

Prevalence and Risk Factor Investigation of *Campylobacter* Species in Retail Ground Beef from Alberta, Canada

SHERRY J. HANNON,^{1*} G. DOUGLAS INGLIS,² BRENDA ALLAN,³ CHERYL WALDNER,¹
MARGARET L. RUSSELL,⁴ ANDREW POTTER,³ LORNE A. BABIUK³ and HUGH G.G. TOWNSEND^{1,3}

¹Dept. of Large Animal Clinical Sciences, Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Drive, Saskatoon, Saskatchewan, S7N 5B4, Canada; ²Agriculture and Agri-Food Canada (Lethbridge Research Centre), 5403–1st Ave. S, Lethbridge, Alberta, T1J 4B1, Canada; ³Vaccine and Infectious Disease Organization, 120 Veterinary Road, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 5E3, Canada; ⁴Dept. of Community Health Sciences, Faculty of Medicine, University of Calgary, 3330 Hospital Drive NW, Calgary, Alberta, T2N 4N1, Canada

ABSTRACT

Campylobacteriosis is the most commonly reported (notifiable) bacterial enteric disease in Alberta, Canada. The purpose of this study was to assess the prevalence of *Campylobacter* species in retail ground beef based on a survey of 60 stores (four supermarket chains, three cities) in southern Alberta. None of the 1,200 retail lean and regular ground beef packages were culture positive. Direct PCR results from a subset of samples (n = 142) indicated that 46% of packages tested were positive for *Campylobacter* DNA. By species, 14.8% (21/142), 26.8% (38/142) and 1.4% (2/142) of packages were PCR positive for *C. jejuni*, *C. coli* and *C. hyointestinalis* DNA, respectively. The presence of campylobacters varied depending on the dates of collection. However, type of package (regular or lean), whether the store cut/packaged poultry in the meat department, type of meat used as the beef source (market trim, coarse grind tubes or a combination of these), whether meat portions were previously frozen, and package weight were not associated with the odds of finding *Campylobacter* spp. DNA by use of PCR. The high levels of *Campylobacter* DNA in the beef suggest that breaks in food safety protocols within slaughter plants, processors or grocery stores could have potentially important public health repercussions.

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* Author for correspondence: Phone: 403.938.5151; Fax: 403.938.5175
Email: sherryh@feedtohealth.com

INTRODUCTION

In Alberta, Canada, campylobacteriosis is the most common bacterial enteric illness, with 36.1 cases per 100,000 people reported in 2005 (25, 27). *Campylobacter jejuni* (*C. jejuni*), the most frequently isolated species in human disease, is responsible for approximately 85% of all human *Campylobacter* infections (21). While consumption of contaminated poultry meat is generally considered the primary source of infection for people (14), other routes of transmission may exist. Similarity between human and domestic livestock *Campylobacter* isolates has been reported based on molecular typing studies. (6, 12, 18, 22).

In studies in Alberta feedlot cattle near the end of the feeding period, fecal prevalences for *Campylobacter* spp. and for *C. jejuni* have been estimated to be up to 87% and 61%, respectively (2, 11, 16). Other species of *Campylobacter* of potential public health importance, including *C. coli*, *C. fetus*, *C. hyointestinalis*, and *C. lanianae*, have also been isolated from cattle feces in Alberta (16, 17). However, research into the prevalence of *Campylobacter* spp. in retail ground beef in Alberta has been limited. In Edmonton, Alberta, a city in northern Alberta which was not part of the sampling area for our study, a recent retail ground beef survey reported no positive samples from the 100 packages tested (4). The prevalence of *Campylobacter* spp. in retail ground beef has ranged from 0–20% worldwide on the basis of culture and biochemical or molecular identification of species; however, commonly less than 5% of samples tested have identified campylobacters (4, 7, 28, 30).

The goals of this project were to assess the prevalence of *Campylobacter* spp. (in particular *C. jejuni*) and to investigate risk factors potentially associated with the presence of *Campylobacter* spp. in retail ground beef. This paper reports the results of a culture survey of retail ground beef ($n = 1,200$) and PCR of a subset of these ($n = 142$) from 60 retail grocers of four major chains in three cities in southern Alberta.

