Journal of Food Protection, Vol. 66, No. 11, 2003, Pages 1978–1986

Seasonal Prevalence of Shiga Toxin–Producing *Escherichia coli*, Including O157:H7 and Non-O157 Serotypes, and *Salmonella* in Commercial Beef Processing Plants†

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MS 03-136: Received 1 April 2003/Accepted 13 June 2003

ABSTRACT

The seasonal prevalence of *Escherichia coli* O157:H7, *Salmonella*, non-O157 *E. coli* (STEC), and *stx*-harboring cells was monitored at three Midwestern fed-beef processing plants. Overall, *E. coli* O157:H7 was recovered from 5.9% of fecal samples, 60.6% of hide samples, and 26.7% of carcasses sampled before the preevisceration wash. This pathogen also was recovered from 1.2% (15 of 1,232) of carcasses sampled at chilling (postintervention) at approximate levels of <3.0 cells per 100 cm^2. In one case, the *E. coli* O157:H7 concentration dropped from ca. 1,100 cells per 320 cm^2 at the preevisceration stage to a level that was undetectable on ca. 2,500 cm^2 at the postintervention stage. The prevalence of *E. coli* O157:H7 in feces peaked in the summer, whereas its prevalence on hide was high from the spring through the fall. Overall, *Salmonella* was recovered from 4.4, 71.0, and 12.7% of fecal, hide, and preevisceration carcass samples, respectively. *Salmonella* was recovered from one postintervention carcass (of 1,016 sampled). *Salmonella* prevalence peaked in feces in the summer and was highest on hide and preevisceration carcasses in the summer and the fall. Non-O157 STEC prevalence also appeared to vary by season, but the efficiency in the recovery of isolates from *stx*-positive samples ranged from 37.5 to 83.8% and could have influenced these results. Cells harboring *stx* genes were detected by PCR in 34.3, 92.0, 96.6, and 16.2% of fecal, hide, preevisceration carcass, and postintervention carcass samples, respectively. The approximate level of non-O157 STEC and *stx*-harboring cells on postintervention carcasses was ≥ 3.0 cells per 100 cm^2 for only 8 of 199 carcasses (4.0%). Overall, the prevalence of *E. coli* O157:H7, *Salmonella,* and non-O157 STEC varied by season, was higher on hides than in feces, and decreased dramatically, along with pathogen levels, during processing and during the application of antimicrobial interventions. These results demonstrate the effectiveness of the current interventions used by the industry and highlight the significance of hides as a major source of pathogens on beef carcasses.

Escherichia coli O157:H7 was first recognized in 1982 as a foodborne pathogen associated with the consumption of ground beef *(43)* and achieved general notoriety following a multistate outbreak in 1993 that resulted in four fatalities *(20).* However, the organism was found on only 0.2% (4 of 2,081) of dressed (postintervention) fed-beef carcasses sampled during 1992 and 1993, and most probable number (MPN) analyses suggested that it was present at very low levels *(29).* In 1994, the U.S. Food Safety and Inspection Service (FSIS) declared *E. coli* O157:H7 to be an adulterant in ground beef, making it the first microorganism given such status under the Federal Meat Inspection Act. The FSIS has now extended that declaration to include ''non-intact" beef, such as mechanically tenderized or reconstructed products *(5).*

Salmonella contamination of raw beef has also garnered attention recently, primarily because of the establishment of *Salmonella* performance standards by the FSIS *(4).* These standards were set up as a measure of sanitation and describe limits for the frequency of *Salmonella* contamination of raw meats, including beef carcasses and ground beef. The data available at the time the standards were established suggested that, like *E. coli* O157:H7, *Salmonella* was present infrequently and at low levels on fed-beef carcasses: 1.0% (20 of 2,081) of postintervention carcasses tested positive at levels of <0.3 cells per cm^2 *(29).* A subsequent study demonstrated a drop in the prevalence of *Salmonella* on postintervention carcasses at large processing plants to 0.1% *(44).*

Non-O157:H7 Shiga toxin–producing *E. coli* (STEC) strains have been associated with human disease, cattle, and beef production *(7, 16).* In the only study of non-O157 STEC on postintervention carcasses that has been carried out in the United States to date, a summertime prevalence of 8% (27 of 326) was found *(6),* but it is not clear whether all of these STEC strains could cause disease. Not all strains of STEC have been associated with human disease *(1, 16, 36).* Although Shiga toxin production is a key virulence factor, other factors also appear to affect pathogenicity.

Human clinical cases of *E. coli* O157:H7, non-O157 STEC, and *Salmonella* tend to peak during the warmer months *(21, 22).* This effect could be caused by a variety

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of factors. For example, a 1999 study conducted by the Animal and Plant Health Inspection Service demonstrated that the prevalence of *E. coli* O157:H7 and *Salmonella* in feedlot cattle feces also increases during the warmer months *(2, 3).* However, no studies have demonstrated seasonal variation in pathogen prevalence during beef processing. The primary objective of this study, therefore, was to measure both the preharvest and the postharvest seasonal prevalence levels of *E. coli* O157:H7, *Salmonella,* and non-O157 STEC during beef processing. In addition, the levels of these pathogens on carcasses were estimated.

