The prevalence of *Listeria*, *Salmonella*, *Escherichia coli* and *E. coli* O157:H7 on bison carcasses during processing

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Abstract

Bison meat is a relatively new, emerging meat species gaining increased popularity in the US and European meat markets, but little is known of its microflora or pathogens that may be present. This study was carried out to determine the incidence of the foodborne pathogens *Listeria*, *Salmonella*, *Escherichia coli*/*E. coli* O157:H7 on slaughtered bison and to evaluate the bison slaughter process. Bison carcass sampling was carried out at monthly intervals over a period of 1 year at a Bison processing facility in the Midwestern United States. A total of 355 Bison carcasses were sampled by surface swabbing the carcasses at five points on the production line: pre-dehiding, post-evisceration, post-USDA inspection, post-washing and 24 h chilled carcass. Overall, the prevalence of *Listeria* spp., *Salmonella* spp., *E. coli* and *E. coli* O157:H7 was 18.3%, 3.94%, 38.3% and 1.13%, respectively. The prevalence of *Listeria* spp. at each sampling point tested was 42.24%, 18.1%, 6.03%, 1.72% and 3.77% while the prevalence of *E. coli* at each sampling point was: 88.79%, 73.28%, 52.59%, 56.89% and 11.3%, respectively. The data obtained suggests that current antimicrobial intervention strategies used at the plant are relatively effective in reducing *Listeria* and *E. coli* contamination on bison carcasses to some extent, however further study is required to determine the influence of current slaughter practices on carcass contamination. The data reported in this study to the authors’ knowledge is some of the first information reporting on the bacteriological status of Bison, and provides some useful baseline information for future research.

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Keywords: Prevalence; *Listeria* spp.; *Salmonella* spp.; *E. coli*; *E. coli* O157:H7; Bison carcasses; Meat; Foodborne; Pathogen

1. Introduction

The American Bison (*Bison bison*) is a relatively new, emerging meat species gaining increased popularity in the US and European meat markets. In 2002, the US Department of Agriculture (USDA) announced a $10 million purchase of bison meat for use in federal food and nutrition programs (Carter, 2002). The meat was considered suitable for the federal program based on its “low-fat, low-cholesterol, high protein and great taste” as fitting well with national nutritional goals. Compared to beef, Bison meat is a highly nutrient-dense food, with a lower fat content (5–7% compared to 25–30% for beef) and a higher protein content (18–20% compared to 10% for beef) (Anon, 2003). In addition, Bisons are typically not subjected to subtherapeutic doses of growth promoting hormones or antibiotics (Anon, 2003) that are often used in other aspects of the meat industry. These animals are usually reared on the open plains on a diet of grass or hay and only grain-fed 90–120 days pre-slaughter (Anon, 2003). This suggests that their microbiological flora may not be exposed to the same selective pressures as seen elsewhere in the meat industry.

In studies of other meat species, three major pathogens have emerged as being of significant importance in terms of human health and disease. These include: *Salmonella* spp., *Listeria monocytogenes* and *E. coli* O157:H7. These organisms have frequently been associated with meat and meat products and linked to a number of cases of human illness (CDC, 1993, 1995a, 2000).
The pathogen *Salmonella*, a causative agent of gastroenteritis, is estimated to be responsible for over 1.4 million cases of human illness annually and approximately 500 deaths (Mead et al., 1999). This organism has frequently been associated with poultry which are considered the primary source (Bryan and Doyle, 1995); however other meats such as pork, lamb, and beef have also been implicated as sources of contamination (Lammerding et al., 1998; Duffy et al., 1999; Madden et al., 2001). Typical symptoms of illness associated with salmonellosis include nausea, vomiting, and diarrhea; additional complications associated with infection include septicemia, or reactive arthritis (D’Aoust et al., 2001).

The pathogen *L. monocytogenes* has been recognized as a causative agent of listeriosis, annually, this organism is estimated to cause approximately 2500 illnesses and 500 associated deaths (Mead et al., 1999). Typical foodstuffs implicated as sources of the organism include salads (Schlech et al., 1983), cooked (ready to eat) and fermented meats, and raw meats such as beef, pork, lamb and poultry (Hudson et al., 1992; Sheridan et al., 1994; Madden et al., 2001). Usually, a listerial infection is linked to well defined high risk groups such as the immuno-compromised or pregnant women (Swaminathan, 2001). Individuals infected with *Listeria* may exhibit ‘flu like symptoms that can progress to septicemia, meningitis, meningoencephalitis in immuno-compromised individuals, newborns and the elderly, and abortion and stillbirth in pregnant women (Farber and Peterkin, 1991).

