Salmonella spp. Shedding by Alberta Beef Cattle and the Detection of Salmonella spp. in Ground Beef

OLE SORENSEN,1* JOYCE VAN DONKERSGOED,2 MARGARET McFALL,1 KEN MANNINEN,1 GARY GENSLER,1 AND GERALD OLLIS3

1Agri-Food Laboratories Branch, Food Safety Division, Alberta Agriculture Food and Rural Development, 5th Floor O. S. Longman Building, 6909-116 Street, Edmonton, Alberta, Canada T6H 4P2; 2Dr. Joyce Van Donkersgoed Veterinary Services, Alberta Castle Feeders Association, 11 Brunns Road, Lacombe, Alberta, Canada T4L 1P1; and 3Agri-Food Surveillance Systems, Food Safety Division, Alberta Agriculture Food and Rural Development, 5th Floor O. S. Longman Building, 6909-116 Street, Edmonton, Alberta, Canada T6H 4P2

MS 01-127: Received 30 March 2001/Accepted 26 October 2001

ABSTRACT

Breeder cows, cattle recently arrived at feedlots, and cattle about to be shipped for slaughter were tested for Salmonella spp. No Salmonella spp. were detected in fecal samples from breeding cows. Nineteen of 1,000 (1.9%) fecal samples from recently arrived feedlot cattle were positive for Salmonella spp. compared to only 2 of 1,000 (0.2%) fecal samples taken within 2 weeks of slaughter. The positive fecal samples were collected in 5 of 50 (10%) “recent arrival” pens tested and in 1 of 50 (2%) pens tested within 2 weeks of slaughter. The serotypes isolated were Salmonella Agona, Salmonella Enteritidis, Salmonella Typhimurium DT104, and Salmonella 4,5,12:i-. Ground beef samples purchased from retail outlets throughout Alberta were processed for Salmonella spp. Thirteen of 1,002 (1.3%) samples were positive for Salmonella spp. The serotypes isolated from ground beef were Salmonella Anatum, Salmonella Heidelberg, Salmonella Montevideo, Salmonella Typhimurium, Salmonella Typhimurium var. Copenhagen, and Salmonella Rough-O:i:1.2. The antibiotic resistance and pulsed-field electrophoresis gel macrorestriction patterns of all isolates were compared.

It has been estimated that more than one third of human foodborne disease outbreaks are due to Salmonella spp. (26), and the incidence of Salmonella spp. infections is increasing in industrialized countries (20). This is of increasing concern with the emergence of multidrug-resistant serotypes such as Salmonella enterica subsp. enterica Typhimurium DT104. For every reported case of salmonellosis, an estimated 60 cases go unreported (12), and yet in 1998, about 40,000 cases of salmonellosis were reported to the U.S. Centers for Disease Control and Prevention, and about 6,000 cases were reported to Canada’s Population and Public Health Branch. FoodNet, an active surveillance network covering 6% of the U.S. population, calculated the incidence of laboratory-confirmed salmonellosis to be 13.7 per 100,000 in 1997 (37), and this was 27.3% of all bacterial foodborne illnesses reported.

Salmonella spp. are ubiquitous in the environment and have been recovered from most vertebrates tested (9, 23, 36). Salmonellosis in humans has been associated with eating raw or improperly prepared foods such as poultry, eggs, or meat including ground beef (1). Cattle have been shown to be carriers of Salmonella spp. (11, 23), and a 1998 U.S. survey found Salmonella spp. in 38% of the feedlots tested (11, 24). The most commonly detected S. enterica subsp. enterica serotypes in cattle (Anatum, Montevideo, Muenster, Kentucky, and Newington) were not the same as the serotypes commonly seen in human infections (Typhimurium, Enteritidis, Heidelberg, Hadar, and Newport) (28); however, 4.8% of clinical isolates from cattle were serotypes associated with human illness (11).

The emergence and increasing prevalence of multidrug-resistant salmonellae such as Salmonella Typhimurium DT104 (19, 31) and other antibiotic-resistant strains (7) have intensified the need to control Salmonella spp. in the cattle industry. In addition, the public’s concern for food safety and the high media profile given to outbreaks of foodborne pathogens have led to an increasing demand that pathogens be controlled in all segments of the food industry, from the farm to the retail outlet (4, 10). Salmonella spp. infections traced back to beef products, particularly ground beef (13, 33–35), have increased the public’s concern for the safety of all beef products.