MATERIALS AND METHODS

Sample collection. Samples were collected during two visits per season to three large Midwestern beef processing plants. The sampling seasons were spring (late April to early May 2001), summer (August 2001), fall (late October to mid-November 2001), and winter (late January to mid-February 2002). To facilitate sampling and to ensure the sampling of several lots, 50 to 65 carcasses (every 8th to 10th carcass) were sampled during each visit.

For individual tagged carcasses, samples were obtained from the hide, from the feces, from the carcass prior to the preevisceration wash, and from the dressed carcass upon entry into the cooler (postintervention). Samples were obtained as previously described, with some modifications *(12, 25).* Hides were sampled by swabbing a ca. 1,700-cm^2 area just to the right of the midline over the brisket. Ten-gram fecal samples were obtained from the distal portion of the colon after evisceration. Preevisceration carcass samples were obtained by swabbing the round and surrounding areas immediately after complete hide removal. Postintervention carcass samples were obtained by swabbing one side immediately prior to or within 2 h of entry into the cooler; the sampled area encompassed the inside round and rump plus a swath approximately 15 cm wide down the midline from the flank-plate juncture to the neck.

Salmonella was not recovered from samples taken during the first four fall visits owing to technical difficulties, so a supplementary visit was made to one of the processing plants. One hundred carcasses were tested for *Salmonella* only. Also, *Salmonella* was not recovered during the first two winter visits.

Enrichment and recovery of *E. coli* O157:H7 and *Salmonella*. Samples were analyzed for the presence of *E. coli* O157:H7 and *Salmonella* by the previously described Meats Research Unit (MRU) and MRU-tetrathionate (MRU-TT) methods *(12).* These methods involve enrichment in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, Md.), immunomagnetic separation (IMS), and selective plating. TSB was added within 3 h of sampling. After enrichment, two 1-ml aliquots were removed for storage at −70°C after 0.5 ml of 50% glycerol (Sigma Chemical Co., St. Louis, Mo.) was added.

The standard MRU method was used to recover *Salmonella* from spring fecal samples and from all hide and carcass samples. The MRU-TT method, which incorporates a TT (Difco) enrichment after the TSB enrichment and prior to IMS, was developed to improve recovery of *Salmonella* from fecal samples *(12).* This method was used to analyze fecal samples collected in summer, fall, and winter.

Confirmation of *E. coli* O157:H7 and *Salmonella*. For *E. coli* O157:H7, up to three suspect colonies (based on colony phenotype on either or both agars) were tested for each sample with DrySpot latex agglutination tests (Oxoid, Ogdensburg, N.Y.). Growth from colonies identified as potentially positive was streaked for isolation on sorbitol MacConkey agar containing cefixime and potassium tellurite *(12)* and stored in nutrient agar stabs *(45)* for further testing. Broth cultures of each isolate were subsequently stored as glycerol stocks at −70°C. *E. coli* O157:H7 confirmation included detection of O157 and H7 antigens by indirect enzyme-linked immunosorbent assay *(25),* multiplex PCR to detect stx_1, stx_2, *eae,* and *hlyA (6, 25, 37),* and verification of *E. coli* with the use of the Sensititre Gram-negative Auto-Identification (AP80) system (Accumed International, Westlake, Ohio) and/or API 20E strips (BioMérieux, Hazelwood, Mo.). As necessary for confirmation, some isolates (e.g., nonmotile isolates) were also analyzed by multiplex PCR for the presence of the $fliC_{H7}$ and $rfbE_{O157}$ genes *(32).* Samples were considered positive if at least one isolate (i) carried at least one *stx* gene, (ii) expressed the O157 antigen or carried the $rfbE_{O157}$ gene, (iii) expressed the H7 antigen or carried the $fliC_{H7}$ gene, and (iv) was identified as *E. coli.* Overall, 91% of samples testing positive by colony screening with DrySpot were positive on the basis of isolate characterization.

For *Salmonella,* up to three suspect (on the basis of colony phenotype on either or both agars) colonies were selected for each sample and streaked for isolation on Hektoen enteric agar containing novobiocin *(12).* Colonies identified as potentially positive on the basis of phenotype were stored in nutrient agar stabs *(45)* for further testing. Broth cultures of each isolate were subsequently stored as glycerol stocks at −70°C. Confirmation included verification of *Salmonella* with the Sensititre AP80 system and/or API 20E strips. Serological identification by agglutination was carried out for positive isolates with the use of poly-group antisera as described by the manufacturer (Difco). Samples were considered positive if at least one isolate (i) was identified as *Salmonella* and (ii) provided a positive agglutination reaction.

PCR detection of *stx*-positive enrichments and recovery and confirmation of non-O157 STEC. *E. coli* O157:H7–negative enrichments were tested with the use of a previously described multiplex PCR to detect the presence of stx_1, stx_2, *eae,* and *hlyA (37).* Samples were prepared for PCR as follows. (i) For postintervention carcass samples, cells were recovered from 100 μl of enrichment culture by centrifugation for 1 min at top speed, washed in 100 μl of 0.9% NaCl, and resuspended in 50 μl of sterile distilled, deionized water. The cell suspension was heated at 100°C for 10 min and was then centrifuged for 30 to 60 s to remove cellular debris. Two-microliter portions of these lysates were used in 30-μl PCR reactions. (ii) For preevisceration carcass samples, 150-μl aliquots were removed from enrichment glycerol stocks (see above) and treated similarly to postintervention samples. (ii) For fecal samples, the TSB enrichments were frozen at −20°C, without glycerol, prior to PCR. Cells recovered by centrifugation from 20 μl of these enrichments were washed in 400 μl of 0.9% NaCl and were then resuspended in 200 μl of InstaGene matrix (Bio-Rad Laboratories, Richmond, Calif.). The DNA was extracted according to the manufacturer's instructions. Forty microliters of the DNA supernatant was carefully removed and used in a 100-μl PCR reaction. (iv) Hide sample enrichments were initially evaluated as described for preevisceration carcass sample enrichments. If no PCR products were detected, then cells from 30 μl of the frozen enrichment (glycerol stocks at −70°C) were treated as described above for fecal sample enrichments. Samples were considered positive for *stx* genes if stx_1, stx_2, or both were detected. *E. coli* O157:H7–positive samples were presumed to be *stx*-positive.

