Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing

Robert O. Elder, James E. Keen, Gregory R. Siragusa, Genevieve A. Barkocy-Gallagher, Mohammad Koohmaraie, and William W. Laegreid*

United States Meat Animal Research Center, United States Department of Agriculture, Agricultural Research Service, Clay Center, NE 68933

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A survey was performed to estimate the frequency of enterohemorrhagic *Escherichia coli* O157:H7 or O157:nonmotile (EHEC O157) in feces and on hides within groups of fed cattle from single sources (lots) presented for slaughter at meat processing plants in the Midwestern United States, as well as frequency of carcass contamination during processing from cattle within the same lots. Of 29 lots sampled, 72% had at least one EHEC O157-positive fecal sample and 38% had positive hide samples. Overall, EHEC O157 prevalence in feces and on hides was 28% (91 of 327) and 11% (38 of 355), respectively. Carcass samples were taken at three points during processing: preevisceration, postevisceration before antimicrobial intervention, and postprocessing after carcasses entered the cooler. Of 30 lots sampled, 87% had at least one EHEC O157-positive preevisceration sample, 57% of lots were positive postevisceration, and 17% had positive postprocessing samples. Prevalence of EHEC O157 in the three postprocessing samples was 43% (148 of 341), 18% (59 of 332) and 2% (6 of 330), respectively. Reduction in carcass prevalence from preevisceration to postprocessing suggests that sanitary procedures were effective within the processing plants. Fecal and hide prevalence were significantly correlated with carcass contamination ($P = 0.001$), indicating a role for control of EHEC O157 in live cattle.

Enterohemorrhagic *Escherichia coli* O157:H7/NM (EHEC O157) may cause severe disease and death in humans (1, 2). Human infection and outbreaks from EHEC O157:H7 have been attributed to the consumption of undercooked beef food products as well as various other foods, such as unpasteurized apple cider (3–5). Since 1982, more than 100 outbreaks of EHEC O157 have been documented (6). Of those outbreaks, 52% have been attributed or linked to foods derived from cattle (6).

Cattle have been implicated as the primary reservoir of EHEC O157 (7, 8). Prevalence surveys conducted on fed cattle estimated the overall fecal prevalence of EHEC O157 to be very low (7, 9). The largest survey of fed cattle conducted to date found only 1.8% of fecal samples to contain EHEC O157 (10). However, it was noted in this study that 63 of 100 feedlots had at least one positive fecal sample, indicating widespread distribution of EHEC O157. Recent studies using improved enrichment and isolation procedures have indicated that the overall prevalence of EHEC O157 infection in cattle may be significantly higher than originally estimated (8, 11, 12). These studies found that peak EHEC O157 fecal shedding rates occur during summer and early fall, and they vary from a low of 0% to as high as 61% on some farms. To date, no factors have been identified, other than season, that consistently affect the EHEC O157 shedding rates of cattle.

Studies have been completed to determine the prevalence of EHEC O157 in cattle feces and on carcasses during slaughter processes (7, 13). From cattle presented for slaughter in the United Kingdom, 0.83% of 6,495 bovine fecal samples were positive for EHEC O157 (13). A study at an abattoir in South Yorkshire found 4% of rectal fecal swabs positive for EHEC O157 (7). Of 23 animals with positive rectal swabs, 30% also tested positive for EHEC O157 on carcasses by sampling neck trimmings and swabbing an adjacent area. Another 8% of adjacent carcasses from fecal negative cattle also tested positive, suggesting another source of carcass contamination (7). A study conducted by the U.S. Department of Agriculture Food Safety and Inspection Service reported only 4 of 2,081 (0.2%) randomly sampled postprocessing beef carcasses contaminated with EHEC O157 (14). Fecal, hide, and carcass prevalence at and during processing may have been underestimated in the past, because of a lack of highly sensitive and specific methods for the isolation EHEC O157 from those matrices.

The origins and subsequent rate at which EHEC O157 carcass contamination occurs have not been well established. Hazard Analysis–Critical Control Point plans can and are being used to decrease the risk of food-borne illness by intervening at stages of processing that pose a plausible risk of carcass contamination. However, these plans require adequate microbiological data if they are to allow confident conclusions regarding the effectiveness of control programs for food-borne pathogens.

