

# Prevalence and Relatedness of *Escherichia coli* O157:H7 Strains in the Feces and on the Hides and Carcasses of U.S. Meat Goats at Slaughter

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**We determined the prevalences of *Escherichia coli* O157:H7 in feces, hide, and carcasses of meat goats at a U.S. processing plant. Prevalences were 11.1%, 2.7%, and 2.7%, respectively. Sixteen pulsed-field gel electrophoresis (PFGE) subtypes were identified among 49 *E. coli* O157:H7 isolates, some of which were present on multiple sample types or collection days.**

Shiga toxin-producing *Escherichia coli* (STEC) bacteria, including the O157 serogroup, are well-described human pathogens associated with bloody diarrhea, hemolytic-uremic syndrome (HUS), and death (1). Illnesses are often attributed to the consumption of contaminated food or water; however, transmission by animal contact may also be important (2, 3). Ruminants, particularly cattle, are considered the primary reservoir for *E. coli* O157:H7, where the organism typically colonizes the lower gastrointestinal tract (4) and is shed in the feces. Recent outbreaks of human illness associated with small-ruminant contact at livestock exhibitions (5) have demonstrated the importance of these animals as reservoirs for STEC, including *E. coli* O157:H7. However, estimates of *E. coli* O157:H7 carriage in U.S. goats is limited.

Previous work in cattle suggests that the prevalence of *E. coli* O157:H7 in the feces is correlated to the prevalence on the hide and carcasses of animals at slaughter (6, 7, 8). An increasing number of goat carcasses are inspected in U.S. packing plants (9), suggesting that goat meat may be an increasingly important transmission vehicle for food-borne pathogens. *Escherichia coli* O157:H7 contamination of goat carcasses represents a public health threat if the contamination is not removed prior to consumption. The objective of our work was to determine the prevalence of *E. coli* O157:H7 in the feces and on the hides and carcasses of meat goats presented for slaughter at a USDA-inspected plant in the southeastern United States. In addition, we wanted to determine the genetic relatedness of the isolates to better understand the ecology of the organism within this environment.

**Animals and sample collection.** Fecal, hide, and carcass samples were collected from 300 goats presented for harvest at a USDA-inspected processing plant in the southeastern United States. Matched samples were collected from each animal. For labeling purposes, fecal, hide, and carcass samples from each animal were given the same number (differentiated by an F, H, or C), and animals were labeled consecutively as they appeared in the plant. Samples were collected on 9 days during a 12-week period from August to October; between 10 and 40 animals were sampled on each collection day (Table 1). The plant had a capacity of approximately 200 goats per week and also processed sheep and cattle. Goats were always processed first on each collection day. There was no available information on the source, age, diet, or previous use (dairy, meat, or pet) of the goats. Retrospectively, information on maximum daily temperature and precipitation for each collection day were obtained from the closest reporting National Oceanic and Atmospheric Administration (NOAA) site.

Hide samples were collected postharvest by swabbing vertically, at least three times, an area of approximately 100 cm<sup>2</sup> along one side and the rump with sterile gauze pads (2 in. × 2 in.), premoistened with 5 ml of sterile water. Similarly, one side and the rump area of carcasses were swabbed with a Speci-Sponge sponge (Nasco, Fort Atkinson, WI) soaked with 15 ml of buffered peptone water. Carcass samples were collected immediately after hide removal and evisceration but prior to USDA inspection. Rectal contents were collected postevisceration by cutting open intact rectums. All samples were placed into Whirl-Pak bags (Nasco) and transported in a cooler with ice packs to the laboratory for processing within 24 h. Sampling personnel wore a new nitrile glove between each animal and sample type, and scissors used to cut rectums were rinsed with water, wiped to remove gross fecal contamination, and soaked in 0.1% chlorhexidine diacetate solution between each animal.