Enrichments that tested positive for *E. coli* O157:H7 or *stx* genes were subjected to colony blotting for the recovery of non-

O157 STEC as previously described *(6),* with the following modifications. Briefly, frozen enrichments (glycerol stocks at −70°C) were diluted and plated to yield 2,000 to 10,000 colonies on 182-mm plates containing EC agar (Difco) supplemented with 1% glucose. Colonies were transferred to Hybond-N+ membranes (Amersham Pharmacia Biotech, Piscataway, N.J.) and treated with a mixed probe to detect stx_1 and/or stx_2. Up to three presumptive positive colonies per sample were streaked for isolation on sorbitol MacConkey agar. Two colonies from each streak then were picked and tested by multiplex PCR *(37).* This assay was carried out strictly to recover non-O157 STEC, so *stx*-positive colonies were screened by the DrySpot O157 latex test and discarded if they tested positive for O157. (Potential *E. coli* O157 *stx*+ colonies were identified for <0.3% of samples that had not been recognized as *E. coli* O157:H7 positive by the MRU method.) Presumptive non-O157 STEC isolates were stored in stabs and subsequently as glycerol stocks at −70°C. Isolates carrying *stx* genes were confirmed to be *E. coli* isolates with the Sensititre AP80 system and/or API 20E strips. Samples were considered positive for non-O157 STEC if at least one isolate (i) carried at least one *stx* gene and (ii) was identified as *E. coli.*

MPN assays. Three-tube MPN assays were used to estimate the levels of *E. coli* O157:H7, *Salmonella,* and *stx*-carrying cells on postintervention carcasses that were identified as presumptive positive. After observing the high prevalence of *E. coli* O157:H7 on preevisceration carcasses in the spring and summer, we also chose to estimate *E. coli* O157:H7 levels for these samples during the fall. Prior to enrichment, three 100-μl aliquots of each sponge sample were transferred to deep-well microtiter trays with each well containing 900 μl of buffered peptone water (Difco) supplemented with 50 μg of ferrioxamine E per ml (BPW-Fe *(41)*). To prepare ferrioxamine E, desferal (Sigma) was mixed with ferric ammonium sulfate (Sigma) at a 1:1 molar ratio and sterilized by filtration. Three serial 1:10 dilutions were prepared from each of the three inoculated wells, for a total of 12 wells per sample. (An extra 1:10 dilution was prepared in case unusual results were obtained *(28)*). The inoculated trays were incubated at 25°C for 2 h, were then transferred to 37°C for 16 to 18 h, and were subsequently stored at 4°C. Wells for samples identified as presumptive positive by the MRU method were processed as described below. Results are reported only for samples that were positive by the isolate criteria outlined above. The approximate number of cells per carcass area and the 95% confidence interval (CI) were determined with the use of an MPN index chart *(28).* For postintervention carcasses, ca. 2,500 cm^2 of each carcass was sampled in 25 ml of BPW–0.05% Tween 20, so the approximate number of cells per 100 cm^2 was determined. For preevisceration carcasses, ca. 8,000 cm^2 of each carcass was sampled, so the approximate number of cells per 320 cm^2 was determined.

If at least one isolate recovered from a sample was presumed to be *E. coli* O157:H7 on the basis of DrySpot screening during the MRU method, then the corresponding BPW-Fe culture wells were analyzed with Meridian ImmunoCard STAT! devices (Meridian Bioscience, Cincinnati, Ohio). The manufacturer's recommended method was adapted by inoculating 350 μl of the BPW-Fe enrichments into 4.5 ml of MacConkey broth (Difco) and incubating the broth at 42°C for 16 to 24 h before testing for the presence of *E. coli* O157:H7.

If at least one isolate recovered from a sample was presumed to be *Salmonella* on the basis of colony phenotype during the MRU method, then the corresponding BPW-Fe cultures were analyzed with VIP for *Salmonella* devices (BioControl Systems, Inc., Bellevue, Wash.). The manufacturer's recommended method was adapted by transferring 350-μl portions of the BPW-Fe enrichments to 10 ml of Rappaport-Vassiliadis broth (Oxoid) for the selective enrichment and transferring 0.5 ml of the Rappaport-Vassiliadis enrichment to 4.5 ml of TSB supplemented with 2,4-dinitrophenol and novobiocin (Sigma) for the postenrichment prior to testing for the presence of *Salmonella.*

If TSB enrichments tested positive for *stx*-carrying cells by PCR, 100-μl portions of the corresponding BPW-Fe cultures were subjected to the multiplex PCR assay described above *(37).*

