

Article

Microbiological baseline study of beef and pork carcasses from provincially inspected abattoirs in Alberta, Canada

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Abstract – In 2006 and 2007 beef and pork carcass swabs from provincially inspected abattoirs in Alberta, Canada were tested to determine the levels of total aerobic bacteria, coliform bacteria, and generic *Escherichia coli*, and the prevalence of *Salmonella* spp., *Campylobacter* spp., and Shiga toxin-producing *E. coli* (STEC). Swabs from beef and pork carcasses from 48 and 34 facilities, respectively, were analyzed. All samples tested were positive for aerobic bacteria with 99.8% of beef and 96.0% of pork samples, having total counts of $\leq 100\,000$ CFU/cm². Coliform bacteria were isolated from 22.4% and 42.0% of beef and pork carcass samples, respectively. Generic *E. coli* were recovered from 14.6% of beef and 33.7% of pork carcass samples. For beef carcasses, positive tests were obtained for 0.1% of 1036 samples tested for *Salmonella* spp., 1.5% of 1022 samples tested for *Campylobacter* spp. and 5.4% of 1018 samples tested for STEC. For pork carcasses, positive tests were obtained for 1.6 % of 1076 samples tested for *Salmonella* spp., 8.8% of 1070 samples tested for *Campylobacter* spp. and 4.8% of 1067 samples tested for STEC.

Résumé – Étude de référence microbiologique des carcasses de bovins et de porcs dans les abattoirs inspectés par les autorités provinciales de l'Alberta, au Canada. En 2006 et en 2007, des écouillons des carcasses de bovins et de porcs provenant des abattoirs inspectés par les autorités provinciales de l'Alberta, au Canada, ont été testés pour déterminer les quantités de bactéries aérobies totales, de bactéries coliformes et d'*Escherichia coli* générique et la prévalence de *Salmonella* spp., de *Campylobacter* spp. et d'*E. coli* produisant de la shiga toxine (STEC). Des écouillons de carcasses de bovins et de porcs provenant de 48 et de 34 établissements, respectivement, ont été analysés. Tous les échantillons testés étaient positifs pour des bactéries aérobies et 99,8 % des échantillons de bovins et 96,0 % des échantillons de porcs présentaient des numérations totales $\leq 100\,000$ CFU/cm². Des bactéries coliformes ont été isolées dans 22,4 % et 42,0 % des échantillons des carcasses de bovins et de porcs, respectivement. *E. coli* générique a été récupéré dans 14,6 % des échantillons de carcasses de bovins et dans 33,7 % des échantillons de carcasses de porc. Pour les carcasses de bovins, des tests positifs ont été obtenus dans 0,1 % des 1036 échantillons testés pour *Salmonella* spp., dans 1,5 % des 1022 échantillons testés pour *Campylobacter* spp. et dans 5,4 % des 1018 échantillons testés pour STEC. Pour les carcasses de porcs, des tests positifs ont été obtenus dans 1,6 % de 1076 échantillons testés pour *Salmonella* spp., dans 8,8 % des 1070 échantillons testés pour *Campylobacter* spp. et dans 4,8 % des 1067 échantillons testés pour STEC.

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Introduction

Consumers, regulatory agencies, governments, and the food industry all consider food safety to be of the utmost importance. The implementation of Hazard Analysis Critical Control Point (HACCP) programs, Good Manufacturing/Production Practices, and various interventions by slaughter and meat processing facilities play a large role in enhancing the safety of meat products. Baseline studies to determine microbial levels of commensal organisms and pathogen prevalence can be used to assess the effectiveness of these programs and interventions. The United

States Department of Agriculture, Food Safety and Inspection Service (USDA/FSIS) conducted several baseline studies of meat and poultry products from federally inspected facilities to determine the prevalence and levels of bacteria of public health concern (1–3). The national baseline data collection programs conducted from June 1997 to May 1998 included testing 1881 sponge samples from beef carcasses and 2127 from pork carcasses for levels of generic *Escherichia coli* and the prevalence of *Salmonella* spp. (2,3). In Canada, the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) conducted baseline studies of poultry, beef, and pork carcasses from provincially inspected abattoirs

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from 1999 to 2002 to determine the counts of total aerobic bacteria, coliform bacteria, and *E. coli*, the prevalence and levels of *Salmonella* spp. and *Campylobacter* spp., and the prevalence of *Listeria monocytogenes* and *E. coli* (4–6).

