, 100–199, 200–299, or $\geq$ 300 head), pen density ($<$ 0.1 or $\geq$ 0.1 animals/m^2), pen weight ($\leq$ 499, 500–599, $\geq$ 600 kg or missing), and feedlot size (10 000–19 999, 20 000–39 999, or $\geq$ 40 000 head), were categorized to assess the linearity of association between each factor and the log odds of the occurrence of *Campylobacter* spp. Categorical variables explored in analysis included pen feed treatment for 7 or more days during the feedlot stay (no feed treatment, monensin/tylosin, monensin/chlortetracycline/tylosin, or monensin/oxytetracycline), regional health authority (geographical location), gender (heifer, steer, or mixed pen), and season of sampling (winter or summer).

Risk factors were each sequentially examined in the null model containing only the random effects for pen and feedlot, and considered for inclusion in the final model if they were associated with the outcome in unconditional analysis at $P \leq 0.25$. Manual backwards stepwise analysis was used to achieve a final model containing statistically significant risk factors ($P \leq 0.05$) or variables that acted as important confounders (accounting for the variable resulted in $>$ 20% change in the measure of association). After establishing the final summary main-effect model, biologically reasonable 1st-order interaction terms were added, assessed for their association with the outcome, and reported if $P \leq 0.05$.

Model fit was evaluated by examining residuals and the impact of outliers. Variance components for both the final and null (constant only) models were approximated using latent variable calculation, which fixed error variance at $\pi^2/3$ (21). The feedlot-level variance components were calculated as: (feedlot variance ÷ total variance), and the pen-level variance components were calculated using: (pen variance ÷ total variance).

Results

Prevalence of *Campylobacter* spp. and *C. jejuni* in fecal samples and feedlot pens

The fecal prevalence of *Campylobacter* spp. in these 7 large commercial feedlots ranged from 76% to 95% by culture (Table I) with an overall study prevalence of 87% (95% CI = 86–88, 2420 of 2776 samples positive). In addition, 1020 of 1486 (69%, 95% CI = 66–71)

Table I. Overall, winter, and summer estimates for *Campylobacter* species[a] and *Campylobacter jejuni*[b] in feedlot cattle feces

	Overall			Winter			Summer		
Feedlot	Pos *(n)*	% Pos	95% CI[c]	Pos *(n)*	% Pos	95% CI[c]	Pos *(n)*	% Pos	95% CI[c]
Campylobacter species									
A	365 (396)	92	89–95	182 (200)	91	86–95	183 (196)	93	89–96
B	362 (400)	91	87–93	180 (200)	90	85–94	182 (200)	91	86–95
C	381 (400)	95	93–97	197 (200)	99	96–100	184 (200)	92	87–95
D	350 (400)	88	84–91	172 (200)	86	81–90	178 (200)	89	84–93
E	366 (400)	92	88–94	180 (200)	90	85–94	186 (200)	93	89–96
F	297 (393)	76	71–80	154 (200)	77	71–83	143 (193)	74	67–80
G	299 (387)	77	73–81	145 (200)	73	66–79	154 (187)	82	76–88
Total	2420 (2776)[d,e]	87	86–88	1210 (1400)	86	85–88	1210 (1376)[d,e]	88	86–90
Campylobacter jejuni									
A	163 (223)	73	67–79	24 (40)	60	43–75	139 (183)	76	43–75
B	148 (222)	67	60–73	24 (40)	60	43–75	124 (182)	68	69–82
C	144 (224)	64	58–71	28 (40)	70	53–83	116 (184)	63	56–70
D	156 (216)	72	66–78	25 (38)	66	49–80	131 (178)	74	66–80
E	159 (226)	70	64–76	24 (40)	60	43–75	135 (186)	73	66–79
F	136 (183)	74	67–80	28 (40)	70	53–83	108 (143)	76	68–82
G	114 (192)	59	52–66	24 (39)	62	45–77	90 (153)	59	51–67
H	1020 (1486)[f,g]	69	66–71	177 (277)[f]	64	58–70	843 (1209)[g]	70	67–72

Pos — positive.

CI — confidence interval.

[a] *Campylobacter* positive by culture.

[b] *Campylobacter* positive isolates identified as *C. jejuni* using hippurate hydrolysis testing.

[c] Binomial exact confidence interval.

[d] Sample not readable due to mold overgrowth ($n = 22$).

[e] Missing data ($n = 2$).

[f] Missing data ($n = 3$).

[g] Missing data ($n = 1$).

Campylobacter positive isolates were identified as *C. jejuni* using hippurate hydrolysis testing (Table I). All of the 280 pens sampled were positive for *Campylobacter* spp. based on culture, and 279 of 280 pens were positive for *C. jejuni* based on hippurate hydrolysis testing. In a model accounting for clustering of observations within pen, the prevalence of *Campylobacter* spp. positive samples was significantly different among feedlots (Wald χ^2 77.97, df 6, $P < 0.001$).