MATERIALS AND METHODS

Sample size calculation

For a survey using simple random sampling, 179 packages of ground beef would have been necessary to measure a 3% expected prevalence of *C. jejuni* (29) with 2.5% precision and 95% confidence (Epi-Info, version 3.01, CDC, USA, 2003). After application of an inflation factor formula (9) to account for clustering of the expected frequency of *Campylobacter* within retail stores, the survey required 1,200 packages from 60 stores (assuming an intraclass correlation coefficient (ICC) of 0.3, an unadjusted sample size of 179, and collection of 20 packages per store). An ICC describing clustering of *C. jejuni* within source was not available from previous publications; the choice of 0.3 was slightly more conservative than previously published ICCs for non-enteric cattle conditions (19).

Sampling protocol

The goal of sampling was to identify grocery chains likely to supply the largest sales volume of ground beef to consumers. Four chains with the highest numbers of retail stores from three cities in southern Alberta were identified, and a sampling frame of individual stores was compiled from telephone book white and yellow pages (chain name and pharmacy headings) and internet searches (chain name). Stratified random sampling (by city and by chain within city) ensured that meat samples were taken from all chains in all cities. Fifteen stores were sampled from chain 1, 22 from chain 2, 16 from chain 3 and seven from chain 4. Forty-six stores were sampled in city 1, six stores in city 2 and eight stores in city 3. Five packages per store per collection were randomly sampled from the 60 stores, using a hand-held randomization program (Handy Randy, Stevens Creek Software, Cupertino, CA, USA), for a total of 1,200 retail packages of regular or lean ground beef. Three hundred packages were purchased during each of four collection periods: two winter (Nov. 21–23, 2004, and Jan. 9–11, 2005) and two summer (May 30–31, June 1, 2005 and July 18–20, 2005). After purchase,

each package of ground beef was placed into a pre-labeled Ziploc bag (SC Johnson, Racine, WI, USA) and then packed into a cooler (The Coleman Company Inc., 5286B, Wichita, KS, USA) with six ice packs (Ice-Pak/Hot-Pak, Montreal, QC, Canada). A Hobo H08 Pro temperature monitor (Onset Computer Corporation, Pocasset, MA, USA) was included in one cooler from each of the 12 meat shipments. Each cooler was sealed and shipped to the Vaccine and Infectious Disease Organization (VIDO, Saskatoon, SK, Canada) by bus (Greyhound Transport Canada Corporation) overnight. Ground beef packages were processed within approximately 24 hours of collection. Transport temperature ranges were evaluated from two hours after closure to two hours before the cooler was opened.

Employees knowledgeable about in-store meat practices were identified by phone inquiry or observed directly working with meat, and were asked questions regarding their meat department practices. Information on the cutting and packaging of raw poultry, the type of meat used to produce the ground beef (coarse tubes, market trim or both) and whether the ground beef contained meat that had previously been frozen were collected.

Experimental inoculation of retail ground beef as sensitivity analysis

A pure culture of *C. jejuni* (NCTC 11168) that had been previously suspended in 25% glycerol/50% Brain Heart Infusion broth and frozen to -70°C was used as the source strain for this experiment. The culture was thawed on ice and plated on a Mueller-Hinton agar plate. The plate was then incubated microaerobically (85% N_2 , 10% CO_2 , 5% O_2) at 42°C for 48 hours and checked to ensure the culture was pure by use of the Gram stain. The culture was then suspended in 0.85% NaCl (normal saline) to an absorbance of 0.5 at 600_{nm} (Ultrospec[®] 3000, Pharmacia Biotech) to form a 10^9 colony forming units (CFU)/ml solution. To create the final 1×10^4 , 1×10^3 , 1×10^2 , or 1×10^1 CFU/g dilutions, *C. jejuni* stock solution was further diluted with normal saline to a total volume of 1 ml, which was added

TABLE 1. *Campylobacter* spp. in retail ground beef (n = 142) based on PCR

Identification	Positive (%)
Genus:	
<i>Campylobacter</i> spp.	65 (45.8)
Species ^{a,b}	
<i>C. jejuni</i> only	20 (14.1)
<i>C. coli</i> only	35 (24.6)
<i>C. jejuni</i> and <i>C. coli</i>	1 (0.7)
<i>C. coli</i> and <i>C. hyointestinalis</i>	2 (1.4)

^aseven isolates could not be identified to the species level.