Unlike the pork and poultry industries, the beef industry is highly fragmented, with cattle passing through several operations between calving and slaughter. Cattle may be commingled with animals from diverse sources at each step of this process. Targeted survey data on the occurrence of Salmonella spp. in beef cattle in the various levels of the Alberta beef cattle industry are not available. Likewise, the occurrence of Salmonella spp. in retail ground beef in Alberta is not known.

To address these unknowns, a study was conducted to determine the occurrence of Salmonella spp. in retail ground beef and beef cattle. Fecal samples were collected from beef cows on cow and calf operations, recently arrived feedlot cattle, and feedlot cattle within 2 weeks of slaughter and were cultured for Salmonella spp. Retail ground beef was purchased throughout Alberta and cultured for Salmonella enterica.
monella spp. The serotypes, phage types, antimicrobial resistance profiles, and pulsed-field gel electrophoretic (PFGE) genotypes were determined for all Salmonella spp. isolated.

MATERIALS AND METHODS

Fecal sampling. Beef cows on cow and calf farms were sampled with the cooperation of herd veterinarians. Veterinarians were contacted in 1998 and 1999 and were asked to collect fecal samples from beef cows during fall pregnancy examinations. A total of 1,000 samples were sought, with the number of samples requested from each county or municipal district equivalent to the proportion of the provincial herd found in that area. Beef cows, 2 years of age or older, were selected at random by the examining veterinarian, and no more than five cows were selected from any single herd. Approximately 60 ml of fecal material was collected from each cow during the pregnancy examination, placed in a sterile container and shipped on ice, not frozen, to the laboratory.

Feedlots were sampled with the cooperation of the Alberta Cattle Feeders Association and its members. All members of the Alberta Cattle Feeders Association were contacted and invited to participate in the survey to determine the prevalence of Salmonella spp. in feedlot cattle. Thirty-eight feedlots, varying from small to large capacity, agreed to participate in the survey. Feedlots were sampled during fall, late fall, and early winter and included both small and large capacity feedlots. All feedlots sampled were located in southern Alberta. Twenty-five feedlots were sampled in May and June 1999, 25 feedlots sampled in December 1999, and 15 feedlots were sampled in January 2000. Each feedlot sample was submitted on ice to the laboratory within 24 h of collection.

Salmonella spp. isolation procedure from fecal samples. Extensive comparisons of Salmonella spp. isolation methodologies from production animal feces were carried out by this laboratory, then known as the Animal Health Laboratories Branch (2). These studies resulted in our current procedures, which optimize our ability to detect those Salmonella spp. usually encountered in production animal feces. These procedures were used in this study. Our method employs a higher than standard inoculum (5 g as opposed to 1 g) and has been shown to be able to detect three Salmonella Muenster cells in a 5-g sample (unpublished data). Thus, we believe that the detection limits of our methods are sufficient to detect even low levels of Salmonella spp. colonies after 24 h. Suspect Salmonella spp. colony was detected by either pathway. All confirmed Salmonella isolates were stored at −70°C in 1 ml of sheep blood for further study.

Ground beef sampling. A total of 1,002 packages of ground beef were purchased from 484 grocery and meat retail outlets (113 independent and 371 major supermarket outlets) located throughout Alberta from March to July 1999. Each store was assigned a laboratory code number upon sample receipt. At each store, one package of regular ground beef and one package of either lean or extra lean ground beef were purchased. The ground beef was either packaged in the retail outlet or was ground and packaged in a chub at a central location. If both in-store and centrally packaged ground beef were available at an outlet, both were purchased. The ground beef was placed in coolers with ice packs and shipped to the laboratory for testing.

