APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 2002, p. 4847–4852 Vol. 68, No. 10
0099-2240/02/$04.00+0 DOI: 10.1128/AEM.68.10.4847–4852.2002

Prevalence and Characterization of Non-O157 Shiga Toxin-Producing *Escherichia coli* on Carcasses in Commercial Beef Cattle Processing Plants

Terrance M. Arthur,* Genevieve A. Barkocy-Gallagher, Mildred Rivera-Betancourt, and Mohammad Koohmaraie

Roman L. Hruska U.S. Meat Animal Research Center, Agricultural Research Service, U.S. Department of Agriculture, Clay Center, Nebraska 68933-0166

Received 6 March 2002/Accepted 16 July 2002

Beef carcass sponge samples collected from July to August 1999 at four large processing plants in the United States were surveyed for the presence of non-O157 Shiga toxin-producing *Escherichia coli* (STEC). Twenty-eight (93%) of 30 single-source lots surveyed included at least one sample containing non-O157 STEC. Of 334 carcasses sampled prior to evisceration, 180 (54%) were found to harbor non-O157 STEC. Non-O157 STEC isolates were also recovered from 27 (8%) of 326 carcasses sampled after the application of antimicrobial interventions. Altogether, 361 non-O157 STEC isolates, comprising 41 different O serogroups, were recovered. O serogroups that previously have been associated with human disease accounted for 178 (49%) of 361 isolates. Although 40 isolates (11%) carried a combination of virulence factor genes (enterohemorrhagic *E. coli hlyA*, *eae*, and at least one *stx* gene) frequently associated with STEC strains causing severe human disease, only 12 of these isolates also belonged to an O serogroup previously associated with human disease. Combining previously reported data on O157-positive samples (R. O. Elder, J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie, and W. W. Laegreid, Proc. Natl. Acad. Sci. USA 97:2999–3003, 2000) with these data regarding non-O157-positive samples indicated total STEC prevalences of 72 and 10% in preevisceration and postprocessing beef carcass samples, respectively, showing that the interventions used by the beef-processing industry effected a sevenfold reduction in carcass contamination by STEC.

Shiga toxin-producing *Escherichia coli* (STEC) has been implicated as the causative agent in several human diseases (22, 27). These diseases range from mild diarrhea to very severe and life-threatening conditions, such as hemolytic-uremic syndrome (HUS). Enterohemorrhagic *E. coli* (EHEC) refers to the subset of STEC strains found to cause human disease. The STEC strain most frequently associated with clinical disease in the United States is serotype O157:H7 (20, 22). However, several other serotypes (O26, O103, O111, O113, and O121) are commonly associated with severe disease outbreaks and, in some countries, are isolated from clinical cases more often than O157 (1, 22). It has been estimated that *E. coli* O157:H7 strains cause two-thirds of the human EHEC infections in the United States, with the remaining cases attributed to the non-O157 STEC population (20). This estimation may be biased toward O157 because several clinical laboratories only screen for *E. coli* O157:H7, leaving non-O157 EHEC-associated diseases underreported (20).

Cattle are considered the primary reservoir of both O157 and non-O157 STEC bacteria (4). Cattle frequently carry STEC without suffering from any pathological symptoms (6, 42). Several human infections have been linked to STEC present in undercooked ground beef (22, 36). During processing, fecal contamination of the carcass or transfer of bacteria found on the animal hide to the carcass can facilitate transmission of STEC into the food supply (2, 3, 13).

The complete list of bacterial virulence determinants necessary for STEC to cause EHEC-related disease is not known. However, the Shiga toxin is a key factor in pathogenesis (1, 27). Two classes of Shiga toxin have been identified. Shiga toxin 1 (Stx_1) is 98% homologous to the Stx produced by *Shigella dysenteriae* type 1 (22, 27). Stx_2 is approximately 60% homologous to Stx_1 and is antigenically distinct (22, 27). Recent studies have indicated that strains possessing stx_2 are potentially more virulent than strains carrying stx_1 or even strains carrying both stx_1 and stx_2 (11, 22, 23, 32, 39). Other virulence factors, such as intimin (*eae*) and EHEC hemolysin (*hlyA*), are thought to enhance pathogenicity but are not required for strains to cause severe disease, including HUS (1, 8, 22).

The objective of this study was to determine the prevalence and virulence profiles of non-O157 STEC on beef carcasses at commercial processing plants in the United States during the summer months. Such data will contribute to the establishment of baseline prevalence levels of non-O157 STEC. The samples used for this study were originally collected by Elder et al. for a survey of O157:H7 prevalence (13). Extrapolation of the data from both studies provides an indication of the total STEC prevalence in U.S. beef-processing plants.

MATERIALS AND METHODS

Carcass samples. Sponge samples were collected by Elder et al. from beef cattle carcasses during processing (13). Briefly, individual carcasses were followed through processing and sampled at various points. Speci-Sponges (Nasco, Fort Atkinson, Wis.) moistened with Butterfield's phosphate diluent and 0.1%

* Corresponding author. Mailing address: USDA, ARS, Roman L. Hruska U.S. Meat Animal Research Center, P. O. Box 166, Spur 18-D, Clay Center, NE 68933-0166. Phone: (402) 762-4227. Fax: (402) 762-4149. E-mail: arthur@email.marc.usda.gov.