The importance of baseline information on pathogen prevalence and levels of commensal bacteria as a benchmark for measuring improvements in provincially inspected abattoirs was also recognized in Alberta. The Food Safety and Animal Health Division of Alberta Agriculture and Rural Development initiated baseline studies of poultry, beef, and pork carcasses in provincially inspected abattoirs starting in November 2004. Poultry carcass rinses, beef, and pork carcasses were tested to determine counts of total aerobic bacteria, coliform bacteria and *E. coli*, and the prevalence of *Salmonella* spp., *Campylobacter* spp., and Shiga toxin-producing *E. coli* (STEC). The results of the baseline study of poultry carcass rinses were recently reported (7). The results of the baseline study for beef and pork carcass sponges collected from provincially inspected Alberta abattoirs are reported herein.

Materials and methods

Experimental design

Samples were collected from provincially inspected cattle and hog slaughter facilities between May 16, 2006 and April 20, 2007. The slaughter facilities were categorized into two strata (high or low numbers of animals slaughtered) based on the annual slaughter numbers from the previous year (2005). The cut-off was a slaughter volume of < 1000 or ≥ 1000 for cattle and < 8000 or ≥ 8000 for hogs. Samples were tested from a total of 50 facilities. Many of the facilities slaughtered both cattle and hogs. Beef carcasses were tested from 35 low-volume and 13 high-volume abattoirs. Pork carcasses were tested from 31 low-volume and 3 high-volume abattoirs.

Sample collection

Beef and pork carcass sponges were collected according to the USDA/FSIS sample collection guidelines (8). Convenience samples were taken based on accessibility of the carcasses in the coolers. Three sterile, pre-moistened sponges (Qualicum Scientific, Ottawa, Ontario) were used to swab adjacent areas on the same side of each carcass. One sponge was used for enumeration of total aerobic bacteria, coliform bacteria, and generic *E. coli*. The second sponge was used for the isolation of *Salmonella* spp. and STEC. The third sponge was used for the isolation of *Campylobacter* spp. The carcass sponges were collected in sterile plastic bags, put on ice in coolers and sent to the laboratory for testing. Samples received at the laboratory at temperatures $> 10^{\circ}\text{C}$ or more than 48 h after collection were rejected.

Culture methods

All media used were Difco brand (Difco Laboratories, Becton Dickinson Microbiology Systems, Sparks, Maryland, USA) unless otherwise indicated. Total aerobic bacteria, coliform bacteria, and generic *E. coli* were enumerated using the pour plate technique. Fifteen milliliters of 0.1% peptone water were added to the sponge samples and mixed for 2 min using a

stomacher (Seward Stomacher 400; Seward Laboratory, London, UK). Ten-fold serial dilutions of carcass sponge diluent were made using 0.1% peptone water, 1-mL aliquots were dispensed into sterile Petri dishes, and molten agar added. Total aerobic bacteria were enumerated on plate count agar incubated at 35°C for 48 h. Coliform bacteria and *E. coli* were enumerated on Violet Red Bile agar with MUG (4-methylumbelliferyl- β -D-glucuronide) after incubation at 35°C for 24 h. Coliform bacteria were confirmed by the production of gas after incubation in 2% Brilliant Green Bile broth at 35°C for 48 h. Generic *E. coli* were confirmed by the production of gas and fluorescence after incubation in EC medium with MUG at 45°C for 48 h. Incubated EC medium with MUG that had gas production but no fluorescence was streaked onto Columbia Blood Agar plates (BAP; Oxoid, Nepean, Ontario), incubated at 35°C for 24 h and the identity of at least 1 colony determined by a Vitek instrument (bioMérieux Canada, St. Laurent, Quebec) using a GNI+ card.