Salmonella spp. isolation procedure from ground beef samples. All ground beef samples were cultured within 48 h of purchase. Ground beef samples were processed by a modification of the method outlined in the Compendium of Analytical Methods: HPB Methods of Microbiological Analysis of Foods (22). Briefly, 25 g of ground beef from each package was mixed with 225 ml of BPW and incubated at 35°C for 24 h. The incubated BPW was then used to inoculate two isolation pathways. In the first pathway, 1 ml of BPW was transferred to Selenite cystine broth (Difco) and incubated at 35°C (21). In the second pathway, 1 ml of BPW was transferred to tetrahionate broth (Difco) (21) and incubated at 43°C. After incubation for 24 h, both broths were streaked for isolated colonies onto brilliant green sulfa (Difco) and XLT4 agar plates. The plates were read for typical Salmonella spp. colonies after incubation at 35°C for 24 h. Suspect Salmonella spp. colonies were forwarded, on Columbia agar (Oxoid) slants, to the Health Canada International Office of Epizootics Reference Laboratory for Salmonellosis (Guelph, Ontario, Canada) for serotyping and phage typing.

Serotyping. Salmonella spp. isolates were forwarded, on Columbia agar (Oxoid) slants, to the Health Canada International Office of Epizootics Reference Laboratory for Salmonellosis (Guelph, Ontario, Canada) for serotyping and phage typing.

Salmonella nomenclature. The Salmonella nomenclature outlined by Brenner et al. (5) has been used throughout this paper.

Antibiotic sensitivity testing. Susceptibility testing was performed by a Sensititre Custom Designed MIC Panel (Trek Diagnostic Systems Ltd., Westlake, Ohio) according to the manufacturer's instructions. Panels were read 18 to 24 h after inoculation. The antibiotics represented in the panel were amikacin (4 to 32 µg/ml), amoxicillin/clavulanic acid (0.5 to 32/16 µg/ml), ampicillin (2 to 64 µg/ml), apramycin (2 to 16 µg/ml), cefetaxone (0.25 to 16 µg/ml), cephalothin (1 to 32 µg/ml), chloramphenicol (4 to 32 µg/ml), ciprofloxacin (0.015 to 2 µg/ml), gentamicin (0.25 to 16 µg/ml), kanamycin (16 to 64 µg/ml), nalidixic acid (4 to 64 µg/ml), streptomycin (32
TABLE 1. Culture results of fecal samples from beef cows on cow and calf operations, recent feedlot arrivals, and preslaughter feedlot cattle

<table>
<thead>
<tr>
<th>Cattle</th>
<th>No. samples (+)</th>
<th>No. feedlots sampled (+)</th>
<th>No. pens sampled (+)</th>
<th>Positive fecal samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow and calf beef cows</td>
<td>827 (0)</td>
<td>NA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NA</td>
<td>21 (91.3)</td>
</tr>
<tr>
<td>Feedlot recent arrivals&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1,000 (19)</td>
<td>32 (4)</td>
<td>50 (5)</td>
<td>A-1 3 (13.0) Agona SuT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>B-1 1 (4.3) Enteritidis DT8 None</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C-1 1 (4.3) Typhimurium DT104 KT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>D-1 1 (4.3) Typhimurium DT104 ACSSuTTi</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>B-1. 2 14 (60.9) 4,5,12:i: None</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C-1 1 (4.3) 4,5,12:i:--:1.2 KT</td>
</tr>
<tr>
<td></td>
<td>1,000 (2)</td>
<td>35 (1)</td>
<td>50 (1)</td>
<td>B-3 2 (8.7) 4,5,12:i:-- None</td>
</tr>
<tr>
<td>Total samples (+)</td>
<td>2,827 (21)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> NA, not applicable.
<sup>b</sup> Ninety days or less on feed; average, 45 ± 28 days.
<sup>c</sup> Within 2 weeks of slaughter; average, 185 ± 43 days on feed.

PFGE. PFGE samples were processed as described by Chang and Chui (6). Briefly, isolated and serotyped Salmonella spp. were grown overnight on blood agar plates, and bacteria were harvested and embedded in low-melting-point agarose plugs (GIBCO-BRL, Rockville, Md.). Embedded salmonellae plugs were lysed by overnight digestion with proteinase K (0.5 mg/ml) in 0.25 M EDTA (pH 9.5), 1% sodium lauryl sarcosine at 50°C. A 1-mm agarose slice was cut from the initial plug for endonuclease digestion with Xba-I for 2 h at 37°C. The slices were then rinsed in 0.5× Tris-borate-EDTA buffer (50 mM Tris, 50 mM boric acid, and 1 mM EDTA) and embedded in a 1% agarose gel in 0.5× Tris-borate-EDTA for fragment separation. Macrestricted DNA was subjected to PFGE for 24 h (initial switch time, 25 s; final switch time, 58 s, 6 V/cm). Banding patterns were photographed and scanned to produce a digital image, which was analyzed by Molecular Analyst Fingerprinting software (Bio-Rad Laboratories, Richmond, Calif.). Banding patterns with ≥85% similarity were considered somewhat related, ≥90% were considered related, ≥95% were considered closely related, and 100% were indistinguishable. Patterns <85% were not considered related.

