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Microbiological Characterization of Imported and Domestic Boneless Beef Trim Used for Ground Beef†

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ABSTRACT

The United States imports lean boneless beef trim from Australia (AUS), New Zealand (NZL), and Uruguay (URY) to meet demand for ground beef production. The reported incidence of and etiological agents responsible for foodborne diseases differ between these countries and the United States. Our objective was to determine whether current U.S. microbiological profiling adequately addresses the potential differences between foreign and domestic beef trim. We compared the hygienic status of imported and domestic (USA) beef trim by enumeration of aerobic bacteria, *Enterobacteriaceae,* coliforms, *Escherichia coli,* and *Staphylococcus aureus.* We also compared the prevalence of pathogens between imported and domestic samples by screening for the presence of *Salmonella, Campylobacter* spp., *Listeria* spp., and non-O157 Shiga toxin–producing *E. coli* (STEC). A total of 1,186 samples (487 USA, 220 AUS, 223 NZL, and 256 URY) of boneless beef trim were analyzed. Results of enumeration revealed significant differences between samples from all countries, with the lowest pathogen numbers in samples from AUS and the highest in samples from URY. Six *Salmonella* isolates (1 NZL, 1 URY, and 4 USA), 79 *L. monocytogenes* isolates (4 AUS, 5 NZL, 53 URY, and 17 USA), and 7 *Campylobacter* isolates (1 NZL, 1 URY, 5 USA) were found among the trim samples tested. Non-O157 STEC prevalence was 10% in NZL samples and about 30% in all of the other samples; 99 STEC strains were isolated. Serotyping of these isolates revealed that serotypes associated with hemolytic uremic syndrome were not different in prevalence between imported and domestic beef trim. Although it may be tempting to do so, these data cannot be used to compare the microbiological quality of beef trim between the countries examined. However, these results indicate that the current pathogen monitoring procedures in the United States are adequate for evaluation of imported beef trim.

The fed beef industry of the United States produces an excess of 50% (50-50) lean-fat beef trim but not enough 90-10 lean-fat beef trim to mix with it to meet the domestic demand for lean ground beef. According to imported beef and veal statistics recorded by the U.S. Department of Agriculture (USDA) Economic Research Service, 3.6 billion pounds (1.6 billion kilograms) of beef were imported into the United States in 2004 *(51).* Much of this beef was lean beef destined to be mixed with domestic 50-50 trim.

More than half of the beef imported annually originates in Australia (AUS), New Zealand (NZL), and Uruguay (URY) *(52),* countries in which the incidence of foodborne disease and etiological agents responsible for it differ substantially from those found in the United States and Canada *(18, 25).* One example of these differences is the variation in serotypes of Shiga toxin–producing *Escherichia coli* (STEC) that have been implicated in several foodborne diseases *(32, 37).* STEC cause symptoms that range from mild diarrhea to a very severe and life-threatening condition, hemolytic uremic syndrome (HUS). The STEC serotype most frequently associated with clinical disease in the United States and other countries of the Northern Hemisphere is O157:H7 *(29, 31, 32).* However, other non-O157 STEC serotypes have been associated with illness and outbreaks of severe disease in countries of the Southern Hemisphere *(1, 32).*

During the production of ground beef, imported beef trim is treated in the same manner as is domestic beef trim. This approach could be problematic because even though foreign sources are required to supply beef that has been tested for *E. coli* O157:H7 and *Salmonella,* once the foreign beef trim is blended and ground with domestic trim it is difficult to trace the origins of any subsequently identified pathogens. Molecular identification techniques are being employed more often in these tracing efforts. Current data regarding the microbial status of imported beef once it is received in the United States are limited. The objective of this project was to determine if and how the hygienic status and pathogen prevalence of imported beef trim from AUS, NZL, and URY differ from those of domestic beef trim. Hygienic status was determined by enumeration of aerobic bacteria, *Enterobacteriaceae,* coliforms, *E. coli,* and *Staphylococcus aureus.* The difference in pathogen prevalence between imported and domestic samples was determined by screening for the presence of *Salmonella, Campylobacter* spp., *Listeria* spp., and non-O157 STEC. Any of these organisms present were isolated and identified to species and/or serotype.

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MATERIALS AND METHODS

Sample processing. Between 1 January and 31 March 2005, 1,186 samples of domestic and imported boneless beef trim were collected and sent to the U.S. Meat Animal Research Center (USMARC) for analysis. The domestic samples were collected from mostly chilled beef from fed and cull beef production facilities. The imported trim originated in AUS, NZL, or URY, was frozen, and may have contained calf or veal trim. The beef trim came from multiple processing plants or establishments within each source country. AUS trim was packed an average of 104 days (53 to 176 days), NZL trim was packed an average of 98 days (46 to 201 days), and URY trim was packed an average of 86 days (39 to 272 days) before samples were collected in the United States. Most U.S. trim (97.5%) was fresh chilled beef that was packed 7 or fewer days (average of 3 days) before samples were collected. The remaining U.S. trim samples (2.5%) were collected from beef trim that had been frozen for 14 to 68 days.

Quality assurance personnel collected samples at each participating supplier by randomly selecting pieces of trim from 27-kg cartons of imported trim or 2,000-lb (907-kg) combos of domestic trim before blending and grinding of ground beef. The pieces of trim were placed in Whirl-Pak bags (Nasco, Fort Atkinson, Wis.) and held frozen (−20°C) until shipment on ice to the USMARC. Samples were received frozen and held at −20°C until processed. Most imported trim was 3- to 4-in. (7.6- to 10.2-cm) long slices, 0.5- to 1-in. (1.2- to 2.5-cm) cubes, or shredded beef. Domestic trim samples were similar but also included coarse ground beef and reduced fat beef. To test the maximal surface area per unit of weight of large or cubed pieces of beef trim, the outside edges of numerous pieces were trimmed away and combined for analysis. Samples received as ground beef, small shredded pieces, or reduced fat beef were used directly in analysis. Once prepared, samples were subdivided for pathogen detection and enumeration assays. A 10-g sample was used to enumerate total aerobic bacteria, *Enterobacteriaceae,* coliform bacteria, *E. coli,* and *S. aureus.* Another 10-g sample was used to determine the prevalence of *Campylobacter.* One 25-g sample was used to determine the prevalence of *Listeria,* and a second 25-g sample was used to determine the prevalence of non-O157 STEC and *Salmonella.*

Enumeration of bacteria. Aerobic plate counts (APCs) were performed, and *Enterobacteriaceae* (EB), coliform bacteria (CF), *E. coli* (EC), and *S. aureus* (SA) were enumerated after preparing a 1:10 ratio of beef trim samples to peptone water (Difco, Becton Dickinson, Sparks, Md.). Each dilution was prepared in a Whirl-Pak filter bag and then stomached in a laboratory blender (BagMixer 400VW, Interscience Laboratories Inc., Weymouth, Mass.) at high speed (nine strokes per second) for 30 s. One milliliter of each sample was taken from the filter side of the bag and plated onto Petrifilm (3M Microbiology, St. Paul, Minn.) aerobic count plates for APC, Petrifilm *Enterobacteriaceae* count plates for EB, Petrifilm *E. coli*/coliform count plates for EC and CF, and Petrifilm Staph Express count plates with Staph Express indicator disks for SA. Petrifilm plates were incubated according to the manufacturer's recommendations, and colonies were counted manually. Results were log transformed for analysis using SAS software (SAS Institute, Inc., Cary, N.C.). EB, CF, EC, and SA plates with no colonies (i.e., less than 10 CFU) were not used for determination of mean log CFU per gram, but a value of 1 CFU/g was assigned in the analysis of APC data when no colonies were present.

