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Research Note

Pulsed-Field Gel Electrophoresis Characterization of Shiga Toxin–Producing *Escherichia coli* O157 from Hides of Cattle at Slaughter

S. M. AVERY, A. SMALL, C.-A. REID, AND S. BUNCIC*

Division of Food Animal Science, Department of Clinical Veterinary Science, University of Bristol, Bristol BS40 5DU, UK

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ABSTRACT

Contamination of the brisket areas of the hides of healthy adult cattle with Shiga toxin–producing *Escherichia coli* O157 at slaughter in England was studied. In total, 73 cattle consignments comprising 584 animals delivered to one abattoir over 3 days during 1 week in July 2001 were studied: 26 cattle consignments arriving on Monday, 32 consignments arriving on Wednesday, and 15 consignments arriving on Friday. Consignment sizes ranged from 1 to 23 animals, with a mean consignment size of 8. The hide of the first animal to be slaughtered in each consignment was sampled by using a sponge swab moistened with 0.85% saline to rub an unmeasured brisket (ventral) area (ca. 30 by 30 cm). The process of isolating *E. coli* O157 from the swabs consisted of enrichment, screening with immunoprecipitation assay kits, and immunomagnetic separation. *E. coli* O157 was found on 24 of 73 (32.9%) cattle hides examined, and 21 of these 24 isolates produced Shiga toxins. The 24 *E. coli* O157 isolates produced six different pulsed-field gel electrophoresis profiles, and 18 (75%) of the isolates were of one prevalent clone. The high prevalence of one *E. coli* O157 clone on the hides of cattle at slaughter could be due to a high prevalence of that clone on the 18 farms involved (not investigated in the current study), in the postfarm transport or lairage environments, or both. Since the lairage environment, but not the farm of origin or the postfarm transport vehicle, was a factor common to all 18 cattle consignments, it could have played an important role in spreading the prevalent *E. coli* O157 clone to the cattle hides. Lairage pen floors and the stunning box floor were identified as the most probable sites along the unloading-to-slaughter route at which the brisket areas of cattle hides could become contaminated.

Shiga toxin–producing *Escherichia coli* O157 can cause outbreaks and sporadic cases of serious human diseases, including hemorrhagic colitis and hemolytic uremic syndrome. Meat and meat products have been identified as vehicles for foodborne disease caused by *E. coli* O157 *(6, 7, 18).*

The contamination of meat can be considered an ultimate consequence of fecal shedding of *E. coli* O157 by healthy animals *(8, 10, 15).* Subsequently, excreted *E. coli* O157 can survive outside animal gastrointestinal tracts for considerable times in various environments, including soils *(4),* waters *(16, 21),* and animal fecal slurries *(4, 13),* and on hard surfaces including stainless steel *(12)* and plastic *(1).* The slaughter and dressing of healthy meat animals, even when high standards of good manufacturing practice and process hygiene are applied, provides many opportunities for *E. coli* O157 to contaminate resultant carcasses, either through direct contamination with fecal material from gastrointestinal tracts or indirectly via contaminated surfaces (e.g., hides, equipment, hands). However, in modern meat production plants, direct contamination of meat from animals' gastrointestinal tracts (brought about by rupture or leakage of guts) is rare. Contamination of carcasses with microorganisms originating from animals' hides and/or hooves is much more likely *(3).*

The brisket areas of cattle hides can be heavily contaminated with microorganisms *(17)* and can have *E. coli* O157 prevalences as high as 10.7 and 28.8% *(10, 19).* Therefore, there is little doubt that the extent and nature of hide contamination is a relevant issue with regard to meat microbial safety. Brisket hide contamination would normally be attributed to direct contact with previously contaminated surfaces taking place while the animal is lying down, either on the farm or in the lairage at the abattoir. Floors and other surfaces in cattle lairages can be frequently contaminated with *E. coli* O157, with an overall average prevalence in three cattle abattoirs of 7.7% recently being found *(22).* Obviously, hide contamination with *E. coli* O157 raises the question of whether the contamination originated on the farm or in the lairage facilities. When the epidemiology of *E. coli* O157 along the meat chain is considered, hide contamination within abattoir lairages is potentially of great concern because it is relatively "unlimited," i.e., the same contaminated lairage environment could spread the pathogen to animals originating from a number of farms (including those whose animals did not harbor *E. coli* O157 at lairaging). In contrast, on-farm hide contamination is more limited, as it is restricted by the pathogen's prevalence and the number of animals on a particular farm.