Comparison of sample and pen prevalence in summer and winter samples

Of the 1400 winter samples collected, 1210 were culture positive for *Campylobacter* spp. (86%, 95% CI = 85–88), as were 1210 of 1376 summer samples (88%, 95% CI = 86–90). Among feedlots, *Campylobacter* spp. prevalences ranged from 73% to 99% and 74% to 93% for winter and summer, respectively (Table I). In addition, 177 of 277 (64%, 95% CI = 57–70) winter and 843 of 1209 (70%, 95% CI = 67–72) summer *Campylobacter* spp. positive isolates were identified as *C. jejuni* based on hippurate hydrolysis testing. In summer, the prevalence of *C. jejuni* was estimated to be 61% (843 of 1376 fecal samples). Table I reports the number of *Campylobacter* isolates identified as *C. jejuni* in each of the 7 feedlots. In a model adjusting only for clustering within pen and feedlot, season was not associated with whether or not a sample was culture positive for *Campylobacter* spp. ($P = 0.40$). Transport temperatures ranged from -5.8°C to 17.5°C in the 7 winter shipments and 5.4°C to 22.8°C in the 7 summer shipments.

PCR of a subsample of *C. jejuni* isolates

In preparation for molecular characterization, 104 isolates, determined to be *C. jejuni* based on culture and hippurate hydrolysis testing, were evaluated using PCR. Isolates were selected randomly (Excel 2007; Microsoft Corporation) from all hippurate positive isolates ($n = 1486$) after stratification by feedlot and by season. One hundred of 104 samples (96%) were identified as *Campylobacter* spp. Of those 100, 68 samples contained *C. jejuni* DNA only, 6 contained *C. coli* DNA only, 17 contained both *C. jejuni* and *C. coli* DNA, and 9 contained *Campylobacter* spp. DNA not identified as either *C. jejuni* or *C. coli.*

Factors associated with *Campylobacter* spp. culture status of fecal samples

In a series of initial models accounting only for clustering by pen and feedlot, days on feed, feed treatment, feedlot size and regional health authority were unconditionally associated ($P \leq 0.25$) with the odds of a positive *Campylobacter* spp. culture (Table II).

Table II. Unconditional analyses of risk factors for whether a sample was positive for *Campylobacter* spp. by culture (adjusting for clustering within pen and feedlot), *n* = 2776

Variable	Level	Number of samples	Percent of samples *Campylobacter* positive (%)	Univariable *P*-value
Density (animal/m^2)	< 0.1[a]	2456	86.9	0.899
	≥ 0.1	320	89.1	
Days on feed (d)	≤ 149[a]	1830	89.5	0.004
	150–299	856	82.8	
	≥ 300	90	82.2	
Feed treatment[c]	None[a]	35	71.4	< 0.001
	Monensin/tylosin	1176	91.6	
	Monensin/ chlortetracycline/tylosin	765	77.1	
	Monensin/oxytetracycline	800	91.0	
Feedlot size (head capacity)	10 000–19 999[a]	1600	91.2	< 0.001
	20 000–39 999	780	76.4	
	$\geq 40\,000$	396	92.2	
Number of head per pen	≤ 99[a]	612	85.8	0.890
	100–199	896	86.7	
	200–299	879	86.9	
	≥ 300	389	91.0	
Regional health authority where feedlot located	5[a]	393	75.6	0.006
	3	387	77.3	
	2	396	91.2	
	1	1600	92.2	
Season of sampling	Winter[a]	1400	86.4	0.401
	Summer	1376	87.9	
Gender	Steer[a]	1828	88.7	0.342
	Heifer	859	85.2	
	Mixed	89	74.2	
Weight (kg)	≤ 499[a]	150	79.3	0.619
	500–599	941	86.9	
	≥ 600	1285	86.8	
	Missing[b]	400	92.0	

[a] Referent category.

[b] Data missing from 2 feedlots for the summer sampling (40 pens).

[c] Feed treatments for ≥ 7 d during the feeding period.

In the final multivariable model accounting for clustering by pen and feedlot, both days on feed, and feedlot size were associated ($P \leq 0.05$) with the odds of positive culture, while regional health authority (geographical location) and feed treatment were not (Table III). No evidence of interaction or confounding was identified. The pen-level variance in the null mixed model was estimated to be 0.22 (22%) and the feedlot-level variance was 0.12 (12%). In the final mixed model, the pen-level variance reduced to 0.13 (13%), and the feedlot-level variance reduced to 0.03 (3%).

