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Microbiological Characterization of Lamb Carcasses at Commercial Processing Plants in the United States†

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ABSTRACT

Although the United States produces 203 million lb (ca. 92.1 kg) of domestic lamb and mutton each year, thorough studies of the microbiological safety during lamb processing are lacking. To address this missing information, a total of 2,548 sponge samples from pelts, preevisceration carcasses, and postintervention carcasses were collected from multiple large commercial lamb processing plants to determine aerobic plate counts, the prevalences of *Escherichia coli* O157:H7, non-O157 Shiga toxin–producing *E. coli* (STEC), and *Salmonella.* The averages of the aerobic plate counts from pelts, the preevisceration carcasses, and the postintervention carcasses were 6.3, 4.4, and 2.4 log CFU/100 cm^2, respectively. The prevalences of *E. coli* O157:H7 from the pelts, the preevisceration carcasses, and the postintervention carcasses were 12.8, 1.6, and 2.9%, respectively. The average *Salmonella* prevalences were 14.4, 4.3, and 1.8% for pelts, preevisceration carcasses, and postintervention carcasses, respectively. The most frequently identified *Salmonella* serotype was Heidelberg. The prevalences of non-O157 STEC from pelts, preevisceration carcasses, and postintervention carcasses averaged 86.2, 78.6, and 81.6%, respectively. A total of 488 non-O157 STEC strains were isolated from postintervention carcasses. Sixty-nine different serotypes of non-O157 STEC were identified. The most frequently detected serotypes were O91:H14 (40.8%), followed by O5:H19 (18.4%). A small number of STEC serotypes associated with severe human illness were isolated from postintervention carcasses. These were serotypes O76:H19, O128:H2 (0.8%), O146:H8 (2.1%), O146:H21, O163:H19, and O174:H8 (1.3%). The results of this study establish a baseline for microbiological quality and prevalences of *Salmonella, E. coli* O157:H7, and STEC in U.S. lamb processing plants.

Foodborne diseases caused by microorganisms are the number one food safety concern among consumers and regulatory agencies *(21).* Illnesses attributed to foodborne microorganisms can cause severe debilitating symptoms, and in some cases, these illnesses may result in death. *Escherichia coli* O157:H7 and *Salmonella* are common human infectious agents throughout the world, and an estimated 1,400,000 and 73,000 cases of *Salmonella* and *E. coli* O157:H7 infection, respectively, occur in the United States annually *(32).* Non-O157 Shiga toxin–producing *E. coli* (non-O157 STEC) can cause diseases similar to those produced by *E. coli* O157:H7 and cause approximately 31,000 cases of foodborne illnesses annually *(2).* In 2000, the U.S. economic costs incurred by *Salmonella,* enterohemorrhagic *E. coli* (EHEC) serotype O157:H7, and non-O157 STEC were estimated to be as much as $3.4 billion dollars *(1).*

Several studies have reported the levels of these pathogens on beef carcasses. It has also been reported that sheep harbor these pathogens in their intestinal tracts at levels similar to those of beef cattle *(7, 9, 40).* However, data on the pathogen contamination of lamb carcasses are lacking.

The prevalence of STEC in feces was reported to be higher in sheep than in cattle *(8).* Several non-O157 STEC serotypes associated with human illnesses, including O91:H^-, O128:H2, O128:H^-, O146:H8, and O146:H21, were isolated from sheep *(9, 14, 30, 40).* Non-O157 STEC strains from sheep feces were found at a higher prevalence than *E. coli* O157:H7 strains *(14).* Approximately 31% of sheep feces in the United States were positive for the presence of *E. coli* O157:H7 *(29),* whereas less than 10% of the sheep feces in Spain, Great Britain, The Netherlands, Scotland, and Italy were positive *(6, 9, 26, 34, 35).*

There have been at least two *Salmonella* outbreaks linked to lamb consumption *(19, 42),* and although lamb products have not been linked to any STEC outbreak, *E. coli* O157:H7 has been isolated from retail lamb products in the United States and Australia *(16, 37).* The objective of this study was to determine the microbiological safety status of lamb processed in the United States and to characterize the virulence factor profiles of *E. coli* O157:H7 and non-O157 STEC, if present. The results from this study establish a baseline for microbiological quality and prevalence levels of *E. coli* O157:H7, *Salmonella,* and non-O157 STEC.

MATERIALS AND METHODS

Samples were collected at three different lamb processing plants (A, B, and C) throughout the United States during the spring and summer of 2005. Samples were collected for 2 or 3

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† Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

consecutive days at each plant visited. Three different samples (pelts, preevisceration carcasses, and postintervention carcasses) were collected on the process line each day with prewetted sponges as follows: (i) pelt, after pelt opening but before pelt removal; (ii) preevisceration carcass, immediately following pelt removal but before any antimicrobial applications; and (iii) postintervention carcass, after antimicrobial interventions were applied. Plant A used acidified sodium chlorite as an antimicrobial rinse, whereas plants B and C used diluted lactic and acetic acid, respectively. Some processing plants used inverted dressing systems to remove pelts. Individual carcasses were tagged and tracked through the entire process. The same carcass was sampled for pelt and preevisceration. To avoid bias caused by repeated sampling of the same area, an immediately adjacent carcass was sampled for the postintervention carcass sample. Ninety-six sponge samples were collected from each of the three sample sites each day at each plant, for a total of 2,592 samples, of which 2,548 were used in analysis.