This study was designed to address the following question: Is the EHEC O157 infection status of beef cattle presented for slaughter reflected in levels of carcass contamination detected after slaughter on a population basis? Specific goals of this study were to (*i*) estimate EHEC O157 frequency in feces and on hides from beef cattle presented for slaughter in the U.S., (*ii*) identify the relative rates of EHEC O157 contamination of beef carcasses during processing, and (*iii*) determine whether a relationship exists between EHEC O157 prevalence in feces and/or on hides to carcass contamination during slaughter processes. Results from these studies in which culture and isolation methods with increased sensitivity were used provide more accurate information regarding the occurrence of EHEC O157 associated with cattle during the slaughter process.

Materials and Methods

Study Design for In-Plant Sampling. Samples were collected from stunned animals and from carcasses at four Midwestern beef processing facilities processing greater than 3,000 head per day (designated plants A through D), during July and August 1999. At each plant, random lots of 35–85 animals were selected. A lot is defined as cattle from a common source (such as a ranch or feedlot) kept as a group through the slaughter process. At least 20% of all animals and carcasses from selected lots were sampled. No attempt was made to ensure that the fecal, hide, and

Abbreviation: EHEC, enterohemorrhagic *Escherichia coli*.

*To whom reprint requests should be addressed at: U.S. Meat Animal Research Center, USDA, ARS, P.O. Box 166, State Spur 18D, Clay Center, NE 68933. E-mail: laegreid@email.marc.usda.gov.

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carcass samples were from exactly the same animals, but all carcass sampling was completed on the same carcasses. Sampling of 3 or 4 lots was completed at each plant on each of two separate occasions. All in-plant sampling was completed from July through August 1999.

Sampling and Bacterial Culture Methods for Feces and Hides. Fecal samples were cultured as previously described with minor modifications (8). For each fecal sample, the distal colon was ligated and transected approximately 750 cm proximal to the rectum, before complete evisceration, and the colorectal tissues were placed in individual clean plastic bags. Within 2 h of sampling, a fecal sample was obtained by aseptically opening the colon, and a 10% fecal suspension was prepared by homogenizing 10 g of feces in 90 ml of GN broth (Fisher Scientific) containing vancomycin (8 mg/liter; Sigma), cefixime (0.5 mg/liter; Lederle Laboratories), and cefsuludin (10 mg/liter; Sigma) (15). The suspension was incubated at 37°C for 6 h followed by immunomagnetic bead enrichment consisting of 30-min incubation of 1 ml of GN enrichment broth with 20 μl of anti-O157 immunomagnetic beads on a rocker (60 cycles per min) at 25°C (Dynal, Lake Success, NY). The immunomagnetic bead suspensions were washed three times in 1 ml of PBS containing 0.05% Tween 20 on a magnetic separation rack (Dynal). After the final wash, the beads were resuspended in 100 μl of PBS/0.05% Tween 20. Fifty microliters of the bead suspension was spread plated on sorbitol MacConkey (SMAC) plates containing cefixime (0.5 mg/liter) and potassium tellurite (2.5 mg/liter, Difco Laboratories; SMACct). After an 18-h incubation at 37°C, up to three sorbitol-negative colonies exhibiting colony morphology typical of EHEC O157 were picked as suspect EHEC O157 (16). Colonies were suspended in 0.2 ml of MacConkey broth (Difco Laboratories) and tested by using ImmunoCard Stat! *E. coli* O157:H7 (Meridian Diagnostics, Cincinnati, OH). Isolates that gave positive results were subjected to a second plating on SMACct and a single colony was picked for further characterization as described below.

Hide samples were obtained from animals after stunning by using two 2-inch × 2-inch sterile gauze pads wetted with sterile H_2O. An area of approximately 450 cm^2 of the ventral brisket was swabbed. The pads were placed into sterile sample bags with the addition of 20 ml of sterile Brilliant Green Bile 2% (60 g/liter, Difco Laboratories), and the mixture was incubated, plated, and processed as described above.