TABLE 1. Summary of STEC prevalence[a]

Sample category	Total no. of samples	Total no. (%) non-O157 STEC positive	95% CI[b]	No. (%) of samples:		Total no. (%) STEC positive[d]
				O157:H7 positive,[c] non-O157 STEC positive	O157:H7 negative,[c] non-O157 STEC positive	
Preevisceration	334	180 (53.9)	48.5–59.2	144, 84 (58.3)	190, 96 (50.5)	240 (71.9)
Postprocessing	326	27 (8.3)	5.3–11.3	6, 0	320, 27 (8.4)	33 (10.1)

[a] Values represent numbers of carcass samples carrying at least one non-O157 STEC isolate.
[b] 95% CI, 95% confidence interval.
[c] See Elder et al. (13).
[d] STEC-positive samples (O157 and non-O157) from this work and that of Elder et al. (13).

Tween 20 were used to sample carcasses just prior to evisceration (preevisceration) and after all antimicrobial interventions when the carcasses were in the cooler (postprocessing). Lots of 35 to 85 animals were sampled. A lot is defined as cattle from a single source and maintained as a group throughout processing. Twenty percent of the carcasses in each lot were sampled. Antimicrobial interventions varied between plants and included steam vacuuming, hot water washing, organic acid washing, and steam pasteurization.

Bacterial culture. Beef carcass sponge samples were enriched by incubation with 90 ml of Brilliant Green Bile 2% (40 g/liter) for 10 h at 37°C (13). Following enrichment, 1 ml of culture was added to 250 μl of 50% glycerol and stored at −70°C. The enrichments were stored frozen for approximately 2 years prior to initiation of this study.

PCR screening of samples. Samples that were not previously determined to be positive for *E. coli* O157:H7 (13) were screened for the presence of stx_1 and stx_2 by PCR. The procedure used is a modification of that described by Paton and Paton (24). Briefly, a 50-μl sample was removed from the frozen enrichment and centrifuged for 1 min at 12,000 × *g* in a microcentrifuge and the supernatant was discarded. The pellet was resuspended in 200 μl of sterile water and centrifuged again, and the supernatant was discarded. The pellet was resuspended in 50 μl of sterile water and boiled for 15 min. The lysate was spun briefly, and 2 μl was used as template DNA in a 30-μl PCR.

Colony hybridization. All samples previously determined to be *E. coli* O157:H7 positive (13), as well as samples determined by PCR to contain either stx_1 or stx_2, were assayed by colony hybridization for STEC detection. Samples from the frozen enrichments were serially diluted in buffered peptone water and plated onto tryptic soy agar in 182-mm-diameter petri dishes to achieve a colony count of ~2,000 CFU per plate. The plates were incubated for 11 h at 37°C and then transferred to 4°C for 30 min. The colonies were blotted to Hybond N+ membranes (Amersham Pharmacia Biotech, Piscataway, N.J.), and hybridization (60°C) and detection were carried out by using the ECL Random Prime Labeling and Detection kit (Amersham) and following the manufacturer's instructions. The membranes were hybridized with 90 ng of both stx_1 and stx_2 probes in 50 ml of hybridization buffer. The DNA probes for random prime labeling were derived via PCR from *E. coli* O157 strain ATCC 43895 by using the following primers: stx_1-F (bp 77 to 102 of Sakai-VT1; 5′-CCTTAGACTTCTCGACTGC AAAGACG-3′), stx_1-R (bp 894 to 913 of Sakai-VT1; 5′-CCAGAGTGGATGA ATCCCAC-3′), stx_2-LP43 (bp 57 to 78 of EDL933; 5′-ATCCTATTCCCGGG AGTTTACG-3′), and stx_2-LP44 (bp 622 to 643 of EDL933; 5′-GCGTCATCG TATACACAGGAGC-3′) (9).

Isolation and characterization of STEC. The developed films from the colony blots were aligned with the original plates, and up to three suspect colonies per sample were patched in a grid on tryptic soy agar. The patch plates were again colony blotted to identify those colonies that were correctly picked. The positive patches were then streaked for isolation on sorbitol MacConkey agar (SMAC). From each SMAC streak plate, two colonies were picked and screened for the EHEC genes stx_1, stx_2, *eae*, and EHEC *hlyA* by multiplex PCR (24). Any colony possessing stx_1 or stx_2 was assayed for expression of the O157 antigen by latex agglutination with the Dryspot *E. coli* O157 Latex Test (Oxoid, Basingstoke, England). Colonies giving a positive agglutination reaction were discarded. All non-O157, *stx*-containing isolates were confirmed to be *E. coli* by biochemical identification with the Sensititre Gram-Negative Autoidentification (AP80) system (Accumed International, Westlake, Ohio) or API 20E strips (bioMérieux, Hazelwood, Mo.).

ETEC and EPEC PCR. Selected strains were screened by PCR for virulence attributes commonly associated with enterotoxigenic *E. coli* (ETEC; heat-stable and heat-labile toxins) and enteropathogenic *E. coli* (EPEC; bundle-forming pilus and EAF plasmid) by using methods described previously (15, 35, 43).