**Isolation of *E. coli* O157:H7 from hides, carcasses, and feces.** Approximately 1 g of fecal pellet (homogenized when possible) was inoculated into 9 ml of Gram-negative broth (Difco; BD, Sparks, MD) supplemented with cefixime (0.5 mg/liter), cefsulodin (10 mg/liter), and vancomycin (GNccv) (8 mg/liter). Samples were vortexed and incubated for 5.5 h at 37°C. Manual immunomagnetic bead separation (IMS) (Dynabeads; Invitrogen Corp., Carlsbad, CA) was performed with 1 ml of enrichment broth, and 50 μl of product was plated on sorbitol MacConkey agar with cefixime (0.5 mg/liter) and potassium tellurite (2.5 mg/liter) (CT-SMAC) (BD). Plates were incubated overnight at 37°C, and up to six sorbitol-negative colonies were selected and streaked onto blood agar plates (BD). After overnight incubation, colonies were tested for indole production (Remel; Lenexa, KS) and latex agglutination with the O157 antigen (Remel). Positive colonies were further characterized by multiplex PCR to detect the *rfbE* (O157), *eae* (intimin), *stx*<sub>1</sub> (Shiga toxin 1), *stx*<sub>2</sub> (Shiga toxin 2), *hlyA* (hemolysin), and *fliC* (H7) genes (10).

Hide and carcass samples were cultured for *E. coli* O157:H7 as previously described (7). Briefly, 20 ml of brilliant green broth

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**TABLE 1** Numbers of processed goats sampled, maximum daily temperatures, and precipitation readings on 9 collection days at a USDA-inspected facility in the southeastern United States

Collection	Sampling date	No. of samples	Temp (°C)	Amt of precipitation (in.)
1	15 August	20	29.4	0.0
2	20 August	40	23.3	0.75
3	27 August	40	27.2	0.0
4	10 September	40	26.1	0.0
5	12 September	30	26.1	0.0
6	17 September	40	23.8	0.11
7	1 October	40	16.7	0.04
8	9 October	40	10.6	0.22
9	15 October	10	22.8	0.57

(BGB) was added to hide swab samples, which were enriched for 6 h at 37°C. Carcass sponges were enriched in 90 ml of BGB for 10 h at 37°C. After enrichment, IMS was performed with 1 ml of enriched BGB, followed by plating onto CT-SMAC plates. Plates were incubated and evaluated as described above. All *E. coli* O157:H7 isolates obtained from feces, hides, and carcasses were frozen at -80°C for future analyses.

**PFGE.** Individual *E. coli* O157:H7 isolates were grown on blood agar plates for 24 h at 37°C for pulsed-field gel electrophoresis (PFGE) analysis. All isolates were subjected to restriction enzyme digestion with XbaI and processed according to the PulseNet protocol (11). Gel images were obtained using a Gel Doc XR+ imaging system (Bio-Rad, Hercules, CA), and the PFGE banding patterns were normalized and evaluated using BioNumerics software (Applied Maths, Inc., Austin, TX). Isolates displaying 100% Dice similarity were considered to be the same PFGE subtype (12).

**Statistical analysis.** The fecal, hide, and carcass prevalences of *E. coli* O157:H7 were the primary outcomes of interest in this study. Descriptive statistics were calculated using the Microsoft Excel program (Redmond, WA). Data were further analyzed utilizing commercially available statistical software (Stata 12; Stata-Corp LP, College Station, TX) to calculate a Fisher exact test to evaluate differences in prevalence by collection day. The odds of a carcass being positive if either the feces or hide was positive was calculated from cross-tabulated tables. Data were considered binomial (positive or negative). A Fisher exact test was used to evaluate the independence of variables associated with one another. Statistical significance was considered to be a *P* value of <0.05.

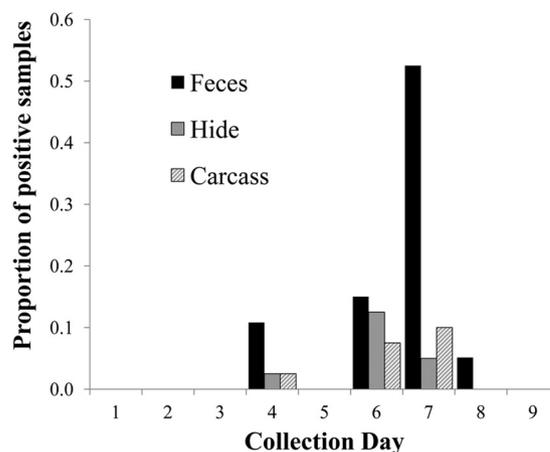
**Prevalence of *E. coli* O157:H7.** The prevalence of *E. coli* O157:H7 in the feces of goats at slaughter was 11.1% (33 of 296; 95% confidence interval [CI], 7.5% to 14.8%). Previous reports of goats within and outside the United States have suggested that fecal prevalence in goats is low, less than 5% (13, 14, 15, 16). Although fecal prevalence in our study was remarkably higher, a large number of positive samples were obtained on one collection day. The mean prevalence for each collection day ranged from 0 (5 of 9 collection days) to 52.5% (Fig. 1) and was significantly different between days (*P* < 0.01). Although our goal was not to specifically evaluate environmental conditions on different collection days, there did not appear to be a large difference in temperatures or amounts of precipitation on the days when we obtained positive samples (Table 1). Daily variation in the prevalence of *E. coli*

O157:H7 in cattle at slaughter has been well described. The impact of collection days on goats in other studies is unclear.