^bzero samples tested positive for DNA of *C. fetus*, *C. lanienae*, *C. concisus* or *C. upsaliensis*.

with each meat sample to the enrichment broth.

For each package of fresh retail ground beef, the plastic wrap over the middle was sliced with a sterile scalpel blade. A deep core sample of 25 g (24–26 g) of raw ground beef was removed with a sterile spoon. Each ground beef sample was placed into a 55-ounce Whirl Pak bag (82007-726, VWR International, Mississauga, ON, Canada) with 1 ml of *C. jejuni* solution and 100 ml of enrichment broth (Bolton broth (# CM0983 Oxoid Ltd., Basingstoke, UK) and 5% horse blood mixture) and mixed thoroughly for 30 seconds (Stomacher Lab Blender 400). The homogenate was then incubated (85% N₂, 10% CO₂, 5% O₂) for 44 hours at 42°C and then streaked onto Karmali selective agar (Oxoid, CM935 with supplement SR0167E, Nepean, ON, Canada) and microaerobically incubated for 48–72 hours. Each culture plate was then examined visually for colonies characteristic of *Campylobacter* spp. (based on growth, color and morphology of the colony, and color of the cell mass).

Ground beef packages were not tested for campylobacters prior to inoculation. Five packages of retail ground beef were tested at each concentration (1×10^4 , 1×10^3 , 1×10^2 , or 1×10^1 CFU/g), and the experiment was repeated on two separate days. Each incubation of test plates included both a negative control plate and a laboratory strain *C. jejuni* plate as positive control. These

experiments were conducted to document our ability to consistently recover *C. jejuni* from ground beef by use of our culture protocol.

Study protocol for detection of campylobacters by use of enrichment culture

The enrichment culture protocol for the study retail ground beef was the same as that already described for the experimental inoculation, except without the addition of the 1 ml of fresh *C. jejuni* solution. Briefly, 25 g of raw ground beef was added to 100 ml of a Bolton broth and 5% horse blood mixture in a 55-ounce Whirl Pak bag and mixed thoroughly for 30 s. The homogenate was then microaerobically incubated for 44 hours at 42°C and then streaked onto Karmali selective agar and re-incubated microaerobically at 42°C for 48–72 hours. Each culture plate was then examined visually for colonies characteristic of *Campylobacter* spp. Each incubation included a laboratory strain *C. jejuni* plate as positive control.

Detection of campylobacters by polymerase chain reaction (PCR)

At the same time as samples were taken for culture, ground beef from approximately 10% of the 1,200 packages collected (52 of 60 stores represented) were frozen for subsequent DNA extrac-

tion and application of taxon-specific PCR for campylobacters. Each subsample (1 g) was thawed and placed in a BagPage 100 filtered blending bag (EW-36840-58; Canadawide Scientific Ltd., Ottawa, ON, Canada) containing 9 ml of Columbia broth (Becton, Dickinson and Company, Sparks, NV, USA), and the sample was homogenized for 120 s at high setting in a Stomacher 80 blender (Seward Ltd., West Sussex, UK). The homogenate was then removed from the bag and centrifuged at $1,750 \times g$ for 10 minutes, the supernatant containing *Campylobacter* cells was collected. To concentrate *Campylobacter* cells, the supernatant was centrifuged at $24,050 \times g$ for 10 minutes, and the supernatant removed and discarded. The pellet was re-suspended in 1 ml of Columbia broth, 200 μ l aliquots were placed in 2 ml tubes, an internal amplification control (IAC; 10 μ l containing 700 copies/ μ l) was added to each tube (15), and DNA was extracted using the DNAeasy Tissue Kit (Qiagen, Mississauga, Canada) according to the manufacturer's protocol. Direct PCR was applied for *Campylobacter* genus, IAC, *C. jejuni*, *C. coli*, *C. fetus*, *C. hyointestinalis*, and *C. lanienae* (15). In addition, nested PCR to detect *C. concisus* and *C. upsaliensis* was applied (Inglis et al., unpublished). In all instances, negative and positive PCR controls were included, and arbitrarily-selected amplicons (including weak amplicons) were sequenced to ensure specificity. Samples were deemed to be negative for *Campylobacter* DNA only if amplification of the IAC occurred (i.e., in the absence of a *Campylobacter* genus amplicon).