Serotyping. All STEC isolates were serotyped in accordance with their O and H antigens by the *E. coli* Reference Center (Pennsylvania State University, University Park).

Statistics. Confidence intervals were calculated by using the method presented by Wardrup (40).

RESULTS

Prevalence of non-O157 STEC. Any sample that produced at least one non-O157 *E. coli* isolate harboring a Shiga toxin gene was considered positive for non-O157 STEC. One hundred eighty (53.9%) of 334 preevisceration samples and 27 (8.3%) of 326 postprocessing samples were positive for non-O157 STEC (Table 1). Multiple isolates were recovered from 90 of the 180 positive preevisceration samples and 17 of the 27 positive postprocessing samples. A total of 361 non-O157 STEC isolates were recovered.

At preevisceration, 58.3% of samples that were previously determined to carry *E. coli* O157:H7 (13) also were positive for non-O157 STEC (Table 1). Of those samples that were negative for O157 (13), 50.5% produced a non-O157 STEC isolate. However, PCR results indicated that 76.8% of these samples contained either one or both of the Shiga toxin genes (data not shown). Twenty-seven (8.4%) of 320 O157:H7-negative postprocessing samples were positive for non-O157 STEC (Table 1). However, PCR results indicated that 43 (13.4%) of the samples contained either one or both of the Shiga toxin genes (data not shown). There were no *E. coli* O157:H7-positive postprocessing samples (13) that also carried a non-O157 STEC isolate (Table 1). Seven of the non-O157 STEC-positive postprocessing samples were from carcasses that were not found to be positive preevisceration.

Twenty-eight (93.3%) of 30 lots produced at least one preevisceration sample that was positive for non-O157 STEC (Table 2). The prevalence of positive preevisceration samples within a lot ranged from 0 to 100%. Eleven lots that had at least one positive preevisceration sample did not have a positive postprocessing sample. Seventeen (56.7%) of 30 lots produced at least one postprocessing sample that was positive for non-O157 STEC (Table 2). All 17 positive postprocessing lots were also positive preevisceration. The prevalence of positive samples within postprocessing lots ranged from 0 to 33.3%.

Total STEC prevalence. Elder et al. originally collected the carcass samples used in this study for a survey of *E. coli* O157:H7 prevalence (13). The *E. coli* O157:H7 prevalence in these samples was determined to be 43.4 and 1.9% for preevisceration and postprocessing, respectively (13). By merging the data from the two studies, the total STEC prevalence on

TABLE 2. Prevalence of non-O157 STEC by lot

Plant	Sampling	Lot	n^a	No. of samples positive/total[b] (%)	
				Preevisceration	Postprocessing
A	1	1	84	6/18 (33.3)	0/18 (0)
		2	35	5/8 (62.5)	1/7 (14.3)
		3	68	1/15 (6.7)	0/16 (0)
	2	1	80	14/18 (77.8)	2/18 (11.1)
		2	41	8/10 (80.0)	2/9 (22.2)
		3	62	11/14 (78.6)	2/13 (15.4)
		4	44	8/13 (61.5)	1/13 (7.6)
B	1	1	46	0/11 (0)	0/9 (0)
		2	37	0/9 (0)	0/9 (0)
		3	39	6/9 (66.7)	1/9 (11.1)
		4	48	3/12 (25.0)	2/12 (16.7)
	2	1	36	2/9 (22.2)	0/9 (0)
		2	36	6/8 (75.0)	0/9 (0)
		3	36	8/9 (88.9)	0/7 (0)
		4	40	10/10 (100)	1/10 (10.0)
C	1	1	41	6/10 (60.0)	0/10 (0)
		2	76	10/17 (58.8)	1/17 (5.9)
		3	42	7/10 (70.0)	1/10 (10.0)
		4	38	10/10 (100)	2/9 (22.2)
	2	1	38	10/10 (100)	0/10 (0)
		2	44	5/9 (55.6)	1/10 (10.0)
		3	40	9/11 (81.8)	2/11 (18.2)
		4	46	9/12 (75.0)	4/12 (33.3)
D	1	1	37	4/7 (57.1)	1/8 (12.5)
		2	39	4/7 (57.1)	1/5 (20.0)
		3	38	2/8 (25.0)	0/8 (0)
		4	42	1/8 (12.5)	2/8 (25.0)
	2	1	65	4/14 (28.6)	0/13 (0)
		2	42	2/11 (18.2)	0/11 (0)
		3	58	9/17 (52.9)	0/16 (0)
Cumulative totals				180/334 (53.9)	27/326 (8.3)

[a] Number of animals in lot.
[b] Values represent the number of positive samples over the total number of carcasses sampled per lot.

carcasses was determined to be 71.9% preevisceration and 10.1% postprocessing (Table 1).