RESULTS

**Beef cows.** During fall pregnancy examinations in 1998 and 1999, 827 fecal samples were collected from beef cows on cow and calf farms. These samples originated from 62 of 70 Alberta counties and municipal districts and represent 94% of the beef cow population of the province. No Salmonella spp. were detected in these fecal samples (Table 1).

**Recent arrival feedlot cattle.** Fecal samples were collected at 32 feedlots from a total of 50 pens of cattle that had been on feed for 90 days or less. The length of time on feed varied from 1 to 90 days, with an average residency of 45 ± 28 days. Salmonella spp. were detected in 1.9% of the fecal samples collected (19 of 1,000) from 10.0% of the sampled pens (5 of 50) distributed among 12.5% of feedlots (4 of 32) (Table 1). In three feedlots (A, C, and D), only one pen from each yielded Salmonella-positive samples. The number of positive samples per pen varied from one in feedlots C and D to three in feedlot A, with an average of 1.7 ± 1.2 samples. In the remaining positive feedlot, B, Salmonella spp. were isolated from two pens, and the number of positive samples collected in those pens was higher (6 and 8; average, 7 ± 1.4).

**Preslaughter feedlot cattle.** Fecal samples were collected from 50 pens at 35 feedlots in which the cattle were within 2 weeks of slaughter. The length of time on feed varied from 80 to 251 days with a mean of 185 ± 43 days. Only 2 of 1,000 fecal samples cultured (0.2%) were positive for Salmonella spp., and these samples were collected from a single pen (2.0% of pens) in feedlot B (2.9% of feedlots) (Table 1).

**Ground beef.** Salmonella spp. were isolated from ground beef purchased at 1 independent and 10 supermarket chain outlets, for a total of 11 of 484 (2.3%) stores. Two samples, a regular and a lean or extra lean ground beef, were positive from independent outlet P. Supermarket chain outlet M also provided two positive samples, and a single sample was positive from each of the remaining nine positive outlets (E, F, G, H, J, K, L, N, and O) (Table 2). Regular ground beef accounted for 373 of the samples purchased, and the remaining 629 samples were either lean or extra lean ground beef. One hundred fifty-six samples were ground and packaged in a chub at a central location. The remaining 846 were ground or reground and packaged in the store of purchase. Four of the 129 (3.1%) centrally ground and packaged lean or extra lean samples were positive, whereas no Salmonella spp. were detected in the 27
TABLE 2. Culture results from retail ground beefa

<table>
<thead>
<tr>
<th>Retail outlet</th>
<th>No. samples (+)</th>
<th>Store code</th>
<th>Type(s)</th>
<th>No. isolates (%)</th>
<th>Salmonella enterica subsp. enterica serotypes</th>
<th>Antibiotic resistance profile</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Major chainb</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>L</td>
<td></td>
<td></td>
<td>1 (6.7)</td>
<td>Anatum</td>
<td>Sensitive</td>
</tr>
<tr>
<td>F</td>
<td>R</td>
<td></td>
<td></td>
<td>1 (6.7)</td>
<td>Heidelberg DT7</td>
<td>Sensitive</td>
</tr>
<tr>
<td>G</td>
<td>Lc</td>
<td></td>
<td></td>
<td>1 (6.7)</td>
<td>Montevideo</td>
<td>Sensitive</td>
</tr>
<tr>
<td>H</td>
<td>Lc</td>
<td></td>
<td></td>
<td>1 (6.7)</td>
<td>Typhimurium</td>
<td>ACcKSTTT1</td>
</tr>
<tr>
<td>H, J</td>
<td>Lc, R</td>
<td></td>
<td></td>
<td>2 (13.3)</td>
<td>Typhimurium</td>
<td>ACcKSSuTT1</td>
</tr>
<tr>
<td>K, L, M, N</td>
<td>XLc, XL, L, R, L</td>
<td></td>
<td></td>
<td>5 (33.3)</td>
<td>Typhimurium var.</td>
<td>Sensitive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Copenhagen DT1</td>
<td></td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td>853 (11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Independentc</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>L, R</td>
<td></td>
<td></td>
<td>1 (6.7)</td>
<td>Rough-O:i:1,2</td>
<td>AKSuTTi</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td>149 (2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total samples (+)</strong></td>
<td>1,002 (13)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a L, lean ground beef packaged in-store; R, regular ground beef packaged in-store; Lc, lean ground beef chub centrally packaged; XLc, extra lean ground beef chub centrally packaged; XL, extra lean ground beef packaged in-store.
b Large store, part of a major supermarket chain, 853 samples purchased from 371 outlets.
c Small independent outlet, 149 samples purchased from 113 outlets.