* Author for correspondence. Tel: +44 117 928 9410; Fax: +44 117 928 9324; E-mail: sava.buncic@bristol.ac.uk.

Molecular typing has been used to study the sources and routes for the spread of *E. coli* O157 contamination before and/or at the abattoir. In a recent study, pulsed-field gel electrophoresis (PFGE) was used to trace *E. coli* O157 isolates from cattle fecal material and from resultant carcasses in Italy, and because they were indistinguishable, it was suggested that carcass contamination was due to improper intestine removal or cross-contamination on the slaughter line *(5).* However, in that study, hide carriage of *E. coli* O157 was not investigated, and therefore hides cannot be excluded as a source of carcass contamination.

To our knowledge, there are no published data on the genetic diversity and potential origins of *E. coli* O157 isolates found on the hides of cattle. Therefore, the current study was initiated to (i) isolate and characterize *E. coli* O157 contaminating cattle hides at slaughter and (ii) investigate the most likely sources of the *E. coli* O157 isolates found.

MATERIALS AND METHODS

Cattle consignments studied. In the current study, the term *cattle consignment* denotes one group of healthy cattle delivered to the abattoir on one lorry, regardless of whether they originated from one farm or were from multiple farms (e.g., if they were previously bought at livestock markets or collection centers). Each consignment was small enough to be transported on a single lorry. In total, 73 cattle consignments comprising 584 animals delivered to one abattoir over 3 days during 1 week in July 2001 were studied: 26 cattle consignments arriving on Monday, 32 consignments arriving on Wednesday, and 15 consignments arriving on Friday. Consignment sizes ranged from 1 to 23 cattle, with a mean consignment size of 8. Each cattle consignment was transported, delivered, and lairaged under normal commercial conditions. On arrival, all cattle consignments were kept in separate pens in the abattoir lairage prior to slaughter.

Hide sampling method. Only the first animal to be slaughtered in each consignment was sampled. Consecutive consignments were sampled on each of the 3 days, and the slaughter of each consignment started only after the slaughter of the previous consignment was completed. Swabs were prepared from kitchen sponges containing no antimicrobial additives, cut in half, and placed in stomacher bags. Swabs in stomacher bags were wetted with 0.85% saline and used to rub an undefined brisket (ventral) area (ca. 30 by 30 cm) of the hide of each animal sampled. Swab samples were stored in a cool box and transported to the laboratory after sampling was completed each day. Care was taken to avoid cross-contamination inside the cool box by segregating swab samples and sealing the tops of the stomacher bags.

Isolation of *E. coli* O157. Each swab sample was enriched in 100 ml of modified tryptone soya broth (tryptone soya broth [Oxoid Ltd., Basingstoke, UK] supplemented with 1.5 g of bile salts per liter, 1.5 g of K_2HPO_4 per liter, and 20 mg of novobiocin per liter) in its stomacher bag. After the addition of modified tryptone soya broth, bags were manually massaged until all visible dirt and hair was removed from the surface of each swab and were then incubated at 37°C for 16 to 20 h. Enrichment broths were screened with VIP-EHEC immunoprecipitation assay kits (BioControl, Bothell, Wash.) following the manufacturer's instructions to detect *E. coli* O157. To isolate *E. coli* O157, immunomagnetic separation (Dynabeads anti *E. coli* O157, Dynalbiotech UK, Wirral, UK) was immediately carried out for all enrichment broths that produced a positive signal with the VIP-EHEC kits. After immunomagnetic separation, the resultant bead-bacteria complexes were streak plated onto sorbitol-MacConkey agar (SMAC; Oxoid) and CT-SMAC (SMAC containing 0.05 mg of cefixime per liter and 2.5 mg of potassium tellurite per liter; Dynalbiotech UK) plates, which were incubated at 37°C for 18 to 24 h.