Sample collection. Pelt and carcass samples were obtained with sterile Speci-Sponges (Nasco, Fort Atkinson, Wis.) moistened with 20 ml of sterile Difco buffered peptone water (Difco, Becton Dickinson, Sparks, Md.). All samples were collected by an aseptic technique. The pelt sample was taken from a 1,000-cm^2 area over the fourth rib area by swabbing five times in a vertical direction on one side of the sponge and five times in a horizontal direction on the other side of the sponge *(4).* A large carcass area (approximately 4,000 cm^2) consisting of the inside and outside leg (~1,000 cm^2) and the breast-foreshank (~3,000 cm^2) was sampled for preevisceration and postintervention carcasses. All sample bags were transported overnight on ice to the laboratory and processed immediately the next morning.

Sample processing and analysis. Bags containing sponge samples were massaged thoroughly by hand a minimum of five times, and then a 1.5-ml aliquot was removed for enumeration studies prior to the addition of enrichment media. For both pelt and preevisceration samples, total aerobic plate counts (APCs) were determined with a Bactometer (bioMérieux, Hazelwood, Mo.). The pelt and preevisceration sample aliquots were 10- and 100-fold diluted with buffered peptone water, respectively, before analyzing with the Bactometer. Each Bactometer sample consisted of 0.1 ml of diluted sample and 0.9 ml of General Purpose Medium-Plus (bioMérieux) supplemented with 18 g of dextrose per liter. The Bactometer samples were incubated at 37°C for 16 h to determine the initial detection time for each sample. Initial detection times were converted to log CFU per milliliter as previously described *(11).* The postintervention samples were expected to have APCs too low for the Bactometer to detect accurately; thus, those samples were directly plated (at undiluted and 100-fold diluted) on Petrifilm Aerobic Count Plates (3M Health Care, St. Paul, Minn.). Petrifilm aerobic count plates were incubated and counted as previously described *(11).*

***E. coli* O157:H7 isolation and characterization.** An 80-ml aliquot of Difco tryptic soy broth (Difco, Becton Dickinson) was added to each of pelt, preevisceration, and postintervention sample bag. All sample bags were incubated at 25°C for 2 h and at 42°C for 6 h and stored at 4°C overnight *(4).* A total of 1 ml of each enrichment were added to 20 μl of anti-O157 immunomagnetic beads (Dynal, Lake Success, N.Y.) and subjected to immunomagnetic separation as previously described *(33).* The bead-bacteria complexes were spread plated on Difco sorbitol MacConkey agar (Difco, Becton Dickinson) supplemented with 0.05 mg of cefixime per liter and 2.5 mg of potassium tellurite per liter (ctSMAC; Dynal) and CHROMagar O157 (DRG International, Mountainside, N.J.) supplemented with 5 mg of novobiocin per liter and 1.0 mg of potassium tellurite per liter (ntCHROMAgar). After the plates were incubated for 16 to 18 h at 37°C, up to three presumptive colonies were tested by latex agglutination (DrySpot *E. coli* O157, Oxoid, Basingstoke, UK). The presumptive colonies were confirmed by PCR *(28, 36),* and one colony was used for characterization. Atypical *E. coli* O157:H7 strains were noted and further tested with the ProSpect Shiga Toxin *E. coli* (STEC) Microplate Assay (Remel, Lenexa, Kans.), according to manufacturer's recommendations, to determine if variable Shiga toxin (*stx*) gene possession was expressed.

***Salmonella* isolation and characterization.** *Salmonella* serotypes were concentrated by immunomagnetic separation from 1 ml of enriched culture with 20 μl of anti-*Salmonella* immunomagnetic beads (Dynal) as described previously *(33).* The bead-bacteria complexes were resuspended in 0.1 ml of phosphate-buffered saline–Tween 20 wash buffer, transferred into 10 ml of Rappaport-Vassiliadis soya broth (Oxoid), and incubated at 42°C for 18 to 24 h. Each Rappaport-Vassiliadis soya broth culture was streaked with a sterile cotton swab over half of each plate containing brilliant green agar with sulfadiazine (Difco, Becton Dickinson) and Hektoen Enteric agar (Difco, Becton Dickinson) supplemented with 15 mg of novobiocin per liter. The other half of each plate was streaked with sterile loops for the isolation of individual colonies. All plates were incubated at 37°C for 16 to 18 h. Two colonies from the Hektoen Enteric agar supplemented with 15 mg of novobiocin per liter plate and one colony from the brilliant green agar with sulfadiazine plate were streaked onto Hektoen Enteric agar supplemented with 15 mg of novobiocin per liter for isolation and confirmation by PCR *(39).* The confirmed isolates were serogrouped with latex agglutination kits (Wellcolex Colour *Salmonella,* Remel). After serogroups were identified, each isolate was serotyped with PCR of common first- and second-phase flagellar antigens *(18, 25).* Results of flagellar antigen PCR were confirmed with specific antisera (Remel) according to the manufacturer's instructions. When PCR did not identify flagellar antigens, the antisera were used to identify serotypes. When unlikely serological identification resulted, the subspecies of the *Salmonella* isolate was confirmed with gram-negative identification plates (Trek Diagnostic Systems, Cleveland, Ohio) according to the manufacturer's recommendations. All S*almonella* isolates were screened for antibiotic resistance by patch plating, onto 150-mm petri dishes, tryptic soy agar (TSA; Difco, Becton Dickinson) supplemented with 32 mg of tetracycline per liter. Plates were incubated at 37°C for 18 to 24 h. The isolates resistant to tetracycline were streaked for isolation on xylose lysine desoxycholate (Difco, Becton Dickinson) agar plates and incubated at 37°C for 18 to 24 h to confirm the *Salmonella* phenotype (black colonies). The isolates were then subcultured onto TSA for MIC determination with National Antibiotic Resistance Monitoring System antibiotic susceptibility panels (Trek Diagnostic Systems). The MICs were set up with a Sensititre auto diluter system (Trek Diagnostic Systems) according to the manufacturer's directions. The National Antibiotic Resistance Monitoring System panels were incubated at 37°C for 18 to 24 h before determining MICs with the Sensititre Autoreader System (Trek Diagnostic Systems).