Characterization of isolates. All non-O157 isolates were confirmed as *E. coli* by biochemical assay, and all isolates were confirmed by PCR to carry the stx_1 and/or stx_2 genes. Eight percent of the isolates (29 of 361) did not ferment sorbitol on the basis of growth on SMAC. The distribution of virulence factors among the non-O157 STEC isolates is summarized in Table 3. With regard to Shiga toxin genes, PCR results indicated that 170 (47.1%) of the isolates carried stx_1, 132 (36.6%) had stx_2, and 59 (16.3%) possessed both stx_1 and stx_2. The *eae* gene was present in 43 isolates (11.9%), and EHEC *hlyA* was found in 98 (27.1%) of the non-O157 STEC isolates. Strains carrying only stx_1, without any other virulence factors for which we tested, accounted for the dominant share of isolates, at 42.1% (152 of 361). Forty isolates (11.1%) possessed the combination of virulence factors (*eae,* EHEC *hlyA*, and at least one Shiga toxin gene) that has been associated with severe disease in humans (1, 12, 14). Only four of these isolates were recovered from postprocessing samples. Of the 90 preevisceration samples that yielded multiple isolates, 25 had isolates of different virulence genotypes. Only one postprocessing sample yielded isolates of different virulence genotypes.

Serotype distribution. Seventy-five percent of the isolates could be serogrouped in accordance with their O antigen, but only 21% could be typed by their H antigen (data not shown). Forty-one O serogroups were identified (Table 4). No isolates of serogroup O26 or O111 were recovered. Eight serogroups (O2, O8, O88, O113, O121, O132, O142, and O171) accounted for 49% of the STEC isolates. O142, a serogroup rarely associated with STEC (34), was the most prevalent serogroup among both preevisceration and postprocessing isolates. Two isolates were recovered that were of a serogroup (OX25) not previously associated with STEC. O serogroups that have been associated with human disease accounted for 178 (49%) of the 361 isolates recovered.

Sixty-two preevisceration samples yielded multiple non-O157 isolates with typeable O antigens. Within 16 of these samples, isolates of different serogroups were identified. In two such samples, all three isolates were of different serogroups.

TABLE 3. Virulence factors of non-O157 STEC isolates

STEC virulence factor(s)	No. of isolates[a]			O serogroups[b]
	Total	Preevis	Post	
stx_1	152	135	17	**2, 3, 8, 45, 68, 74, 81, 119, 121, 128, 132,** 142, **172, NT, rough**
stx_2	93	78	15	**2, 6, 8,** 10, **15, 20, 55,** 88, **113, 132, 168, 171, x3,** x6, **NT, rough**
stx_1, stx_2	15	15	0	**8, 15, 113,** 117, **171, NT**
stx_1, *eae*	2	2	0	59, **119**
stx_1, *hlyA*	8	3	5	**104, NT**
stx_2, *hlyA*	19	17	2	**2, 8,** 88, **113,** 139, x25, **NT**
stx_1, stx_2, *hlyA*	31	23	8	**2, 55,** 87, 88, 106, **113,** 139, **153, NT, rough**
stx_1, stx_2, *eae*	1	1	0	**NT**
stx_1, *eae*, *hlyA*	8	6	2	**103,** 109, 136, **145, NT**
stx_2, *eae*, *hlyA*	20	20	0	**6,** 10, 117, **165, NT**
stx_1, stx_2, *eae, hlyA*	12	10	2	21, 88, **121,** 131, **153,** 162, **165, NT**
Total	361	310	51	

[a] Preevis, preevisceration; Post, postprocessing.
[b] O serogroups of isolates from each virulence genotype are listed. NT, O antigen nontypeable; rough, autoagglutinable strains. Bold indicates a serogroup previously associated with human disease.

TABLE 4. Serogroup distribution of non-O157 STEC isolates[a]

Serogroup	No. of isolates[b]		
	Total	Preevis	Post
O142	54	46	8
O121	31	31	0
O2	22	19	3
O171	18	18	0
O113	15	12	3
O132	14	13	1
O8	13	11	2
O88	10	10	0
O6	9	8	1
O139	9	5	4
O172	9	7	2
OX3	9	3	6
O104	5	1	4
O117	5	5	0
O15	4	4	0
O165	4	4	0
O3	3	3	0
O55	3	3	0
O153	3	3	0
O168	3	0	3
O10	2	2	0
O45	2	2	0
O103	2	2	0
O109	2	0	2
O119	2	2	0
O145	2	2	0
OX25	2	2	0

[a] Bold indicates a serogroup previously associated with human disease. Serogroups with only one isolate: **O20,** O21, O59, **O68, O74, O81,** O87, O106, **O128,** O131, O136, O162, and OX6. Not listed are 87 **Ont** and 4 **Orough** isolates.
[b] Preevis, preevisceration; Post, postprocessing.

Fifteen postprocessing samples produced multiple isolates of detectable O serogroups. Within each sample, all of the isolates belonged to a single serogroup.

One isolate was of a serotype (O6:H6) that has been previously associated with ETEC. PCR testing indicated that this isolate did not harbor genes for either the heat-labile or the heat-stable toxin that is indicative of ETEC (data not shown). Several isolates of serogroups commonly associated with EPEC were screened by PCR for the presence of the EAF plasmid or the gene coding for bundle-forming pili (*bfp*), both of which are usually found in EPEC strains. None of the STEC strains tested were positive for either marker (data not shown).