One of the most significant foodborne pathogens that has gained increased attention in recent years is *E. coli* O157:H7. Mead et al. (1999) estimate that this organism is responsible for approximately 73,000 cases of human illnesses and 61 deaths per year in the United States. Typical illness as a result of an *E. coli* O157:H7 infection can be life threatening, and susceptible individuals show a range of symptoms including hemolytic colitis and other complications, including hemolytic-uremic syndrome and thrombocytopenic purpura (Griffin et al., 1988; Bell et al., 1994; Meng et al., 2001). Typical sources of this pathogen have been significant, one of the primary hosts implicated are cattle (Meng et al., 2001)—hence beef and other such products have been associated with a large number of outbreaks of human illness (CDC, 1993). Other foodstuffs implicated include other meat types, fermented meats such as salami and pepperoni, cheese, fresh fruit, unpasteurized fruit juice and vegetables (Griffin and Tauxe, 1991; CDC, 1995b; Chapman et al., 1996; Faith et al., 1996; CDC, 1996; Buchanan and Doyle, 1997; Todd, 1997; Mead and Griffin, 1998).

Overall, meat and meat products have been implicated as significant sources of all of the pathogens described above. However, as Bison is a relatively new emerging meat species there is currently, little information in the literature regarding the microbiological safety of Bison meat destined for human consumption. The current study was carried out to address the following questions in relation to the safety of Bison meat: (i) estimate the incidence of the foodborne pathogens: *Listeria, Salmonella* and *E. coli* O157:H7 on slaughtered Bison, and (ii) evaluate the Bison slaughter process and determine its influence on pathogen contamination and spread on carcasses during processing. In addition, a study on Bison feces was carried out to determine if Bison are potential carriers of *E. coli* O157 strains.

2. Materials and methods

2.1. Carcass sample collection and preparation

Bison carcass samples were collected from a Midwestern Bison processing plant, which slaughters approximately 60 bison daily. Sampling visits were made over a period of 1 year on a monthly basis (from February 2002 to January 2003) on a randomly selected day. Five sampling points on the processing line were selected for study: (i) pre-dehiding: where samples were taken prior to complete hide removal; (ii) post-evisceration: where samples were obtained after evisceration and part splitting of carcass; (iii) post-USDA inspection: samples at this point were collected after splitting, trimming and a visual inspection by USDA personnel; (iv) post-washing: where the carcasses were washed with hot water at 80°C for approximately 30 s in a carcass washer. This point is the last step of the process line before the carcasses are placed in the chillier; (v) chilled carcasses: which were stored in a chill room for approximately 24 h. Animals were followed through the slaughter line and sampled at the first four sites, whereas chilled carcasses were 24 h old when sampled.

Carcass samples were obtained at all sites using a similar swabbing method. Briefly, a sterile sponge was moistened with 20 ml of sterile water in a sample bag (WHIRL-PAK, Nasco, Fort Atkinson, WI). Surface swabbing of the carcasses was carried out using a non-destructive non-invasive technique previously described by Lasta et al. (1992) for sampling beef carcasses. Briefly, a sterile bag was inverted over the operator’s hand and the sterile moistened swab grasped in the covered hand. Carcasses were swabbed along the ventral midline in a downward motion (Modified FSIS method). Once swabbing was complete, the bag was returned to its normal position with the sponge inside. All bags containing swabs were tagged, and placed together in Ziploc® easy zipper bags (SC Johnson, USA) coded to identify the point on the production line where the
samples were taken. All swabs were transported to the lab in cooler boxes containing ice packs.

2.2. Bacterial culture and isolation

On arrival at the lab, all samples were logged and a sample number assigned. 100 ml of Maximum Recovery Diluent (MRD), (CM 783, Oxoid, Ogdenberg, NY, USA) was added to each swab and the sample homogenized in a stomacher (IUL Masticator, Tiorrent de Lastadella, Barcelona, Spain) for 90 s. Isolation and identification of the pathogens was carried out using methods described in the Food and Drugs Administration (FDA) Bacteriological Analytical Manual (BAM) with some modifications (BAM, 1998).