centrally packaged regular ground beef samples tested. Thus, in total, 4 of the 156 centrally ground samples tested (2.6%) were positive for Salmonella spp. Five (1.5%) of the 346 regular in-store packaged ground beef samples were positive, as were 4 (0.8%) of the 500 in-store packaged lean or extra lean ground beef samples. Thus, 9 (1.1%) of the 846 in-store packaged ground beef samples tested were positive for Salmonella spp. Overall, 13 (1.3%) of 1,002 ground beef samples were positive for Salmonella spp. (Table 2).

Salmonella isolates. Salmonella colonies were considered different isolates if they originated in different samples, were identified as different serotypes, or displayed different antibiotic resistance profiles. When multiple colonies from a single sample were shown to be the same serotype with the same antibiotic resistance profile, they were considered a single isolate. In general, multiple colonies picked from a single positive sample yielded a single Salmonella isolate.

Multiple Salmonella isolates were detected in fecal samples from only two recent arrival pens in two feedlots (B-1 and C-1). PFGE analysis of DNA macrorestriction patterns from the isolates from pen C-1 suggested that the isolates, Salmonella Typhimurium DT104 and Salmonella 4,5,12::−:1,2, were related genotypes (92.3% similarity). PFGE analysis also demonstrated that two unrelated Salmonella genotypes, Salmonella Enteritidis and Salmonella 4,5,12::−:− (52.2% similarity), were present in pen B-1. Salmonella 4,5,12::−:− was somewhat related to both Salmonella Typhimurium var. Copenhagen DT1 (88.0% similarity) and Salmonella Typhimurium DT104 (88.9% similarity) (Fig. 1). In two instances (one recent arrival pen and one preslaughter pen in feedlot B), multiple positive samples were identified from a single feedlot pen, but only a single Salmonella serotype (Salmonella 4,5,12::−:−) was identified.