***Salmonella* isolation and characterization.** *Salmonella* was isolated by enriching a 25-g portion of each sample in 225 ml of tryptic soy broth (TSB; Difco, Becton Dickinson) according to established USMARC protocols *(18)* with the following modifications. Immunomagnetic separation was performed without the addition of protamine. The captured anti-*Salmonella* separation beads were selectively enriched in Oxoid Rappaport-Vassiliadis Soya peptone broth (Remel, Lenexa, Kans.) instead of Rappaport-Vassiliadis broth before plating to Hektoen enteric agar (HE) and brilliant green agar with sulfadiazol (BGS). Suspect colonies on either of these agar plates were confirmed to be *Salmonella* using PCR for the *invA* gene *(39).*

All confirmed *Salmonella* isolates were serogrouped using Wellcolex Colour *Salmonella* tests (Remel) according to the manufacturer's recommendations and were serotyped *(3)* using antisera for the identification of somatic and flagellar antigens (Remel). The antibiotic susceptibility of all *Salmonella* isolates was determined by performing MIC tests with the defined National Antimicrobial Resistance Monitoring System *Salmonella* antibiotic panels (CMV1AGNF, Trek Diagnostic Systems, Inc., Cleveland, Ohio) and a Sensititre AutoInoculator and AutoReader (Trek). The antibiotics in this panel were amikacin, amoxicillin–clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim-sulfamethoxazole.

***Campylobacter* isolation and identification.** The *Campylobacter* isolation procedure was performed using procedures found in the *Bacteriological Analytical Manual (21).* One hundred milliliters of 30°C prewarmed Bolton broth base (Neogen Corp., Lansing, Mich.) containing 5% sheep blood was added to 10 g of trim in a Whirl-Pak bag. This sample was then gently suspended by stomaching at the lowest speed (six strokes per second) for 1 min in a laboratory blender (Interscience Laboratories). Because trim samples had been frozen for storage and transit, a preenrichment step was used, which consisted of shaking (50 rpm) for 4 h at 37°C in a microaerobic atmosphere of 10% CO_2, 5% O_2, and 85% N. A selective enrichment followed, which consisted of the addition of antibiotics (20 mg/liter cefoperazone, 20 mg/liter trimethoprim, 20 mg/liter vancomycin, and 50 mg/liter cycloheximide) and incubation at 42°C for 48 h under microaerobic conditions. The enriched samples were streaked for isolation on modified CCDA plates (*Campylobacter* Blood-Free Selective Agar, Neogen) that were prepared as recommended by the manufacturer. These plates were incubated at 42°C for up to 48 h under microaerobic conditions. Suspect *Campylobacter* on CCDA plates were confirmed by a genus-specific PCR assay *(22)* and a species-specific multiplex PCR assay *(28)* that identified the thermotolerant *Campylobacter* species (*C. jejuni, C. coli, C. lari,* and *C. upsaliensis*).

***Listeria* isolation and characterization.** The prevalence of *Listeria* spp. in trim samples was determined using standard enrichment procedures *(19)* and PCR assay confirmation *(13).* Portions of trim (25 g) were placed in Whirl-Pak bags with 225 ml of University of Vermont medium (Neogen) and stomached (nine strokes per second) for 30 s in a laboratory blender (Interscience Laboratories). Each sample was then incubated at 35°C for 20 h. An aliquot (0.1 ml) of this enrichment was subcultured in 10 ml of Fraser broth (Neogen) that was incubated at 35°C for 40 h; this subculture was then streaked for isolation onto Oxford agar (Neogen) to isolate presumptive *Listeria* colonies. A multiplex PCR assay *(13)* was used to identify *Listeria* spp. (*L. monocytogenes, L. grayii, L. innocua, L. seeligeri, L. welshimeri,* and *L. ivanovii*). Isolates of *L. monocytogenes* (LM) were further characterized by serogrouping, using a combination of PCR assay *(14)* and specific antisera (Difco, Becton Dickinson) according to established protocols *(9).*

Non-O157 STEC isolation and characterization. The TSB enrichments for *Salmonella* were also used for the isolation of STEC as described previously *(6)* with minor modifications. Two 1-ml aliquots of each enrichment were mixed with 0.5 ml of 50% glycerol and stored at −70°C, and a 5-μl aliquot was used in a multiplex PCR assay to detect the *stx* genes *(35).* Enrichments of samples with positive signals for stx_1 and/or stx_2 were used for isolation of STEC. The corresponding −70°C glycerol stock of each stx_1- and/or stx_2-positive sample was diluted and spread plated to yield approximately 1,000 colonies per plate on 150-mm petri dishes of modified EC broth (Difco, Becton Dickinson) containing 1.5% Difco agar (Becton Dickinson) and 1% glucose. Colonies were lifted to Hybond-N+ membranes (GE Healthcare, Piscataway, N.J.) and used in hybridizations with combined stx_1 and stx_2 DNA probes *(6)* labeled using the ECL Direct Nucleic Acid Labeling and Detection System (GE Healthcare) according to the manufacturer's recommendations. Positive colonies were subcultured and confirmed to be STEC by PCR assay *(35).* Biochemical assays using Fluorocult LMX Broth (Merck KGaA, Darmstadt, Germany) and Sensititre Gram-negative ID panels (Trek) confirmed the organism to be *E. coli.* The serotype of each STEC isolate was determined by serologic identification of the O serogroup and molecular identification of the H group performed by the Gastroenteric Diseases Laboratory (College of Animal Sciences, Pennsylvania State University, University Park, Pa.).

Statistical analyses. Results from the enumeration of bacterial groups were analyzed by analysis of variance using the GLM procedures of SAS. The model included the main effect of country of origin. For significant main effects ($P < 0.05$), least squares means separation was carried out with the PDIFF option (a pairwise *t* test). Data for enumerations were log transformed before the analysis of variance. Pairwise comparisons of frequencies of EB, CF, EC, SA, and other pathogens were made using the PROC FREQ and Mantel-Haenszel chi-square analysis of SAS.

RESULTS

Enumerations. Populations of aerobic bacteria, EB, EC, CF, and SA (Table 1) revealed that URY samples had the highest concentrations of each indicator organism and AUS and NZL samples had the lowest. The APCs were different ($P < 0.05$) for each country. URY beef trim had the highest mean APC (2.8 log CFU/g), and U.S., NZL, and AUS trim had mean APCs of 2.5, 2.2, and 1.6 log CFU/g, respectively. Mean EB concentrations were the same ($P > 0.05$) for AUS, NZL, and U.S. trim (1.4, 1.5, and 1.5 log CFU/g, respectively) but were significantly higher ($P < 0.05$) for URY trim (2.0 log CFU/g). The frequency of samples with EB concentrations higher than 10 CFU/g was lowest and not different ($P > 0.05$) in AUS and NZL samples (8.2 and 9.0%, respectively). However, this frequency was significantly higher in both URY and U.S. samples (31.3 and 37.8%, respectively). The CF and EC results were similar in pattern to those for EB.

