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Comparison of the Molecular Genotypes of *Escherichia coli* O157:H7 from the Hides of Beef Cattle in Different Regions of North America†

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ABSTRACT

Cattle hides become contaminated with *Escherichia coli* O157:H7 via pathogen transmission in the feedlot, during transport, and while in the lairage environment at the processing facility, and the bacteria can be transferred to beef carcasses during processing. Several studies have shown that *E. coli* O157:H7 strains possessing indistinguishable restriction digest patterns (RDPs) can be isolated from distant locations. Most of these studies, however, examined RDPs from strains isolated within a single region of the United States or Canada. The experiment described in the present study was designed to identify the molecular genotypes of *E. coli* O157:H7 isolates from beef cattle hides in nine major cattle-producing regions of North America. Prevalence for *E. coli* O157:H7 in beef cattle hide samples ranged from 9 to 85%. Pulsed-field gel electrophoresis (PFGE) analysis of *Xba*I-digested genomic DNA from 1,193 *E. coli* O157:H7 isolates resulted in 277 unique RDPs. Of the 277 unique *Xba*I RDPs, 54 contained isolates collected from multiple regions. After two subsequent rounds of PFGE analysis (*Bln*I and *Spe*I), there were still many isolates ($n = 154$) that could not be distinguished from others, even though they were collected from different regions separated by large geographical distances. On multiple occasions, strains isolated from cattle hides in Canada had RDPs that were indistinguishable after three enzyme digestions from cattle hide isolates collected in Kansas and Nebraska. This information clearly shows that strains with indistinguishable RDPs originate from multiple sources that can be separated by large distances and that this should be taken into account when the source tracking of isolates is based on PFGE.

Escherichia coli O157:H7 is a foodborne pathogen that has the potential to cause severe human disease *(17).* In many instances, the source of infection with *E. coli* O157:H7 has been determined to be bovine related. Cases of hemorrhagic colitis caused by *E. coli* O157:H7 were associated with consumption of undercooked ground beef in the early 1980s *(24).* During 1992 and 1993, a ground beef–related *E. coli* O157:H7 outbreak in the United States caused hundreds of illnesses and four deaths *(29).* These events led the Food Safety and Inspection Service to declare the *E. coli* O157:H7 organism an adulterant in ground beef and to require that meat processors establish hazard analysis critical control point plans for their plants *(14).*

Several studies have shown that *E. coli* O157:H7 present on cattle hides is the major source of beef carcass contamination during processing *(4, 5, 8, 21).* Cattle hides become contaminated with *E. coli* O157:H7 via fecal-to-hide, hide-to-hide, and environment-to-hide transmission in the feedlot, during transport, and in the lairage environment at the processing facility *(1, 3, 9).* Several studies have shown that *E. coli* O157:H7 strains that possess indistinguishable restriction digest patterns (RDPs) can be isolated from distant locations *(13, 19, 22, 23, 31, 32).* However, most of these studies examined RDPs from strains isolated within a single region of the United States or Canada. Lee et al. *(19)* compared RDPs from strains collected from dairy cattle across the United States but were able to isolate only 26 strains for the comparison. Even with this relatively limited strain set, *E. coli* O157:H7 isolates from New York, Ohio, and Washington were found to have indistinguishable RDPs *(19).* In accordance with this finding, Davis et al. *(11)* have shown that *E. coli* O157:H7 strains are frequently transmitted over wide geographic distances, even on a global scale. To date, there are few data on the diversity of *E. coli* O157:H7 across the major cattle-producing regions of the United States and Canada.

The study described herein was designed to identify the molecular genotypes of *E. coli* O157:H7 isolates from beef cattle hides in nine major cattle-producing regions of North America. In addition, the hide prevalence of *E. coli* O157:H7 for beef cattle in these regions was examined.

MATERIALS AND METHODS

Hide sponge samples were obtained from fed cattle at beef processing plants in nine regions of North America during the

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TABLE 1. *Cattle-producing regions sampled*

Region	Location of region	Months of sampling
1	Western Kansas	June
2	Central and eastern Nebraska	June
3	Oklahoma panhandle, southwest Kansas, and northern Texas panhandle	July
4	Idaho, Utah, and Montana	August
5	Northeastern Colorado and western Nebraska	October
6	Washington and Oregon	August
7	Central California	September
8	Alberta, Canada	September
9	Southern Texas panhandle	October

summer and fall of 2003 (Table 1). One processing plant located within each region was chosen as the sample collection point. At each plant, 300 samples were to be collected for a total of 2,700 samples. To ensure that the cattle sampled were representative of the region, sampling was limited to lots of cattle that came from feedlots located within the region represented by the processing plant. Samples were transported (4°C) to the U.S. Meat Animal Research Center (Clay Center, Nebr.) and assayed to determine the prevalence of *E. coli* O157:H7. *E. coli* O157:H7 isolates obtained from hide samples were analyzed for relatedness by pulsed-field gel electrophoresis (PFGE).