2.2.1. Listeria isolation

10 ml of homogenate was added to an equal volume of double-strength University of Vermont Medium (UVMI), (CM 863 and SR 142, Oxoid) and incubated at 30°C for 36–48 h. Following incubation, Immuno-magnetic Separation (IMS) was performed to concentrate Listeria spp. according to manufacturer’s instructions. 1 ml of UVMI broth was added to a sterile centrifuge vial containing 20 μl anti-Listeria magnetic beads (Dynal, Oslo, Norway) and then rotated on a rocker (Dynal) mixer at 18 rpm for 10 min. The vial was then placed in a magnetic separator (Dynal) for 3 min, rotating the separator back and forth occasionally to concentrate the cells. Once concentrated, the liquid was discarded and the immuno-magnetic beads re-suspended in 1 ml of phosphate buffered saline (PBS), (BR 14a, Oxoid) containing Tween 20 (0.05%, # 9480, EM Science, Gibbstown, NJ). The washing procedure was carried out three times. After the final wash, the beads were re-suspended in 100 μl of PBS and Tween 20. Fifty microliters of the concentrated sample was streaked onto Palcam (CM 877 and SR150, Oxoid) and Oxford (CM 856 and SR 140) selective agars and incubated at 30°C for 36–48 h. Suspect colonies of typical morphology (black/gray with a dimpled center) from the Palcam and Oxford plates were picked and streaked onto Tryptic Soy Agar (TSA) for purity and confirmed biochemically by inoculating into urea broth (#0272-17, Difco), lysine decarboxylase broth and triple sugar iron (TSI) (#0265-15, Difco) slants. Presumptive isolates were identified using the AP80® Gram-negative auto-identification plates (Trek Diagnostics, Westlake, OH) and serotyped at National Veterinary Services Laboratories (NVSL, Ames, IA).

2.2.2. Salmonella

Isolation of Salmonella was carried out by adding 10 ml of homogenate to 10 ml double-strength Buffered Peptone Water (BPW), (#1810-17, Difco, Becton and Dickinson, Sparks, MD) and incubated at 37°C for 18–24 h. Following enrichment, samples were subjected to IMS to concentrate Salmonella as described above. 100 μl of the concentrated sample was transferred to Rappaport Vassiliadis (RV) broth (#1858-17, Difco) and incubated at 42°C for 18–24 h. Following incubation, samples were streaked out onto Modified Brilliant Green Agar (MBGA) (#1880-17, Difco) plates and Mannitol Lysine Crystal Violet Brilliant Green (MLCB) (CM 783, Oxoid) plates with incubation at 37°C for 18–24 h. Suspect colonies were selected and streaked onto TSA for purity and confirmed biochemically by inoculating into urea broth (#0272-17, Difco), lysine decarboxylase broth and triple sugar iron (TSI) (#0265-15, Difco) slants. Presumptive isolates were identified using the AP80® Gram-negative auto-identification plates (Trek Diagnostics) by the indole test and triple sugar iron slants. Identification of E.coli isolates was confirmed by AP80® Gram-negative auto-identification plates (Trek Diagnostics).

2.2.3. E. coli

Isolation of E. coli was carried out by adding 10 ml of homogenate to 10 ml double-strength E. coli Enrichment (EE) broth (CM 317, Oxoid) and incubated at 37°C for 18–24 h. Following incubation, the enrichment samples were streaked out on Levine Eosin Methylene Blue agar (EMB) (#0005-15, Difco) plates and incubated at 37°C for 18–24 h. Suspect colonies (green color, with a metallic sheen) were streaked onto TSA for purity and screened by the indole test and triple sugar iron slants. Identification of E. coli isolates was confirmed by AP80® Gram-negative auto-identification plates (Trek Diagnostics).

2.2.4. E. coli O157:H7

E. coli O157:H7 were isolated by enrichment of 10 ml of the homogenate in 10 ml double-strength E. coli (EC) broth (#314-15, Difco) supplemented with 0.04 mg/ml novobiocin (N1628, Sigma-Aldrich, USA) for 18–24 h at 37°C. Following enrichment, 1.0 ml of the sample was removed and subjected to IMS to concentrate the organism as described above. 100 μl of the concentrated sample was spread onto the surface of Sorbitol MacConkey (SMAC, CM 813, Oxoid) plates supplemented with cefixime (0.5 mg/l, Dynal) and potassium tellurite (2.5 mg/l, Dynal) and incubated for 18–24 h at 37°C. Suspect colonies (i.e. Sorbitol-negative) were streaked for purity and screened by the indole test and then were confirmed as E. coli O157 using the Dry Spot® test (DR0120M, Oxoid) and AP80® Gram-negative auto-identification plates (Trek Diagnostics). Confirmed isolates were serotyped at the E. coli Reference Center (Pennsylvania State University, PA).