Sampling and Bacterial Culture Methods for Carcasses. Beef carcass samples were obtained by using a Speci-Sponge (Nasco, Fort Atkinson, WI) moistened with 25 ml of Butterfield's phosphate diluent, with 0.1% Tween 20 in a stomacher bag. Sponges were wrung out as much as possible within the bag, withdrawn, and used to swab each area. Carcass sponge sampling areas were as follows: (*i*) Preevisceration samples were taken immediately after complete hide removal encompassing an area of both inner and outer hocks, then across the perineum. (*ii*) Postevisceration samples were collected after evisceration, splitting, and trimming but before final antimicrobial interventions. Three approximately 10 × 25 cm areas from one half of each carcass were sampled (rump, midline, and brisket/neck) according to standard protocols, with minor modifications (17). (*iii*) Postprocessing samples were taken after final plant antimicrobial intervention from the carcasses hanging in the cooler no more than 2 h after final intervention. Sampling was performed on the same sites and areas as described for postevisceration sampling but on the opposite half of the same carcass. Thus, the preevisceration, postevisceration, and postprocessing samples were matched by carcass. Antimicrobial interventions were specific for each plant and included steam pasteurization, hot water washes, organic acid washes, or combinations of these treatments.

Carcass samples were enriched as described above with minor modifications. Sponges were incubated with 90 ml of Brilliant Green Bile 2% (40 g/liter) for 10 h at 37°C, followed by anti-O157 immunomagnetic bead enrichment as described above. Fifty microliters of immunomagnetic bead enrichment cultures was plated onto SMACct and BCM (Biosynth International, Naperville, IL) agar plates and incubated for 18 h at 37°C (18). Presumptive EHEC O157 colonies on plates were picked, up to eight colonies per sample, and tested by using ImmunoCard Stat! *E. coli* O157:H7 tests. On those plates that did not have isolated individual colonies but had presumptive EHEC O157 colonies, one to three sweeps approximately 0.5 cm long were resuspended in MacConkey broth and tested as described above. Separate picks were made from each location on the plate included in a positive pool and streaked for isolation on SMAC agar, then incubated at 37°C overnight. Presumptive positive colonies were picked on the basis of a positive serologic reaction with O157 latex reagents or Dry Spot tests (Unipath/Oxoid, Ogdensburg, NY), and isolated colonies were transferred to stab vials for storage and further testing.

Isolate Characterization. ImmunoCard Stat! *E. coli* O157:H7 positive suspect EHEC O157 isolates were subjected to biochemical, genetic, and serological characterization. Biochemical identification of isolates was completed with the Sensititre Gram-negative AutoIdentification (AP80) system (Accumed International, Westlake, OH) or API 20E strips (BioMerieux, Hazelwood, MO). Genetic profiling for EHEC O157 markers (*stx1*, *stx2*, *ehxA*, *eaeA*, rfb_{O157}) was completed by PCR as previously described (19). Isolates were confirmed as O157:H7 by an indirect ELISA using monoclonal antibodies specific for O157 lipopolysaccharide and H7 flagellar antigen (15, 20). Isolates that were H7-negative were examined for motility by wet-mount microscopy or agar stab methods. Motile isolates that were H7-negative were considered EHEC O157-negative. An isolate was classified as confirmed EHEC O157 if it fit the biochemical profile of *E. coli*, was serologically O157 and H7 or nonmotile, and was PCR-positive for rfb_{O157}, *ehxA*, *eaeA,* and either *stx1 or stx2* or both.

Statistical Analysis. Confirmed bacteriological results were compiled and entered into a database and analyzed. The proportion of positive samples and the exact binomial 95% confidence interval for these proportions were calculated by using public domain software (EPI INFO 6.0; Centers for Disease Control and Prevention, Atlanta). Comparisons between proportions and correlations were made by using the ASTUTE software module (University of Leeds, U.K.) for Excel 5.0 (Microsoft).

Results

Overall Prevalence of EHEC O157-Positive Samples. Of 341 carcasses sampled, 158 (46.3%) were positive at at least one site. The numbers of individual positive samples for feces, hide, and carcasses at preevisceration, postevisceration, and postprocessing were 91 of 327 (28%), 38 of 355 (11%), 148 of 341 (43%), 59 of 332 (18%), and 6 of 330 (2%), respectively (Table 1). Positive isolates were identified within every lot, except lot 1 plant B first sampling and lot 3 plant D first sampling.