After adjusting for feedlot size, the odds of a sample (within the same pen and feedlot) testing positive for *Campylobacter* spp. was 1.59 × greater ($P = 0.003$) among animals that had been in the feedlot for < 150 d than it was for animals that had been in the feedlot for 150–299 d, and 2.13 × greater ($P = 0.054$) in animals on feed for < 150 d than it was for those animals that had been in the feedlot for > 300 d. After accounting for days on feed, the odds of yielding a positive culture for *Campylobacter* spp. were 3.45 × greater ($P < 0.001$) in pens from smaller-sized feedlots (10 000 to 19 999 head) compared with pens from mid-sized feedlots (20 000 to 39 999 head), and not statistically different when pens from smaller-sized feedlots (10 000 to 19 999 head) were compared with pens from large feedlots (≥ 40 000 head) (Table III).

Table III. Final multivariable model (accounting for clustering within pen and feedlot) showing the association between risk factors and whether a fecal sample was positive for *Campylobacter* spp. by culture (2420 of 2776 positive, 280 pens, 7 feedlots)

Pen-level risk factors	OR[b]	95% CI	Specific *P*-values	Overall *P*-value
Days on feed (d)				0.005
	≤ 149[a]			
	150–299	0.63	0.46–0.86	0.003
	≥ 300	0.47	0.22–1.02	0.054
Feedlot size (head capacity)				< 0.001
	< 19 999[a]			
	20 000 to 39 999	0.29	0.15–0.53	< 0.001
	≥ 40 000	0.93	0.39–2.21	0.862

[a] Referent category.

[b] For ease of interpretation odds ratios have been inverted in results and discussion text (for example, 1/0.63 = 1.59).

CI — confidence interval; OR — odds ratio.

Discussion

The current study targeted cattle from large commercial feedlots as a means of establishing *Campylobacter* levels just prior to animals entering the food chain. The isolation rates of viable (culturable) *Campylobacter* spp. and *C. jejuni* yielded by this study were similar to or greater than those published for other commercial feedlot surveys in Alberta (10,13). The overall culture prevalence of *Campylobacter* spp. in cattle feces was 87% (95% CI = 86%–88%). Sixty-nine percent (95% CI = 66%–71%) of tested isolates were classified as *C. jejuni*, the most common cause of clinical campylobacteriosis in people (1). Among feedlots, *Campylobacter* species isolation rates in cattle feces varied from 75%–95%. Using mixed models, the feedlot sampled, the number of days cattle were on feed, and feedlot size were all associated with *Campylobacter* spp. isolation rates, while season of the year was not. Our data suggest that cattle sent to slaughter from these 7 feedlots would have contained large numbers of individuals shedding campylobacters, potentially relevant to food safety.

Several strategies were implemented to try and optimize recovery and maintain viability of campylobacters collected in this study. The extended survival of *Campylobacter* spp. in fecal pats on the ground may be limited due to exposure to air, drying, and extreme temperatures (24). Hoar et al (25) found statistically significant differences in *Campylobacter* spp. isolation rates from cattle rectal samples and fecal pats on the ground (5.0% and 0.5%, respectively). Research has also shown that *E. coli* shed in cattle feces may not be evenly distributed through fecal pats (26). As a result, we chose to sample only fresh and "steaming" fecal pats, each swab was inserted into 5 different areas of the pat at the time of collection, and samples were immersed in charcoal transport media to try and maximize *Campylobacter* recovery. In this study, the use of swabs for sampling fresh, pen-floor fecal pats was found to be a convenient, efficient, economically feasible and non-invasive collection technique.

While sampling fresh feces, protecting campylobacters in transport media, and plating samples within 24 h of collection may have supported *Campylobacter* recovery, some study design limitations should be considered. Our sampling strategy involved the use of swabs rather than obtaining larger amounts of feces from each fecal pat. It is possible that the collection of larger amounts of fecal matter may have resulted in increased recoveries of *Campylobacter* spp. and, therefore, higher prevalence estimates compared to swab sampling. The use of the hippurate hydrolysis test to identify *C. jejuni* may also have affected results. False positives and false negative results have been reported in the literature using this test, and some *C. jejuni* strains have been found to be hippuricase negative (27). Weak hippurate hydrolysis reactions were considered positive in this study to maximize the number of *C. jejuni* isolates available for later molecular characterization. Polymerase chain reaction testing of a subsample of isolates indicated that the hippurate hydrolysis test may have overestimated the prevalence of *C. jejuni* by misclassifying some *C. coli* isolates as *C. jejuni*. In addition, only one colony was selected for hippurate hydrolysis testing from each culture positive plate, which may have underestimated the prevalence of *C. jejuni* in the fecal samples. During the winter sampling, colonies from only 2 positive plates per pen were subjected to hippurate hydrolysis testing compared to the summer collection where the decision was made to test colonies from all *Campylobacter* spp. positive plates, resulting in wider confidence intervals for the winter prevalence estimates.