URY trim had the highest frequency of SA-positive samples (29.5%). The frequency of SA-positive samples was second highest in NZL samples (8.2%) and slightly different ($P < 0.05$) from U.S. trim (4.2%), but the same ($P > 0.05$) as AUS trim (4.0%). The level of SA contamination was statistically similar for URY (1.61 log CFU/g) and U.S. (1.35 CFU/g) samples but was significantly higher

TABLE 1. *Frequency and concentration of bacterial indicators of contamination in imported and domestic boneless beef trim destined for ground beef*[a]

Country[b]	Aerobic bacteria		EB			EC			CF			SA		
	No. of samples	Mean log CFU/g	No. of samples	No. (%) of samples above LOD[c]	Mean log CFU/g	No. of samples	No. (%) of samples above LOD	Mean log CFU/g	No. of samples	No. (%) of samples above LOD	Mean log CFU/g	No. of samples	No. (%) of samples above LOD	Mean log CFU/g
AUS	219	1.6 D	219	18 (8.2) B	1.4 B	198	2 (1.0) B	1.2[d]	198	9 (4.5) B	1.4 AB	198	8 (4.0) BC	1.1 B
NZL	223	2.2 C	223	20 (9.0) B	1.5 B	219	1 (0.5) B	1.0[d]	219	10 (4.6) B	1.5 AB	219	18 (8.2) B	1.3 B
URY	256	2.8 A	256	80 (31.3) A	2.0 A	241	23 (9.5) A	1.8 A	241	63 (26.1) A	2.0 A	241	71 (29.5) A	1.6 A
USA	486	2.5 B	487	184 (37.8) A	1.5 B	377	27 (7.2) A	1.2 B	377	96 (25.5) A	1.6 B	377	16 (4.2) C	1.4 AB

[a] Manual counts were done on appropriate Petrifilm count plates (3M Microbiology). EB, *Enterobacteriaceae*; EC, *E. coli*; CF, coliforms; SA, *Staphylococcus aureus*. Bacterial concentrations and frequencies within a column followed by the same letter are not significantly different ($P > 0.05$).

[b] Frozen beef trim from Australia (AUS), New Zealand (NZL), and Uruguay (URY) was compared with domestic (USA) beef trim (97.5% chilled and 2.5% frozen).

[c] Number of samples for which plate counts were above the limit of detection (LOD) of the 10 CFU/g.

[d] No statistical analysis was conducted when only one or two samples were above the LOD.

TABLE 2. *Frequency and characterization of* Salmonella *in imported and domestic boneless beef trim destined for ground beef*[a]

Country[b]	No. of samples	No. (%) of positive samples[c]	No. of samples resistant to antibiotics[d]
AUS	220	0	NA[e]
NZL	223	1 (0.4)[f]	1[g]
URY	256	1 (0.4)[h]	0
USA	487	4 (0.8)[i]	0

[a] *Salmonella* strains were isolated by immunomagnetic separation and selective enrichment. Suspect colonies on HE and BGS were confirmed by PCR assay, serogrouped, and serotyped.
[b] Frozen beef trim from Australia (AUS), New Zealand (NZL), and Uruguay (URY) was compared with domestic (USA) beef trim (97.5% chilled and 2.5% frozen).
[c] Prevalences for each country are not significantly different ($P > 0.05$).
[d] Antibiotic resistance was determined using NARMS MIC panels and the Trek Diagnostics Sensititre.
[e] NA, not applicable.
[f] New Zealand *Salmonella* isolate was serotype Typhimurium O5 null (Copenhagen).
[g] Isolate was resistant to ampicillin, chloramphenicol, streptomycin, sulphazoxazole, and tetracycline.
[h] Uruguay *Salmonella* isolate was serotype Newport.
[i] Domestic *Salmonella* isolates were serotypes Anatum, Enteritidis, and Montevideo. One isolate was untypeable.

TABLE 3. *Frequency and characterization of* Campylobacter *isolates from imported and domestic boneless beef trim destined for ground beef*[a]

Country[b]	No. of samples	No. (%) of positive samples[c]	No. of samples infected with: Undetermined species	*C. jejuni*	*C. coli*
AUS	151	0			
NZL	216	1 (0.5)	1	0	0
URY	250	1 (0.4)	0	1	0
USA	393	5 (1.3)	0	4	1

[a] *Campylobacter* strains were isolated by selective enrichment in Bolton broth followed by selective growth on modified CCDA. Suspect colonies were confirmed by latex agglutination and species-specific PCR. Thermotolerant *Campylobacter* species were identified by PCR assay of the *lpxA* gene.
[b] Frozen beef trim from Australia (AUS), New Zealand (NZL), and Uruguay (URY) was compared with domestic (USA) beef trim (97.5% chilled and 2.5% frozen).
[c] Prevalences are not significantly different ($P > 0.05$).

in URY samples than in both AUS and NZL samples ($P < 0.05$).

Salmonella. The prevalence of *Salmonella* was less than 1% in all samples and the same ($P > 0.05$) in the samples from all countries (Table 2). One *Salmonella* strain was isolated from NZL samples and one was isolated from URY samples. Four *Salmonella* strains were isolated from U.S. samples. No *Salmonella* was found in the 220 samples from Australia. Each *Salmonella* isolate was of a different serotype. The NZL *Salmonella* was serotype Typhimurium O5 null (Copenhagen), and the URY *Salmonella* isolate was serotype Newport. One of the *Salmonella* isolates in the U.S. samples was untypeable. The others were identified as *Salmonella* Anatum, *Salmonella* Enteritidis, and *Salmonella* Montevideo. All of the *Salmonella* isolates were characterized for antibiotic resistance; one isolate possessed resistance to multiple antibiotics. The *Salmonella* Typhimurium from New Zealand was resistant to ampicillin (MIC > 32 μg/ml), chloramphenicol (MIC > 32 μg/ml), streptomycin (MIC $= 64$ μg/ml), sulfamethoxazole (MIC > 256 μg/ml), and tetracycline (MIC $= 32$ μg/ml). This isolate was also intermediately resistant to amoxicillin–clavulanic acid (MIC $= 16$ μg/ml).

Campylobacter. The prevalence of *Campylobacter* was low and was not different ($P > 0.05$) among the samples tested. Some difficulties in maintaining microaerophilic conditions existed during these experiments, leading to the loss of some samples; seven *Campylobacter* were isolated (Table 3). Four *C. jejuni* and one *C. coli* strains were isolated from 393 U.S. samples. One *C. jejuni* strain was isolated from 250 URY samples. One *Campylobacter* strain that was neither *C. jejuni* nor *C. coli* also was isolated from 216 NZL samples. No *Campylobacter* strains were isolated from 151 AUS samples.