Non-O157 STEC screening, hybridization, isolation, and characterization. Two 1-ml aliquots of each enrichment were mixed with 0.5 ml of 50% glycerol and stored at −70°C, while a third 5-μl aliquot was used in a multiplex PCR to detect the *stx* genes *(36).* Enrichments of postintervention carcass samples found to have positive signals for stx_1, stx_2, or both were used for the isolation of STEC as described previously *(3),* with minor

TABLE 1. *Aerobic plate counts of lamb pelts and carcasses at various stages of processing*

Processing plant	*n*	Sampling site[a]		
		Pelt	Preevisceration	Postintervention
A	282	7.0 A	5.3 A	3.0 A[b]
B	282	6.3 B	3.7 B	1.2 B
C	282	5.6 C	4.2 C	3.0 A
Overall	846	6.3	4.4	2.4[c]

[a] Values represent the mean log CFU/100 cm^2 determined with a Bactometer. Means within the same column with the same letter are not significantly different ($P < 0.05$).

[b] Postintervention samples from plant A = 188 rather than 282.

[c] Postintervention samples are $n = 752$ because of the number collected at plant A.

modifications. The corresponding −70°C glycerol stock of each stx_1- or stx_2-positive sample was diluted and spread plated to yield approximately 1,000 colonies per plate on 150-mm petri dishes of Difco modified *E. coli* broth (Difco, Becton Dickinson) containing 1.5% Difco agar (Difco, Becton Dickinson) and 1% glucose. Colonies were lifted to Hybond-N+ membranes (GE Healthcare, Piscataway, N.J.) and used in hybridizations with combined stx_1 and stx_2 DNA probes *(3),* labeled with the ECL Direct Nucleic Acid Labeling and Detection System (GE Healthcare) according to the manufacturer's recommendations. Positive colonies were subcultured and confirmed to possess *stx* genes by PCR *(36).* Biochemical assays with Fluorocult LMX broth (Merck KGaA, Darmstadt, Germany) and Sensititre gram-negative identification panels (Trek Diagnostic Systems) were used to confirm the organisms as *E. coli.* The serotype of each STEC isolate was determined by the serologic identification of the O serogroup, and the molecular identification of the H group was performed by the Gastroenteric Disease Center, The Pennsylvania State University.

Statistical analysis. Colony counts were transformed to log CFU/100 cm^2 values, and the prevalence levels of *E. coli* O157:H7, *Salmonella,* and non-O157 STEC were reported as the percentage of samples that tested positive for each pathogen. The data were analyzed by analysis of variance for a completely randomized design for the main effect of plant. PEPI software (version 2, USD, Inc., Stone Mountain, Ga.) was used to calculate the pairwise differences in means. 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 *(27).* All differences were reported with a significance level of $\alpha = 0.05$.

RESULTS

Aerobic plate counts. Samples were collected from multiple lots and on multiple days to generate a representative sample set for evaluation. The bacterial populations on sheep differed ($P < 0.05$) among the processing plants. Overall mean APCs (log CFU/100 cm^2) across all three plants for pelts, preevisceration carcasses, and postintervention carcasses were 6.3, 4.4, and 2.4, respectively (Table 1). Pelts from plant A had higher ($P < 0.05$) APCs than plant B, which had higher ($P < 0.05$) APCs than plant C (Table 1). Immediately after pelt removal, the overall mean APC of lamb carcasses was 1.9 log CFU/100 cm^2 less than the APC from the pelts. After preevisceration sampling, the lamb carcasses were washed, eviscerated, trimmed, inspected, rinsed with an antimicrobial compound, and chilled. The lamb carcasses were sampled immediately prior to entering the cooler for chilling. Postintervention carcasses from plants A and C had the same APC, which was higher ($P < 0.05$) than plant B. The overall mean APC of postintervention carcasses was 2.0 log CFU/100 cm^2 lower than the overall mean APC of preevisceration carcasses.

Prevalence of *E. coli* O157:H7. The prevalence of *E. coli* O157:H7 on lamb pelts and carcasses differed ($P < 0.05$) among processing plants (Table 2). The prevalence of *E. coli* O157:H7 found on pelts ranged from 0.4 to 28.7% among the three plants. Plant A pelts had a higher ($P < 0.05$) prevalence of *E. coli* O157:H7 than plant B, which had a higher ($P < 0.05$) prevalence of *E. coli* O157:H7 than plant C (Table 1). Immediately following pelt removal, the *E. coli* O157:H7 prevalence on carcasses ranged from 0.7 to 3.5%. Similar to the pelt samples, the preevisceration samples from plant A had a higher ($P < 0.05$) prevalence of *E. coli* O157:H7 than did samples from plants B and C. The prevalence of *E. coli* O157:H7 found on postintervention carcasses varied from 0 to 4.6%. The overall prevalences of *E. coli* O157:H7 (for the three plants) on pelts, preevisceration carcasses, and postintervention carcasses were 12.8, 1.6, and 2.9%, respectively.