Salmonella spp. were identified using the USDA/FSIS method with modifications (8). Briefly, 50 mL of Buffered Peptone Water (BPW) were added to the carcass sponge. The sample was mixed thoroughly and incubated at 35°C for 24 h ensuring the sponge was immersed in the pre-enrichment broth. One milliliter and 0.1 mL of BPW pre-enrichment culture were transferred to 10 mL of tetrathionate (TT) broth and 10 mL of Rappaport Vassiliadis (RV) broth (EM Science, Merck, Darmstadt, Germany), respectively, and incubated at 42°C for 24 h. One hundred and fifty microliters of each selective enrichment broth (TT and RV) were combined for DNA extraction followed by real-time polymerase chain reaction (PCR) (9). Culture was continued on those samples that were positive by real-time PCR. Enrichment cultures (TT and RV) from positive samples were streaked onto Brilliant Green Sulfa agar (BGS), Xylose-Lysine-Tergitol 4 agar (XLT4) and Rambach agar (Merck, Darmstadt, Germany) and incubated at 35°C . Plates were examined after 24 h of incubation and suspect colonies were selected for biochemical testing. Plates were incubated for an additional 24 h and reexamined for additional suspect colonies.

Suspect colonies were streaked on BAP and MacConkey agar plates and inoculated into Urea agar, Triple Sugar Iron agar and Lysine Iron agar slants. Plates were examined for purity and reactions of the slants and MacConkey agar plates were noted. Isolates that appeared to be *Salmonella* spp. were further tested using polyvalent O and O1 antisera (Denka Seiken, Tokyo, Japan). Samples that were positive by the real-time PCR assay but negative by the culture method previously described were retested using immunomagnetic separation (IMS). One milliliter each of BPW, TT and RV enrichment broths were mixed with immunomagnetic beads (Dynabeads[®] anti-*Salmonella*; Dynal, Lake Success, New York, USA) on a semi-automated magnetic particle processor (KingFisher[™] mL, Fisher Scientific, Nepean, Ottawa) according to the manufacturer's instructions. The bead-bacteria complexes were inoculated into TT, incubated and then plated onto XLT4 and Rambach agar plates and examined for typical *Salmonella* colonies. Typical colonies were tested using biochemical and serological tests as previously described.

Table 1. Descriptive statistics of microbiological counts (CFU/cm²) of beef and pork carcasses from high- and low-volume abattoirs

Bacteria	Summary statistics	Beef		Pork	
		Low volume	High volume	Low volume	High volume
Total aerobic bacteria	Geometric Mean	240.9	317.2	1060.9	1525.3
	CI ^b Geometric Mean	207.3 to 280.1	273.7 to 367.6	876.7 to 1283.8	1259.8 to 1846.7
	Log ₁₀ Mean	2.38	2.50	3.02	3.18
	CI Log ₁₀ Mean	2.32 to 2.45	2.44 to 2.56	2.94 to 3.11	3.10 to 3.27
	Median Value	225.0	291.7	916.7	1416.7
	10th percentile	28.5	39.3	66.7	83.3
	90th percentile	2416.7	2666.7	18333.3	28083.3
Coliform bacteria ^a	Geometric Mean	0.4	0.6	1.4	2.0
	CI Geometric Mean	0.3 to 0.6	0.4 to 0.8	1.0 to 1.9	1.4 to 2.8
	Log ₁₀ Mean	-0.37	-0.23	0.14	0.29
	CI Log ₁₀ Mean	-0.50 to -0.25	-0.38 to -0.07	0.00 to 0.27	0.14 to 0.44
	Median Value	0.3	0.4	0.8	1.2
	10th percentile	0.1	0.1	0.1	0.1
	90th percentile	3.9	13.0	46.8	141.7
<i>E. coli</i> ^a	Geometric Mean	0.3	0.6	1.0	1.7
	CI Geometric Mean	0.2 to 0.4	0.4 to 0.9	0.7 to 1.5	1.1 to 2.5
	Log ₁₀ Mean	-0.54	-0.23	0.01	0.23
	CI Log ₁₀ Mean	-0.68 to -0.40	-0.43 to -0.02	-0.15 to 0.16	0.06 to 0.40
	Median Value	0.2	0.3	0.5	0.8
	10th percentile	0.1	0.1	0.1	0.1
	90th percentile	2.2	17.5	122.5	189.2

^a For values above detection value.

^b 95% confidence intervals.