Five Salmonella enterica subspecies enterica serotypes (Agona, Enteritidis DT8, Typhimurium DT104, 4,5,12::−:1,2, and 4,5,12::−:−) were isolated from recent arrival feedlot cattle in feedlots A, B, C, and D. Salmonella Agona was isolated from three samples collected from pen A-1. Salmonella Typhimurium DT104 was isolated from one pen at each of feedlots C and D. These isolates, however, were not related by PFGE (81.5% similarity) (Fig. 1) and had distinct antibiotic resistance profiles (Table 1). In addition, pen C-1 also yielded an isolate of Salmonella 4,5,12::−:1,2 that PFGE analysis indicated was related to the Salmonella Typhimurium DT104 isolated from the same sample (92.3% similarity), but it was unrelated to the Salmonella Typhimurium DT104 isolated from pen D-1 (64% similarity) (Fig. 1). Salmonella 4,5,12::−:− was isolated from 14 samples collected from recent arrival pens B-1 and B-2 and from 2 samples collected from preslaughter pen B-3. PFGE analysis indicated that these isolates could be divided into two groups, A and B (Fig. 1), with high similarity within each group but only a limited similarity between the groups. In group A, PFGE similarities ranged from 91.7 to 100%, whereas all group B isolates were indistinguishable (100%) (Fig. 1). PFGE analysis also indicated that these isolates were related or closely related (87 to 100% similarity) to the two distinct Salmonella Typhimurium var. Copenhagen DT1 genotypes and somewhat related to one of the two Salmonella Typhimurium DT104 isolates (88.9% similarity) (Fig. 1). Group A Salmonella 4,5,12::−:− isolates were closely related to the Salmonella Typhimurium var. Copenhagen DT1 isolated from ground beef purchased from stores K, M, and N (Fig. 1). By contrast, the Salmonella 4,5,12::−:− isolates in group B were indistinguishable by PFGE from the Salmonella Typhimurium var. Copenhagen DT1.
Six *S. enterica enterica* serotypes were isolated from ground beef: Anatum, Heidelberg DT7, Heidelberg DT10, Montevideo, Typhimurium, Typhimurium var. Copenhagen DT1, and Rough-O:i:1.2. *Salmonella Typhimurium* was isolated from both cattle and ground beef. The cattle isolates were phage type 104, whereas the ground beef isolates, purchased in stores H and J, were not phage typeable and were not related to the DT104 isolates by PFGE (similarity, 76.9 and 80%). PFGE analysis failed to show a close association between the *Salmonella Rough-O:i:1.2* and the other serotypes isolated from ground beef or fecal samples. Two distinct *Salmonella Typhimurium* var. Copenhagen DT1 were identified in ground beef by PFGE analysis (52.2 to 87% similarity). A *Salmonella Typhimurium* var. Copenhagen DT1 genotype isolated from samples from stores K, M, and N was related to the DT104 isolates by PFGE (similarity, 76.9 and 80%). PFGE analysis failed to show a close association between the *Salmonella Rough-O:i:1.2* and the other serotypes isolated from ground beef or fecal samples. Two distinct *Salmonella Typhimurium* var. Copenhagen DT1 were identified in ground beef by PFGE analysis (52.2 to 87% similarity). A *Salmonella Typhimurium* var. Copenhagen DT1 genotype isolated from samples from stores K, M, and N was related to

---

**FIGURE 1.** *The relatedness of the Salmonella isolates as determined by pulsed-field gel electrophoresis and analysis with Molecular Analyst Fingerprinting software.*
the group A Salmonella 4,5,12:i-- (88.0 to 96.0% similarity) (Fig. 1). The other Salmonella Typhimurium var. Copenhagen DT1 genotype was indistinguishable (100% similarity) from the group B Salmonella 4,5,12:i-- genotype (Fig. 1).

Antibiotic resistance. The majority (75%) of Salmonella isolates from feedlot fecal samples were sensitive to all antibiotics tested, and only one isolate was resistant to more than two antibiotics. All isolates of Salmonella 4,5,12:i-- except for one (16 of 17), were sensitive to all antibiotics in the test panel, and the remaining isolate had intermediate sensitivity to kanamycin. All Salmonella Agona isolates were resistant to sulfamethoxazole and tetracycline (Table 1). Only one of the two Salmonella Typhimurium DT104 isolates displayed the multiresistant phenotype (ACSSuST) commonly associated with this serotype. This isolate was resistant to six antibiotics: ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, tetracycline, and ticarcillin (ACSSuTTi) (Table 1). This isolate also had intermediate sensitivity to cephalothin. The other Salmonella Typhimurium DT104 and the closely related Salmonella 4,5,12:i--:1,2 isolate were resistant to kanamycin and tetracycline (Table 1).

Eight of 15 (53%) Salmonella isolates from ground beef were sensitive to all antibiotics in the test panel (Table 2). Those isolates that were resistant tended to be resistant to many antibiotics. Isolates of Salmonella Anatum, Salmonella Heidelberg DT7, Salmonella Montevideo, and Salmonella Typhimurium var. Copenhagen DT1 from ground beef were sensitive to all test antibiotics (Table 2). Salmonella Heidelberg DT10 isolates were resistant to streptomycin and tetracycline (Table 2). Two non-DT104 Salmonella Typhimurium isolates from ground beef were resistant to eight and nine antibiotics: ampicillin, cephapentin, chloramphenicol, kanamycin, streptomycin, tetracycline, ticarcillin, and trimethoprim/sulfamethoxazole with and without resistance to sulfamethoxazole, respectively (ACCeKSTTTiM and ACcEKSuTTiM) (Table 2). These isolates also had intermediate sensitivity to amoxicillin/克拉维酸。Two serotype Rough-O: i:1,2 isolates from a single ground beef sample were resistant to ampicillin, kanamycin, sulfamethoxazole, tetracycline, and ticarcillin (AKSuTTi), and one of the isolates was also resistant to streptomycin (Table 2).