The prevalence of *E. coli* O157:H7 on hides of goats at slaughter was 2.7% (8 of 300; 95% CI, 0.8% to 4.5%) and ranged between collection days from 0 (6 of 9 collection days) to 12.5%, which was significantly different (*P* = 0.02). The prevalence of *E. coli* O157:H7 on meat goat carcasses was 2.7% (8 of 299; 95% CI, 0.8% to 4.5%), similar to a previous report on Ethiopian goat carcasses (16). Prevalences in carcasses ranged from 0 (6 of 9 collection days) to 10.0% and tended to be different between collection days (*P* = 0.052). In addition, for 43 of 300 goats (14.3%) sampled in our study, at least 1 site (feces, hide, or carcass) was positive for *E. coli* O157:H7. The risk of an animal having at least one collection site positive was different between collection days (*P* < 0.01). For 6 goats, 2 sites were culture positive for *E. coli* O157:H7; for 3 goats, positive isolates were found for the feces and carcass; 2 goats were feces and hide positive; and for 1 goat, *E. coli* O157:H7 was isolated from both the hide and carcass.

It is well established in beef production that a specific sampling day and/or load of cattle with high fecal prevalence is correlated with carcass contamination with *E. coli* O157:H7 (6, 7, 17). The days of higher hide and carcass prevalence also correlated with days with high fecal prevalence in our study (Fig. 1). A previous study of sheep and goats in Ethiopia identified a significant association between the carcass contamination of an individual animal and the fecal presence of *E. coli* O157:H7 in the same animal (16). We found this same correlation; the odds of a goat carcass being positive for *E. coli* O157:H7 if the feces from that animal was positive or negative were different (*P* < 0.01). In our study, a goat with a positive fecal culture was 5.2 times (95% CI, 1.15 to 23.1) more likely to be carcass positive.

Studies in cattle have also suggested that hides are the major source of *E. coli* O157:H7 for carcasses at slaughter, and controlling hide contamination will reduce prevalences in carcass (18, 19). Interestingly, we did not observe a strong association between hide and carcass prevalences in our study. The odds of a hide being positive if the animal was fecal positive was 2.8 (95% CI, 0.5 to 14.4), which was not statistically significant (*P* = 0.21), and an animal with a positive hide tended to have an increased odds (5.8; 95% CI, 0.6 to 54.5) of being carcass positive (*P* = 0.08). A lack of



**FIG 1** Proportions of *Escherichia coli* O157-positive samples collected from the feces, hides, and carcasses of meat goats at a southeastern U.S. abattoir over nine collection days.