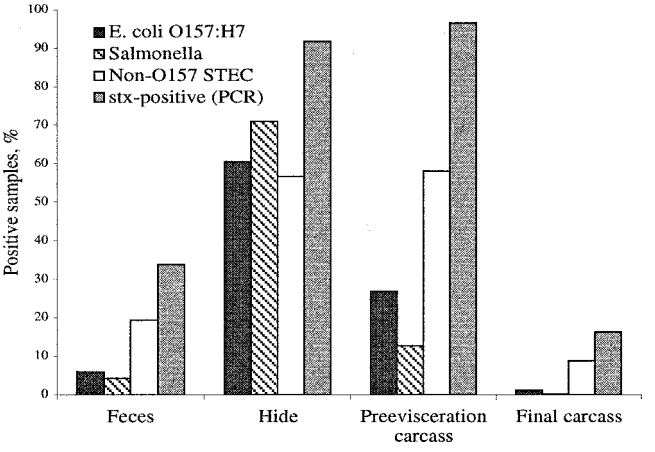

FIGURE 1. *Overall prevalence of* E. coli *O157:H7,* Salmonella, *non-O157 STEC, and* stx*-positive cells by sampling site.*

Statistical analyses. For each pathogen, prevalence was estimated for each type of sample (feces, hide, preevisceration carcass, and postintervention carcass) by season; data were pooled across all three processing plants. (The number of positive samples was divided by the total number of samples tested.) The exact binomial 95% CIs were calculated for each prevalence point estimate with PEPI software (version 2; USD, Inc., Stone Mountain, Ga. *(27)*). In order to test for sample type–specific prevalence differences between seasons for each pathogen, PEPI was used to calculate the pairwise difference in prevalence (spring versus summer, spring versus fall, spring versus winter, summer versus fall, summer versus winter, and fall versus winter; $P < 0.05$). To avoid inflated type I error rates due to multiple comparisons, the pairwise P values were adjusted with the use of Hommel's modification of the Bonferroni procedure *(31).*

RESULTS

Pathogen prevalence in feces. Feces were sampled to measure pathogen prevalence for individual animals. Overall and in each season, the prevalence of each pathogen was lower in feces than on hides or on preevisceration carcasses (Fig. 1 and Table 1). *E. coli* O157:H7 prevalence in fecal samples was highest in the summer (12.9%) and lowest in the winter (0.3%) ($P < 0.05$). *Salmonella* prevalence in fecal samples also was highest in the summer ($P < 0.05$). However, the prevalence of *Salmonella* as detected in the spring could have been lower by a factor of up to one-half relative to the prevalence in the other seasons because of a change in methodology to improve recovery (see "Materials and Methods") *(12).* Unlike *E. coli* O157:H7 and *Salmonella,* non-O157 STEC and *stx* genes were found in more fecal samples in the spring and fall than in the summer and winter ($P < 0.05$).

TABLE 1. *Seasonal variation in the prevalence of* E. coli *O157:H7,* Salmonella, *non-O157 STEC, and* stx*-positive cells in feces, on hides, and on preevisceration and postintervention carcasses*[a]

	Prevalence of *E. coli* O157:H7			Prevalence of *Salmonella*			Prevalence of non-O157:H7 STEC			Prevalence of *stx* (PCR)			Non-O157:H7 STEC isolation efficiency[b]	
Season	*n*	% positive	95% CI	*n*	% positive	95% CI	*n*	% positive	95% CI	*n*	% positive	95% CI	% isolated	95% CI
Feces														
Spring	285	3.9 B	1.9–6.8	285	2.1 B[c]	0.8–4.5	285	22.5 A	17.7–27.8	285	36.8 A	31.2–42.4	61.0 A	51–70
Summer	287	12.9 A	9.2–17.3	287	9.1 A	6.0–13.0	287	13.9 B	10.2–18.5	287	26.1 B	21.1–31.6	53.3 A	41–65
Fall	310	6.8 B	4.2–10.2	218	2.8 B	1.0–5.9	310	27.1 A	22.2–32.4	310	45.8 A	40.2–51.5	59.2 A	51–67
Winter	307	0.3 C	0.0–1.8	197	2.5 B	0.8–5.8	307	14.0 B	10.3–18.4	307	26.1 B	21.2–31.3	53.8 A	42–65
Hides														
Spring	305	73.8 A	68.5–78.6	306	61.4 C	55.7–66.9	305	43.0 C	37.3–48.7	305	88.5 B	84.4–91.9	48.5 C	42–55
Summer	321	73.5 A	68.3–78.3	321	91.6 B	88.0–94.4	321	56.1 B	50.5–61.6	321	97.2 A	94.7–98.7	57.7 B	52–63
Fall	332	67.2 A	61.8–72.2	219	97.7 A	94.8–99.3	332	77.7 A	72.9–82.1	332	92.8 AB	89.4–95.3	83.8 A	79–88
Winter	330	29.4 B	24.5–34.6	220	27.7 D	21.9–34.1	330	48.5 BC	43.0–54.0	330	88.2 B	84.2–91.5	55.0 B	49–61
Preevisceration carcasses														
Spring	303	38.9 A	33.4–44.7	305	3.0 B	1.4–5.5	304	60.2 A	54.5–65.7	304	99.7 A	98.2–99.9	60.4 A	55–66
Summer	319	40.8 A	35.3–46.4	319	19.7 A	15.5–24.6	319	64.9 A	59.4–70.1	319	98.7 A	96.8–99.7	65.7 A	60–71
Fall	330	27.3 B	22.5–32.4	217	24.9 A	19.3–31.1	330	65.5 A	60.0–70.6	330	97.6 A	95.3–99.0	67.1 A	62–72
Winter	329	1.2 C	0.3–3.1	219	4.1 B	1.9–7.7	329	41.6 B	36.3–47.2	329	90.3 B	86.6–93.3	46.1 B	40–52
Postintervention carcasses														
Spring	294	3.1 A	1.4–5.7	295	0.0 A	0.0–1.2	294	13.6 A	9.9–18.1	294	23.5 A	18.7–28.7	58.0 A	45–70
Summer	301	1.0 AB	0.2–2.9	301	0.3 A	0.0–1.8	301	4.0 B	2.1–6.9	301	10.6 B	7.4–14.7	37.5 A	21–56
Fall	312	1.0 AB	0.2–2.8	205	0.0 A	0.0–1.8	312	12.2 A	8.8–16.3	312	21.8 A	17.3–26.8	55.9 A	43–68
Winter	325	0.0 B	0.0–1.1	215	0.0 A	0.0–1.7	325	6.2 B	3.8–9.3	325	9.5 B	6.6–13.3	64.5 A	45–81