Listeria. The prevalence of *Listeria* spp. and LM was significantly higher ($P < 0.05$) in URY samples than in samples from other countries (Table 4). Twenty-nine percent (66 of 226) of URY trim samples contained at least one species of *Listeria.* Of the 66 *Listeria*-positive samples, 53 (almost 25% of all the URY samples tested) contained LM. Fifteen of the 66 *Listeria*-positive samples contained multiple species of *Listeria* and/or multiple serovars of LM. Other *Listeria* species identified in URY samples were *L. innocua and L. seeligeri,* and four isolates were untypeable. Twenty-two (6.5%) of the U.S. samples contained *Listeria.* Seventeen of these samples contained LM, and the other five contained other *Listeria* species (*L. ivanovii, L. welshimeri,* and *L. innocua*). One U.S. sample contained *L. innocua* and two serovars of LM. Six NZL and six AUS samples contained *Listeria*; five and four of these samples, respectively, were positive for LM. The only other *Listeria* species isolated from NZL and AUS samples was *L. innocua.*

Different serovars of LM were predominant in U.S., NZL, and AUS samples. All NZL LM isolates were serovar 1/2c, and these five isolates originated from two different establishments on five different pack dates. Three of four AUS LM strains were of the 4b serovar, and these three originated from one establishment but pack dates spanned a 3-month period. The predominant LM serovar in U.S. samples was 1/2a; 10 of these isolates originated from six different establishments. No LM serovar predominated in the URY samples, where serovars 1/2b, 1/2c, and 4b were equally prevalent ($P > 0.05$). Serovar 4b was found in 22 of the 58 LM-positive URY samples. These 22 serovar 4b LM stains originated from 7 of the 17 URY establishments

TABLE 4. *Frequency and characterization of* Listeria *and* L. monocytogenes *(LM) isolates from imported and domestic boneless beef trim destined for ground beef*[a]

Country[b]	No. of samples	No. (%) of samples positive for[c]:			No. of LM isolates of each serovar[d]:				
		LM	Other *Listeria*	Total	1/2a	1/2b	1/2c	4b	3a or 3b
AUS	198	4 (2.0) B	2 (1.0) B	6 (3.0) BC	0 Y	0 Y	1 XY	3 X	0 Y
NZL	219	5 (2.3) B	1 (0.5) B	6 (2.7) C	0 Y	0 Y	5 X	0 Y	0 Y
URY	226	53 (24) A	13 (5.8) A	66 (29) A	3 Y	16 X	17 X	22 X	0 Y
USA	341	17 (5.0) B	5 (1.5) B	22 (6.5) B	10 X	4 Y	0 Z	0 Z	4 Y

[a] *Listeria* detection was performed using standard culture techniques, and LM was identified by PCR assay of the *iap* and *prs* genes.
[b] Frozen beef trim from Australia (AUS), New Zealand (NZL), and Uruguay (URY) was compared with domestic (USA) beef trim (97.5% chilled and 2.5% frozen).
[c] Some samples contained multiple species of *Listeria* and/or serovars of LM. Within the same column values followed by the same letter are not significantly different ($P > 0.05$).
[d] LM serovars were determined using a combination of serology and PCR assay for specific sequences within Imo0737, Imo1118, ORF 2819, and ORF 2110. Within the same row in this section, values followed by the same letter are not significantly different ($P > 0.05$).

that provided beef trim. Serovar 1/2b originated from multiple establishments, whereas over 75% of serovar 1/2c strains originated from the same establishment.

Non-O157 STEC. The prevalence of Shiga toxin genes (*stx*) in each sample was determined by PCR assay (Table 5). Two different *stx* genes were evaluated, and trim samples were positive for stx_1, stx_2, or both stx_1 and stx_2. About 30% of the U.S., AUS, and URY samples were PCR positive for the *stx* genes. NZL samples were least often positive (10% of samples; $P < 0.05$). The distribution of stx_1 and stx_2 genes among the *stx*-positive samples varied by country of origin. The frequency of stx_1 alone was lower in *stx*-positive URY samples ($P < 0.05$) than in *stx*-positive AUS, NZL, and U.S. samples, whereas the frequency of stx_2 alone was not different ($P > 0.05$) among all source countries. The frequency of both genes in a single sample was highest in *stx*-positive URY samples ($P < 0.05$) and lowest in *stx*-positive AUS and NZL samples ($P < 0.05$).

The samples that were PCR positive in the *stx* gene screen were then further processed in an effort to isolate the source of the *stx* gene(s) (Table 6). At least one STEC was isolated from 81 of the 309 samples that were positive for *stx* genes. A total of 99 STEC were isolated. In 10 samples, two different STEC were isolated, and in four samples three different STEC were isolated. We were able to confirm the source of the *stx* gene(s) in 28 U.S., 9 AUS, 4 NZL, and 40 URY samples by isolation of an STEC. The STEC isolates were serotyped, and 63 different serotypes were identified. At least one serotype associated with human illness was isolated in samples from each country. Thirteen serotypes associated with HUS were isolated from U.S., URY, and NZL samples. No HUS-related strains were isolated from AUS samples. The prevalence of HUS-related serotypes among the isolated STEC strains from U.S. samples was not different ($P > 0.05$) from the prevalence in NZL, AUS, and URY samples.

TABLE 5. *Frequency of Shiga toxin 1 and Shiga toxin 2 genes in imported and domestic boneless beef trim destined for ground beef*[a]

Country[b]	No. of samples	No. (%) of samples[c] positive for:			
		stx_1	stx_2	stx_1 and stx_2	Total
AUS	220	44 (66) A	15 (22) A	8 (12) BC	67 (30) A
NZL	223	16 (70) A	5 (22) A	2 (8) BC	23 (9.7) B
URY	256	14 (20) B	24 (33) A	34 (47) A	72 (28) A
USA	487	77 (52) A	38 (26) A	32 (22) B	147 (30) A

[a] Shiga toxin genes were detected by PCR assays from TSB enrichments of each sample.
[b] Frozen beef trim from Australia (AUS), New Zealand (NZL), and Uruguay (URY) was compared with domestic (USA) beef trim (97.5% chilled and 2.5% frozen).
[c] Within each column, values followed by the same letter are not significantly different ($P > 0.05$).

DISCUSSION

Approximately 40 countries are approved to export to the United States (listed in the U.S. Code of Federal Regulations 9 CFR 327.2[b] and 381.196[b]). Countries that export the most beef to the United States are Australia, Canada, and New Zealand followed by Uruguay, Denmark, Argentina, Brazil, Nicaragua, and Costa Rica *(52)* In 2004, the United States imported 3.6 billion pounds (1.6 billion kilograms) of beef and veal, about 1 billion pounds (0.5 billion kilograms) of which originated in Canada *(51).* Although Canada is a significant source of imported beef, it does not primarily provide lean beef trim destined for ground beef. The North American cattle herds are similar with regard to relevant foodborne pathogens *(18).*

The number of tests performed in these studies consumed the entire sample, and in some cases insufficient sample was available. In situations where results were inconclusive, no results were recorded. For this reason, only 341 of the 487 U.S. samples produced results for *Listeria* (Table 4). This situation also was encountered with other enumerations (Table 1). While processing samples for *Campylobacter* (Table 3), the inability to maintain the microaerophilic atmosphere for a number of samples precluded valid results. Ap-

TABLE 6. *Serotypes of STEC isolated from imported and domestic boneless beef trim destined for ground beef*[a]