DISCUSSION

The data presented here show that 58.3% of the beef carcasses tested in large processing plants in the United States carried at least one type of non-O157 STEC prior to evisceration, but the prevalence was reduced, by using various antimicrobial intervention strategies, to 8.3% of the carcasses carrying non-O157 STEC at postprocessing. The samples surveyed were collected during the summer months, which have been associated with a peak in the prevalence of bovine STEC carriage (37). Therefore, STEC carcass contamination may be much less frequent at other times of the year.

Rogerie et al. reported a lower postprocessing non-O157 STEC prevalence (1.9%) on carcasses sampled during the summer at processing plants in France (29). Similarly, the non-O157 STEC prevalence on carcasses processed in Hong Kong was reported to be 1.7% (18). These apparently lower prevalence levels could come from a variety of factors but are most likely due to the use of dissimilar methods of STEC isolation. The methods used in both of these reports screened 100 or fewer colonies per sample, whereas we screened approximately 2,000 colonies per sample. In the previous studies, 10.7 and 11.4% of the postprocessing carcass samples were identified by PCR as carrying Shiga toxin genes, indicating a prevalence level similar to that described here (8.3% by colony hybridization, 13.4% by PCR) (18, 29). Neither of the previous studies reported STEC prevalence on carcasses prior to the application of antimicrobial interventions.

The hides and feces of animals presented for slaughter have been shown to be major sources of pathogens in processing plants (2, 13). To our knowledge, there have been no previous studies reporting non-O157 STEC prevalence on cattle hides to date. The prevalence of non-O157 STEC in the feces of beef cattle in the United States has scarcely been studied. One report using a small sample of heifers ($n = 23$) found fecal prevalence to range from 0 to 10% (33). Reported levels of non-O157 STEC in the feces of dairy cattle in the United States have ranged from 5.8 to 19.0% (10, 38, 41). There have been several studies of fecal prevalence of non-O157 STEC in beef cattle in other countries. Two studies testing healthy cattle in France recovered non-O157 STEC from 7.9 and 34% of the samples tested (28, 29). PCR results reported by these groups indicate that even higher levels of STEC were actually present, as *stx* genes were identified in 18 and 70% of the samples tested (28, 29). Kobayashi et al. detected *stx* genes, by using a nested PCR approach, in 100% of the cattle fecal samples obtained at an abattoir in Japan (17).

It is not clear what proportion of non-O157 STEC bacteria detected in cattle feces or on beef carcasses is able to cause disease in humans. Gyles et al. put forward the idea that STEC virulence is likely to be on a continuum, implying that virtually all STEC bacteria could be pathogenic under the proper circumstances (16). This would mean that less virulent strains could cause the same types of disease as highly virulent strains, given a sufficiently large dose and a sufficiently low immune status of the infected individual. Although there are most certainly nonpathogenic STEC bacteria in the continuum, there are not enough data to allow a determination of which STEC bacteria in the food supply represent a potential threat to human health.

Even though the set of virulence factors necessary to cause EHEC-related disease has not been defined strictly, associations between the carriage of certain genes and the ability to cause severe disease in humans have been made. Toxin-profiling studies of O157:H7 clinical isolates by Ostroff et al. showed that patients infected with isolates carrying only stx_2 were 6.8 times more likely to develop severe disease than those infected with strains carrying stx_1 or both stx_1 and stx_2 (23). Another study determined that, within a particular serotype, the carriage of stx_2 increases an isolate's likelihood of association with severe disease fivefold (7). It has also been demonstrated that Stx_2 has a lower 50% lethal dose than does Stx_1 when administered to mice (32). Therefore, isolates carrying stx_2 potentially represent an increased threat to human health. Of the non-O157 STEC isolates recovered in this study, 36.6% carried

stx$_2$ either alone or in combination with the accessory virulence factors intimin and/or EHEC hemolysin.

Several epidemiological studies have associated the possession of *eae* by STEC with severe disease (1, 5, 8). In addition, EHEC *hlyA* has been found in a high proportion of non-O157 STEC strains causing human disease (12, 16, 30). Therefore, irrespective of serotype, carriage of the combination of *stx, eae*, and EHEC *hlyA* may be a good indicator of the pathogenic potential of STEC strains (21). We identified 36 isolates (11.6%) from 31 preevisceration samples and 4 isolates (7.8%) from 2 postprocessing samples that carried *stx, eae*, and EHEC *hlyA*.

HUS cases have been reported in which the STEC isolates did not contain *eae* (8, 26, 44). It has been assumed that these isolates used other means of attachment to the intestinal epithelium (25). Without knowing the ingested dose in these cases and the immunological status of the persons preinfection, the virulence of such isolates cannot be estimated. We isolated 58 strains, 12 from postprocessing samples, that possessed *stx*$_2$ and matched the serogroups (O2, O6, O55, O113, O168, and OX3) of *eae*-negative strains isolated from HUS patients (8, 26, 44).

Serotype has been reported to be the most consistent factor for predicting the disease-causing potential of an STEC strain (16). More than 150 serotypes have been associated with human disease (www.sciencenet.com.au/vtectable.htm), while more than 200 serotypes of STEC have been isolated from cattle (16, 22). Non-O157 serogroups commonly identified in clinical cases in the United States include O111, O26, O121, and O103 (1). Surprisingly, no isolates of serogroup O111 or O26 were recovered from beef carcasses in this study. The serogroup (O142) that was most common among the isolates we identified has rarely been associated with STEC (34).