Campylobacter spp. were isolated and enumerated using a modification of the FDA method (10). The sample sponge was added to 90 mL of *Campylobacter* Enrichment Broth, Bolton formula (Oxoid) with laked horse blood. The sample was placed under microaerobic conditions in an anaerobic jar filled with 10% CO₂, 5% O₂, 85% N₂ and incubated at 42°C for 48 h. After 24 and 48 h of incubation, the enrichment broth was plated onto Campy-line agar (CLA; Dalynn Biologicals, Calgary, Alberta) and Modified *Campylobacter* Blood-Free Selective agar (CCDA; Oxoid) plates using a 10 µL loop and the plates were incubated microaerobically at 42°C. Plates were examined for typical colonies after 24 h and 48 h incubation. Typical smooth, convex, translucent, colorless to cream colonies on CCDA plates and deep red to magenta, smooth, shiny and convex colonies with a defined edge or flat with an irregular edge on CLA plates were streaked onto BAP. The BAP was incubated microaerobically at 42°C for 24 h. *Campylobacter* species were identified by wet mount, oxidase, catalase, hippurase, and naladixic acid/cephalothin susceptibility tests.

Shiga toxin-producing *E. coli* (STEC) were identified following the method cited in Renter et al (11). Briefly, DNA was extracted from the BPW enrichment broths that were used for the isolation of *Salmonella* spp. Extracted DNA was tested for the presence of the genes for Shiga toxin 1 (*stx1*) and Shiga toxin 2 (*stx2*) with the use of PCR in a multiplex assay. Enrichment broths positive for the *stx1* and/or *stx2* genes were streaked onto MacConkey and Violet Red Bile Agar with MUG and incubated at 42°C for 24 h. Twenty isolated colonies from each plate were stab inoculated onto a MacConkey plate and incubated at 35°C for 24 h. Deoxyribonucleic acid was extracted from the isolates and tested by PCR for the *stx1* and *stx2* genes. If no positive isolates were identified, the enrichment broth was restreaked onto agar plates and an additional 40 colonies were tested.

Only samples in which a positive isolate could be identified were reported as positive. STEC isolates were characterized for O157 antigen using Prolex™ *E. coli* O157 latex test kit (Pro-Lab Diagnostics, Richmond Hill, Ontario) and verotoxin (Shiga toxin) production using GLISA Duopath™ Verotoxin test kit (EM Science, Merck).

Positive and negative controls were included with each set of samples tested. Positive controls were ATCC strains (*S. Typhimurium* ATCC 14028, *C. jejuni* ATCC 33250, and *E. coli* O157:H7 ATCC 35150) inoculated into the appropriate enrichment broths. Negative controls were uninoculated enrichment broths.

Enumeration data were recorded as colony forming units (CFU)/mL and then converted to CFU/cm² by dividing the bacterial counts by 12. This was derived from:

total carcass surface area swabbed: 3 × 100 cm² = 300 cm²

total volume of fluid for the sponge samples:

10 mL + 15 mL = 25 mL

conversion factor calculation: 25 mL for 300 cm², then

300/25 = 12

Statistical analysis

The chi-squared (χ²) test was used to compare the proportion of positive samples for *Salmonella* spp. and *Campylobacter* spp. across the 2 strata for beef and pork samples. Similarly, two-sample Wilcoxon rank-sum (Mann-Whitney) tests were used to compare microbial counts. The statistical analyses were carried out using statistical software (Stata Statistical Software: version 10; StataCorp, College Station, Texas, USA).

Results

From May 16, 2006 to April 20, 2007, 2245 samples from 50 facilities were collected; however, not all samples met testing