[a] Within a sample type, values with no common letter that are in the same column are significantly different ($P \geq 0.05$).

[b] Recovery of non-O157 STEC expressed as a percentage of *stx*-positive samples.

[c] In the spring, *Salmonella* was recovered from feces without the secondary TT broth enrichment used in the other seasons (see "Materials and Methods").

Pathogen prevalence on hides. Pathogen prevalence on hides may reflect several sources of contamination. Feces from one animal can contaminate multiple hides, and hides can be contaminated with feces from multiple animals, so these samples may reflect both lot and individual contamination. It has also been suggested that lairage may be a source of pathogens on hides *(8, 46)*. Overall, *E. coli* O157:H7 was isolated from 781 (60.6%) of 1,288 hides sampled throughout all four seasons (Fig. 1). *E. coli* O157:H7 prevalence in the spring did not differ significantly from that in the summer ($P > 0.05$) but fell slightly in the fall ($P > 0.05$) and dropped to its lowest point (29.4%) in the winter ($P < 0.05$; Table 1). *Salmonella* was recovered from 757 (71.0%) of 1,066 hides sampled throughout the four seasons. *Salmonella* prevalence was highest in the summer and fall ($P < 0.05$) and lowest in the winter ($P < 0.05$). The prevalence of non-O157 STEC on hides appeared to peak at 77.7% (258 of 332 samples) in the fall ($P < 0.05$). However, non-O157 STEC isolates were recovered from more of the *stx*-positive hide samples (83.8%) in the fall than in any of the other three seasons ($P < 0.05$). The prevalence of *stx*-positive hide samples was highest in the summer ($P < 0.05$) and fall ($P > 0.05$) and remained above 85% throughout all four seasons.

Pathogen prevalence and levels on preevisceration carcasses. Preevisceration carcasses were sampled to measure the transfer of pathogens from hides to the carcasses. At this point, immediately after hide removal and before the preevisceration wash, carcasses had been subjected to minimal antimicrobial interventions. The seasonal variation in the prevalence of *E. coli* O157:H7 and *Salmonella* on preevisceration carcasses mirrored that of hides but at much lower levels (Table 1). The prevalence of *E. coli* O157:H7 was highest in the spring and summer, lower in the fall ($P < 0.05$), and lowest in the winter (1.2%; $P < 0.05$). The prevalence of *Salmonella* was higher in the summer and fall than in the spring or winter ($P < 0.05$). The prevalence of non-O157 STEC did not differ significantly across the spring, summer, and fall ($P > 0.05$) but appeared to be lower in the winter ($P < 0.05$). A small but significant ($P < 0.05$) decrease in the prevalence of *stx*-positive samples in the winter was also observed. At least 90% of the preevisceration carcass samples were *stx* positive in all four seasons, with an overall prevalence of 96.5% (Fig. 1).

MPN assays were used to estimate levels of *E. coli* O157:H7 on preevisceration carcasses found to be culture positive during the fall sampling period (Table 2). Sixty-two (68.9%) of the 90 carcasses identified as positive had an MPN index of <3.0 *E. coli* O157:H7 cells per 320 cm^2. MPN indices for the other 28 positive carcasses ranged from 3.0 to 1,100 cells per 320 cm^2, with a mean and median of 54.3 and 3.6 cells per 320 cm^2, respectively. Eighteen (64.3%) of these 28 carcasses were estimated to carry fewer than 4 cells per 320 cm^2. With one exception, all preevisceration carcasses with MPN indices of >3.6 cells per 320 cm^2 were identified during one sampling visit, and *E. coli* O157:H7 was not detected on any of these nine carcasses at the end of processing (postintervention samples; see Table 3).