O121 was the most common serogroup we recovered that has been associated with human disease previously (19, 31). Only 1 of the 31 O121 isolates carried *stx*$_2$, *eae*, and EHEC *hlyA* in addition to *stx*$_1$. Overall, of the 361 isolates described here, 178 (49%) belonged to an O serogroup previously associated with human disease. Twelve isolates belonged to serogroups previously associated with human disease (O6, O103, O121, O145, O153, and O165) and carried *eae* and EHEC *hlyA*. However, none of these 12 isolates were recovered from postprocessing samples.

A comparison of the number of samples that were positive for *stx* genes by PCR and the number of samples from which STEC were isolated indicates that the STEC prevalence levels presented here underestimate the actual STEC prevalence. PCR screening showed that 146 (76.8%) of 190 O157-negative samples possessed Shiga toxin genes; however, isolates were only recovered from 96 (50.5%) of these samples. Secondly, PCR results for several samples indicated the presence of both *stx*$_1$ and *stx*$_2$ while the isolates recovered from those samples contained *stx*$_1$ or *stx*$_2$ but not both. These differences are not surprising, since the sensitivity of PCR is greater than that of colony hybridization. The PCR method used here has been shown to detect one STEC cell in a background of 10^4 cells, which is at least 10-fold more sensitive than the colony hybridization method used herein to isolate STEC (24). In addition, the samples used in this study were from enrichments in selective media that had been frozen for 2 years. Under such conditions, it is likely that some STEC bacteria would no longer be viable. The nonviable organisms would contribute to the number of PCR-positive samples but would not be isolated by colony hybridization. Therefore, a possibility exists that the prevalence of non-O157 STEC has been underestimated.

In addition, the variation of serotypes and virulence genotypes detected would likely have been greater if more isolates had been characterized. As was seen with some carcasses in this study, several different serogroups can be present on a single carcass. Since we only selected, at most, three presumptive STEC isolates from each sample, the serogroup and virulence genotype populations were certainly not exhausted for some carcasses.

In summary, we report here that a non-O157 STEC prevalence of greater than 50% exists on beef carcasses prior to evisceration during processing in U.S. plants. The non-O157 STEC load on carcasses was reduced to 8.3% by in-plant antimicrobial interventions. Subsequently, non-O157 STEC bacteria possessing accessory virulence factors indicative of pathogenic strains were identified on only two carcasses (0.6%) postprocessing.

ACKNOWLEDGMENTS

We thank Dee Kucera and Frank Reno for technical assistance and Carol Grummert for secretarial assistance.

Names are necessary to report factually on available data; however, the U.S. Department of Agriculture neither guarantees nor warrants the standard of the product and the use of the name by the U.S. Department of Agriculture implies no approval of the product to the exclusion of others that may also be suitable.

REFERENCES

1. **Acheson, D. W.** 2000. How does *Escherichia coli* O157:H7 testing in meat compare with what we are seeing clinically? J. Food Prot. **63:**819–821.
2. **Barkocy-Gallagher, G. A., T. M. Arthur, G. R. Siragusa, J. E. Keen, R. O. Elder, W. W. Laegreid, and M. Koohmaraie.** 2001. Genotypic analyses of *Escherichia coli* O157:H7 and O157 nonmotile isolates recovered from beef cattle and carcasses at processing plants in the midwestern states of the United States. Appl. Environ. Microbiol. **67:**3810–3818.
3. **Bell, R. G.** 1997. Distribution and sources of microbial contamination of beef carcasses. J. Appl. Microbiol. **82:**292–300.
4. **Bettelheim, K. A.** 2000. Role of non-O157 VTEC. J. Appl. Microbiol. **88:** 38S–50S.
5. **Beutin, L., S. Zimmermann, and K. Gleier.** 1998. Human infections with Shiga toxin-producing *Escherichia coli* other than serogroup O157 in Germany. Emerg. Infect. Dis. **4:**635–639.
6. **Blanco, M., J. E. Blanco, J. Blanco, A. Mora, C. Prado, M. P. Alonso, M. Mourino, C. Madrid, C. Balsalobre, and A. Juarez.** 1997. Distribution and characterization of faecal verotoxin-producing *Escherichia coli* (VTEC) isolated from healthy cattle. Vet. Microbiol. **54:**309–319.
7. **Boerlin, P., S. A. McEwen, F. Boerlin-Petzold, J. B. Wilson, R. P. Johnson, and C. L. Gyles.** 1999. Associations between virulence factors of Shiga toxin-producing *Escherichia coli* and disease in humans. J. Clin. Microbiol. **37:**497–503.
8. **Bonnet, R., B. Souweine, G. Gauthier, C. Rich, V. Livrelli, J. Sirot, B. Joly, and C. Forestier.** 1998. Non-O157:H7 Stx2-producing *Escherichia coli* strains associated with sporadic cases of hemolytic-uremic syndrome in adults. J. Clin. Microbiol. **36:**1777–1780.
9. **Cebula, T. A., W. L. Payne, and P. Feng.** 1995. Simultaneous identification of strains of *Escherichia coli* serotype O157:H7 and their Shiga-like toxin type by mismatch amplification mutation assay-multiplex PCR. J. Clin. Microbiol. **33:**248–250.
10. **Cray, W. C., Jr., L. A. Thomas, R. A. Schneider, and H. W. Moon.** 1996. Virulence attributes of *Escherichia coli* isolated from dairy heifer feces. Vet. Microbiol. **53:**369–374.
11. **Dorn, C. R., S. M. Scotland, H. R. Smith, G. A. Willshaw, and B. Rowe.** 1989. Properties of Vero cytotoxin-producing *Escherichia coli* of human and animal origin belonging to serotypes other than O157:H7. Epidemiol. Infect. **103:**83–95.
12. **Eklund, M., F. Scheutz, and A. Siitonen.** 2001. Clinical isolates of non-O157 Shiga toxin-producing *Escherichia coli*: serotypes, virulence characteristics,