Confirmation of *E. coli* O157. Typical colonies (colorless, pale, or with brown centers) chosen from the SMAC or CT-SMAC plates were tested for agglutination with a commercial latex antibody test for O157 antigen (Oxoid). Those colonies that agglutinated were streaked onto plate count agar and confirmed to be *E. coli* colonies with Gram staining, oxidase and catalase tests, and API 20E kits (bioMerieux UK Ltd., Basingstoke, UK).

Detection of Shiga toxin. Each confirmed *E. coli* O157 isolate was examined for the production of Shiga toxins (Stx) 1 and 2 with a reverse passive latex agglutination kit (VTEC-RPLA, Oxoid) following the manufacturer's instructions. Briefly, brain heart infusion (Oxoid) was inoculated from isolated colonies grown on plate count agar (Oxoid) and incubated at 37°C for 18 to 20 h with shaking at 120 rpm, and 1-ml volumes were centrifuged at 14,000 rpm (Hermle Z 160 M, Hermle Laborotechnik, Wehingen, Germany). Each resultant supernatant was diluted four times in two series (25 μl in doubling dilutions with the manufacturer's diluent) in V-form microtiter plates, and 25 μl of anti–Stx 1 or anti–Stx 2 latex agglutination reagent was added to the wells. The plates were gently agitated and left at room temperature for 20 to 24 h. Agglutination reactions, indicating the presence of Stx 1 or Stx 2, were read as instructed by the manufacturer.

PFGE. Isolated colonies obtained after overnight growth on plate count agar at 37°C were inoculated into 1-ml volumes of Luria-Bertani broth with glucose (10 g of Bacto tryptone [Difco Laboratories, Detroit, Mich.] per liter, 5 g of yeast extract per liter, 10 g of NaCl per liter, 1 g of glucose per liter [final pH of 7.0]) in screw-cap microcentrifuge tubes. After overnight growth at 37°C, bacterial cells were heat inactivated with a water bath (Grant Instruments, Cambridge, UK) for 20 min at 80°C. Inactivation was confirmed by spread plating 200-μl volumes in duplicate onto plate count agar (Oxoid) plates, which were incubated at 37°C overnight and examined for the absence of colony growth. The remaining 600-μl volumes of heat-inactivated cultures were stored at 4°C overnight, after which the heat-inactivated bacterial cells were pelleted by centrifugation (MSE Microcentaur), washed twice in 0.85% saline, resuspended in 480 μl of 0.85% saline, and equilibrated at 56°C. Bacterial suspensions were mixed with equal volumes of melted 2% agarose (Clean Cut, Bio-Rad, Hemel Hempstead, UK) and molded in disposable plug molds (Bio-Rad) (three plugs prepared for each *E. coli* O157 isolate). Bacterial cells in each set of three plugs were lysed by incubation in 2 ml of ESP buffer (0.5 M EDTA, 1% *N*-lauroylsarcosine [pH 9.0], containing 250 μg of proteinase K per ml) overnight at 56°C, followed by a second overnight incubation in fresh ESP buffer. Lysed plugs were washed six times in 4-ml volumes of TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]) before being stored at 4°C. Sections (3 mm) were cut from the plugs, and the chromosomal DNAs were digested with *Xba*I (Promega) for 14 to 16 h at 37°C in buffer D according to the manufacturer's instructions. Digestion was stopped by adding 1-ml volumes of TE buffer to the plugs, which were stored at 4°C. Digested DNA plug sections were sealed into the wells of 150-ml gels containing 1% pulsed-field gel certified agarose (Bio-Rad) in 0.5× TBE extended-range buffer (Bio-Rad). The lambda ladder PFG marker (New England Biolabs, Hitchin, UK) was used in two outside lanes and one internal

TABLE 1. *Characteristics of and epidemiological data for* E. coli *O157 isolated from the brisket areas of cattle hides from 24 separate cattle consignments*