TABLE 2. *MPN indices and 95% CIs for* E. coli *O157:H7 recovered from preevisceration carcasses sampled during the fall*

Visit no.	No. of samples[a]	Results[b]	No. of cells per 320 cm^2	
			MPN index	95% CI
13	9	0, 0, 0	<3.0	0.0–9.5
	2	0, 1, 0	3.1	0.2–10.7
14	10	0, 0, 0	<3.0	0.0–9.5
	2	0, 0, 1	3.0	0.2–9.6
	1	0, 1, 0	3.1	0.2–10.7
	1	1, 0, 0	3.6	0.2–18.1
15	7	0, 0, 0	<3.0	0.0–9.5
	2	0, 0, 1	3.0	0.2–9.6
	1	1, 2, 0	11.4	3.6–42.0
16	16[c]	0, 0, 0	<3.0	0.0–9.5
	1	0, 0, 1	3.0	0.2–9.6
17	9	0, 0, 0	<3.0	0.0–9.5
18	11	0, 0, 0	<3.0	0.0–9.5
	3	0, 1, 0	3.1	0.2–10.7
	6	1, 0, 0	3.6	0.2–18.1
	1	0, 2, 0	6.2	1.2–18.1
	1	1, 0, 1	7.2	1.3–18.2
	1	1, 1, 0	7.4	1.3–20.3
	1	2, 0, 0	9.2	1.4–37.5
	1	2, 1, 0	14.7	3.7–42.0
	1	3, 0, 0	23.1	4.6–94.5
	1	3, 1, 0	42.7	9.0–183.0
	1	3, 3, 0	240	42.0–1,000.0
	1	3, 3, 2	1,100	180.0–4,100.0

[a] Number of samples per visit with the indicated MPN index.
[b] Numbers of positive wells (100 μl, 10 μl, 1 μl).
[c] Two of these carcasses were also positive at the postintervention sampling site. The third positive postintervention carcass was negative at the preevisceration sampling site.

Pathogen prevalence and levels on postintervention carcasses. Pathogen prevalence was determined for carcasses that had received a full complement of antimicrobial interventions. *E. coli* O157:H7 was recovered from 15 (1.2%) of the 1,232 postintervention carcasses sampled (Fig. 1). Nine of these carcasses were identified as positive in the spring (3.1% prevalence), three were identified as positive in the summer (1.0% prevalence), and three were identified as positive in the fall (1.0% prevalence; Table 1). The positive carcasses were detected during 5 of the 24 sampling visits (see Table 3); five of the positive carcasses were from the same lot. Seven of the 15 *E. coli* O157:H7–positive carcasses also carried non-O157 STEC (data not shown). For these 15 carcasses, *E. coli* O157:H7 was also recovered from 13 hide and 3 fecal samples; 8 of the 15 carcasses were *E. coli* O157:H7 positive prior to evisceration as well (data not shown). All 15 *E. coli* O157:H7–positive postintervention carcasses carried <3.0 cells per 100 cm^2 as determined by MPN assays (Table 3). *E. coli* O157:H7 levels were available for two of these carcasses at the preevisceration stage; these MPN indices were <3.0 cells per 320 cm^2.

TABLE 3. *MPN indices and 95% CIs for* E. coli *O157:H7,* Salmonella, stx, *and STEC recovered from postintervention carcasses*

Season	Visit no.	No. of samples[a]	Results[b]	No. of cells per 100 cm^2 MPN index	95% CI
E. coli O157:H7					
Spring	3	2	0, 0, 0	<3.0	0.0–9.5
	5	6	0, 0, 0	<3.0	0.0–9.5
	6	1	0, 0, 0	<3.0	0.0–9.5
Summer	12	3	0, 0, 0	<3.0	0.0–9.5
Fall	16	3	0, 0, 0	<3.0	0.0–9.5
Salmonella					
Summer	10	1	0, 0, 0	<3.0	0.0–9.5
stx (non-O157 STEC)					
Spring	1	1 (0)	0, 0, 0	<3.0	0.0–9.5
	2	12 (7)	0, 0, 0	<3.0	0.0–9.5
	2	1 (1)	2, 3, 2	38.2	17.7–82.6
	3	21 (10)	0, 0, 0	<3.0	0.0–9.5
	4	4 (2)	0, 0, 0	<3.0	0.0–9.5
	5	18 (13)	0, 0, 0	<3.0	0.0–9.5
	5	1 (0)	1, 1, 0	7.4	1.3–20.3
	6[c]	10 (7)	0, 0, 0	<3.0	0.0–9.5
	6	1 (0)	1, 0, 0	3.6	0.2–18.1
Summer	8	9 (4)	0, 0, 0	<3.0	0.0–9.5
	10	3 (3)	0, 0, 0	<3.0	0.0–9.5
	12	20 (5)	0, 0, 0	<3.0	0.0–9.5
Fall	13	1 (0)	0, 0, 0	<3.0	0.0–9.5
	13	1 (1)	0, 0, 1	3.0	0.2–9.6
	14	1 (0)	0, 0, 0	<3.0	0.0–9.5
	15	28 (24)	0, 0, 0	<3.0	0.0–9.5
	15	1 (1)	0, 0, 1	3.0	0.2–9.6
	15	1 (1)	1, 0, 0	3.6	0.2–18.1
	16	30 (10)	0, 0, 0	<3.0	0.0–9.5
	16	2 (0)	1, 0, 0	3.6	0.2–18.1
	17	1 (0)	0, 0, 0	<3.0	0.0–9.5
	18	2 (1)	0, 0, 0	<3.0	0.0–9.5
Winter	20	1 (1)	0, 0, 0	<3.0	0.0–9.5
	21	1 (1)	0, 0, 0	<3.0	0.0–9.5
	22	4 (3)	0, 0, 0	<3.0	0.0–9.5
	24	17 (11)	0, 0, 0	<3.0	0.0–9.5
	25	8 (4)	0, 0, 0	<3.0	0.0–9.5

[a] Number of samples per visit with the indicated MPN index; for *stx*-positive samples, the number in parentheses is the number of those samples from which a non-O157 STEC isolate was recovered.