Characterization of *E. coli* O157:H7 isolates. All *E. coli* O157:H7 isolates were confirmed by PCR for the presence of *rfbE, fliC, eae, hlyA,* stx_1, and stx_2 genes *(27, 36).* A total of 148 isolates were characterized in this study, with 109 isolates from pelts, 14 isolates from preevisceration carcasses, and 25 isolates from postintervention carcasses (Table 3). A total of 147 of 148 isolates were positive for the presence of at least one Shiga toxin gene. PCR results showed that 142 isolates carried stx_2 and that 5 isolates possessed both stx_1 and stx_2. Of 148 isolates, 147 and 142 isolates had virulence factor genes coding for intimin (*eae*) and EHEC hemolysin (*hlyA*). One preevisceration isolate that did not harbor Shiga toxin genes carried both *eae* and *hlyA* genes. Numerous *E. coli* O157:H7 isolates had atypical phenotypes on ntCHROMAgar but not on ctSMAC. The colonies on ntCHROMAgar were smooth, circular, and greenish-blue instead of mauve, indicating these isolates did not lack the characteristic β-glucuronidase activity of *E. coli* O157:H7 *(20).* Meanwhile, the colonies on ctSMAC were the straw color characteristic of the negative sorbitol fermentation *E. coli* O157:H7. A small subset of these atypical *E. coli* O157:H7 isolates was further characterized by PCR. The PCR results indicated that these atypical *E. coli* O157:H7 isolates carried the stx_2 gene; however, no Shiga toxin protein was produced from these isolates (data not shown). Additional experiments were not conducted to determine the reason for not producing the toxin.

Prevalence of *Salmonella.* The prevalence of *Salmonella* was similar to the prevalence of *E. coli* O157:H7 on pelts and carcasses. The overall *Salmonella* prevalences on pelts, preevisceration carcasses, and postintervention carcasses were 14.4, 4.3, and 1.8%, respectively. The preva-

TABLE 2. *Prevalences of* E. coli *O157:H7,* Salmonella, *and non-O157 STEC from pelts and carcasses at various stages of processing*[a]

Plant	No. (%) of *E. coli* O157:H7				No. (%) of *Salmonella*				No. (%) of non-O157 STEC			
	n	Pelt	Pre	Post	*n*	Pelt	Pre	Post	*n*	Pelt	Pre	Post
A	282	81 (27.8) A	10 (3.5) A	13 (4.6) A	282	92 (32.6) A	35 (12.4) A	13 (4.6) A	282	260 (92.2) A	265 (94.0) A	219 (77.6) A
B	284	27 (9.5) B	2 (0.7) B	0 (0) B	284	30 (10.6) B	1 (0.4) B	0 (0) B	282	282 (100.0) B	276 (97.8) A	234 (83.0) B
C	285	1 (0.4) C	2 (0.7) B	12 (4.2) A	285	1 (0.4) C	1 (0.4) B	2 (0.7) B	282	187 (66.3) C	124 (44.0) B	237 (84.0) B
Overall	851	109 (12.8)	14 (1.6)	25 (2.9)	851	123 (14.4)	37 (4.3)	15 (1.8)	846	729 (82.6)	665 (78.6)	690 (81.6)

[a] Values within the same column showing a common letter are not significantly different ($P < 0.05$). Pre, preevisceration carcass samples; post, postintervention carcass samples.

TABLE 3. *Incidence and characteristics of* E. coli *O157:H7 isolates from lamb samples*

Processing plant	Site[a]	No. of isolates	*stx* absent	*stx* present	Virulence-associated factors			
					stx_2	stx_{1+2}	*eae*	*hlyA*
A	Pelt	81	0	81	80	1	80	77
	Pre	10	1	9	9	0	10	10
	Post	13	0	13	13	0	13	13
B	Pelt	27	0	27	27	0	27	27
	Pre	2	0	2	2	0	2	2
	Post	0	0	0	0	0	0	0
C	Pelt	1	0	1	0	1	1	1
	Pre	2	0	2	2	0	2	2
	Post	12	0	12	9	3	12	10
Overall		148	1	147	142	5	147	142

[a] Pre, preevisceration carcass samples; post, postintervention carcass samples.

lence differed ($P < 0.05$) among plants (Table 2). Pelts from plant A had a higher ($P < 0.05$) *Salmonella* prevalence than plant B, which had a higher *Salmonella* prevalence ($P < 0.05$) than plant C (Table 2). After pelt removal, *Salmonella* was recovered from 0.4 to 12.4% of the preevisceration carcasses. The level of *Salmonella* on preevisceration carcasses was higher ($P < 0.05$) from plant A than from plant B, which did not differ from plant C (Table 2). The prevalence of *Salmonella* on postintervention carcasses ranged from 0 to 4.6%. Postintervention carcasses from plant A had a higher *Salmonella* ($P < 0.05$) prevalence than plant B, which was less than but not significantly different from plant C.