Sample collection. Sampling with wetted sponges was done after hide opening before hide removal *(2)*. All samples were obtained with Speci-Sponges (Nasco, Fort Atkinson, Wis.) moistened with 20 ml of buffered peptone water (Difco, Becton Dickinson, Sparks, Md.). Sponges were wrung out in the bag and then removed from the bag and used to swab the hides and carcasses. The hide sample was collected by swabbing a 1,000-cm^2 area over the plate region with five vertical and five horizontal passes (up and down or side to side is considered one pass), flipping the sponge over midway through taking the sample.

***E. coli* O157 detection.** Eighty milliliters of tryptic soy broth (Difco, Becton Dickinson) was added to the sample bags. All sample bags were incubated, subjected to immunomagnetic separation, and plated as previously described by Barkocy-Gallagher et al. *(5)*. After the plates were incubated, up to three suspect colonies were picked and tested by latex agglutination (DrySpot *E. coli* O157, Oxoid, Basingstoke, UK). Suspect colonies were those that were nonsorbitol fermenting and straw-colored on sorbitol MacConkey agar (Difco, Becton Dickinson) supplemented with 0.05 mg of cefixime per liter and 2.5 mg of potassium tellurite per liter and mauve on CHROMagar O157 (DRG International, Mountainside, N.J.) supplemented with 5 mg of novobiocin per liter and 1.0 mg of potassium tellurite per liter. PCR was used to confirm that each isolate harbored genes for the O157 antigen, the H7 flagella, and at least one of the Shiga toxins *(15)*. Isolates were maintained as frozen stocks for later use in strain typing by PFGE.

PFGE analyses. *E. coli* O157:H7 isolate molecular genotypes generated and analyzed in this study were based on PFGE separation of *Xba*I-, *Bln*I (*Avr*II)-, and *Spe*I-digested genomic DNA by methods developed by members of PulseNet (http://www.cdc.gov/ncidod/dbmd/pulsenet/pulsenet.htm). Pulsed-field gel certified agarose was obtained from Bio-Rad (Hercules, Calif.); Tris-borate-EDTA running buffer and lysozyme were purchased from Sigma (St. Louis, Mo.). *Xba*I, *Bln*I, and *Spe*I were purchased from New England Biolabs (Beverly, Mass.). *Salmonella* serotype Braenderup strain H9812 was used as a control and for standardization of gels *(16)*. Banding patterns were analyzed, and comparisons were made by Bionumerics software (Applied Maths, Sint-Martens-Latem, Belgium), employing the Dice similarity coefficient in conjunction with the unweighted pair group method by arithmetic averages for clustering. Isolates were grouped into types that likely had the same origin based on the similarities between the RDPs. Types were defined strictly as isolates that grouped together and had indistinguishable PFGE patterns (approximately 99.99% Dice similarity).

Statistical analysis. Differences of proportions were calculated by PEPI differ (PEPI software version 2, USD, Inc., Stone Mountain, Ga.).

RESULTS AND DISCUSSION

Hide samples (n = 2,591) were collected from nine commercial, fed-beef processing plants located in major cattle-producing regions of North America from June to October 2003 (Table 1). Three hundred samples were collected from each of eight regions. Because of cattle supply issues at the time of sampling, only 181 animals met the project sampling requirements in region 6.

In this study, PFGE analysis of *Xba*I-digested genomic DNA was performed for 1,193 *E. coli* O157:H7 isolates collected from cattle hide samples. This analysis detected 277 unique RDPs resulting from digestion with the *Xba*I restriction endonuclease. The numbers of unique RDPs collected from each region are given in Table 2. Of the total 277 unique *Xba*I RDPs, 54 (19.5%) contained isolates collected from multiple regions. To further distinguish the strains from those 54 RDPs, DNA digests with two additional restriction enzymes, *Bln*I and *Spe*I, were performed. After the two subsequent rounds of PFGE analysis, there was still a large population of isolates (n = 154) that had indistinguishable patterns, even though they were collected from different regions separated by distances of up to 1,400 mi. (2,253 km). The 154 strains were divided among nine RDP groups (Table 3). Within each group, all strains had indistinguishable RDPs for the three enzymes used. Two of these RDPs, MRUREG 1179 and 521821, contained isolates collected from three separate regions. On multiple occasions, strains isolated from cattle hides in Canada had RDPs that were indistinguishable from cattle hide isolates collected in Kansas and Nebraska.