2.3. Fecal sample collection and microbiological analysis

Fifty bison fecal samples were collected from a bison farm in September 2003. The rancher is a supplier to the bison plant where the carcass samples were collected.
Approximately 300 bison are reared on the ranch. The field where the fecal samples were obtained was reported to have only ever grazed bison over the previous 10-year period, and the site where the samples were obtained had grazing bison in the previous 12 h period.

Briefly, a sterile sample bag was inverted over the hand of the sampler and approximately 50 g of fecal material was collected from a fecal pat on the ground. Most of the fecal pats sampled were relatively dry on the outside and “opened” to remove material from the center. The bagged samples were transported to the lab, logged and assigned an identification number. In the lab, 10 g portions of the fecal sample were removed and added to a sterile jar containing 90 ml of *E. coli* (EC) (#314-15, Difco) broth supplemented with 0.02 mg/ml of novobiocin (Sigma), and the mixture incubated at 37°C for 18–24 h. Following incubation, the enriched samples were subjected to IMS and 100 m l of the concentrated sample spread on the surface of SMAC plates supplemented with cefiximine (0.5 mg/l, Dynal) and potassium tellurite (2.5 mg/l, Dynal) and incubated at 37°C for 18–24 h. Suspect colonies (i.e. Sorbitol negative) were streaked for purity and screened by the indole test and confirmed as *E. coli* O157 by using the Dry Spot® test (Oxoid) and AP80® Gram-negative autoidentification plates (Trek Diagnostics).

2.4. *E. coli* O157 isolate characterization

Dry Spot® test positive *E. coli* isolates were confirmed as *E. coli* O157:H7 by PCR assay using flagellar H7 gene (*flIC*) generic primers as described before (Gannon et al., 1997). Isolates that were H7-negative were examined for motility using agar stabs of motility test agar (Difco). PCR assays were also performed for *E. coli* O157 to identify genes encoding Shiga-like toxin I and II (*stx1* and *stx2*), intimin (*eaeA*) and Enterohemorrhagic *E. coli* hemolysin (EHEC *hly*) as described before (Gannon et al., 1992, 1993; Fratamico et al., 1995). Primer sequences used are shown in Table 1.

### Table 1: Primers for PCR assays

<table>
<thead>
<tr>
<th>Primers</th>
<th>Direction</th>
<th>Primer sequence (5’–3’)</th>
<th>Product size (bases)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>flIC17</em></td>
<td>Forward</td>
<td>GCGCTGTAGGTTCTCTCGGCAC</td>
<td>625</td>
<td>Gannon et al. (1997)</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>CAACGGTGACCTTATGCGACCTTCC</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>stx1</em></td>
<td>Forward</td>
<td>ACACTGATGATCTGACTGTTG</td>
<td>614</td>
<td>Gannon et al. (1992)</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>CTGAATCCCTCTCATTATG</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>stx2</em></td>
<td>Forward</td>
<td>CCAAGAACGGAGACAGCATGTT</td>
<td>779</td>
<td>Gannon et al. (1992)</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>CCGTACACTGAGCACTATTGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>eaeA</em></td>
<td>Forward</td>
<td>GTGGCGAATTGCGAGCT</td>
<td>890</td>
<td>Gannon et al. (1993)</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>CCCCATTCTTTCTACCGTCG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EHEC</td>
<td>Forward</td>
<td>AGCATGTCGGTATATCTGGA</td>
<td>166</td>
<td>Fratamico et al. (1995)</td>
</tr>
<tr>
<td><em>hly</em></td>
<td>Reverse</td>
<td>CTCACAGTGACACGATCATAT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.5. Statistical analysis

Data obtained from the samples collected were analyzed using the MINILAB 13 for windows.

3. Results

Over the 12-month period of the study, a total of 355 bison carcasses were sampled, of these, 116 were from the slaughter line and 239 were 24 h chilled carcasses. A total of 703 carcass swabs were obtained from the four points sampled on the slaughterline (i.e. pre-dehiding, post-evisceration, post-USDA inspection and post-washing) and 24 h chilled carcasses.