Characterization of *Salmonella* isolates. Eighty-nine of the 175 characterized isolates of *Salmonella* were *Salmonella enterica* subsp. *enterica* (subspecies I) and were of six different serotypes (Table 4). The remaining *Salmonella enterica* isolates were either *Salmonella enterica* subsp. *salamae* (subspecies II) or *Salmonella enterica* subsp. *arizonae* (subspecies III), which are both associated with cold-blooded hosts. Of the 175 isolates, 4 and 82 isolates were in subspecies II and IIIa, respectively, whereas 8 isolates were untypeable (Table 4). One hundred seventy-four isolates were susceptible to the antibiotics tested, with only one isolate, from a preevisceration carcass, being multidrug-resistant (MDR) *Salmonella.* The MDR *Salmonella* was serotype Typhimurium and was resistant to amoxicillin–clavulonic acid, ampicillin, chloramphenical, streptomycin, sulfisoxazole, and tetracycline (AmACSST). Two strains of *Salmonella* Newport (one from a pelt from plant B and one from a postintervention carcass from plant A) were isolated, but both were susceptible to all antibiotics tested. The most frequently identified *Salmonella* isolates on pelts and carcasses were *S. enterica* subsp. *arizonae* and *Salmonella* serotype Heidelberg.

Prevalence of non-O157 STEC. Any sample for which at least an *stx* gene was detected but an isolate was not previously determined as *E. coli* O157:H7 by PCR was considered positive for non-O157 STEC. The overall prev-

TABLE 4. *Incidence and characteristics of* Salmonella *isolated from lamb samples*

Processing plant	Sites[a]	No. of isolates	Subspecies[b]			Serotypes[c]							MDR[d]	Antibiotic-resistant patterns[e]
			I	II (Sa)	IIIa (Ar)	He	In	Mu	Ne	Po	Ty	UT		
A	Pelt	92	46	4	42	41	0	0	0	0	0	5	—	—
	Pre	35	3	0	32	2	0	0	0	0	0	1	—	—
	Post	13	6	0	7	5	0	0	1	0	0	0	—	—
B	Pelt	30	30	0	0	0	14	14	1	0	0	1	—	—
	Pre	1	1	0	0	0	0	0	0	0	0	1	—	—
	Post	0	0	0	0	0	0	0	0	0	0	0	—	—
C	Pelt	1	0	0	1	0	0	0	0	0	0	0	—	—
	Pre	1	1	0	0	0	0	0	0	0	1	0	+	AmACSSuT
	Post	2	2	0	0	0	0	0	0	2	0	0	—	—
Overall		175	89	4	82	48	14	14	2	2	1	8		

[a] Pre, preevisceration carcass samples; post, postintervention carcass samples.
[b] Sa, *Salmonella enterica* subsp. *salamae* (II) strains were not serotyped; Ar, *S. enterica* subsp. *arizonae* (IIIa) strains were not serotyped.
[c] He, Heidelberg; In, Infantis; Mu, Muenster; Ne, Newport; Po, Poona; Ty, Typhimurium; UT, untypeable.
[d] MDR, multidrug resistant.
[e] Am, amoxicillin–clavulonic acid; A, ampicillin; C, chloramphenical; S, streptomycin; Su, sulfisoxazol; T, tetracycline.

alences of non-O157 STEC on pelts, preevisceration carcasses, and postintervention carcasses were 86.2, 78.6, and 81.6%, respectively (Table 2). The prevalence of non-O157 STEC differed ($P < 0.05$) among plants (Table 2). Pelts from plant A had lower ($P < 0.05$) prevalences of non-O157 STEC than plant B, which had higher prevalences than plant C. Preevisceration carcasses from both plants A and B had higher ($P < 0.05$) non-O157 STEC prevalences than from plant C, whereas postintervention carcasses from plants B and C had higher ($P < 0.05$) non-O157 STEC prevalences than plant A (Table 2).

Characterization of non-O157 STEC isolates. The results of the PCR assay and the serologic identification of O and H antigens of isolates from postintervention carcasses of each plant are presented in Table 5. A total of 488 isolates were recovered from 690 postintervention samples at three processing plants. Non-O157 STEC isolates from three plants belonged to 30 O serogroups and 69 O:H serotypes. The most common serotypes identified in this study were O91:H14 (199 isolates), followed by O5:H19 (90 isolates). Three serotypes associated with human STEC that caused hemolytic-uremic syndrome (HUS) (O128:H2, O146:H8, and O163:H19) and three serotypes previously isolated from humans (O76:H19, O146:H21, and O174:H8) *(7)* were isolated from postintervention carcasses as well. At least one serotype linked to a previously reported incidence of HUS was isolated from each processing plant. Serotypes O128:H2 and O146:H8 were isolated from plant A, while serotype O146:H8 was isolated from plant B (Table 5). Serotypes O146:H8 and O163:H19 were isolated from plant C (Table 5).

The distribution of virulence-associated factors among the non-O157 STEC isolates is presented in Table 5. Among the 488 non-O157 STEC isolates, the most common virulence factor profile found was a combination of stx_1 and stx_2. Sixty-four of 488 isolates carried only the stx_1 gene. Seven isolates had only the stx_2 gene. A total of 225 isolates harbored both stx_1 and stx_2 genes. Only two isolates, one from plant A and one from plant C, carried the *eae* gene, while 163 isolates possessed the EHEC *hlyA* gene. Of the non-O157 STEC recovered from postintervention in this study, 375 of 488 isolates carried stx_2, either alone or in combination with other virulence-associated factors.