In light of recent reports detailing the contribution of the lairage environment to the prevalence and levels of *E. coli* O157:H7 on the hides of cattle at slaughter *(1, 3, 28)*, it is logical to conclude that the *E. coli* O157:H7 isolates that were found to be common between different regions are not necessarily endemic in those cattle-producing regions. Cattle are frequently transported great distances for processing. It is likely that these cattle contaminate the lairage environment with strains of multiple genetic types *(1, 3, 9, 28)*. These strains subsequently are transferred to the hides of other cattle in the lairage environment.

On the contrary, it has been reported that *E. coli* O157:H7 strains with indistinguishable RDPs have been isolated

TABLE 2. E. coli *O157:H7 data by region*

	Region:									
	1	2	3	4	5	6	7	8	9	Overall
n[a]	300	300	300	300	300	181	300	300	300	2,591
Prevalence[b]	45.7 Y	72.7 X	48.7 Y	85.3 W	67.0 X	48.1 Y	9.0 Z	53.0 Y	45.3 Y	53.0
No. of isolates[c]	137	117	107	248	192	86	27	151	127	1,193
No. of RDPs[d]	44	56	31	58	32	21	12	43	43	277

[a] Number of hide samples collected from each region.
[b] Values given are the number of hide samples that were positive divided by the total number of hides sampled. Values within rows with same letter are not significantly different ($P \geq 0.05$).
[c] Number of *E. coli* O157:H7 isolates obtained from hide samples that were processed by PFGE.
[d] Number of unique *Xba*I restriction digestion patterns (RDPs) produced from region isolates.

from cattle herds separated by large distances *(13, 19, 22, 23, 31, 32).* Rice et al. *(23)* isolated *E. coli* O157:H7 strains with indistinguishable RDPs from multiple farms in the Pacific Northwest United States. The authors suggested that cattle movement between farms was the mechanism of *E. coli* O157:H7 transfer, but they were unable to definitively prove this *(23).* Davis et al. *(11)* identified a low but significant correlation between geographic distance and genetic similarity and concluded that transmission of *E. coli* O157:H7 strains occurs even over global distances. Both Davis et al. and Rice et al. *(10, 23)* listed cattle feed as a potential vehicle for *E. coli* O157:H7 transmission over large geographic distances. *E. coli* O157:H7 and *Salmonella* have been detected in feed mill and component feed samples *(10, 23).* Another potential vehicle for *E. coli* O157:H7 strain dissemination over large distances is the wild bird population. Wetzel and LeJeune *(32)* identified strains with indistinguishable RDPs isolated from wild bird feces on two separate farms. Van Donkersgoed et al. *(31)* also suggested wild birds as a transfer vehicle for their finding of *E. coli* O157:H7 strains with indistinguishable RDPs on two Alberta farms separated by 100 km.

In addition to comparing the cattle hide isolate RDPs among themselves, the *Xba*I RDPs for all 1,193 isolates were compared with the 10 most common *Xba*I RDPs in the PulseNet database of the Centers for Disease Control and Prevention (CDC) from isolates in 2003. Indistinguishable RDPs were identified for 9 of the top 10 CDC RDPs. The number of plants from which these matching RDPs were isolated ranged from one to seven (Table 4). The frequencies with which the various RDPs were identified in human clinical cases were similar to the frequencies with which we identified the RDPs in the North American cattle population. It is noteworthy that the *Xba*I RDP identified most frequently in this study (n = 127 [10.6%] collected from plants 4 and 5) was not among the top 10 strains isolated from clinical cases. Although the data presented provide only a snapshot of the *E. coli* O157:H7 strain diversity, this finding is consistent with recent studies that have reported large amounts of genetic diversity in the *E. coli* O157:H7 population associated with cattle, where

TABLE 3. *RDP (restriction digestion pattern) groups containing isolates from multiple regions*

RDP group[a]	Regions[b]
MRUREG 145	4 (6), 5 (93)
MRUREG 1143	4 (5), 5 (3)
MRUREG 1173	1 (4), 8 (20)
MRUREG 1179	1 (1), 2 (2), 8 (1)
MRUREG 1774	3 (1), 8 (1)
MRUREG 12411	4 (1), 8 (3)
MRUREG 521819	5 (1), 9 (2)
MRUREG 521821	1 (1), 2 (3), 9 (3)
MRUREG 541820	1 (1), 4 (2)

[a] Isolates within a group were indistinguishable following PFGE analysis with three enzymes (*Bln*I, *Spe*I, and *Xba*I).
[b] Regions are listed by number. The number of isolates matching an RDP group per region is given in parentheses.