Consignment no.[a]	No. of cattle in consignment	Day of sampling	Stx production[b]	PFGE profile (X type)
5	10	Monday	2	1
6	10	Monday	2	2
8	14	Monday	2	2
10	6	Monday	2	2
12	14	Monday	2	2
13	5	Monday	2	2
16	18	Monday	2	1
17	10	Monday	2	2
19	14	Monday	2	2
21	4	Monday	2	2
26	9	Monday	2	2
34	15	Wednesday	2	3
37	8	Wednesday	1, 2	4
38	10	Wednesday	1, 2	4
40	1	Wednesday	2	3
41	6	Wednesday	ND[c]	5
42	6	Wednesday	ND	6
45	12	Wednesday	2	2
47	3	Wednesday	2	2
49	3	Wednesday	2	2
50	1	Wednesday	2	2
57	8	Wednesday	2	2
58	14	Wednesday	2	2
72	1	Friday	ND	2

[a] Cattle consignments in the study were numbered consecutively from 1 to 73.
[b] Reverse passive latex agglutination.
[c] ND, Stx not detected.

lane for each gel. Gels were subjected to PFGE with the CHEF DRIII apparatus (Bio-Rad) in 0.5× TBE, with pulse times ramped from 2.2 to 54.2 s for 22 h at 6 V/cm with an angle of 120°, and cooled to 14°C *(14)*. Gels were stained in ethidium bromide (80 μl of 10 mg/ml solution in 1 liter of distilled water) for 30 min prior to destaining for 5 h and image capture under UV light transillumination. Band position differences, but not band intensity differences, were used to allocate PFGE profiles (X types).

RESULTS AND DISCUSSION

E. coli O157 was found on 24 of the 73 cattle hide briskets examined (32.9%), a prevalence that is higher than those previously found (22.2% *(19)* and 10.7% *(10)*). This higher *E. coli* O157 prevalence could be due to more extensive fecal shedding of *E. coli* O157 by animals in the current study (but we did not investigate this possibility), the different sizes of the areas swabbed (ca. 900 cm^2 in the current study compared with 100 cm^2 *(19)*), or the different methods used to isolate *E. coli* O157. Additionally, daily variations in *E. coli* O157 isolation rates (6.7% to 42.3% in the current study) can contribute to different prevalences of *E. coli* O157 on hides between different studies.

Only 1 of 24 *E. coli* O157 isolates showed nontypical phenotypic characteristics by fermenting sorbitol in the API 20E test but looking pale on CT-SMAC. Most (19) of the *E. coli* O157 isolates produced Stx 2, and two produced both Stx 1 and Stx 2, but three did not produce any Stx detectable by the method used (Table 1). Significant public health risks may be associated with the consumption of Stx-producing *E. coli* O157 on meat/meat products, although the public health significance of non-Stx-producing *E. coli* O157 may be low.

The 24 *E. coli* O157 isolates produced just six different PFGE profiles (Fig. 1 and Table 1). Two of the PFGE profiles were represented by one isolate each, while four PFGE profiles included multiple isolates. X2, the most prevalent PFGE profile, included 16 of the 24 *E. coli* O157 isolates (Fig. 1 and Table 1). X1 included two isolates and differed from X2 by the absence of one band. Studies involving both PFGE and ribotyping showed that one- or two-band differences in PFGE profiles among epidemiologically related *E. coli* O157 isolates can be insignificant *(2)*. Therefore, these two X1 isolates could be considered closely related to the 16 X2 isolates. The 18 X1 and X2 isolates comprised 75% of the isolates and can together be regarded as a prevalent *E. coli* O157 clone found on the briskets of the cattle hides examined.