Country[b]	No. of positive samples[c]	No. of STEC isolated	Serotypes[d]					No. of HUS-related serotypes[e]
AUS	9	10[f]	O33:H11	O73:H35	O113:H36 (3)	O113:H51	O147:H7	0 B
			O171:H$^+$	ONT:H$^+$[g]	ONT:H2			
NZL	4	4	O26:H8	O26:H11[g,h]	O64:H9	O163:H19[g,h]		2 A
URY	40	52[i]	O2:H25	O6:H30	O6:H34	O8:H3	O8:H19 (2)[g,h]	6 B
			O15:H27 (3)[g]	O20:H19 (4)[g,h]	O39:H14	O55/83:H15	O74:H28 (2)	
			O82:H8	O82:H15	O83:H8 (2)	O83:H11	O88:H38 (2)	
			O113:H21[g,h]	O113:H36	O116:H36	O130:H11	O163:H19 (3)[g,h]	
			O163:H26	0168:H$^+$[g,h]	O174:H11	O174:H28 (2)	O174:H36 (2)[g]	
			ONT:H$^+$ (2)[g]	ONT:H11 (2)[g,h]	ONT:H18[g]	ONT:H19 (2)[g]	ONT:H32	
			ONT:H34	ONT:H46 (4)[g]	ONT:H51	ONT:H52		
USA	28	32[j]	O5:H36	O8:H19 (3)[g,h]	O20:H19[g,h]	O55/83:H15	O73:H$^+$	5 AB
			O73:H18	O79:H7[g,h]	O83:H$^+$	O83:H38	O83/132:H2	
			O88:H38	O113:H4[g]	O113:H51	O116:H21	O117:H$^+$	
			O132:H$^+$	O132:H38	O142:H34	O150:H2 or 35	O165:Hneg[g,h]	
			O171:H2	O172:H10	O174:H36	OX25:H11	ONT:H2	
			ONT:H7	ONT:H32	ONT:H51	Orough:Hneg[g,h]		

[a] Serotyping of STEC isolates was performed by the Gastroenteric Diseases Laboratory (Pennsylvania State University).
[b] Frozen beef trim from Australia (AUS), New Zealand (NZL), and Uruguay (URY) was compared with domestic (USA) beef trim (97.5% chilled and 2.5% frozen).
[c] Number of Shiga toxin 1– or Shiga toxin 2–positive samples from which an STEC isolate was recovered.
[d] Numbers in parentheses are the number of samples in which this serotype was found when more than 1.
[e] Values followed by the same letter are not different ($P > 0.05$).
[f] One sample contained two STEC serotypes.
[g] Serotype associated with human illness.
[h] Serotype associated with HUS *(11)*.
[i] Five samples contained two STEC serotypes, and one sample contained three STEC serotypes.
[j] Seven samples contained two STEC serotypes, and three samples contained three STEC serotypes.

proximately 200 or more samples from each country were available in each assay for statistical analyses, except for AUS samples tested for *Campylobacter*.

In this study, the foreign samples destined for export to the United States were packed from September through December, which are spring months in the Southern Hemisphere. The samples arrived and were compared with U.S. samples that were collected from December through February, which are winter months in the Northern Hemisphere. The seasonality of pathogens such as *Salmonella (7), E. coli (7),* and *Campylobacter (44)* is well documented. Pathogens increase in the spring, become highly prevalent through the summer and into the autumn, and then decrease during the winter. Therefore, the U.S. samples in this study were obtained during the low-prevalence season whereas the imported samples were from a high-prevalence season. These seasonal effects may be responsible for some of the differences observed between URY and U.S. samples, but when the results from the AUS and NZL samples are considered, the differences between the URY and U.S. samples seem more likely to be associated with the processing environment and processes in use rather than seasonal variations in bacterial prevalences.

The concentrations of these target indicator bacteria in boneless beef trim appear to have changed little since the publication of earlier studies. In previous studies, U.S. trim samples had a mean APC of 2.7 log CFU/g (range, 1.9 to 3.6 log CFU/g) and a mean CF count of 1.7 log CFU/g (range, 0.8 to 2.5 log CFU/g) *(26, 27)*. The differences in these populations between countries of origin are likely due to the number of specific instances of high counts. APCs for U.S. trim ranged as high as 4.85 log CFU/g as did APCs of URY trim, but the number of instances in which this concentration was observed in the U.S. trim was half that for the URY trim (4 and 9%, respectively). Both NZL and AUS trim had individual APCs that were higher than 4 log CFU/g, but the occurrence of these high concentrations was less frequent (1%) than it was in U.S. or URY trim samples. This frequency of putative contamination events also was reflected by the number of EB, CF, and EC colonies that were countable in all the samples.

All AUS processing establishments are required to operate under a single standard for the production, transportation, and export control of meat products for human consumption *(43)*. In a recent report on the microbiological quality of beef carcasses and frozen beef in Australia *(38),* beef produced at 24 establishments was examined. The results of that report correlate well with the results of our study for mean counts of total viable cells (APCs), coliforms, *E. coli,* and *Enterobacteriaceae*. The exception was the SA frequency. Coagulase-positive staphylococci were detected in 20.3% of the samples at a mean concentration of 0.80 log CFU/g and a maximum concentration 2.32 log CFU/g. We detected SA at a frequency of only 4% in AUS

samples. This discrepancy may be due to differences in the methodologies used to detect and identify SA *(38).*

C. jejuni is the most commonly reported bacterial cause of foodborne infection in the United States *(2).* The major risk factors for human campylobacteriosis are mishandling of raw poultry and consumption of undercooked poultry. The possible presence of *Campylobacter* previously has not been considered a significant food safety issue associated with beef. However, in beef cattle *Campylobacter* has been reported to be chronically shed by 85% of animals, and *C. jejuni* prevalence as high as 38% was reported *(23, 24).* Because of these high prevalences before processing, *Campylobacter* could be a significant carcass and subsequent trim contaminant. Despite the potential for contamination, a very low prevalence was found in the U.S. beef trim samples. Retail beef *Campylobacter* prevalence of <1% has been reported *(2, 54).*

We expected to find a higher prevalence of *Campylobacter* in AUS and NZL samples, as indicated by a 2001 Australian (OzFoodNet) survey performed in conjunction with the U.S. counterpart (FoodNet) in which the incidence of culture-confirmed *Campylobacter* infections was more than six times higher in Australia than in the United States *(53).* In New Zealand, *Campylobacter* infections were reported to have quadrupled in the last 15 years, and the incidence of *Campylobacter* infections was reported to be about five times that in Australia *(5).* We found a very low incidence of *Campylobacter* in the AUS and NZL samples, as has been previously reported for retail red meat in these two countries *(33).* Little is known about *Campylobacter* prevalence in URY, but this pathogen is recognized as a significant cause of diarrhea in that country *(46).* Our results indicate that *Campylobacter* does not appear to be a significant contaminant of beef trim and ground beef.