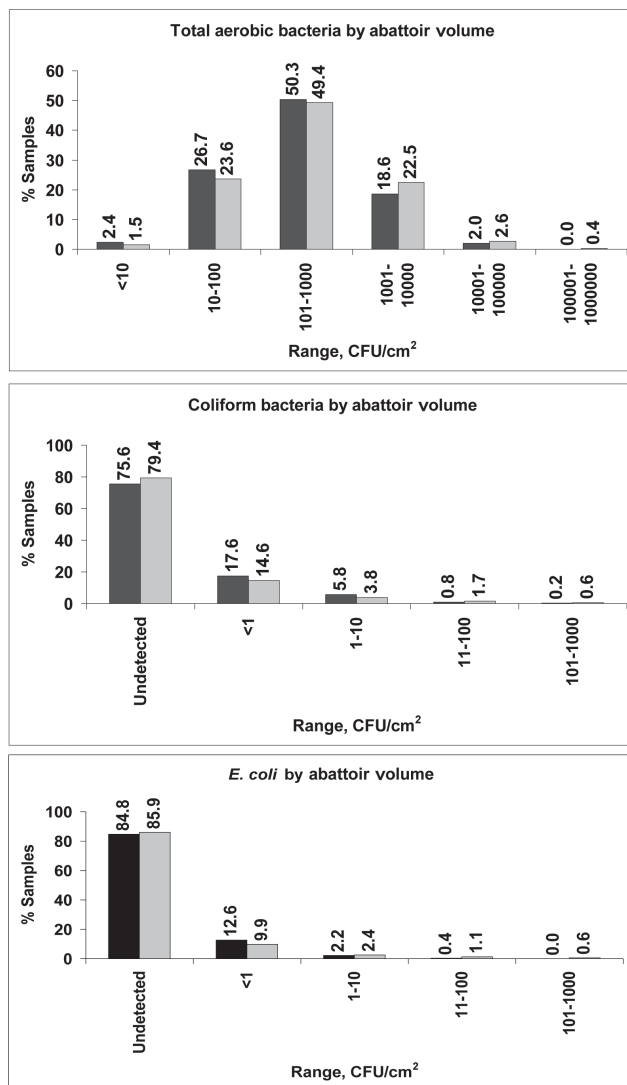


Figure 1. Frequency distribution of total aerobic bacteria, coliform bacteria, and generic *E. coli* from beef carcass sponge samples by abattoir volume. Low volume abattoirs are shown with black bars and high volume abattoirs are shown with grey bars.

criteria. All beef and pork carcass samples tested for total aerobic bacteria (2084) were positive. Coliform bacteria were recovered from 22.4% of 1034 beef carcass samples and 42.0% of 1069 pork carcass samples ($P < 0.01$) and *E. coli* were recovered from 14.6% of beef carcasses and 33.7% of pork carcasses ($P < 0.01$). There was no significant difference between the positive rates for these organisms in high and low-volume abattoirs. Table 1 displays descriptive statistics for counts of commensal organisms on pork and beef carcasses, from both high and low volume abattoirs. The log means for total aerobic bacteria, coliform bacteria, and *E. coli* were significantly higher in pork carcass samples (all $P < 0.01$). The frequency distribution of samples according to the levels of these organisms is shown in Figures 1 and 2. Total aerobic bacteria were recovered from 100% of the samples tested, with 99.8% and 96.0% of beef and pork carcass samples, respectively, having counts of $\leq 100\,000$ CFU/cm². Most (75.0%) of the beef carcass samples