[b] Numbers of positive wells (100 μl, 10 μl, 1 μl).

[c] The MPN index was not determined for one sample recovered during visit 6.

Salmonella was recovered from one postintervention carcass sampled in the summer, resulting in an overall prevalence of 0.1% (Fig. 1 and Table 1). This postintervention carcass tested negative for *E. coli* O157:H7, non-O157 STEC, and *stx* genes. Fewer than 3.0 *Salmonella* cells per 100 cm^2 of the sampled area were detected (Table 3). *Salmonella* was also recovered from the hide and feces of the same animal and from the same carcass prior to evisceration (data not shown).

Shiga toxin genes were detected in 200 (16.2%) of 1,232 postintervention carcass samples, and non-O157 STEC strains were recovered from 110 (8.9%) of these 200 carcasses (Fig. 1). The prevalence levels for both *stx*-carrying cells and non-O157 STEC on postintervention carcasses were highest in the spring and fall ($P < 0.05$), similar to the pattern observed for fecal prevalence (Table 1). The majority of the *stx*-positive postintervention carcasses (191 of 199; 96.0%) harbored fewer than 3.0 *stx*-carrying cells per 100 cm^2 (Table 3). The MPN indices for the remaining eight carcasses for which data were available ranged from 3.0 to 38.2 cells per 100 cm^2, with only two carcasses carrying more than 3.6 cells per 100 cm^2.

DISCUSSION

Cattle hides have been identified as a source of general microbial contamination on carcasses *(15, 30, 34, 42),* and it has been shown that *E. coli* O157:H7 can be transferred from hides to carcasses during processing *(17).* In the present study, *E. coli* O157:H7, *Salmonella,* and non-O157 STEC were found predominantly on hides, suggesting that hides are a more significant source of carcass contamination than feces. *E. coli* O157:H7 and *Salmonella* were recovered more frequently from preevisceration carcasses than from feces, and clear seasonal trends in the prevalence of these organisms on hides were mimicked on preevisceration carcasses. Pathogen prevalence on hides may reflect several sources of contamination, such as soils, feces from other animals, and possibly lairage *(8, 46).* For both *E. coli* O157:H7 and *Salmonella,* methodological differences could account for the discrepancies between the results of previous reports and the data presented here *(12, 23).* Previous reports have suggested substantially lower prevalence rates (ranging from 4.5 to 18.0%) for *E. coli* O157:H7 on hides in the United States at slaughter *(10, 25, 40).* In a summertime study of four feedlot pens, 51% (71 of 139) of ventral hides were found to test positive for *E. coli* O157:H7 *(33). Salmonella* prevalence rates of 15.4 to 86.9% have been found for hides in previous U.S. studies *(9, 10, 14, 40).* In addition, and in contrast to the results of the present study, Sofos et al. *(47)* reported a higher prevalence for *Salmonella* in the late fall and the winter (November to January) than in the spring (May to June).

In the present study, hides, not feces, appeared to be the primary source of the tested pathogens on carcasses. Previous studies had suggested that *E. coli* O157:H7 prevalence in feces was correlated with the pathogen's prevalence on carcasses *(11, 18, 24, 25),* and the prevalence levels of both *E. coli* O157:H7 and *Salmonella* in cattle feces have been investigated in depth. Although it is difficult to compare results from various studies because of differences in methodology, preliminary data have suggested that the MRU and MRU-TT methods are at least as sensitive as previously employed methods *(12).* Peak prevalence rates for *E. coli* O157:H7 in feces have been reported to occur in summer and to range from 17 to 37% *(2, 13, 35, 49),* comparable to the 12.9% peak summertime rate reported here. The results of the present study are similar to those of a 1999 feedlot study *(3)* demonstrating a peak in *Salmonella* prevalence in feces in the summer but contrast with

those of a study carried out at slaughter in 1995 and 1996 suggesting that *Salmonella* prevalence was higher from November to January (14.2%) than from May to June (8.3%) *(47).* The overall fecal prevalence rate for *Salmonella* (4.4%) was in good agreement with reports of 5.5 to 6.7% for fed cattle in the United States *(3, 10, 26, 40).*

Interestingly, the relative proportions of positive preevisceration carcasses versus those of positive hides were noticeably higher for *Salmonella* than for *E. coli* O157:H7 except in the winter. Assuming that hides are the major source of pathogens, this observation suggests that there may be a significant difference in carcass contamination rates for the two organisms. It is possible that the data reflect a disparity between the method's sensitivity for the recovery of *E. coli* O157:H7 and its sensitivity for the recovery of *Salmonella,* and this possibility should be investigated. Alternatively, levels of hide contamination may vary by site *(33),* so the data could reflect relative prevalence differences between the brisket area (hide sampling site) and the rump area (preevisceration carcass sampling site). It is also possible, but intuitively less likely, that *E. coli* O157:H7 is more successful at contaminating carcasses. Steam vacuuming was the only direct antimicrobial intervention administered prior to preevisceration carcass sampling, and *E. coli* O157:H7 and *Salmonella enterica* Typhimurium have been shown to be equally susceptible to this antimicrobial process *(38).* An alternative explanation is that *E. coli* O157:H7 may be present on hides in larger numbers than *Salmonella,* making *E. coli* O157:H7 more likely to be transferred to the carcass.