DISCUSSION

The aim of this study was to determine the microbiological status of lamb processed in the United States and to characterize the virulence factor profiles of *E. coli* O157:H7 and non-O157 STEC, if present. Bacterial contamination of carcasses is a common occurrence during processing, and such a contamination of fresh meat has important implications for food safety and product shelf life. An APC is generally accepted as a criterion for the microbial contamination of carcasses *(22).* The APC levels suggested that cross-contamination had occurred from the pelts to the carcasses during pelt removal *(4, 22).* The average APC of finished carcasses in our study (2.4 log CFU/100 cm^2) was lower than the APCs of lamb carcasses previously reported *(17, 37, 46),* which ranged from 4.5 to 6.4 log CFU/100 cm^2. However, comparison is quite difficult because of seasonal involvement, different carcass chill times, and different sampling sites in some studies *(17, 37, 46).* Furthermore, dressing processes may contribute to the lower bacterial counts of lamb carcasses. Inverted dressing systems (carcass hangs by the forelegs during pelt removal) have been shown to reduce visible and microbial contamination on carcasses when compared with conventional dressing systems (carcass hangs by the hind legs during pelt removal) *(23, 44).* The pelts are cut to open from the forequarter area in inverted dressing rather than from the hindquarter area, which is a region of high contamination. The pelt is pulled down from the forequarter, preventing the contamination from the hind legs and anal area from being transferred to the carcass.

Most of the studies on the prevalence of *E. coli* O157:

TABLE 5. *Serotype and virulence factor profiles among non-O157 STEC isolates from the postintervention carcass samples at different processing plants*[a]

Serotype[b]	Plants	No. of isolates	Virulence factor profiles (no.)
OUT:H2	A	3	*stx*$_1$, *stx*$_2$, *hlyA*; *stx*$_1$, *stx*$_2$ (2)
OUT:H2/35	A	4	*stx*$_1$, *stx*$_2$
OUT:H3	A	2	*stx*$_1$, *stx*$_2$
OUT:H7	A	1	*stx*$_1$, *stx*$_2$
OUT:H10	A	1	*stx*$_1$, *stx*$_2$, *hlyA*
OUT:H12	A	3	*stx*$_1$, *stx*$_2$; *stx*$_1$ (2)
OUT:H14	A	2	*stx*$_1$, *stx*$_2$, *hlyA*; *stx*$_1$, *stx*$_2$
OUT:H38	A	1	*stx*$_1$, *eae*, *hlyA*
O5:H19	A	35	*stx*$_1$ (3); *stx*$_1$, *stx*$_2$ (6); *stx*$_1$, *stx*$_2$, *hlyA* (25); *stx*$_1$, *hlyA*
O5:HUT	A	1	*stx*$_1$, *stx*$_2$, *hlyA*
O8:H8	A	1	*stx*$_1$, *stx*$_2$
O15:HUT	A	1	*stx*$_1$
O76:H19	A	2	*stx*$_1$; *stx*$_1$, *hlyA*
O81:HUT	A	1	*stx*$_1$
O83:H7	A	1	*stx*$_1$, *stx*$_2$, *hlyA*
O84/O172:H34	A	1	*stx*$_1$, *stx*$_2$
O87:H16	A	1	*stx*$_2$
O91:H14	A	74	*stx*$_1$ (5); *stx*$_1$, *stx*$_2$ (63); *stx*$_1$, *stx*$_2$, *hlyA* (6)
O91:HUT	A	2	*stx*$_1$, *stx*$_2$, *hlyA*
O128:H2	A	3	*stx*$_1$, *stx*$_2$
O128:H2/35	A	20	*stx*$_1$, *stx*$_2$
O128:H3	A	2	*stx*$_1$; *stx*$_1$, *stx*$_2$
O128:HUT	A	1	*stx*$_1$, *stx*$_2$
O146:H8	A	2	*stx*$_1$
O146:H11	A	1	*stx*$_1$
O146:HUT	A	1	*stx*$_1$
O158:H28	A	1	*stx*$_1$
O174:H8	A	4	*stx*$_1$, *hlyA*; *stx*$_1$, *stx*$_2$; *stx*$_1$ (2)
O174:HUT	A	3	*stx*$_1$ (2); *stx*$_1$, *stx*$_2$
O$^-$:H5	B	1	*stx*$_1$
O5:H19	B	15	*stx*$_1$; *stx*$_1$, *hlyA*; *stx*$_1$, *stx*$_2$ (3); *stx*$_1$, *stx*$_2$, *hlyA* (10)
O6:H10	B	4	*stx*$_1$
O15:H12	B	1	*stx*$_1$, *stx*$_2$, *hlyA*
O91:H14	B	75	*stx*$_1$ (3); *stx*$_2$; *stx*$_1$, *stx*$_2$ (34); *stx*$_1$, *hlyA*; *stx*$_1$, *stx*$_2$, *hlyA* (36)
O112:H2/35	B	1	*stx*$_1$, *stx*$_2$
O128:H2/35	B	11	*stx*$_1$, *hlyA*; *stx*$_1$, *stx*$_2$, *hlyA* (2); *stx*$_1$, *stx*$_2$ (8)
O146:H8	B	8	*stx*$_1$
O146:H36	B	3	*stx*$_1$; *stx*$_1$, *hlyA* (2)
OX18:H2/35	B	1	*stx*$_1$, *hlyA*
OX18:H36	B	3	*stx*$_1$
OUT:H2/35	C	1	*stx*$_1$, *stx*$_2$
OUT:H4	C	1	*stx*$_1$, *hlyA*
OUT:H8	C	1	*stx*$_1$
OUT:H10	C	8	*stx*$_1$, *stx*$_2$ (2); *stx*$_1$, *stx*$_2$, *hlyA* (6)
OUT:H16	C	1	*stx*$_2$
O−:H2/35	C	2	*stx*$_1$, *stx*$_2$
O−:H10	C	1	*stx*$_1$, *stx*$_2$, *hlyA*
O5:H19	C	40	*stx*$_1$ (6); *stx*$_1$, *hlyA* (2); *stx*$_1$, *stx*$_2$ (5); *stx*$_1$, *stx*$_2$, *hlyA* (27)
O6:H5	C	1	*stx*$_1$
O6:H10	C	3	*stx*$_1$
O8:H7	C	1	*stx*$_1$
O8:H9	C	3	*stx*$_2$
O8:H16	C	1	*stx*$_2$
O15:H27	C	5	*stx*$_1$; *stx*$_1$, *stx*$_2$ (4)
O36:H7	C	4	*stx*$_1$
O73:H19	C	1	*stx*$_1$, *stx*$_2$
O76:H19	C	5	*stx*$_1$, *stx*$_2$, *hlyA*; *stx*$_1$, *hlyA* (4)
O85:H10	C	1	*stx*$_1$
O85/O167:H34	C	1	*stx*$_1$, *stc*$_2$, *hlyA*
O87:H16	C	1	*stx*$_2$
O91:H14	C	50	*stx*$_1$ (8); *stx*$_1$, *hlyA*; *stx*$_1$, *stx*$_2$ (25); *stx*$_1$, *stx*$_2$, *hlyA* (16)
O103:H38	C	2	*stx*$_1$; *stx*$_1$, *hlyA*
O104:H7	C	1	*stx*$_1$, *stx*$_2$
O104/OX18:H7	C	1	*stx*$_1$
O109:H30	C	2	*stx*$_1$
O128:H2/35	C	33	*stx*$_1$ (4); *stx*$_2$; *stx*$_1$, *stx*$_2$, *hlyA*; *stx*$_1$, *stx*$_2$ (27)
O136:H9	C	1	*stx*$_1$
O141:H16	C	1	*stx*$_1$, *stx*$_2$
O145:HUT	C	1	*stx*$_1$, *eae*, *hlyA*
O146:H8	C	1	*stx*$_1$
O146:H21	C	1	*stx*$_1$, *stx*$_2$, *hlyA*
O146:H36	C	1	*stx*$_1$, *stx*$_2$, *hlyA*
O163:H19	C	1	*stx*$_2$, *hlyA*
O174:H8	C	6	*stx*$_1$
OX18:H36	C	4	*stx*$_1$
OX25:H11	C	2	*stx*$_1$; *stx*$_1$, *hlyA*