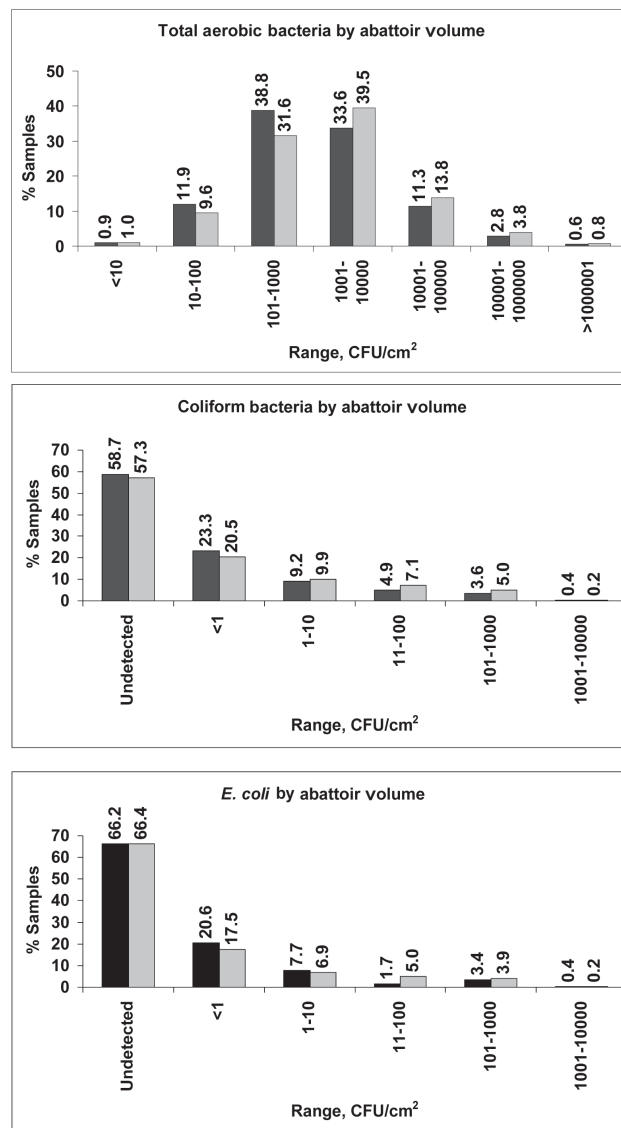


Figure 2. Frequency distribution of total aerobic bacteria, coliform bacteria, and generic *E. coli* from pork carcass sponge samples by abattoir volume. Low volume abattoirs are shown with black bars and high volume abattoirs are shown with grey bars.

had counts in the range of 10 to 1000 CFU/cm². Most (71.8%) of the pork carcass samples had aerobic bacteria counts in the range of 101 to 10 000 CFU/cm². For coliform bacteria, most samples (98.4% for beef and 89.5% for pork) had levels of ≤ 10 CFU/cm². Most samples (98.9% for beef and 92.7% for pork) had levels of *E. coli* of ≤ 10 CFU/cm².

For foodborne pathogens, the overall results [and 95% confidence intervals (CI)] were as follows: 0.8% (0.5% to 1.3%) of 2112 samples that were tested for *Salmonella* spp. were positive; 5.2% (4.3% to 6.2%) of 2092 samples that were tested for *Campylobacter* spp. were positive; and 5.1% (4.2% to 6.1%) of 2085 samples that were tested for STEC were positive. The prevalence of *Salmonella* spp. and *Campylobacter* spp. by carcass type is shown in Table 2. The difference between high- and low-volume abattoirs was not significant; therefore, aggregated results are reported. The difference in *Campylobacter* spp. and *Salmonella* spp. prevalence between beef and pork samples was

Table 2. Proportion of beef and pork carcass sponge samples positive for *Salmonella* spp., *Campylobacter* spp., and Shiga toxin-producing *E. coli* (STEC)

	Number of samples (Number positive)			% Positive (95% CI)		
	Beef	Pork	Total	Beef	Pork	Total
<i>Salmonella</i>	1036 (1)	1076 (17)	2112 (18)	0.1 ^a (0.0–0.5)	1.6 ^a (0.9–2.5)	0.8 (0.5–1.3)
<i>Campylobacter</i>	1022 (15)	1070 (94)	2092 (110)	1.5 ^a (0.8–2.4)	8.8 ^a (7.2–10.6)	5.2 (4.3–6.2)
STEC	1018 (55)	1067 (51)	2085 (106)	5.4 (4.1–7.0)	4.8 (3.6–6.2)	5.1 (4.2–6.1)

CI — 95% confidence intervals; ^a $P < 0.01$; STEC — Shiga toxin-producing *E. coli*.

statistically significant ($P < 0.01$ in both cases). This was not the case with STEC ($P = 0.52$). Only one of the STEC isolates from a beef carcass was determined by agglutination assays to be *E. coli* O157. Isolates from samples that were positive for *Campylobacter* spp. were further characterized to the species level with 84.4% of the isolates identified as *C. coli*, 13.8% as *C. jejuni*, and 1.8% of the isolates identified as *C. lari*. There were differences between beef and pork carcasses in the species of *Campylobacter* identified from positive samples. For beef carcass samples 73.3% and 2.7% of the positive isolates were identified as *C. jejuni* and *C. coli*, respectively. For pork carcass samples, 4.3% and 93.6% of the positive isolates were identified as *C. jejuni* and *C. coli*, respectively.

Discussion

Microbiological studies are needed to assess the effectiveness of HACCP programs, Good Manufacturing/Production Practices, and interventions used in meat slaughter and processing facilities. In the absence of ongoing microbiological monitoring programs, such as those conducted in some European countries, baseline studies can serve as a means of benchmarking the current levels of hygiene in slaughter plants (12). Prior to this study there was no information on the levels of pathogens or commensal bacteria on beef and pork carcasses processed in Alberta provincially inspected meat facilities.