Characterization of Isolates. All of the isolates were confirmed as *E. coli* by biochemical profile and were confirmed as EHEC O157:H7 or O157:NM by their reaction with monoclonal antibodies directed to O157 lipopolysaccharide and the H7 flagella or were classified as nonmotile by microscopic examination. All serologically O157:H7/NM isolates were positive by PCR for *ehxA*, *eaeA*, rfb_{O157}, and either *stx1* or *stx2* or both *stx1* and *stx2*, except three preharvest isolates, two of which were rfb_{0157} and *eaeA* negative and one which was *ehxA* negative, and one

Table 1. Data summary for all samples and lots

			Carcass		
	Fecal	Hide	Preevisceration	Postevisceration	Postprocessing
Total samples	91/327	38/355	148/341	59/332	6/330
Percent positive	27.8 (23.0–33.0)	10.7 (7.7–14.4)	43.4 (38.1–48.8)	17.8 (13.8–22.3)	1.8 (0.7–3.9)
Lots sampled	21/29	11/29	26/30	17/30	5/30
Percent lots positive	72.4 (52.5–86.6)	37.9 (20.7–57.7)	86.7 (69.3–96.2)	56.7 (37.4–74.5)	16.7 (5.6–34.7)
Mean positive/lot, %	26.2 (15.9–36.5)	13.0 (3.5–22.5)	43.4 (31.5–55.3)	18.3 (10.3–26.3)	1.9 (0.2–3.7)
Range, %	0–100	0–89	0–100	0–78	0–22

Values are number of samples positive for EHEC O157/total samples taken and percent positive (95% confidence interval).

postprocessing carcass sample that was *stx* negative (Table 2). The distribution of shigatoxin types was as follows: *stx1*, 1.4% of isolates, *stx2*, 41.2%, and both *stx1* and *stx2*, 57.4% of all 342 isolates tested.

Prevalence of EHEC O157 in Feces and on Hides. EHEC O157 was isolated from at least one sample in 21 of 29 lots (72%, Tables 1 and 2). Prevalence of positive fecal samples within lots ranged from 0% to 100%, with a mean prevalence of 26% (Tables 1 and 2). Eleven of 29 lots (38%) were hide positive for EHEC O157, with prevalence ranging from 0% to 89%, with a mean prevalence of 13% (Tables 1 and 2). Ten lots that had at least one positive fecal sample also had at least one positive hide sample (34%). Seven lots were negative for EHEC O157 in both feces and hides (24.1%). One fecal and hide sampling set was omitted from the study because of sampling error. However, valid carcass samples were collected from that lot (lot 3 plant A second sampling).

Prevalence of EHEC O157 on Carcasses. Twenty-seven of 30 lots (90%) had at least one positive sample from a carcass within a lot (Tables 1 and 2). Three lots were negative for EHEC O157 for all carcass samples taken (10%). Of 30 lots sampled, 26 (87%), 17 (57%), and 5 (17%) were positive for EHEC O157