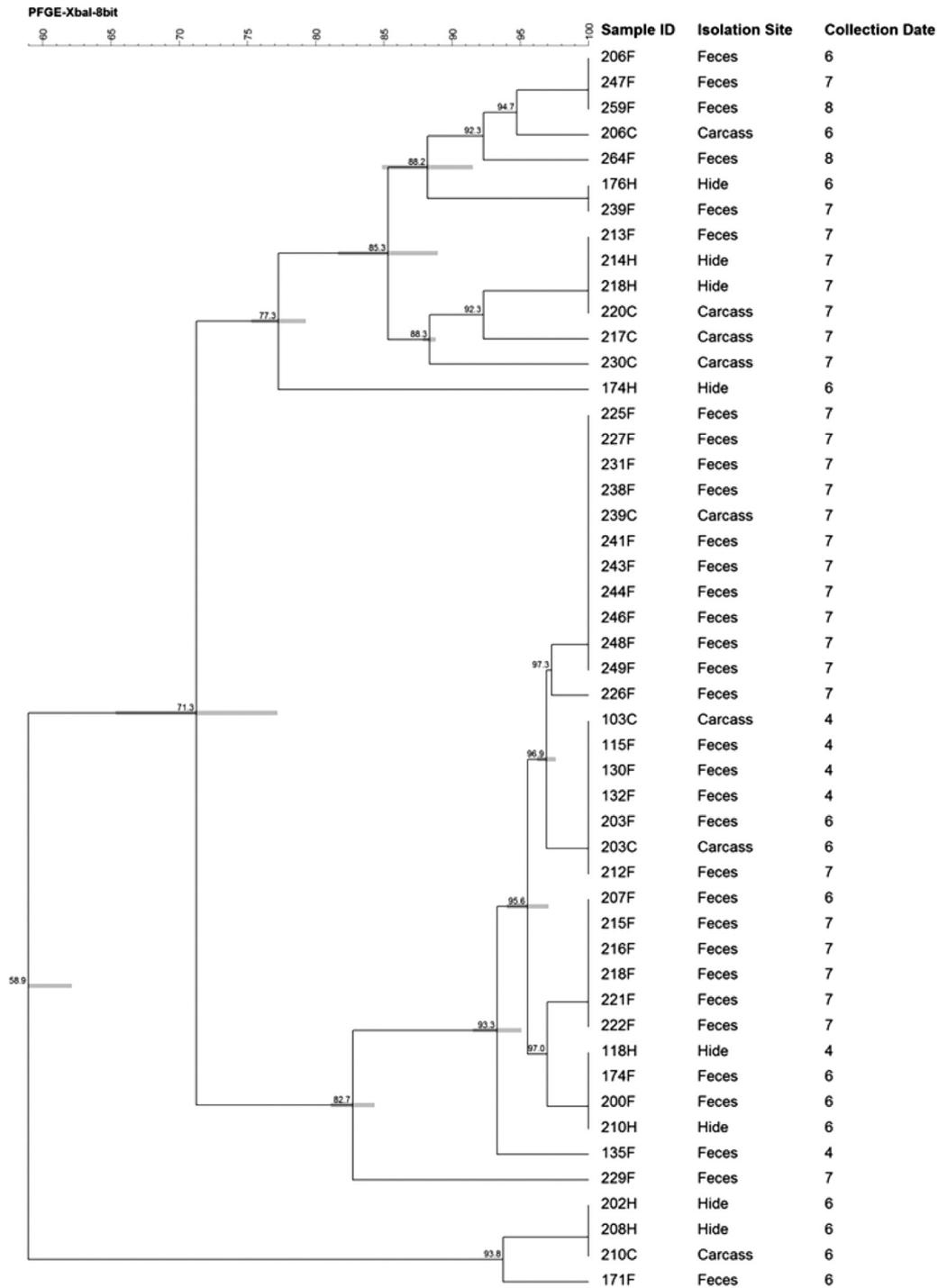


FIG 2 Dendrogram describing the relatedness of *Escherichia coli* O157 isolates obtained from the feces, hides, and carcasses of meat goats at a southeastern U.S. abattoir over nine collection days. Animals were consecutively numbered as they entered the slaughter plant. The same number for fecal, hide, or carcass samples would indicate that the samples came from the same animal.

power could have contributed to our ability to fully understand this association.

**Genetic relatedness of isolates.** All 49 *E. coli* O157:H7 isolates obtained in this study were positive for *stx*<sub>1</sub>, *rfbE*, *fliC*, *eae*, and *hlyA*. One isolate obtained from the hide of an animal was positive for *stx*<sub>2</sub>; all other isolates were negative. The distribution of Shiga

toxin-producing genes from *E. coli* O157:H7 isolates in this study resembles reports from outside the United States, which suggest that sheep and goats more frequently carry *stx*<sub>1</sub> or *stx*<sub>1</sub> and *stx*<sub>2</sub> than *stx*<sub>2</sub> alone (20, 21, 22, 23, 24). The distribution of virulence genes, including Shiga toxin genes, in STEC is important, and the presence of *stx*<sub>2</sub> has been associated with more-severe human dis-

ease (25, 26, 27). The complete virulence profile of the *E. coli* O157:H7 isolates obtained in our study are not known; however, all isolates were also positive for the *eae* gene (encoding intimin) and the *hlyA* gene (encoding hemolysin). Taken together, it is likely that at least some of the *E. coli* O157:H7 bacteria isolated from these goats could be important human pathogens.