The percentages of carcass swab samples positive for total aerobic bacteria in this study were similar to those in the 1998 OMAFRA study (7,9), in which all the carcasses tested contained aerobic bacteria. The percentage of carcasses containing coliform bacteria and *E. coli* were slightly lower in Alberta than that reported in Ontario where 27.8% and 18.6% of beef carcasses and 61.3% and 39.5% of pork carcasses were positive for coliform bacteria and *E. coli*, respectively (4,6). The USDA baseline studies measured the levels of generic *E. coli* on beef and pork carcasses and these were very similar to those found in this study. The USDA reported that 98.9% of beef and 91.5% of pork carcasses contained ≤ 10 CFU/cm² generic *E. coli* (13,14) and we found that 98.9 and 92.7% of beef and pork carcasses, respectively, had 10 or fewer CFU/cm² *E. coli*.

In regards to the percentage of carcasses positive for food-borne pathogens, there was more variation among the different studies. In this study, *Salmonella* spp. were isolated from 0.1% and 1.6% of beef and pork carcasses, respectively, whereas the

OMAFRA baseline found 1.6% of beef carcasses and 4.8% of pork carcasses positive (4,6). The USDA also reported higher results with 1.2% and 6.9% of beef and pork carcasses, respectively, positive for *Salmonella* spp. (2,3). However, a national survey in Australia in 2004 did not isolate any *Salmonella* spp. from 1147 chilled beef carcasses (15). Several other countries have also investigated the level of *Salmonella* spp. on pork carcasses and the reported results revealed that there is much variation in the percentage of positive carcasses. A study in Great Britain found that 5.3% were positive (13), a baseline of Swedish slaughterhouses revealed that no *Salmonella* spp. were isolated from pre-chilled carcasses (14), a study in Belgian abattoirs found an overall prevalence of 16.1% from carcasses sampled from the chill-room 2 to 4 h after slaughter (16), and a baseline study of pre-chill carcasses in Taiwan showed that 1.7% were positive for *Salmonella* spp. (17).

In Alberta, 1.5% of beef carcasses were positive for *Campylobacter* spp. and this is the same positive rate that was reported by OMAFRA (4). However, only 8.8% of pork carcasses from Alberta contained *Campylobacter* spp. whereas Ontario reported a positive rate of 26.7% (6). The 2004 Australian national survey found that 0% of chilled beef carcasses were positive for *Campylobacter* spp. (13) which is similar to the low positive rate found in the Canadian studies. Baseline surveys of pre-chill pork carcasses in Sweden (14) and Taiwan (17) found that 1.0% and 13.8%, respectively, were positive for *Campylobacter* spp., which indicates wide variation in reported positive rates.

We determined that 5.4% of beef and 4.8% of pork samples were positive for STEC. However, only 1 of the isolates from a beef carcass (0.1%) was toxigenic *E. coli* O157. Other countries have also reported low positive rates for *E. coli* O157:H7. For beef carcasses, 0.1% were positive in Australia (15) and 2.7% were positive in Mexico (18). For pork carcasses, no *E. coli* O157:H7 were isolated from baseline studies in Sweden (14) or Taiwan (17) as was the case in this study. The OMAFRA study also identified pathogenic *E. coli*; however, they reported that only 0.3% of beef carcasses and 2.1% of pork carcasses were positive for verotoxigenic *E. coli* (4,6). The differences between the Alberta and Ontario results may be due to the different methodologies used. In this study PCR was used to identify STEC, whereas Ontario used a Vero cell assay to identify verotoxigenic *E. coli*.

Caution must be used when comparing results from different studies as variations in results may be due to differences in contamination rates, differences in laboratory methodologies, variations in sampling technique, time during the process when samples were obtained (for example, pre-chill, post-chill), study design, and seasonal or yearly effects.

The results of this baseline study indicate that improvements need to be made in Alberta's provincially inspected abattoirs and/or other sections of the beef and pork production chain to reduce the prevalence of pathogenic bacteria and lower the levels of bacteria on beef and pork carcasses. Efforts are currently underway in Alberta to improve Good Manufacturing Practices and implement interventions in provincially inspected abattoirs. Subsequent studies will determine the effectiveness of these efforts in reducing microbial levels and pathogen prevalence.

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