When potential sources of hide contamination are considered, a high prevalence of one *E. coli* O157 clone on cattle hides at slaughter can be attributed to a high prevalence of that clone on all of the farms involved. The 18 farms involved were not known to have any contact and were widely spread geographically over areas including Devon, Dorset, Somerset, Wales, and Wiltshire (data not shown). *E. coli* O157 isolates from unlinked farms usually

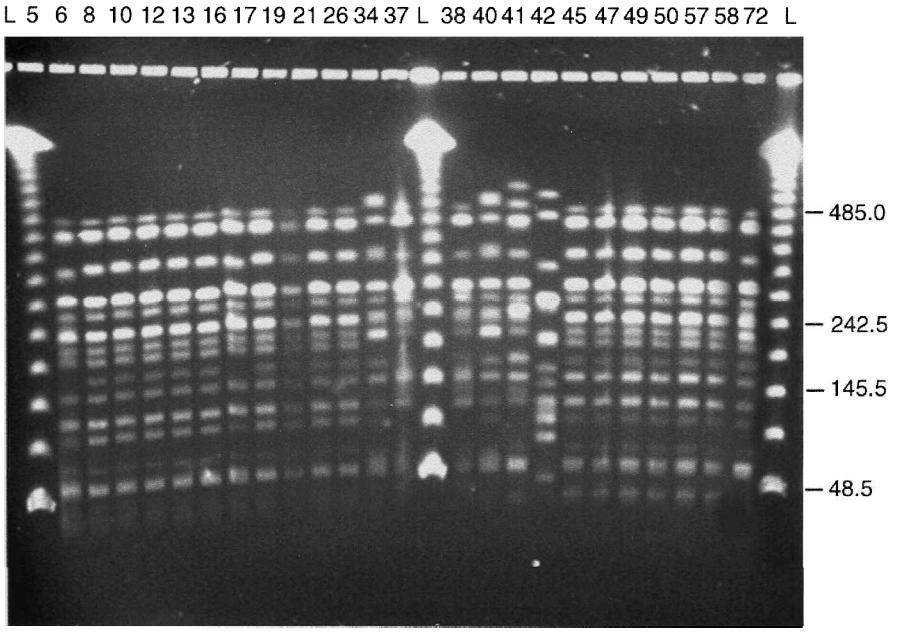

FIGURE 1. *PFGE patterns of the 24* E. coli *O157 isolates from the briskets of cattle hides. The number above each of the lanes is the cattle consignment number. Lane L, lambda ladder. Selected marker sizes (in kilobases) are indicated to the right.*

have distinguishable PFGE profiles *(2, 11, 15, 20),* although indistinguishable profiles are occasionally found *(9, 20).* Further on-farm studies are necessary to determine whether this prevalent *E. coli* O157 clone exists on all 18 farms.

Another source of the prevalent *E. coli* O157 clone on hides may have been the postfarm transport environment. The 18 cattle consignments were hauled by 17 different haulage companies (or farm lorries) over three different days (data not shown). The predominance of the single *E. coli* O157 clone with that number of different companies and lorries is unlikely.

The lairage environment was common to all 18 cattle consignments and likely contributed to the spread of the prevalent *E. coli* O157 clone on the hides. Direct hide-to-hide cross-contamination with fecal material harboring *E. coli* O157 was not a major factor in this spread because (i)