### 3.1. Prevalence of *Listeria* spp. on bison carcasses sampled

Of the 355 carcasses sampled, 65 (18.3%) were positive for *Listeria* spp. (Carcasses which were positive at more than one sampling site were only counted as being positive once in overall prevalence). The prevalence of the organism at pre-dehiding, post-evisceration, post-USDA inspection and post-washing was: 42.24% (49/116), 18.1% (21/116), 6.03% (7/116) and 1.72% (2/116) respectively (see, Table 2). Significant reductions in the proportion of carcass samples positive for *Listeria* spp. were observed between pre-dehiding and post-evisceration points (*p* ≤0.05), and between post-evisceration and post-USDA inspection points (*p* <0.05). No significant differences were observed between the post-USDA and the post-washing points (*p* >0.05). Nine of the 239 (3.77%) chilled carcass samples were positive for *Listeria* spp., a little higher than the prevalence at the post-washing sampling point.

Of the 355 carcasses tested, 4 (1.13%) carcasses were positive for *L. monocytogenes*, 52 were positive for *L. innocua*, 6 were positive for *L. welshimeri*, 1 was positive for *L. grayi*, 1 was positive both for *L. ivanooi*
and L. grayi and 1 was positive both for L. ivanovii and L. innocua. One of the four L. monocytogenes isolates found was recovered from 24 h chilled carcass; the other three were recovered from the pre-dehiding and post-evisceration stages.

3.2. Prevalence of Salmonella spp. on bison carcasses sampled

Fourteen (3.94%) of the 355 carcasses sampled were positive for Salmonella. Unlike the other organisms tested for, no Salmonella isolates were found at the pre-dehiding stage. At post-evisceration and post-USDA inspection points, Salmonella was recovered from 3 (2.59%) carcasses at each point respectively. At the post-washing point, Salmonella was recovered from 4 carcasses. Of the 239 chilled carcasses sampled, 7 (2.93%) were positive for Salmonella. The prevalence is higher than that of other points sampled. Serotype analysis showed that all of the Salmonella isolates recovered were S. enterica serovar Hadar strains.

3.3. Prevalence of E. coli/E. coli O157:H7 on bison carcasses sampled

The prevalence of E. coli was much higher than that of any other micro-organism tested. Of the 355 carcasses sampled, 136 (38.30%) were positive for E. coli. Approximately 90% (103/116) of the samples tested were positive for E. coli at the pre-dehiding point, indicating a high rate of carcass fecal contamination. The prevalence E. coli at post-evisceration, post-USDA inspection and post-washing was: 73.3% (85/116), 52.6% (61/116), and 56.9% (66/116) respectively. Significant differences in the proportion of carcass samples positive were observed between pre-dehiding and post-evisceration points ($p<0.05$), and between post-evisceration and post-USDA inspection points ($p<0.05$). No significant differences were observed between post-USDA and post-washing points ($p>0.05$). 27 of the 239 (11.3%) chilled carcass samples were positive for E. coli.

Of the 355 carcasses sampled, five E. coli O157:H7 isolates were recovered from 4 carcasses. Four of the five E. coli O157:H7 isolates were recovered from carcasses at the pre-dehiding stage, while one was recovered at the post-evisceration point. E. coli O157:H7 was not recovered from any of the 24 h chilled carcasses sampled.

PCR analysis of the E. coli O157 strains recovered showed that all five isolates were positive for eaeA and EHEC hly genes. Two of the isolates were positive for both stx 1 and stx 2, one isolate was positive for stx 1 only and two isolates were positive for stx 2 only.

3.4. E. coli O157:NM on bison fecal samples

Two E. coli O157:NM were recovered from the 50 bison fecal samples collected, demonstrating that bison are potential carriers of E. coli O157:H7. PCR assay results showed that both of the isolates were positive for the EHEC hly gene and stx 2 gene, while one was positive for the eae gene. Both were negative for motility using motility medium.