Salmonella is a major cause of foodborne illness in the United States, resulting in an estimated 1.3 million human cases, 15,600 hospitalizations, and 550 deaths each year *(31).* The most common *Salmonella* serotypes associated with human illness in the United States are Typhimurium, Enteritidis, Newport, Heidelberg, and Muenchen *(31).* We isolated *Salmonella* serotypes Anatum, Montevideo, and Enteritidis from the U.S. trim samples in this study. According to the USDA National Animal Health Monitoring System, *Salmonella* Montevideo was one of the five serotypes most frequently shed by feedlot cattle *(49),* and *Salmonella* Anatum was one of the five serotypes most frequently shed by dairy cattle *(48, 50). Salmonella* Enteritidis is usually associated with eggs and poultry and is uncommon in cattle *(42).*

Isolates of *Salmonella* serotypes Newport and Typhimurium have exhibited various antimicrobial susceptibility patterns, and many have shown resistance to multiple antimicrobials *(10, 55).* A multidrug-resistant *Salmonella* Newport of particular concern is resistant to eight antimicrobials: ampicillin, chloramphenicol, streptomycin, sulfonamides, tetracycline, amoxicillin–clavulanic acid, ceftiofur, and cephalothin. Multidrug-resistant *Salmonella* Typhimurium can have either the same antibiotic resistance as *Salmonella* Newport or can be resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT). The *Salmonella* Newport isolated from a URY trim sample was not resistant to these antimicrobials. We isolated *Salmonella* Typhimurium, a commonly identified serotype from bovine sources in New Zealand *(4),* from one NZL trim sample, and it was ACSSuT resistant.

LM is the causative agent of epidemic and sporadic listeriosis, and the consequences from contracting listeriosis can be particularly severe in pregnant women, newborns (<1 year of age), the elderly (>65 years of age), and immunocompromised individuals *(16, 30, 41).* LM is present throughout the environment and is routinely isolated from numerous animal sources, including cattle, but comprehensive information on the prevalence of LM in U.S. and foreign beef trim is minimal *(40).* LM is considered a public health threat in ready-to-eat foods (e.g., cold cuts, cheese, and ice cream), whereas its presence in raw meat, which will presumably be cooked, is not considered as great a threat. In our study, the prevalence of LM in beef trim was generally below 5%, except for samples from Uruguay, where almost one in four samples contained LM.

Thirteen serovars of LM have been described, but four serovars (1/2a, 1/2b, 1/2c, and 4b) make up 95% of the isolates recovered from foods and infected humans *(14, 45).* Serovar 4b has been described as the cause of major outbreaks of invasive listeriosis *(14, 30).* We isolated LM 4b only from AUS and URY trim samples. Three of the four LM isolates from AUS samples were serovar 4b and were traceable to a single establishment, which provided only four samples for this study. Thirty-eight percent of the LM isolated from URY samples were serovar 4b, and two thirds of these isolates could be traced back to four establishments. Such high prevalence of a single serotype suggest that its source is the processing environment, but additional tracking data are needed for confirmation. The five LM isolates found in the NZL samples came from 2 of the 44 establishments that provided samples. The 17 LM isolates from U.S. samples could be traced back to nine establishments. These data suggest that URY processors need to examine their production environments for sources of LM contamination so that contamination with this organism can be reduced.

STEC strains have been implicated as the causative agents in human diseases ranging from mild diarrhea to life-threatening HUS *(32, 37).* STEC strains can contain either or both of the *stx* genes; however, severe disease is more commonly associated with strains that possess the stx_2 gene *(37).* A large difference in the distribution of the stx_2 gene was observed in the trim samples. Only 10% of NZL samples contained an *stx* gene, and in 70% of the cases it was the less pathogenic stx_1 gene. URY, AUS, and U.S. samples had similar frequencies of *stx* gene carriage, but 80% of the *stx*-positive URY samples carried the more pathogenic stx_2 gene, whereas only 34% of AUS and 48% of U.S. samples carried stx_2.

Statistical analysis of recovery rates for STEC isolates from samples that were PCR positive for the *stx* genes revealed that URY trim was different from U.S., AUS, and NZL trim. The rate of recovery of STEC isolates from U.S.,

AUS, or NZL samples was less than 20%, whereas STEC isolates were recovered from 56% of URY samples that were PCR positive for *stx*. The PCR assay used may have detected *stx* genes carried by other organisms such as *Citrobacter freundii* or *Enterobacter cloacae (34, 47)*; however, it is more likely that the *E. coli* contamination in the URY samples, based on the results of *E. coli* enumeration, is the explanation for the higher rate of recovery of STEC in the URY samples.

Many STEC serotypes have been isolated, characterized, and catalogued. Some of the non-O157 STEC serotypes associated with clinical disease are O8, O26, 045, O103, O111, O113, O121, and 0145 *(1, 12, 15, 32)*. However, many STEC strains have been neither isolated from humans nor associated with human disease *(11)*. Statistical analysis revealed that the serotype distribution of the HUS-associated strains from imported and U.S. beef trim was not different.

The STEC serotype most frequently isolated from the AUS trim in our study was O113:H36. Although STEC O113 is described as a common disease-related serotype, the H36 antigen associated with this isolate in the AUS samples has not been associated with HUS *(11, 36)*. In our study, only 4 of the 223 NZL samples examined contained an isolatable STEC, and two of the four isolates had HUS-related serotypes. A much larger sample set would be needed to determine whether NZL beef trim posed any higher risk of infection than does U.S. trim. In our study, the incidence of HUS-associated serotypes in NZL samples (two of the four isolates) was not different ($P > 0.05$) from that in U.S. samples (7 of 32 isolates). In studies performed for the New Zealand Food Safety Authority (NZFSA), the majority of STEC isolates from NZL red meats were of serotypes that were infrequently a cause of disease *(29)*. In that study, STEC contamination was low in New Zealand by international standards, and infections were rare. The majority of STEC infections in New Zealand were attributed to serotype O157:H7, and only one death was attributed to a non-O157 STEC infection *(29)*. We isolated STEC serotypes O163:H19 and O26:H11 from NZL samples. Serotype O163:H19 has been isolated by NZFSA from minced beef *(20)*, and serotype O26:H11 was previously isolated from NZL bobby veal *(17)*. The presence of veal or calf trim in the imported beef trim from NZL could not be determined. According to previous reports and ongoing surveillance, the other serotypes of STEC we isolated from NZL samples, O26:H8 and O64:H9, were unique.

To the best of our knowledge, this is the first report of STEC serotypes O5:H36, O8:H3, O33:H11, O39:H14, O73:H35, O82:H15, O83:H8, O83:H38, O113:H36, O113:H51, and O163:H26. Three of these serotypes (O88:H38, O113:H36, and O113:H51) were isolated from beef trim originating in different countries. These results indicate that STEC is common in multiple countries, it is likely that there are many STEC serotypes yet to be identified.

The results of this study indicate that microbiological differences exist between imported and U.S. boneless beef trim destined for ground beef, but these differences do not necessitate changes to the current monitoring system used in the United States. Although it may be tempting to use these data to compare the microbiological quality of beef trim from the countries examined, such comparisons are best made using data collected before export or data from agencies such as OzFoodNet or the New Zealand National Microbiological Database. Generally, AUS and NZL lean beef trim had lower levels of contamination than did U.S. trim, whereas in URY samples levels of some contaminants were higher after arrival in the United States. Compared with U.S. trim, URY trim also had a higher frequency of illness-related serovars of LM and HUS-associated STEC. During the collection of these data, preliminary results were shared with the participants. The importers who supplied the beef trim for this work communicated with their URY suppliers in an effort to reduce contamination and improve slaughter practices. It has been reported to us that URY processors have adopted improved methods and that current imports of URY beef trim into the United States have improved in microbial quality.