Data analysis

Descriptive analyses were conducted using SPSS (version 15.0; SPSS, Chicago, US). A second commercial software package (MLwiN version 2.02; Centre for Multilevel Modeling, Institute of Education, London, UK) was utilized for the hierarchical model analysis. The hierarchical models (9) were specified with a logit link, binomial distribution, restricted iterative generalized least square and second order penalized quasi-likelihood nonlinear estimation. The outcome was whether or not a ground beef sample was positive for *Campylobacter* spp. DNA. Variables included "poultry cutting" (whether or not poultry was cut or

TABLE 2. Unconditional analyses of risk factors for whether a sample was positive for *Campylobacter* spp. by direct PCR (n = 140)

Variable	Level	# of packages	% packages <i>C. spp.</i> positive at each level	P-value
Chain	1 ^a	28	42.9	0.936
	2	45	46.7	
	3	47	51.1	
	4	20	35.0	
City	1 ^a	109	45.0	0.891
	2	9	55.6	
	3	22	45.5	
Collection period	1 ^a	30	30.0	< 0.001
	2	30	66.7	
	3	31	80.7	
	4	49	20.4	
Frozen portions	No ^a	124	47.6	0.459
	Yes	16	31.3	
Package type	Lean ^a	86	40.7	0.158
	Regular	54	53.7	
Poultry cutting ^b	No ^a	94	48.9	0.937
	Yes	40	45.0	
Trim type	Coarse grind tube ^a	56	41.1	0.876
	Market trim	50	50.0	
	Both	34	47.1	
Weight_c	≤ 0.499 kg ^a	17	35.3	0.343
	0.500–0.999 kg	113	48.7	
	≥ 1.000 kg	10	30.0	

^aReference category; ^bData unavailable for one store (six packages)

C. spp.: *Campylobacter* species

packaged in the meat department), “trim type” (what source of ground beef was used in the grinding; coarse grind tubes, market trim or a combination), “city” (1, 2 or 3), “collection” (collection period 1, 2, 3, 4), “package type” (lean or regular ground beef), and “weight” (kg, the only continuous variable). The scale of the “weight” variable was explored and categorized into “weight_c” (package less than 0.5 kg, package 0.5 to 0.999 kg, or package 1.0 kg or greater) to evaluate model linearity assumptions. Random effects (e.g. chain or store levels) were kept in the model if more than one variable at that level was entered as a fixed effect, if the amount of variability explained at that level was greater than 10%, or if the level was believed to be important to the data structure *a priori*.

RESULTS

Experimental inoculation

Of the 40 ground beef samples inoculated, only one sample (1×10^2 CFU/g) did not yield *C. jejuni*. Positive control plates and all other samples, including 100% of samples inoculated with 1×10^1 CFU/g, were positive for *C. jejuni* using the study protocol. None of the negative control plates grew *Campylobacter* spp.

Prevalence survey using culture

All 60 stores reported that they did a final grind of beef in-store, and that the source beef for grinding came from local (Alberta) slaughter plants or processors.