DISCUSSION

No Salmonella spp. were detected in the fecal samples from beef cows on cow and calf farms. This result was not unexpected, since these cows are generally free-ranging during the summer months and are only confined in smaller pastures during the winter. The dispersal of the herd during the summer months, traditionally the peak period for Salmonella spp. isolation (12, 27, 37), may effectively control the spread of infection to other members of the herd.

A survey of U.S. feedlots found Salmonella spp. in 38% of the operations and in 5.5% of the individual samples tested (11). It also showed that the rate of infection was lower in more northerly states. By contrast, our study found Salmonella spp. in only 4 of 38 (10.5%) Alberta feedlots and in 21 of 2,000 (1.1%) of the individual samples tested. The difference between the results reported by Fedorka-Cray et al. (11) and the infection rates reported here may, in part, be due to Alberta’s more northerly location. As with the U.S. survey (11), samples for this study were collected at one time point, but Salmonella spp. shedding is intermittent (32); thus, the level of shedding reported here may underestimate the true prevalence in Alberta beef cattle. The samples, however, were collected during the summer months, which is the high seasonal risk period for Salmonella shedding. This survey was also conducted at feedlots that responded to an invitation to participate, not a random sample of all Alberta feedlots. These feedlots, however, were representative of feedlots with various cattle procurement and management practices.

Cattle are transported from diverse locations, and then are commingled and confined in close proximity in the feedlot. Transport is stressful to cattle, and commingling creates the possibility for Salmonella-naive cattle to come in close contact with, and be infected by, shedding animals. This infectious cycle is reflected in our detection of Salmonella spp. in 10% of the recent arrival feedlot pens and in 1.9% of the individual samples tested but in only 2.0% of pens and 0.2% of samples tested by the time the cattle were ready for slaughter. In contrast, data from U.S. feedlots suggest that the rate of infection increases over the first 180 days on feed from 3.5 to 7.4% (11). Others, however, have suggested that cattle clear Salmonella infections within 3 months (32). All recent arrival pens sampled in this study were 90 days or less on feed (mean, 45 ± 28 days), whereas the preslaughter samples were collected, on average, at 185 ± 43 days on feed. As with the U.S. survey (11), most positive feedlots in our study had very low numbers of positive samples, and all but one feedlot had less than three positive samples/pen.

Salmonella 4,5,12:i-- was the most frequently isolated S. enterica serotype from feedlot fecal samples, even though this serotype was isolated at only one feedlot. The other serotypes isolated from feedlot samples were Salmonella Agona, Salmonella Typhimurium DT104, Salmonella Enteritidis DT8, and Salmonella 4,5,12:i--:1,2. In the United States, by contrast, the most commonly identified feedlot serotypes were Salmonella Anatum, Salmonella Kentucky, Salmonella Mbandaka, Salmonella Montevideo, and Salmonella Muenster (10). Others have identified Salmonella Dublin, Salmonella Typhimurium, Salmonella Newport, and Salmonella Montevideo as the most common cattle serovars (9).

Salmonella spp. were recovered from 1.3% of the ground beef samples tested compared to 7.5% of U.S. ground beef in 1993 and 1994 (13, 15). Salmonella Typhimurium var. Copenhagen DT1 was the most common serovar isolated from ground beef, followed by Salmonella Typhimurium, Salmonella Heidelberg DT10, Salmonella Heidelberg DT7, Salmonella Anatum, Salmonella Montevideo, and Salmonella Rough-O: i:1,2. Studies in the United States identified Salmonella Anatum, Salmonella Hadar, Salmonella Muenster, Salmonella Meleagridis, and Salmonella...
nella Typhimurium var. Copenhagen as the most commonly isolated serotypes in ground beef (17).