[a] Total number of isolates was 488.

[b] Serotypes in italics indicate names of serotypes previously found in human STEC strains; serotypes in boldface indicate names of serotypes previously associated with human STEC-causing HUS; UT, untypeable; −, negative.

H7 from lamb were obtained from fecal samples *(6, 9, 13, 26, 29, 34, 40).* Only one study reported the prevalence of *E. coli* O157:H7 from pelts *(41).* In our study, the overall prevalence of *E. coli* O157:H7 among three plants on pelts was 7.3% higher than the *E.* coli O157:H7 on pelts from slaughter in the United Kingdom, as reported by Small et al. *(41).* In that study, 90 slaughtered lambs were sampled before pelt removal, and the overall prevalence of *E. coli* O157:H7 was 5.5%. Similarly, a higher *E. coli* O157:H7 prevalence in the fecal samples (31%) of U.S. sheep was reported *(29),* while only 0.5 to 6.5% of the sheep feces in Spain, Great Britain, The Netherlands, and Scotland were found to have *E. coli* O157:H7 *(9, 26, 34, 35).* The *E. coli* O157:H7 found on pelts may not always be correlated with the pathogen found in feces. However, the *E. coli* O157:H7 in feces indicates that at least one animal shedding the pathogen in its feces could subsequently contaminate the pelts of several animals. The prevalence of *E. coli* O157:H7 found on the postintervention carcasses from our study varied from 0 to 4.6%. Variations of the prevalence level of *E. coli* O157:H7 also were reported in other countries. Zweifel and Stephan *(46)* reported that there was no *E. coli*