Feedlots entered this study based on willingness to participate (8 approached, 1 declined) and were approached based on large capacities and representation of a number of geographical areas within Alberta. It is possible that the non-random selection of feedlots could have affected results. Feedlots willing to participate might be different from other feedlots in the province in characteristics such as size, management practices, or use of veterinary services. *Campylobacter* spp. prevalences differed significantly among feedlots, which may be due to factors such as geography, environment (such as, temperature, precipitation), management (treatment protocols, cattle purchasing preferences), reservoir prevalence (flies, birds) or other unidentified factors. In this study, isolation rates for

campylobacters were greater in the smaller-sized feedlots compared to mid-sized, and the effect of size may reflect management factors between feedlots that were not evaluated in this study. Research into reasons for differences among feedlots should be pursued, and future risk factor studies should include a larger number of randomly selected feedlots to minimize potential bias and increase power in the study.

Care must be taken in interpreting seasonal point estimates as indicators of seasonal fluctuation. However, *Campylobacter* spp. isolates were obtained from all pens of cattle regardless of season, and summer and winter isolation rates were not statistically different. Longitudinal studies have shown that feedlot cattle shed *Campylobacter* spp. chronically (12,13,16), suggesting that within the feedlot it may be difficult for animals to clear these bacteria from their intestinal tracts. This may be due to high stocking densities in feedlot pens, presence of biofilm within pens, constant exposure to fresh feces [on pen boards, feed troughs, water troughs (16)], or stabilized *Campylobacter* populations related to finishing diets. Cattle have a relatively long lifespan compared to poultry and, as campylobacters seem able to adapt successfully to the ruminant digestive system, it may not be surprising that our summer and winter prevalence estimates were similar. Further, the persistence of *Campylobacter* spp. within reservoirs such as water sources, wild birds, and flies may help to propagate the animal-host-environment cycle.

Cattle may stay in feedlots for variable lengths of time depending on body weight at arrival and the goals of the respective feeding programs. *Campylobacter* spp. isolation rates were higher in pens of animals that had been in the feedlot for shorter periods of time. This finding may reflect animal or bacterial physiological factors, the effects of antimicrobials in feed, or the use of growth promotants. Although some longitudinal studies report chronic and rising carriage of *Campylobacter* spp. over time (10,12,13,16), Lefebvre et al (28) found a decreasing prevalence of *Campylobacter* spp. in a longitudinal study on the use of growth promotants in Canadian feedlot cattle.

As this research was designed at the pen-level, individual animal information on injectable antimicrobials was not collected, and it is possible that treatments at the individual animal level affected shedding of campylobacters. In addition, dosages of antimicrobials in feed were not accounted for in the analyses, and it is possible that the broad classifications used to compare feed treatments in this study may have masked true medicated feed differences. Future study designs should incorporate individual animal treatment data and specific feed dosages into feedlot cattle risk factor analyses.

The estimates of pen-level and feedlot-level proportions of variation in *Campylobacter* spp. sample status reported here may be used in future research for sample size calculations in multistage designs. The results suggest that the amount of clustering within feedlots is small, and is explained, to a large extent, by the variables in the risk factor model. Most of the clustering was found at the pen-level, and while some of this was explained by variables in the final model, inclusion of a greater number of pen-level risk factors is warranted in the future.

The risk of human exposure to *Campylobacter* spp. from cattle is not fully known. In addition to *C. jejuni*, other species of thermophilic campylobacters, including *C. coli*, *C. hyointestinalis*, and *C. lanienae* have been identified in cattle and may have implications for public health (2,10,29). Human cases of campylobacteriosis are usually sporadic, and outbreaks have been linked only rarely to direct or indirect contact with cattle or to the consumption of red meat (5,30). In North America, surveys of retail ground beef found less than 1% of packages culture positive for campylobacters (31,32,33), and a small survey of feedlot employees in Alberta did not find evidence of active *Campylobacter* shedding in the 60 participants (34). Worldwide, outbreaks of campylobacteriosis have been linked to water contamination (30), and the ability of these microbes to persist in a viable but non-culturable state, in biofilm, and in untreated water (24,35) may contribute to their potential as environmental pathogens.

Beef cattle in Alberta are an integral part of the landscape and economy, and exist in close proximity to people in many rural areas. The high prevalence levels found in this study suggest that a large proportion of feedlot cattle near slaughter weight may be shedding campylobacters, and that seasonal effects may be small. These findings may have important implications for food safety, public health, and environmental transmission of campylobacters in the province, and further investigations will be required to fully understand the role of cattle in the epidemiology of campylobacters in Alberta.

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