and molecular profiles of strains of the same serotype. J. Clin. Microbiol. **39:**2829–2834.

13. **Elder, R. O., J. E. Keen, G. R. Siragusa, G. A. Barkocy-Gallagher, M. Koohmaraie, and W. W. Laegreid.** 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. Proc. Natl. Acad. Sci. USA **97:**2999–3003.
14. **Fey, P. D., R. S. Wickert, M. E. Rupp, T. J. Safranek, and S. H. Hinrichs.** 2000. Prevalence of non-O157:H7 Shiga toxin-producing *Escherichia coli* in diarrheal stool samples from Nebraska. Emerg. Infect. Dis. **6:**530–533.
15. **Franke, J., S. Franke, H. Schmidt, A. Schwarzkopf, L. H. Wieler, G. Baljer, L. Beutin, and H. Karch.** 1994. Nucleotide sequence analysis of enteropathogenic *Escherichia coli* (EPEC) adherence factor probe and development of PCR for rapid detection of EPEC harboring virulence plasmids. J. Clin. Microbiol. **32:**2460–2463.
16. **Gyles, C., R. Johnson, A. Gao, K. Ziebell, D. Pierard, S. Aleksic, and P. Boerlin.** 1998. Association of enterohemorrhagic *Escherichia coli* hemolysin with serotypes of Shiga-like-toxin-producing *Escherichia coli* of human and bovine origins. Appl. Environ. Microbiol. **64:**4134–4141.
17. **Kobayashi, H., J. Shimada, M. Nakazawa, T. Morozumi, T. Pohjanvirta, S. Pelkonen, and K. Yamamoto.** 2001. Prevalence and characteristics of Shiga toxin-producing *Escherichia coli* from healthy cattle in Japan. Appl. Environ. Microbiol. **67:**484–489.
18. **Leung, P. H., W. C. Yam, W. W. Ng, and J. S. Peiris.** 2001. The prevalence and characterization of verotoxin-producing *Escherichia coli* isolated from cattle and pigs in an abattoir in Hong Kong. Epidemiol. Infect. **126:**173–179.
19. **McCarthy, T. A., N. L. Barrett, J. L. Hadler, B. Salsbury, R. T. Howard, D. W. Dingman, C. D. Brinkman, W. F. Bibb, and M. L. Cartter.** 2001. Hemolytic-uremic syndrome and *Escherichia coli* O121 at a lake in Connecticut, 1999. Pediatrics **108:**E59.
20. **Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe.** 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. **5:**607–625.
21. **Meng, J., S. Zhao, and M. P. Doyle.** 1998. Virulence genes of Shiga toxin-producing *Escherichia coli* isolated from food, animals and humans. Int. J. Food Microbiol. **45:**229–235.
22. **Nataro, J. P., and J. B. Kaper.** 1998. Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. **11:**142–201.
23. **Ostroff, S. M., P. L. Tarr, M. A. Neill, J. H. Lewis, N. Hargrett-Bean, and J. M. Kobayashi.** 1989. Toxin genotypes and plasmid profiles as determinants of systemic sequelae in *Escherichia coli* O157:H7 infections. J. Infect. Dis. **160:**994–998.
24. **Paton, A. W., and J. C. Paton.** 1998. Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx_1, stx_2, *eaeA*, enterohemorrhagic *E. coli hlyA*, rfb_{O111}, and rfb_{O157}. J. Clin. Microbiol. **36:**598–602.
25. **Paton, A. W., P. Srimanote, M. C. Woodrow, and J. C. Paton.** 2001. Characterization of Saa, a novel autoagglutinating adhesin produced by locus of enterocyte effacement-negative Shiga-toxigenic *Escherichia coli* strains that are virulent for humans. Infect. Immun. **69:**6999–7009.
26. **Paton, A. W., M. C. Woodrow, R. M. Doyle, J. A. Lanser, and J. C. Paton.** 1999. Molecular characterization of a Shiga toxigenic *Escherichia coli* O113:H21 strain lacking *eae* responsible for a cluster of cases of hemolytic-uremic syndrome. J. Clin. Microbiol. **37:**3357–3361.
27. **Paton, J. C., and A. W. Paton.** 1998. Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections. Clin. Microbiol. Rev. **11:**450–479.
28. **Pradel, N., V. Livrelli, C. De Champs, J. B. Palcoux, A. Reynaud, F. Scheutz, J. Sirot, B. Joly, and C. Forestier.** 2000. Prevalence and characterization of Shiga toxin-producing *Escherichia coli* isolated from cattle, food, and children during a one-year prospective study in France. J. Clin. Microbiol. **38:**1023–1031.