This study was designed to determine the prevalence of Salmonella spp. shedding in beef cattle and to determine the occurrence of Salmonella spp. in ground beef. The study was not designed to trace the contamination from beef cattle to ground beef. The results reported, however, may suggest that sources other than beef cattle are important contributors to ground beef contamination. These sources may include other sources of meat, postslaughter breaking and grinding, and potential human contact. Other sources of meat, including cull dairy cows, were not included in this survey. Cull dairy cows, accounting for approximately 17% of the U.S. ground beef production (3), have been reported to have Salmonella infection rates as high as 18.1% (18, 38), which is much higher than the rate found in U.S. beef cattle. Baseline surveys by the U.S. Food Safety and Inspection Service of carcass contamination have also shown that cow and bull carcasses are contaminated with Salmonella spp. 2.7 times as frequently as heifers and steers (14, 16). Antibiotic resistance data from ground beef Salmonella isolates in this study more closely reflect the antibiotic resistance profiles from Alberta dairy cow Salmonella isolates (unpublished data) than from the feedlot isolates identified in this survey. Thus, the mix of sources represented in any sample of ground beef and the postslaughter processing may be the prime determinants of potential contamination.

Antibiotic resistance profiles and PFGE analysis showed some interesting relationships between the Salmonella serovars isolated in this study. Salmonella Typhimurium DT104 was isolated from two feedlots; however, these serologically identical isolates showed only 81.5% relatedness by PFGE. These Salmonella Typhimurium DT104 isolates were no more closely related to each other by PFGE than they were related to two Salmonella Typhimurium (phage untypeable) isolates from ground beef (76.9 to 83.3%). The antibiotic resistance profiles of these Salmonella Typhimurium DT104 isolates were also radically different. One isolate was resistant only to kanamycin and tetracycline, whereas the other showed the full penta-resistant R-type profile (ACSSuT) plus resistance to ticarcillin and was the only feedlot isolate resistant to more than two antibiotics. By contrast, a higher proportion of ground beef isolates (5 of 15) were resistant to five or more antibiotics. All antibiotic-resistant isolates from ground beef were Salmonella Typhimurium or Salmonella Rough-O:1,1,2 and were resistant to between five and nine antibiotics. The frequent isolation of multiantibiotic-resistant Salmonella spp. from ground beef was unexpected. By contrast, only one (1 of 23) fecal isolate was resistant to more than two antibiotics.

Salmonella Typhimurium var. Copenhagen DT1 isolated from ground beef could be allocated into two groups by PFGE. Within each group, there was a very close PFGE relatedness (100%), but the groups were much less closely related (87%). Interestingly, one representative from each group was isolated from ground beef purchased at outlet N. The above two Salmonella Typhimurium var. Copenhagen DT1 groups were related by PFGE to Salmonella 4,5,12:i− isolates from feedlot fecal samples. Sixteen isolates of Salmonella 4,5,12:i− were identified in fecal samples from two recent arrival pens and one preslaughter pen of feedlot B. These isolates could be differentiated into two relatedness groups by PFGE. Each of the two Salmonella 4,5,12:i− groups were isolated from both recent arrival pens, and each group was closely related to a different Salmonella Typhimurium var. Copenhagen DT1 PFGE grouping.

This study has shown that Salmonella spp. were present at low levels in both the Alberta beef cattle population and the Alberta retail ground beef. Different spectrums of S. enterica enterica serovars were identified in cattle and ground beef. This study, however, was designed to determine the current situation in cattle and ground beef, not to trace bacterial isolates from beef cattle to retail ground beef. The multiresistant S. enterica strains in ground beef are of concern, and further study should be undertaken to confirm these results, to identify potential contamination sources, and to identify control strategies.

ACKNOWLEDGMENTS

The authors thank the Alberta Veterinary Medical Association members for their cooperation and assistance in obtaining samples from cow and calf herds. The authors thank the Alberta Cattle Feeder’s Association and their members who participated in this survey for their cooperation and assistance, and we thank Darren Malchow for fecal sample collection. The authors also acknowledge the laboratory expertise provided by Evelyn Bowibly, Rachel Cantelon, Rashed Cassis, Suzanne Gibson, Carol Goertz, Lindsey Haines, Louise Hawker, Robin King, Sonja Marshall, Arlene Otto, Catherine Taschuck, Barb Tomik, Cheryl Turner, and Annette Viser. Funding for this survey was provided by Alberta Agriculture and Food and Rural Development and the Government of Canada, Western Economic Diversification, through a Western Economic Partnership Agreement.

REFERENCES

1.1. Fedorka-Cray, F. J., D. Durgatz, L. Thomas, and J. Gray. 1998. Sur-
vey of *Salmonella* serotypes in feedlot cattle. J. Food Prot. 61:525–530.