Table 2. Data summary by plant and lot

						Carcass		
Plant	Sample	Lot	*n*	Fecal	Hide	Preevisceration	Postevisceration	Postprocessing
A	1	1	84	13/17 (76.5%)	2/18 (11.1%)	10/18 (55.6%)	3/18 (16.7%)	0/18 (0.0%)
		2	35	0/9 (0.0%)	0/9 (0.0%)	6/8 (75.0%)	0/7 (0.0%)	0/8 (0.0%)
		3	68	2/16 (12.5%)	1/16 (6.3%)	9/16 (56.3%)	0/16 (0.0%)	0/16 (0.0%)
	2	1	80	4/18 (22.2%)	0/18 (0.0%)	0/18 (0.0%)	0/18 (0.0%)	0/18 (0.0%)
		2	41	NT	NT	1/10 (10.0%)	1/9 (11.1%)	0/10 (0.0%)
		3	62	0/12 (0.0%)	0/14 (0.0%)	3/14 (21.4%)	0/14 (0.0%)	0/14 (0.0%)
		4	44	1/13 (7.7%)	0/10 (0.0%)	2/13 (15.4%)	0/13 (0.0%)	0/13 (0.0%)
B	1	1	46	0/12 (0.0%)	0/12 (0.0%)	0/11 (0.0%)	0/11 (0.0%)	0/9 (0.0%)
		2	37	0/9 (0.0%)	0/15 (0.0%)	1/9 (11.1%)	0/8 (0.0%)	0/9 (0.0%)
		3	39	1/10 (10.0%)	0/16 (0.0%)	6/10 (60.0%)	3/8 (37.5%)	0/9 (0.0%)
		4	48	4/12 (33.3%)	1/20 (5.0%)	7/12 (58.3%)	0/12 (0.0%)	0/12 (0.0%)
	2	1	36	2/9 (22.2%)	8/9 (88.9%)	8/9 (88.9%)	4/9 (44.4%)	0/9 (0.0%)
		2	36	4/9 (44.4%)	7/9 (77.8%)	8/9 (88.9%)	7/9 (77.8%)	2/9 (22.2%)
		3	36	1/9 (11.1%)	7/9 (77.8%)	5/9 (55.6%)	2/9 (22.2%)	0/9 (0.0%)
		4	40	0/10 (0.0%)	5/10 (50.0%)	3/10 (30.0%)	0/10 (0.0%)	0/10 (0.0%)
C	1	1	41	2/10 (20.0%)	0/10 (0.0%)	4/10 (40.0%)	3/9 (33.3%)	1/10 (10.0%)
		2	76	10/17 (58.8%)	2/17 (11.8%)	17/17 (100.0%)	11/17 (64.7%)	1/17 (5.9%)
		3	42	3/10 (30.0%)	0/10 (0.0%)	8/10 (80.0%)	6/10 (60.0%)	0/10 (0.0%)
		4	38	7/9 (77.8%)	3/10 (30.0%)	9/10 (90.0%)	5/10 (50.0%)	0/10 (0.0%)
	2	1	38	10/10 (100.0%)	1/10 (10.0%)	2/10 (20.0%)	2/10 (20.0%)	1/10 (10.0%)*
		2	44	4/10 (40.0%)	0/11 (0.0%)	2/10 (20.0%)	2/10 (20.0%)	0/10 (0.0%)
		3	40	1/10 (10.0%)	0/10 (0.0%)	4/11 (36.4%)	3/11 (27.3%)	0/11 (0.0%)
		4	46	7/10 (70.0%)	0/12 (0.0%)	12/12 (100.0%)	2/12 (16.7%)	1/12 (8.3%)
D	1	1	37	0/7 (0.0%)	0/10 (0.0%)	3/8 (37.5%)	0/8 (0.0%)	0/8 (0.0%)
		2	39	0/6 (0.0%)	0/10 (0.0%)	2/8 (25.0%)	0/6 (0.0%)	0/5 (0.0%)
		3	38	0/8 (0.0%)	0/10 (0.0%)	0/8 (0.0%)	0/8 (0.0%)	0/8 (0.0%)
		4	42	1/8 (12.5%)	0/10 (0.0%)	0/8 (0.0%)	1/8 (12.5%)	0/8 (0.0%)
	2	1	65	2/20 (10.0%)	0/15 (0.0%)	2/15 (13.3%)	1/15 (6.7%)	0/13 (0.0%)
		2	42	6/11 (54.5%)	1/11 (9.1%)	10/11 (90.9%)	3/11 (27.3%)	0/11 (0.0%)
		3	58	6/16 (37.5%)	0/14 (0.0%)	4/17 (23.5%)	0/16 (0.0%)	0/16 (0.0%)

Values are number of samples positive for EHEC O157/total samples taken (percent positive). *n*, Number of cattle in lot; NT, not tested.
*This isolate was negative for *stx* but was O157:H7 and had all other EHEC virulence markers.