We identified 16 unique PFGE subtypes (Dice similarity < 100%) among the 49 *E. coli* O157:H7 isolates; a dendrogram describing their relatedness is provided (Fig. 2). There were 13 unique PFGE types, with a Dice similarity of  $\geq 95\%$ , and 8 types with a similarity of  $\geq 90\%$ . Some PFGE types differed across and between days and sample types. On collection day 4, there were 6 *E. coli* O157:H7 isolates obtained and 3 unique PFGE subtypes, or 2 with a Dice similarity of  $\geq 95\%$ . Four of the 6 isolates, including 1 carcass isolate, had the same PFGE subtype. On collection day 6, there were 14 *E. coli* O157:H7 isolates and 9 unique PFGE subtypes: 7 PFGE types with a similarity of  $\geq 95\%$ . The same subtypes were detected on hides and carcasses, feces and hides, and feces and carcasses. Similar results were seen on collection day 7, with 27 *E. coli* O157:H7 isolates and 10 unique PFGE subtypes. There were 7 PFGE types with a similarity of  $\geq 95\%$ . On collection day 8, 2 unique subtypes were detected from 2 isolates, both from feces. These were not recovered on the hide or carcasses of any animal. There were 5 different PFGE subtypes that appeared on 2 or more collection days.

Within each sample type, there was also a distribution of unique PFGE patterns. Of 33 fecal sample *E. coli* O157:H7 isolates, 13 unique PFGE subtypes (9 with  $\geq 95\%$  Dice similarity) were identified. Twenty-four fecal isolates (72%) had a Dice similarity of  $\geq 95\%$ . There were 5 unique PFGE types from hide isolates ( $n = 8$ ), all with Dice similarities of < 90%. *Escherichia coli* O157:H7 isolates from carcasses ( $n = 8$ ) were obtained on 3 collection days, and 7 unique PFGE subtypes (6 with  $\geq 95\%$  Dice similarity) were found. For animals with more than 1 sample type positive, only 1 animal had an identical PFGE pattern identified for both sample types, feces and carcass. For all other animals, 2 sites were positive, with unique *E. coli* O157:H7 PFGE subtypes (Dice similarity < 95%).

Unfortunately, the origins of the goats and their duration of mingling before processing are not known for this study; therefore, the number of goats arriving at the abattoir with the same *E. coli* O157:H7 PFGE subtype is not known. Still, this study demonstrates the potential for spread of *E. coli* O157:H7 throughout a processing facility, as evidenced by isolate PFGE profiles and their relatedness to one another. Within a collection day, an individual *E. coli* O157:H7 PFGE subtype could be isolated from the feces, hide, or carcass of multiple goats. In only one instance was the same subtype recovered from multiple sites on the same animal. There was no obvious relationship between where a specific PFGE type was first identified in the slaughter order and where it might be found again downstream (Fig. 2). The presence of the same PFGE subtype in the feces of animals on multiple collection days (up to 3 weeks apart) is interesting and suggests that there may be a common source of *E. coli* O157:H7 immediately prior to slaughter, that the goat population received by this abattoir is integrated, or perhaps that the plant received animals from the same operation on multiple days. Identical PFGE subtypes of *E. coli* O157:H7 on cattle carcasses and in feces at slaughter have also been found to cross loads and collection days (12). There is evidence in cattle feces that more than one PFGE type can be present within the

same sample (28, 29). We did not evaluate the number of *E. coli* O157:H7 PFGE subtypes present in goat feces or on hides and carcasses in this study. However, if we expect these samples to contain multiple subtypes, we may be failing to capture a complete understanding of how *E. coli* O157:H7 is spread in the goat processing environment.

Because of an increasing demand for goat meat in the United States, determining the risk of *E. coli* O157:H7 contamination of goat meat products is warranted. We found preliminary evidence to suggest that *E. coli* O157:H7 exists throughout the production chain of meat goats, including on carcasses intended for consumption. The prevalence and distribution of PFGE subtypes within and between animals suggest that contamination within the plant may be widespread and not dependent solely on the fecal status of an individual animal. In addition, the virulence profiles of U.S. goat isolates suggest they may be important human pathogens. Further work to characterize the virulence profile of *E. coli* O157:H7 bacteria isolated from goats and to determine their potential as human pathogens, as well as understanding mitigation efforts, is warranted.

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## AUTHOR CORRECTION

# Correction for Jacob et al., Prevalence and Relatedness of *Escherichia coli* O157:H7 Strains in the Feces and on the Hides and Carcasses of U.S. Meat Goats at Slaughter

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Volume 79, no. 13, p. 4154–4158, 2013. Page 4156, column 1, line 4: “*stx*<sub>1</sub>” should read “*stx*<sub>2</sub>.”

Page 4156, column 1, line 6: “*stx*<sub>2</sub>” should read “*stx*<sub>1</sub>.”

Page 4156, column 2, line 2: “resembles” should read “does not resemble.”

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