O157:H7 detected on 580 sheep carcasses from three Swiss abattoirs, whereas *E. coli* O157:H7 was found on 0.7% of finished Australian lamb carcasses *(37).* Typically, during processing, the microbial contamination trend is to decline from pelt to finished carcass. In this study, a higher prevalence of *E. coli* O157:H7 was found on finished carcasses than on preevisceration carcasses. Gill et al. *(23)* reported that the number of generic *E. coli* isolates recovered from inverted dressing was less than the number of *E. coli* isolates from conventional dressing. Therefore, the higher prevalence of *E. coli* O157:H7 suggested occasional loss of process control. We hypothesized that the increasing number of carcasses positive for *E. coli* O157:H7 at plant A from preevisceration (3.5%) to postintervention carcasses (4.6%) had occurred because of adjacent carcass sampling. However, the prevalence data from plant B (lower postintervention prevalence than preevisceration prevalence) did not support this hypothesis. Other factors may contribute to the higher prevalence on postintervention carcasses, one of which may be the potential cross-contamination from the personnel handling the viscera *(22)* and then the carcass without washing in between, as observed in one of the processing plants. The majority of the *E. coli* O157:H7 isolated (95.6%) from pelts, preevisceration carcasses, and postintervention carcasses of three plants carried stx_2, and 3.4% harbored both stx_1 and stx_2. The results were complementary to the studies from Spain, Italy, and the United Kingdom, where the majority of *E. coli* O157:H7 isolated from sheep carried stx_2 *(6, 9, 13, 40).* In contrast, 75% of lamb *E. coli* O157:H7 from a flock in the United States had both stx_1 and stx_2 *(29).* The STEC strains carrying stx_2 have been associated with HUS more often than those carrying only stx_1 *(10, 43).* Our isolates also carried both intimin (*eae*) and EHEC hemolysin (*hlyA*) genes. Severe diarrhea, especially hemorrhagic colitis and HUS, was closely associated with STEC carrying the *eae* gene *(10).*

The prevalence of *Salmonella* on postintervention carcasses of this study (1.8%) and the finding in a previous study of the prevalence of *Salmonella* (1.5%) is in agreement *(17).* Although MDR *Salmonella* was previously isolated from lamb *(24),* no MDR *Salmonella* was recovered from postintervention carcasses in this study. Interestingly, of all the isolates collected from three plants, 46.8% were *S. enterica* subsp. *arizonae,* which is associated with cold-blooded animals. Two studies have shown that *S. enterica* subsp. *arizonae* is commonly associated with sheep in North America *(38, 45),* and it is considered host adapted *(31).* Approximately 27% of *Salmonella* Heidelberg was isolated from lambs in our study. The isolation of *Salmonella* Heidelberg was also reported from sheep in Canada *(45).*

Unlike *E. coli* O157:H7 and *Salmonella,* non-O157 STEC strains were found at a high frequency at all sites sampled. A higher prevalence of non-O157 STEC than *E. coli* O157:H7 is not uncommon and was previously reported on sheep. In one study, non-O157 STEC was found in sheep at a frequency of 67%, while *E. coli* O157:H7 was found at a frequency of 21% *(9).* In another study, 535 lamb carcasses were examined by PCR for the Shiga toxin genes, and 36.6% were *stx* positive, with no *E. coli* O157:H7 found *(46).* A higher prevalence of non-O157 STEC than of *E. coli* O157:H7 and *Salmonella* also has been observed on cattle hides and beef carcasses *(3, 5).* In our study, the non-O157 STEC serogroups O5 and O91 were frequently identified. In a previous study, serogroups O5, O91, O128, and O146 also were frequently isolated from sheep *(9),* indicating that these serogroups are specifically associated with sheep. The most common serogroups of non-O157 STEC isolated from clinical laboratories in the United States are O26, O45, O103, O111, O121, and O145, with almost 50% of the outbreaks caused by serogroup O111, which accounted for most cases of non-O157 HUS in the United States *(12).* In this study, non-O157 STEC serogroups O26, O45, O111, and O121 were not isolated from postintervention carcasses. The majority of isolates from our study carried both stx_1 and stx_2 genes, followed by the stx_1 gene alone, the stx_2 gene alone, and the *eae* gene alone. In contrast, a higher percentage of non-O157 STEC carried the stx_1 gene (42.9 to 56%) in sheep from Spain and Switzerland *(9, 46).* The Spanish and Swiss sheep also harbored 1, 42, 5, and 27% of stx_2, both stx_1 and stx_2, *eae,* and EHEC *hlyA* genes, respectively. None of the isolates from this study possessed a combination of *eae* and EHEC *hylA* and at least one Shiga toxin gene that has been previously reported as the genetic determinants linked to cause of illness. The severe illnesses caused by non-O157 STEC are comparable to those caused by EHEC O157:H7. Although there is no specific combination of virulence-associated factors necessary to cause EHEC-related diseases, stx_2 was strongly associated with an increasing risk of HUS, and *eae* was strongly associated with an increasing risk of bloody diarrhea *(12).*

In conclusion, our studies provided a baseline of microbial contamination and prevalences of *E.* coli O157:H7, *Salmonella,* and non-O157 STEC during processing. Samples were collected on the processing line from three stages (pelts, preevisceration carcasses, and postintervention carcasses). Overall, *E. coli* O157:H7 (2.9%), *Salmonella* (1.8%), and non-O157 STEC (81.6%) were all found on the postintervention carcasses. Some non-O157 STEC serotypes previously associated with illnesses also were isolated from the postintervention carcasses. The high frequency of *E. coli* O157:H7, *Salmonella,* and non-O157 STEC isolation from postintervention carcasses raises a safety concern for finished lamb products. All of the processing plants studied herein used either a diluted organic acid or an acidified sodium chlorite rinse as a final antimicrobial intervention. An alternative antimicrobial intervention, such as hot water (70 to 96°C), may be more effective in reducing these pathogens, as it has been shown to be an effective means to reduce bacteria both on beef and lamb carcasses *(15)* and more effective than 2% lactic acid *(11).*

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