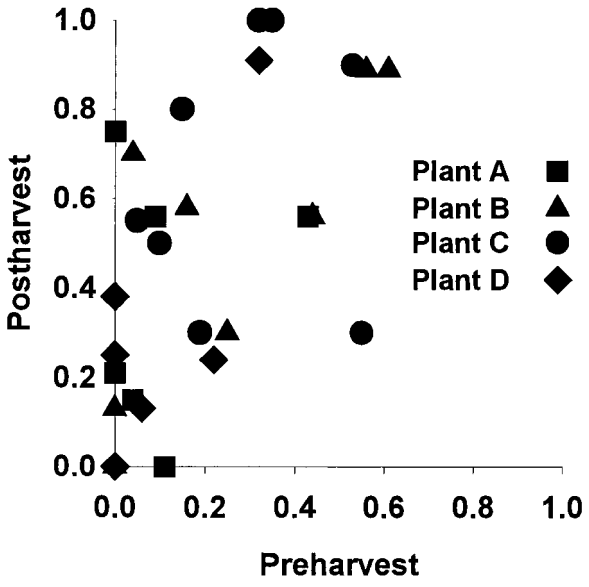

Fig. 1. Spearman rank correlation of EHEC O157 prevalence in all fecal and hide samples (preharvest) versus prevalence of carcasses positive on any sample (postharvest), by lot. Spearman rank correlation coefficient (r_s) = 0.58 (95% confidence interval 0.27–0.78), $P = 0.001$, $n = 29$.

preevisceration, postevisceration, and postprocessing, respectively (Table 2). Sixteen of the 17 lots that had positive postevisceration carcasses also had positive preevisceration carcasses (94%) and only one carcass of eight sampled was positive postevisceration in the remaining lot.

Ninety-five carcasses (27.8%) had only preevisceration positive samples, and 47 (13.8%) had both pre- and postevisceration positive samples. Ten carcasses (3.0%) had only postevisceration positive samples. Of the six carcasses that sampled positive postprocessing, all were positive preevisceration and three were positive postevisceration. Similarly, 79.6% of EHEC O157-positive postevisceration samples came from preevisceration positive carcasses. Significant reductions in the proportion of carcass samples positive were observed between pre- and postevisceration samples ($P < 0.0001$, $n = 333$) and between postevisceration and postprocessing samples ($P < 0.0001$, $n = 332$).

Relationship Between Fecal or Hide Prevalence and Carcass Contamination. Nineteen of 21 lots (90%) with positive fecal samples also had positive preevisceration samples (Table 2). One of the remaining two fecal-positive lots had a positive postevisceration sample, leaving only one lot that had a positive fecal sample without corresponding carcass contamination. All 11 lots that had positive hide samples had positive preevisceration samples, although this was only 42% of the lots with positive preevisceration samples. There was no significant difference between the proportion of lots positive on fecal and hide samples and those positive on carcass samples ($P = 0.2207$, $n = 29$).

A significant positive correlation was observed between the total fecal and hide prevalence within a lot (sum of all positive samples) and the lot prevalence of carcasses that were ever classified as positive for EHEC O157 (i.e., had a positive isolate on any sample pre- and postevisceration, or postprocessing; $P = 0.001$, Fig. 1). No clustering of fecal and hide prevalence and carcass contamination by processing plant was evident (Fig. 1).

Discussion

This study indicates the overall prevalence of EHEC O157 in cattle and on carcasses during processing is much higher than previous studies have suggested. Isolation methods and time of year are the likely reasons for the difference between the results of this study and those previously reported. In other EHEC O157 studies, immunomagnetic separation and enrichment broth selection played a key role in the isolation of the target organism (8, 12, 21). In the absence of a reliable "gold standard" for the presence of EHEC O157 in samples, it is difficult to directly compare the methods used in this study with other culture techniques (8, 12, 21, 22). In addition, because operator experience can have a significant effect on the outcome obtained by different culture techniques, comparisons should be made on split samples in laboratories experienced in performing a given culture method. Large-scale studies of this type have not been completed for EHEC O157 culture methods, although a small study on split fecal samples, comparing the fecal culture method used in this study to two other methods used by veterinary diagnostic laboratories, showed the method used in this study to have significantly higher sensitivity (J.E.K. and R.O.E., unpublished data).

Studies on North American cattle have demonstrated that peak prevalence occurs in the late summer and early fall, the same period in which this study was performed (9, 23). This is also the time frame in which most human outbreaks occur in North America, July through August (24). The dynamic nature of EHEC O157 prevalence in cattle relative to season should be taken into account in risk factor and intervention studies in processing plants to avoid misleading conclusions.