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REFERENCES

1. Acheson, D. W. 2000. How does *Escherichia coli* O157:H7 testing in meat compare with what we are seeing clinically? *J. Food Prot.* 63:819–821.
2. Altekruse, S. F., N. J. Stern, P. I. Fields, and D. L. Swerdlow. 1999. *Campylobacter jejuni*—an emerging foodborne pathogen. *Emerg. Infect. Dis.* 5:28–35.
3. Andrews, W. H., and T. S. Hammack. 1998. *Salmonella,* chap. 5. *In* Bacteriological analytical manual, 8th ed., rev. A. Available at: http://www.cfsan.fda.gov/~ebam/bam-toc.html. Accessed 6 January 2006.
4. Anonymous. 2005. Non-human *Salmonella* isolates, 2004. Public Health Surveillance Report. Available at: http://www.surv.esr.cri.nz/enteric_reference/nonhuman_salmonella.php?we_objected=579. Accessed 6 January 2006.
5. Anonymous. 2005. Notifiable and other diseases in New Zealand. Annual report 2004. Available at: http://www.surv.esr.cri.nz/PDF_surveillance/AnnSurvRpt/2004AnnualSurvRpt.pdf. Accessed 6 January 2006.
6. Arthur, T. M., G. A. Barkocy-Gallagher, M. Rivera-Betancourt, and M. Koohmaraie. 2002. Prevalence and characterization of non-O157 Shiga toxin–producing *Escherichia coli* on carcasses in commercial beef cattle processing plants. *Appl. Environ. Microbiol.* 68:4847–4852.
7. Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2003. Seasonal prevalence of Shiga toxin–producing *Escherichia coli,* including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. *J. Food Prot.* 66:1978–1986.
8. Barkocy-Gallagher, G. A., E. D. Berry, M. Rivera-Betancourt, T. M. Arthur, X. Nou, and M. Koohmaraie. 2002. Development of methods for the recovery of *Escherichia coli* O157:H7 and *Salmonella* from beef carcass sponge samples and bovine fecal and hide samples. *J. Food Prot.* 65:1527–1534.
9. Bennett, R. W., and R. E. Weaver. 1998. Serodiagnosis of *Listeria*