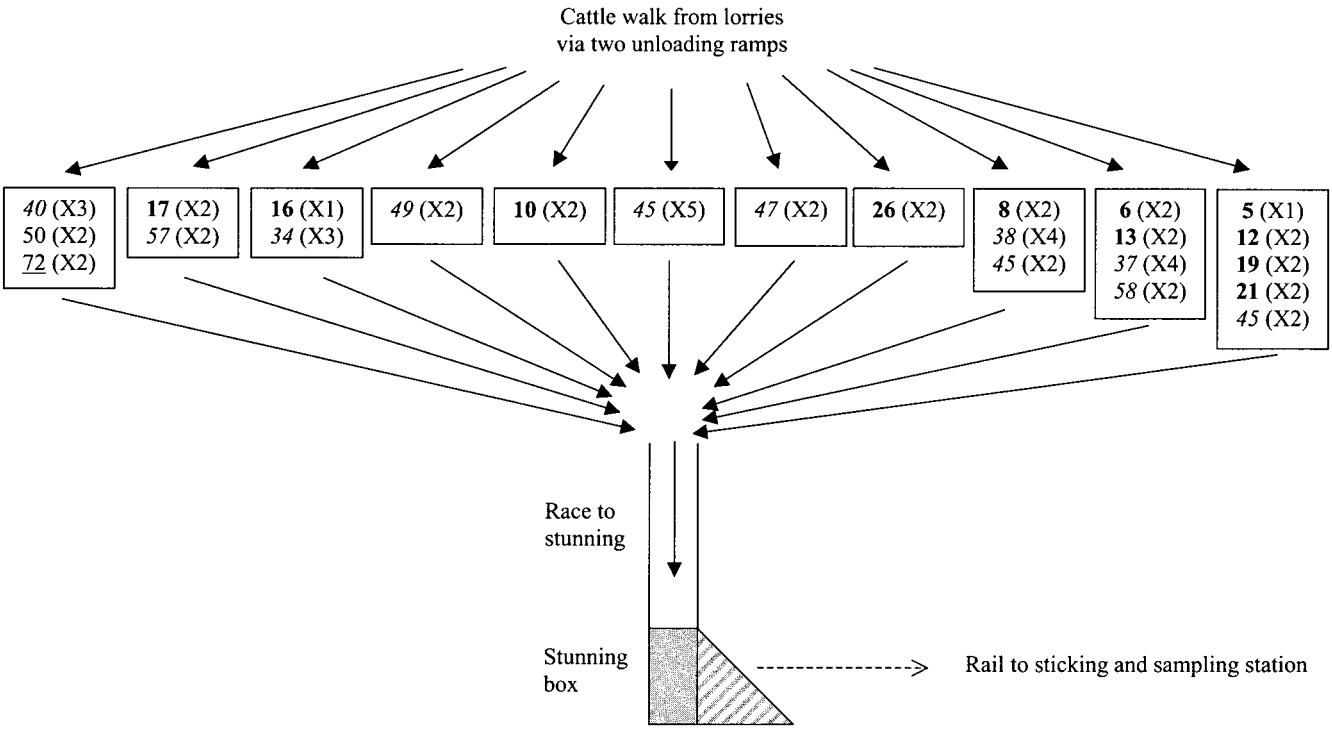

FIGURE 2. *Scheme of the lairage area showing the 24 cattle consignments in which* E. coli *O157 was found. The directions of walking cattle are indicated by solid arrows. The 11 lairage pens are indicated by enclosed boxes. Cattle consignments are numbered; the consignments from Monday are in bold, the consignments from Wednesday are in italics, and the consignment from Friday is underlined.* E. coli *O157 X-types found in each consignment are in brackets. The stunning box floor and the roll-down ramp to the hoist are shaded gray and hatched, respectively.*

cattle carrying *E. coli* O157 on their hides were penned separately (11 pens were used to house the 24 *E. coli* O157–positive consignments over the 3 days; Fig. 2); (ii) cattle consignments were killed on separate days (Fig. 2); (iii) only the first animal in each consignment was sampled; and (iv) no direct contact between the hides of animals occurred after stunning and slaughter, when carcasses were hanging on the rail. Therefore, the lairage environment is a likely source of, and a vector for, the prevalent *E. coli* O157 clone found on the contaminated hides.

Lairage pens are implicated in the spread of *E. coli* O157 from animal to animal if cattle consignments are mixed prior to slaughter *(5),* but in the current study, cattle consignments were not mixed directly prior to slaughter. However, *E. coli* O157 can contaminate lairage pen floors and stunning box floors *(22),* so brisket areas of hides may have become contaminated while cattle were lying on lairage pen floors. Moreover, brisket areas of all cattle hides sampled touched the same spot on the stunning box floor when each animal was stunned (Fig. 2), so the stunning box floor and the roll-down ramp to the hoist area is a focal point for cross-contamination of the hides. In addition, neither *Salmonella* in pig lairages nor *E. coli* O157 in cattle lairages was eliminated after routine cleaning *(22, 23).* These factors make it likely that the cattle hides were cross-contaminated with the prevalent *E. coli* O157 clone in the unloading-to-stunning area in the abattoir.

Since the contamination of hides with *E. coli* O157 can affect the microbial safety of carcass meat, further research is necessary for a better understanding of the lairage epidemiology of this pathogen and its potential control strategies.

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