29. **Rogerie, F., A. Marecat, S. Gambade, F. Dupond, P. Beaubois, and M. Lange.** 2001. Characterization of Shiga toxin producing *E. coli* and O157 serotype *E. coli* isolated in France from healthy domestic cattle. Int. J. Food Microbiol. **63:**217–223.
30. **Schmidt, H., L. Beutin, and H. Karch.** 1995. Molecular analysis of the plasmid-encoded hemolysin of *Escherichia coli* O157:H7 strain EDL 933. Infect. Immun. **63:**1055–1061.
31. **Stock, K. J., M. A. Scott, S. F. Davis, R. N. Pierson III, and J. S. Dummer.** 2001. Hemorrhagic colitis due to a novel *Escherichia coli* serotype (O121:H19) in a transplant patient. Transpl. Int. **14:**44–47.
32. **Tesh, V. L., J. A. Burris, J. W. Owens, V. M. Gordon, E. A. Wadolkowski, A. D. O'Brien, and J. E. Samuel.** 1993. Comparison of the relative toxicities of Shiga-like toxins type I and type II for mice. Infect. Immun. **61:**3392–3402.
33. **Thran, B. H., H. S. Hussein, M. R. Hall, and S. F. Khaiboullina.** 2001. Shiga toxin-producing *Escherichia coli* in beef heifers grazing an irrigated pasture. J. Food Prot. **64:**1613–1616.
34. **Todd, E. C., R. A. Szabo, J. M. MacKenzie, A. Martin, K. Rahn, C. Gyles, A. Gao, D. Alves, and A. J. Yee.** 1999. Application of a DNA hybridization-hydrophobic-grid membrane filter method for detection and isolation of verotoxigenic *Escherichia coli*. Appl. Environ. Microbiol. **65:**4775–4780.
35. **Tsen, H. Y., and L. Z. Jian.** 1998. Development and use of a multiplex PCR system for the rapid screening of heat labile toxin I, heat stable toxin II and Shiga-like toxin I and II genes of *Escherichia coli* in water. J. Appl. Microbiol. **84:**585–592.
36. **Tuttle, J., T. Gomez, M. P. Doyle, J. G. Wells, T. Zhao, R. V. Tauxe, and P. M. Griffin.** 1999. Lessons from a large outbreak of *Escherichia coli* O157:H7 infections: insights into the infectious dose and method of widespread contamination of hamburger patties. Epidemiol. Infect. **122:**185–192.
37. **Van Donkersgoed, J., T. Graham, and V. Gannon.** 1999. The prevalence of verotoxins, *Escherichia coli* O157:H7, and *Salmonella* in the feces and rumen of cattle at processing. Can. Vet. J. **40:**332–338.
38. **Wachsmuth, I. K., J. A. Kiehlbauch, C. A. Bopp, D. N. Cameron, N. A. Strockbine, J. G. Wells, and P. A. Blake.** 1991. The use of plasmid profiles and nucleic acid probes in epidemiologic investigations of foodborne, diarrheal diseases. Int. J. Food Microbiol. **12:**77–89.
39. **Wadolkowski, E. A., L. M. Sung, J. A. Burris, J. E. Samuel, and A. D. O'Brien.** 1990. Acute renal tubular necrosis and death of mice orally infected with *Escherichia coli* strains that produce Shiga-like toxin type II. Infect. Immun. **58:**3959–3966.
40. **Wardrup, R. L.** 1995. Inference for a population, p. 185–227. *In* P. Heighton and D. Bruflodt (ed.), Statistics: learning in the presence of variation. Wm. C. Brown Publishers, Dubuque, Iowa.
41. **Wells, J. G., L. D. Shipman, K. D. Greene, E. G. Sowers, J. H. Green, D. N. Cameron, F. P. Downes, M. L. Martin, P. M. Griffin, S. M. Ostroff, M. E. Potter, R. V. Tauxe, and I. K. Wachsmuth.** 1991. Isolation of *Escherichia coli* serotype O157:H7 and other Shiga-like-toxin-producing *E. coli* from dairy cattle. J. Clin. Microbiol. **29:**985–989.
42. **Wieler, L. H., R. Bauerfeind, and G. Baljer.** 1992. Characterization of Shiga-like toxin producing *Escherichia coli* (SLTEC) isolated from calves with and without diarrhoea. Zentbl. Bakteriol. **276:**243–253.
43. **Wieler, L. H., E. Vieler, C. Erpenstein, T. Schlapp, H. Steinrück, R. Bauerfeind, A. Byomi, and G. Baljer.** 1996. Shiga toxin-producing *Escherichia coli* strains from bovines: association of adhesion with carriage of *eae* and other genes. J. Clin. Microbiol. **34:**2980–2984.
44. **Willshaw, G. A., S. M. Scotland, H. R. Smith, and B. Rowe.** 1992. Properties of Vero cytotoxin-producing *Escherichia coli* of human origin of O serogroups other than O157. J. Infect. Dis. **166:**797–802.