3.5. Seasonality component

Fig. 1 shows the prevalence of Listeria, Salmonella, E. coli and E. coli O157:H7 on carcasses during the 12 month period of the study. E. coli O157:H7 strains were found in August (3.33%) and October (10.34%). The highest incidence of Listeria spp. (50%) occurred in May, with high isolation rates (>30%) also noted in February (30%), August (36.66%) and October (31.03%). The organism failed to be detected in December and January. Salmonella spp. were only detected in October with a prevalence of 24.13%. The highest incidence of E. coli occurred in March (53.33%), while the incidence during the other months of the study were also relatively high with an overall incidence.
Escherichia coli O157:H7  
Listeria spp.  
Salmonella spp.  
Escherichia coli

Fig. 1. The Incidence of foodborne pathogens on bison.

generally greater than 30%. One significant observation was that October was the only month of the study where bison carcasses sampled were positive for all four organisms tested (i.e. Salmonella, Listeria, E. coli and E. coli O157:H7).

4. Discussion

Contaminated ground beef and other bovine products are particularly important in transmitting E. coli O157:H7. Since 1982, more than 100 outbreaks of Enterohemorrhagic E. coli O157 have been documented. Of these outbreaks, 52% have been attributed or linked to foods derived from cattle (Elder et al., 2000). Thus far, many studies have been completed to determine the prevalence of E. coli O157:H7/EHEC O157 on beef carcasses. A study conducted by the USDA Food Safety and Inspection Service from October 1992 to September 1993 reported only 0.2% of the 2081 randomly sampled post-processing beef carcasses contaminated with EHEC O157 (Anon, 1994). While in another study conducted by FSIS from December 1993 to November 1994, reported no E. coli O157:H7 were recovered from any of the 2112 cow and bull carcasses sampled (Anon, 1996). This contrasts sharply with a survey performed in the Midwestern USA in July and August 1999 by Elder et al. (2000) who reported EHEC O157 was found in 43% of pre-evisceration, 18% of post-evisceration and 2.0% of post-processing beef carcasses. The study indicated that the overall prevalence of EHEC O157 in cattle may be much higher than previous estimates.

Many factors are thought to contribute to the variations among the studies, including the improved enrichment and isolation procedures (i.e. immunomagnetic separation), differences in the numbers of the samples collected, the type of sample and how it was collected, and when samples were collected (i.e. seasonality), as the prevalence of E. coli appears to peak in the late summer and early fall (Byrne et al., 2003).

In the current study, only 4.31% of pre-dehiding and 0.86% of post-evisceration bison carcasses were positive for E. coli O157:H7, and the organism was not detected on any of the 24 h chilled carcasses sampled. The highest incidence was recorded in October at the pre-dehiding point where 30% (3/10 carcasses sampled) were positive for the organism. This number is still lower than that reported by Elder et al. (2000). The present study seems to suggest that E. coli O157:H7 may not be as common on bison carcasses as compared to what is seen elsewhere in the beef industry.

The prevalence of Listeria on beef carcasses varies widely in different countries. A British survey by Fenlon et al. (1996) found 7% cattle carcasses were positive for L. monocytogenes. In the USA, 4.1% of steer and heifer carcasses were found positive for the organism in a survey conducted by FSIS (Anon, 1994), while 11.3% of cow and bull carcasses were positive in another survey (Anon, 1996). In Australia, Listeria spp. were recovered from 15% beef carcasses in domestic meat plants while the organism was only recovered from 0.77% of carcasses in export meat plants (Vanderlinde et al., 1998). In Northern Ireland, L. monocytogenes was absent from beef carcasses sampled (Madden et al., 2001), but L. innocua, and L. seeligeri were found on 3% of carcasses (3/200 sampled). The present study found that the prevalence of L. monocytogenes on chilled bison carcasses was 0.42%. However, the presence of L. monocytogenes in chilled bison carcasses is still a food safety concern as Listeria is capable of significant growth at refrigeration temperatures (Hudson and Mott, 1993; Sheridan et al., 1995).

This study found that the prevalence of Salmonella on chilled bison carcasses was 2.79%, which is similar to the 2.7% found in cow and bull carcasses in a study conducted by FSIS (Anon, 1996). However, the contamination rate of the organism in other studies was much lower. 1.0% of steer and heifer carcasses were positive for Salmonella in another survey performed by FSIS (Anon, 1994). In a study performed in seven United States slaughtering plants (Sofos et al., 1999), Salmonella spp. were recovered from 1.5% of steer and heifer carcasses, which is relatively similar (1.5%) to that reported in Northern Ireland by Madden et al. (2001). Vanderlinde et al. (1998) reported that 1.4% beef carcasses were positive for Salmonella in Australian domestic meat plants while 0.27% were positive in export meat plants.