Twenty-seven stores used coarse ground tubes, 17 stores used market trim, and 16 stores used a combination of both for their second in-store grind. Forty stores did not package or cut raw poultry in the department, 19 stores reported cutting or packaging some poultry products (e.g. wings), and for one store data were unavailable. Fifty-six stores used fresh meat only, while in four stores the retail ground beef may have included previously frozen portions. Of the 1,200 packages of retail ground beef, 726 were lean and 474 were regular ground beef. Twenty-eight packages were labeled as a “discount”. By weight, 121 packages were less than 0.500 kg, 1,030 packages were 0.500 kg to 0.999 kg, and 49 packages were greater than or equal to 1.000 kg. Transport temperatures ranged

from 3.31°C to 9.03°C in the six summer shipments and -2.44°C to 9.42°C in the six winter shipments. *Campylobacter* species were not isolated from any of the 1,200 packages of retail ground beef.

PCR detection of campylobacters

Of the 142 samples tested using PCR, 65 (46%) were positive for DNA of *Campylobacter* spp. origin while 77 were negative (Table 1). Two of the 142 samples tested with use of PCR could not be linked to store or chain and were omitted from all subsequent analyses. The remaining 140 ground beef samples represented 52 different stores. Twelve stores had more than one meat sample tested from the same collection period. Of these 12 stores, only four stores had more than one meat sample positive for DNA of *Campylobacter* spp. origin. Ten of these 12 stores had either four or five samples from the same collection period tested with PCR, and the most any store had positive for DNA of *Campylobacter* spp. origin was two samples.

Factors associated with PCR detection of *Campylobacter* spp.

For one sample, data were missing for whether or not the source store cut poultry. This sample was included in risk factor analysis, and designated 'missing' in the "poultry" analysis. Supermarket chain did not explain an important part of the variance in the null model (chain level variance 0.000, standard error 0.000) and was not included as a random effect in the final analysis. After accounting for clustering within the store of origin, only the package type and the collection period variables were selected for consideration in the development of a final model ($P \leq 0.25$) (Table 2). None of the other risk factors considered (chain, city, inclusion of frozen portions, on-site poultry cutting practices, kinds of trim in the ground beef or package weight) were associated with the odds of detecting campylobacters by PCR (Table 2).

When package type (regular or lean) and collection period (1: Nov 21–23, 2004, 2: Jan 9–11, 2005, 3: May 30–31, June 1, 2005, and 4: July 18–20, 2005) were examined together, only the collection period was significantly associated ($P \leq 0.05$) with detection of

Campylobacter spp. by PCR. The odds of a retail ground beef package testing positive for *Campylobacter* spp. DNA was 5.6 times greater if the package was from collection period 2 than if it was from collection period 1 (OR 5.6, 95% CI 1.8–17.5). Further, a package had 12 times greater odds of testing positive for *Campylobacter* spp. DNA if it was from collection period 3 than if it was from collection period 1 (OR 12.0, 95% CI 3.5–42.0). Ground beef from collection period 4 was not statistically different from beef from collection period 1 (OR 0.6, 95% CI 0.2–2.0).

DISCUSSION

The samples from this large retail ground beef survey represented four different supermarket chains and three cities in southern Alberta. Random selection of packages in stores, multiple collection periods, and limiting the number of packages purchased per store were used to avoid oversampling the same meat batches. In 2005, source beef for ground beef likely came from the six federally inspected slaughter plants in Alberta (1), or from provincially inspected facilities. Because retail chains likely purchased meat from the same plants or processors, it was expected that variation within each chain would be small. As a result, only five packages of ground beef were purchased from each store at each collection time.

Hazard analysis critical control points (HACCP) have been identified and programs implemented in all federally registered beef slaughter plants in Canada (5). In previous surveys in cattle, poultry and swine, significant reductions in *Campylobacter* isolation rates from slaughter to post-chill have been reported (20, 24, 26). Protocols in cattle slaughter plants, including hide-on-carcass, lactic acid, hot water, and carcass washes, chilling, and the ability to remove potentially contaminating components (e.g., hides and intestinal tracts) quickly and intact may have all contributed to bacterial numbers below detectable levels in the retail ground beef surveyed here.

It can be difficult to compare laboratory protocols with other published research because many incubation and temperature protocols, culture media, and antimicrobial supplements are avail-

able, and because viable but non culturable *Campylobacter* strains may exist (8, 23). Using the culture technique described, we were able to isolate *C. jejuni* at 1×10^1 CFU/g in experimentally inoculated ground beef samples; this level is below the estimated dose required for human infection (3, 14). However, none of the 1,200 packages of retail ground beef collected as part of this study were culture positive for viable *Campylobacter* spp., an encouraging finding for public health in Alberta.