Non-O157 STEC and *stx*-carrying cells were highly prevalent among all types of samples. To the best of our knowledge, this is the first study examining non-O157 STEC prevalence on hides. Previously, non-O157 STEC prevalence in fecal samples was examined for 23 range cattle in the United States, and one animal per season was found to carry these pathogens *(48).* The reported fecal prevalence of non-O157 STEC for dairy cattle in the United States and other countries ranges from 5.8 to 19.0% (for a recent review, see Arthur et al. *(7)*). In the present study, the overall prevalence of non-O157 STEC in feces was found to be at the high end of this range (19.3%). Variations in seasonal and sample type prevalence should be interpreted with some caution, however. The relative sensitivity of the PCR protocols for each type of sample has not been established, and the efficiency of our protocols in isolating non-O157 STEC from *stx*-positive samples varied significantly by season ($P < 0.05$). Differences in the types and levels of background growth in the samples, among other things, could have affected both the PCR and the colony hybridization assays. As detection methods continue to improve, non-O157 STEC likely will be recovered increasingly more often from feces, hides, and other sources, judging from the number of samples testing positive for *stx* genes in this and other studies *(7, 39).*

One primary indicator of beef-product contamination is the prevalence of carcass contamination after processing (postintervention). As was previously shown for *E. coli* O157:H7 *(25),* the number of carcasses contaminated with *E. coli* O157:H7, *Salmonella,* and non-O157 STEC dropped significantly from the preevisceration stage to the postintervention stage ($P < 0.0001$). The overall prevalence of *E. coli* O157:H7 on postintervention carcasses (1.2%) was similar to the prevalence reported in other studies that also used sensitive methods incorporating IMS *(13).* Two studies have shown higher prevalence rates for *Salmonella* on dressed carcasses (1.0 and 1.3%, compared with the rate of 0.1% observed in this study). However, one of these studies was carried out during the summer, when prevalence rates may be higher *(9).* The other study was completed prior to the introduction of hazard analysis critical control point regulations *(3),* and a follow-up report has suggested that *Salmonella* prevalence in large beef processing plants is now lower (0.1%) *(44).* Non-O157 STEC prevalence rates (4.0%) on postintervention carcasses in the summer were lower than expected on the basis of previous data (8%) *(6),* possibly reflecting a lower efficiency of recovery, as noted above.

In addition to prevalence, pathogen levels on postintervention carcasses also are significant to public health issues. The data presented here suggest that only low levels of pathogens survive the application of antimicrobial interventions. Small numbers of *E. coli* O157:H7, *Salmonella,* and non-O157 STEC were recovered from the few contaminated, dressed carcasses (postintervention). Furthermore, data for *E. coli* O157:H7–positive preevisceration carcasses suggest that, like prevalence, the level of contamination drops between the preevisceration and postintervention processing sites. Different areas were sampled at each site, relatively few samples were analyzed, and some of the MPN scores (e.g., 0, 2, 0; 0, 0, 1; and 0, 1, 0) appear more often than would be expected by chance *(17),* so the data must be interpreted cautiously. However, the drop in *E. coli* O157:H7 levels from the preevisceration stage to the postintervention stage appears to have been substantial in some cases; for example, there was a drop from ca. 240 and ca. 1,100 cells per 320 cm^2 on two preevisceration carcasses to levels undetectable on ca. 2,500 cm^2 of the same carcasses at the postintervention stage (see Table 3). In conjunction with the prevalence rates, these data suggest that in general, few pathogen cells are introduced into beef products produced from fed-cattle carcasses.

The data presented here suggest that hides are the major source of contamination of beef carcasses with *E. coli* O157:H7, *Salmonella,* and non-O157 STEC. Significant seasonal differences in the prevalence levels of *E. coli* O157:H7 and *Salmonella* were found both preharvest (in feces and on hides) and postharvest (on carcasses). In addition, throughout the year the prevalence of cells carrying *stx* genes was higher than that of *E. coli* O157:H7 or *Salmonella.* These observations are consistent with seasonal rates for human clinical cases reported for *E. coli* O157:H7 and *Salmonella* in previous years but not with seasonal rates reported for non-O157 STEC *(21, 22).* The discrepancy could reflect underreporting of non-O157 STEC disease cases, an inability of all STEC to cause disease, or a myriad of other factors *(7).* However, these data cannot be used to establish a direct cause-and-effect relationship be-

tween pathogen prevalence during beef processing and disease cases caused by *E. coli* O157:H7, *Salmonella,* and non-O157 STEC. Further studies are needed to determine the relevance of these implications.

ACKNOWLEDGMENTS

We thank the participating beef processing plants for their cooperation. We also thank the entire staff of the Meats Research Unit for excellent support throughout this project, particularly Carol Grummert and Marilyn Bierman (the clerical staff) and Patty Beska, Julie Dyer, Troy Gramke, Sue Hauver, Bruce Jasch, Brooke Korell, Dee Kucera, Kim Kucera, Frank Reno, Audrey Smidt, Greg Smith, Jon Wendell, and Nick Woods (the technical staff).

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