monocytogenes, chap. 11. *In* Bacteriological analytical manual, 8th ed., rev. A. Available at: http://www.cfsan.fda.gov/~ebam/bam-toc.html. Accessed 6 January 2006.
10. Berge, A. C., J. M. Adaska, and W. M. Sischo. 2004. Use of antibiotic susceptibility patterns and pulsed-field gel electrophoresis to compare historic and contemporary isolates of multi-drug-resistant *Salmonella enterica* subsp. *enterica* serovar Newport. *Appl. Environ. Microbiol.* 70:318–323.
11. Bettelheim, K. A. 2006. Serotypes of VTEC. The VTEC table. Available at: http://www.microbionet.com.au/vtectable.htm. Accessed 20 January 2006.
12. Brooks, H. J., K. A. Bettelheim, B. Todd, and M. D. Holdaway. 1997. Non-O157 vero cytotoxin producing *Escherichia coli*: aetiological agents of diarrhoea in children in Dunedin, New Zealand. *Comp. Immunol. Microbiol. Infect. Dis.* 20:163–170.
13. Bubert, A., I. Hein, M. Rauch, A. Lehner, B. Yoon, W. Goebel, and M. Wagner. 1999. Detection and differentiation of *Listeria* spp. by a single reaction based on multiplex PCR. *Appl. Environ. Microbiol.* 65:4688–4692.
14. Doumith, M., C. Buchrieser, P. Glaser, C. Jacquet, and P. Martin. 2004. Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *J. Clin. Microbiol.* 42:3819–3822.
15. Friedrich, A. W., M. Bielaszewska, W. L. Zhang, M. Pulz, T. Kuczius, A. Ammon, and H. Karch. 2002. *Escherichia coli* harboring Shiga toxin 2 gene variants: frequency and association with clinical symptoms. *J. Infect. Dis.* 185:74–84.
16. Gasanov, U., D. Hughes, and P. M. Hansbro. 2005. Methods for the isolation and identification of *Listeria* spp. and *Listeria monocytogenes*: a review. *FEMS Microbiol. Rev.* 29:851–875.
17. Hathaway, S. (Programme Development Group, New Zealand Food Safety Authority). 2005. Personal communication.
18. Health Canada. 2003. Canadian integrated surveillance report: *Salmonella, Campylobacter,* pathogenic *E. coli,* and *Shigella* from 1996 to 1999. CCDR 2003;29S1. Available at: http://www.phac-aspc.gc.ca/publicat/ccdr-rmtc/03vol29/29s1/index.html. Accessed 6 January 2006.
19. Hitchins, A. D. 2002. Detection and enumeration of *Listeria monocytogenes* in foods, chap. 10. *In* Bacteriological analytical manual, 8th ed., rev. A. Draft rev., September 2002. Available at: http://www.cfsan.fda.gov/~ebam/bam-toc.html. Accessed 6 January 2006.
20. Hudson, J. A., C. Nicol., J. Capill, and J. Bennett. 2000. Isolation of Shiga toxin–producing *Escherichia coli* (STEC) from foods using EHEC agar. *Lett. Appl. Microbiol.* 30:109–113.
21. Hunt, J. M., C. Abeyta, and T. Tran. 1998. *Campylobacter,* chap. 7. *In* Bacteriological analytical manual, 8th ed., rev. A. Available at: http://www.cfsan.fda.gov/~ebam/bam-toc.html. Accessed 6 January 2006.
22. Inglis, G. D., and L. D. Kalischhuk. 2003. Use of PCR for direct detection of *Campylobacter* species in bovine feces. *Appl. Environ. Microbiol.* 69:3435–3447.
23. Inglis, G. D., L. D. Kaliscuk, and H. W. Busz. 2003. A survey of *Campylobacter* species shed in faeces of beef cattle using polymerase chain reaction. *Can. J. Microbiol.* 49:655–661.
24. Inglis, G. D., L. D. Kaliscuk, and H. W. Busz. 2004. Chronic shedding of *Campylobacter* species in beef cattle. *J. Appl. Microbiol.* 97:410–420.
25. Kaferstein, F. K., Y. Motarjemi, and D. W. Bettcher. 1997. Foodborne disease control: a transnational challenge. *Emerg. Infect. Dis.* 3:503–510.
26. Kang, D. H., M. Koohmaraie, W. J. Dorsa, and G. R. Siragusa. 2001. Development of a multiple-step process for the microbial decontamination of beef trim. *J. Food Prot.* 64:63–71.
27. Kang, D. H., M. Koohmaraie, and G. R. Siragusa. 2001. Application of multiple antimicrobial interventions for microbial decontamination of commercial beef trim. *J. Food Prot.* 64:168–171.
28. Klena, J. D., C. T. Parker, K. Knabb, J. C. Ibbitt, P. M. L. Devane, S. T. Horn, W. G. Miller, and M. E. Konkel. 2004. Differentiation of *Campylobacter coli, Campylobacter jejuni, Campylobacter lari,* and *Campylobacter upsaliensis* by a multiplex PCR developed from the nucleotide sequence of the lipid A gene *lpxA. J. Clin. Microbiol.* 42:5549–5557.
29. Lake, R., A. Hudson, and P. Cressey. 2002. Risk profile: Shiga toxin–producing *Escherichia coli* in red meat and meat products. Institute of Environmental Science and Research, Ltd., Porirua, New Zealand.
30. Mead, P. S., E. F. Dunne, L. Graves, M. Wiedmann, M. Patrick, S. Hunter, E. Salehi, F. Mostashari, A. Craig, P. Mshar, T. Bannerman, B. D. Sauders, P. Hayes, W. Dewitt, P. Sparling, P. Griffin, D. Morse, L. Slutsker, and B. Swaminathan. 2006. Nationwide outbreak of listeriosis due to contaminated meat. *Epidemiol. Infect.,* in press.
31. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* 5:607–625.
32. Nataro, J. P., and J. B. Kaper. 1998. Diarrheagenic *Escherichia coli. Clin. Microbiol. Rev.* 11:142–201.
33. OzFoodNet Working Group. 2005. Reported foodborne illness and gastroenteritis in Australia: annual report of the OzFoodNet network, 2004. *Commun. Dis. Intell.* 29:164–190.
34. Paton, A. W., and J. C. Paton. 1996. *Enterobacter cloacae* producing a Shiga-like toxin II–related cytotoxin associated with a case of hemolytic-uremic syndrome. *J. Clin. Microbiol.* 34:463–465.
35. Paton, A. W., and J. C. Paton. 1998. Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for *stx1, stx2, eaeA,* enterohemorrhagic *E. coli hlyA, rfb*O111, and *rfb*O157. *J. Clin. Microbiol.* 36:598–602.
36. Paton, A. W., R. M. Ratcliff, R. M. Doyle, J. A. Lanser, and J. C. Paton. 1996. Molecular microbiological investigation of an outbreak of hemolytic-uremic syndrome caused by dry fermented sausage contaminated with Shiga-like toxin–producing *Escherichia coli. J. Clin. Microbiol.* 34:1622–1627.
37. Paton, J. C., and A. W. Paton. 1998. Pathogenesis and diagnosis of Shiga toxin–producing *Escherichia coli* infections. *Clin. Microbiol. Rev.* 11:450–479.
38. Phillips, D., D. Jordan, S. Morris, I. Jenson, and J. Sumner. 2006. A national survey of the microbiological quality of beef carcasses and frozen boneless beef in Australia. *J. Food Prot.* 69:1113–1117.
39. Rahn, K., S. A. De Grandis, R. C. Clarke, S. A. McEwen, J. E. Galan, G. Ginocchio, R. Curtiss, and C. L. Gyles. 1992. Amplification of an *invA* gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella. Mol. Cell. Probes* 6:271–279.
40. Rivera-Betancourt, M., S. D. Shackelford, T. M. Arthur, K. E. Westmoreland, G. Bellinger, M. Rossman, J. O. Reagan, and M. Koohmaraie. 2004. Prevalence of *Escherichia coli* O157:H7, *Listeria monocytogenes,* and *Salmonella* in two geographically distant commercial beef processing plants in the United States. *J. Food Prot.* 67:295–302.
41. Schlech, W. F. 2000. Foodborne listeriosis. *Clin. Infect. Dis.* 31:770–775.
42. Sorensen, O., J. V. Donkersgoed, M. McFall, K. Manninen, G. Gensler, and G. Ollis. 2002. *Salmonella* spp. shedding by Alberta beef cattle and the detection of *Salmonella* spp. in ground beef. *J. Food Prot.* 65:484–491.
43. Standards Australia. 2002. Australian standard for production and transportation of meat and meat products for human consumption, AS 4696. SAI Global, Sydney.
44. Stanley, K. N., J. S. Wallace, J. E. Currie, P. J. Diggle, and K. Jones. 1998. The seasonal variation of thermophilic campylobacters in beef cattle, dairy cattle and calves. *J. Appl. Microbiol.* 85:472–480.
45. Tappero, J., A. Schuchat, K. A. Deaver, L. Mascola, and J. D. Wenger. 1995. Reduction in listeriosis: effectiveness of prevention efforts? *JAMA* 273:1118–1122.
46. Torres, M. E., M. C. Pírez, F. Schelotto, G. Varela, V. Parodi, F. Allende, E. Falconi, L. Dell'Acqua, P. Gaione, M. V. Méndez, A. M. Ferrari, A. Montano, E. Zanetta, A. M. Acuña, H. Chiparelli, and E. Ingold. 2001. Etiology of children's diarrhea in Montevideo, Uruguay: associated pathogens and unusual isolates. *J. Clin. Microbiol.* 39:2134–2139.
47. Tschape, H., R. Prager, W. Streckel, A. Fruth, E. Tietze, and G. Bohme. 1995. Verocytotoxigenic *Citrobacter freundii* associated

with severe gastroenteritis and cases of haemolytic uraemic syndrome in a nursery school: green butter as the infection source. *Epidemiol. Infect.* 114:441–450.

48. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services. 1994. *Salmonella* in dairy calves. Centers for Epidemiology and Animal Health, U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, Fort Collins, Colo.
49. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services. 1995. *Salmonella* shedding by feedlot cattle. Centers for Epidemiology and Animal Health, U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, Fort Collins, Colo.
50. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services. 1998. *E. coli* O157:H7 and *Salmonella* status on U.S. dairy operations. Centers for Epidemiology and Animal Health, U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, Fort Collins, Colo.
51. U.S. Department of Agriculture, Economic Research Service. 2005. Background statistics: U.S. beef and cattle industry. Available at: http://www.ers.usda.gov/news/BSECoverage.htm. Accessed 6 January 2006.
52. U.S. Department of Agriculture, Foreign Agricultural Service. 2005. BICO import commodity aggregations. Available at: http://www.fas.usda.gov/USTRADE/USTImBICO.asp?QI=. Accessed 6 January 2006.
53. Vally, H., M. D. Kirk, E. Scallan, F. J. Angulo, and G. V. Hall. 2004. Higher incidence of *Campylobacter* infection in Australia compared with the United States in 2001. FoodNet publication. Available at: www.cdc.gov/foodnet/pub/publications/2004/vally_2004.pdf. Accessed 6 January 2006.
54. Zhao, C., B. Ge, J. De Villena, R. Sudler, E. Yeh, S. Zhao, D. G. White, D. Wagner, and J. Meng. 2001. Prevalence of *Campylobacter* spp., *Escherichia coli,* and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the greater Washington, D.C. area. *Appl. Environ. Microbiol.* 67:5431–5436.
55. Zhao, S., P. J. Fedorka-Cray, S. Friedman, P. F. McDermott, R. D. Walker, S. Qaiyumi, S. L. Foley, S. K. Hubert, S. Ayers, L. English, D. A. Dargatz, B. Salamone, and D. G. White. 2005. Characterization of *Salmonella* Typhimurium of animal origin obtained from the National Antimicrobial Resistance Monitoring System. *Foodborne Pathol. Dis.* 2:169–181.