The very low prevalence of culturable *Campylobacter* levels in retail ground beef observed in this study is similar to those seen in other North American ground beef surveys (4, 28) and lower than the 60–90% prevalences reported in raw retail chicken (4, 30, 31). In a survey in the United States from 2002–2005, campylobacters were identified in only 1 of 2,073 packages of ground beef using culture (28), and a smaller Alberta survey found zero of 100 packages positive (4). However, it is possible that the laboratory sensitivity of the culture method used here may not have been high enough to pick up very low numbers of organisms. Further, if campylobacters were sufficiently stressed, it is possible the method was not able to resuscitate these pathogens sufficiently for growth with culture. Three of the meat shipments dipped below the 0°C mark during shipping; however, campylobacters have been isolated from ground beef frozen at -18°C for 90 days (10), and culture recovery in our study did not vary between summer and winter samplings.

Traditionally, PCR has been used to confirm isolates as campylobacters rather than as a survey tool in retail meat studies (13, 30, 31). This is because from a food safety point of view, viable campylobacters are usually the targets of interest and the identification of *Campylobacter* DNA by use of PCR does not ensure viability. However, from our direct PCR results, *C. jejuni*, *C. coli*, and *C. hyointestinalis* were identified in the retail ground beef. None of the samples were positive for *C. fetus* or *C. lanienae*, species which may be carried by cattle, or for *C. concisus* or *C. upsaliensis*, which are pathogens responsible for infections in people but are putatively not carried by livestock (14, 21). Finding 27% (38/142) of samples PCR positive for

C. coli and only 15% (21/142) of samples PCR positive for *C. jejuni* was interesting. *C. jejuni* is the most frequently isolated species from cattle (11, 17), while *C. coli* is the most common *Campylobacter* species found in swine (21, 24). Stores were asked about the cutting and packaging of raw poultry, but not raw pork, and this may be a consideration for future research.

We initially considered that cross-contamination of surfaces and equipment from raw poultry cutting and packaging in grocery stores might lead to ground beef contamination. However, 2/3 of stores did not cut poultry onsite and brought in pre-packaged poultry cuts for consumers. No association was found between poultry cutting and the presence of *Campylobacter* DNA in retail ground beef in the risk factor evaluation.

Approximately 10% of retail ground beef packages were tested by use of PCR. Initially, every 10th ground beef sample was selected and frozen for later testing, but this systematic approach did not continue for the entire study. However, 52 of the 60 stores were represented, 60 samples from winter and 82 from summer were selected, and samples were tested from all chains and most stores in all three cities. Hierarchical models were likely hampered by the small sample size tested with PCR (n = 142). However, individual collection periods were associated with the presence of *Campylobacter* spp. The results did not indicate a seasonal difference, as one winter and one summer collection period were significantly different from the others. However, these findings do indicate that differing levels of *Campylobacter* spp. contamination may occur between slaughter and retail sale. Descriptive analyses found that from the five packages collected at the same store on the same day, one package might be positive for *Campylobacter* DNA and the others negative. This may reflect differing package contamination levels, within package *Campylobacter* distribution (as only 1 g of ground beef was collected from the centre of each package), or possible dilution effects from the PCR process. Further, variables within the control of slaughter plants, processors or grocery meat departments (e.g., carcass cleanliness, hygiene practices,

cross-contamination) may have contributed to variability between collections.

CONCLUSIONS

None of the 1,200 packages were culture positive for campylobacters in this retail ground beef survey, supporting the adequacy of food safety practices in the province. The prevalence of *Campylobacter* DNA with PCR detection, however, was moderate to high (46%); thus continued research into potential interventions in the slaughter-to-retail continuum could be of use. The high levels of *Campylobacter* DNA in the beef suggest that breaks in food safety protocols within slaughter plants, processors or grocery stores could have potentially important public health repercussions.

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