In the current study, all Salmonella isolates obtained from bison carcasses were serotyped as S. enterica serovar Hadar (S. hadar) strains. S. hadar was the fifth most frequently reported Salmonella serotype in humans in the United States from 1986 through 1990 (CDC, 1992), while other studies by Logue et al. (2003) found
that this serotype was common on turkeys. In recent years however, the incidence of *S. hadar* in humans has decreased markedly. There were 307 isolates of *S. hadar* reported from human sources in 2001 as compared with 1970 in 1991 (Anon, 2001). Typical symptoms associated with *S. hadar* infection in humans are usually exhibited as gastroenteritis, while some clinical complications such as epididymoorchitis (Noble and Chan, 1988), peritonitis (Pascall et al., 1980), endovascular infection (Grey et al., 1991) and pneumonia (Wallet et al., 1999) have been reported in the immunocompromised and the elderly.

The prevalence of *E. coli* on pre-dehiding bison carcasses in the present study was high at 88.79%, indicating a high rate of fecal contamination. *E. coli* was found at a level of 11.30% on chilled bison carcasses, which is a little lower than the 20.9% reported in Australian domestic meat plants and 15.9% in export meat plants (Vanderlinde et al., 1998). In the current study, the overall prevalence of *E. coli* on bison carcasses was significantly reduced from processing, washing and chilling, i.e. 88.79% to 11.3%. This suggests that current antimicrobial intervention strategies employed at the plant are effective in reducing *E. coli* on carcasses to some extent, however, 53 (51.45%) of the 103 carcasses positive at pre-dehiding point were still positive for *E. coli* further down the line (results not shown) suggesting that the development of new strategies or interventions are necessary to continue to ensure the overall safety of bison meat.

Examination of the carcasses for *Listeria* indicated a higher prevalence of the organisms in chilled carcasses than in post-washing point. Several possible reasons may be responsible for this finding. First, the carcasses sampled were 24h old and had been processed on a previous day to the carcasses sampled on the production line, and may therefore may have had an overall different level of contamination present. Secondly, *Listeria* spp. are capable of growth at refrigeration temperatures (Hudson and Mott, 1993; Sheridan et al., 1995) and the organism may have grown during the carcass chill phase, alternatively, cross-contamination between carcasses may have occurred due to close proximity of the carcasses during chilling.

*E. coli* O157:H7 was found on carcasses sampled in August and October, which is in agreement with the previous studies that reported peak prevalence occurs in the later summer and early fall (Hancock et al., 1997; Elder et al., 2000). In a surveillance study carried out in Canada (Anon, 1998), the prevalence of *S. hadar* exhibited a multi-seasonal distribution with a peak in the summer months, while in the present study, the organism was only recovered in the fall (October). Seasonal variation in the incidence of *Listeria* spp. in animals has been described. It was reported that most cases of listerial abortion in cattle occurred between December and May in the Netherlands (cited by Yoshida et al., 1998). Studies by Gray and Killinger (1966) (cited by Yoshida et al., 1998) also indicated that the number of animals infected with *L. monocytogenes* generally increased from late November to early May, with the highest incidence occurring during February and March in the Northern hemispheres. However, the current study found that the highest incidence of *Listeria* spp. occurred in May, while a second peak incidence was noted in August and October. Many factors may contribute to this difference, such as the source of animals, the plant environment, and seasonal factors such as climate, temperature, relative humidity etc. (Fenlon and Wilson, 1989; Lovett et al., 1987; Yoshida et al., 1998, Chasseignaux et al., 2002). It was interesting to note that all four organisms were only isolated in one month (October). This suggests that there may be other factors that need to be considered including farm prevalence, which was outside the scope of this study but would be interesting to investigate in the future.

Overall, the current study may be one of the first to report findings on the incidence of *Listeria, Salmonella, E. coli* and *E. coli* O157:H7 on bison carcasses. The data from this study provides some baseline information that could be used in future studies of this unique species. The present study demonstrated that the prevalence of *Listeria, E. coli* and *E. coli* O157:H7 is lower than those observed on beef, while the prevalence of *Salmonella* is higher than that reported in previous studies. The study suggests that Bison meat may not be a significant source of the foodborne pathogens that are seen in other meat industries. Data obtained from this study also show that current in-plant process practices appear to reduce the level of carcass contamination rates. However, some new interventions should be developed in order to ensure the overall safety of bison meat.

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**References**