The hide is often implicated as a major source of microbiologic contamination of carcasses, although there are scant data in the literature to support this assertion. In fact, one study found no association between visible cleanliness of hides and carcass contamination at slaughter (25). The data from this study suggest a lack of association between hide prevalence and carcass contamination (Tables 1 and 2). However, a surprising result of this study was the relatively low hide prevalence for EHEC O157 compared with feces. Preliminary studies had indicated good concordance in EHEC O157 isolation rates between fecal and hide samples on individual cattle (data not shown). Furthermore, it was expected that with grouping of cattle in close quarters during transport and holding that significant cross-contamination of hides should occur, thus increasing the apparent prevalence on hides relative to feces. One explanation for this apparent discrepancy is choice of sampling site. Hide samples were taken from the ventrum of the animal over the sternum (brisket) on the assumption that as cattle rested in sternal recumbency this site would be in contact with fecal matter on the ground, in effect swabbing the pen floor and maximizing hide contamination. It is possible that other sites on the hide have higher levels of contamination and are, therefore, greater risks for generating direct or airborne carcass contamination. It is also possible that survival rates of EHEC O157 differ by site on the hide. It is clear that hides do contribute to the total bacterial load, which may contribute to carcass contamination. Further studies are required to address the relative importance of hides as a source of carcass contamination by EHEC O157.

EHEC O157 was recovered from almost half of the carcasses tested in this study (45.5%), particularly from the posterior region of the carcass (preevisceration sample, 43%). This is much higher than previous estimates of carcass contamination, presumably because of the culture methodology and the sampling sites used in this study (14). However, it is also important to remember that this study was conducted during peak cattle EHEC O157 shedding season. One would predict that the level of carcass contamination would drop dramatically at other times during the year based on the apparent correlation between EHEC O157-positive cattle feces and carcass contamination. Furthermore, the number of positive carcass samples was reduced by about 50% after the carcasses were eviscerated and

split (22%, $P < 0.001$), and dropped significantly (2%, $P < 0.001$) when carcass samples were taken after final intervention strategies were executed. This result suggests, but certainly does not prove, that current antimicrobial intervention strategies are effective in reducing EHEC O157 on carcasses. Other factors, such as drying or cooling of carcasses during processing, could be responsible for the observed effect. The present results indicate that preevisceration carcass contamination may be a critical control point where additional intervention strategies could be implemented.

A significant positive correlation was present between prevalence in feces and hides, and prevalence of carcass contamination within lots (Fig. 1). Given the variability of the data, studies incorporating a much greater number of lots will be required to estimate the function relating fecal prevalence and initial carcass contamination.

The overall prevalence of carcass contamination with EHEC O157 was significantly greater than that of fecal and hide prevalence (Tables 1 and 2). Furthermore, in some lots even when no animals were fecal or hide positive, carcass samples within the same lot were found to be positive (Table 2). This finding suggests that cross-contamination of carcasses may be occurring in processing plants. Finding EHEC O157 at these levels during processing is not completely unexpected, given the potential for bacterial contamination to occur when large numbers of animals infected with EHEC O157 are being processed. Several mechanisms may be responsible for dissemination of EHEC O157 during processing. Contamination through direct contact with personnel, knives, or other equipment may occur. Carcasses may be directly in contact with each other during processing with the potential to transfer microorganisms. In addition, air and/or water-borne contamination is possible. Careful microbiologic studies in processing plants are required to determine the actual sources of carcass contamination with EHEC O157.

Three major conclusions can be drawn from the data in this study. First, the prevalence of EHEC O157 in live animals presented for slaughter, as well as on carcasses, is considerably higher than previously estimated. The prevalence estimates from this study provide a sound microbiological basis for risk assessment modeling, which was previously lacking (26). Furthermore, these data may serve as a baseline for evaluation of possible intervention strategies both within processing plants and on the farm. Second, current in-plant processing practices appear to reduce the level of carcass contamination with EHEC O157. However, development of additional strategies aimed at preventing preevisceration carcass contamination is indicated. Third, there appears to be a correlation between fecal prevalence and initial carcass contamination. The association between fecal prevalence and carcass contamination indicates a role for control of EHEC O157 in cattle on the farm toward reducing the risk of human infection from ingestion of undercooked beef or cross-contamination of other foods. Obviously, such a control program would also reduce the risk of environmental contamination, another potential source of human infection. Unfortunately, no effective control methods are currently available for producers to use in reducing prevalence of EHEC O157 in cattle. Development of such control methods remains an area of active research in this and other laboratories.

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