TABLE 4. *Comparison of the 10 most common* E. coli *O157:H7* Xba*I RDPs from the PulseNet national* E. coli *database with those from this experiment*[a]

CDC RDPs	Ranks (%)[b]	% of isolates matching RDPs[c]	Count of regions with indistinguishable patterns[d]
EXHX01.0047	1 (9.8)	3.4	3
EXHX01.0074	2 (7.1)	5.8	7
EXHX01.0224	3 (4.4)	0	0
EXHX01.0097	4 (1.6)	0.1	1
EXHX01.0087	5 (1.5)	2.8	5
EXHX01.1343	6 (1.3)	1.0	3
EXHX01.0011	7 (0.9)	2.8	5
EXHX01.0125	8 (0.9)	0.1	1
EXHX01.0248	9 (0.6)	1.6	4
EXHX01.0079	10 (0.2)	1.7	3

[a] Data are courtesy M. Joyner, PulseNet CDC.
[b] Ranks of the frequency with which *E. coli* O157:H7 *Xba*I restriction digestion patterns (RDPs) are found in the PulseNet national *E. coli* database. Percents given are the number of isolates with indicated *Xba*I patterns submitted to PulseNet in 2003, of a total number of *Xba*I patterns submitted to PulseNet in 2003 (n = 2,563).
[c] Percentage of isolates (n = 1,193) collected in this study that match the CDC RDP patterns.
[d] Count of regions where *E. coli* O157:H7 isolates of the same RDP as the CDC RDP were isolated.

many genotypes commonly found in cattle either have not been associated with human disease or are associated at very low frequencies *(7, 18, 20).* These observations have led to the hypothesis that several *E. coli* O157:H7 strains have reduced virulence potential for humans *(7).*

Note that although two strains may have indistinguishable RDPs after digestion with one restriction endonuclease, it does not necessarily mean that those strains are identical or even related. Davis et al. *(10)* have suggested that PFGE analysis based on DNA digestion patterns of six enzymes is required to give an accurate estimation of strain relatedness. It also has been reported that for source tracking studies related to foodborne illness outbreaks, one enzyme PFGE analysis is useful only if supported by reliable epidemiological data *(6, 26).* This information clearly shows that strains with matching RDPs, even after digestion with three restriction enzymes, can originate from multiple sources, and those sources may be separated by large distances.

In analyzing the cattle hide samples for *E. coli* O157:H7 strains, the hide prevalence for *E. coli* O157:H7 also was examined. Prevalence for *E. coli* O157:H7 in sponge samples collected from beef cattle hides sampled for this study ranged from 9% in region 7 to 85% in region 4 (Table 2). Although the differences in the hide prevalence of *E. coli* O157:H7 in some regions were statistically significant, we do not believe that these differences were due to regional effects. The reason for this belief comes, in part, from the day-to-day variation observed in sampling. Large fluctuations (e.g., 79 to 27%, region 1) in *E. coli* O157:H7 prevalence in hide samples occurred from one day to the next. Every region, except for one, had at least one sampling day when the hide prevalence of *E. coli* O157:H7 was over 60% (data not shown). Region 7 (central California), the exception, was sampled for previous studies and was found to have 80 and 90% hide prevalences for *E. coli* O157:H7 (data not shown).

Circumstantial evidence has led some members of the beef industry to speculate that the prevalence of *E. coli* O157:H7 in southwestern Kansas is lower than for the rest of the country. In addition, *E. coli* O157:H7 has been isolated at a significantly higher rate from clinical cases involving patients in the northern regions of the United States than from such cases in the southern regions *(27).* Previous reports of *E. coli* O157:H7 prevalence in cattle have produced conflicting results on the issue of ''regional'' bias in *E. coli* O157:H7 prevalence *(12, 25, 30).* Dewell et al. *(12)* reported an association between geographic location and *E. coli* O157:H7 fecal prevalence in beef cattle, suggesting that cattle in central Nebraska are nine times more likely to have *E. coli* O157:H7–positive fecal samples than cattle in feedlots located in eastern Colorado. A drawback of this study was that the number of feedlot pens analyzed was quite low (four in central Nebraska and nine in eastern Colorado). As the number of locations sampled was limited, it is possible that the effect seen was not indicative of the broader population. Rivera-Betancourt et al. *(25)* collected hide samples from multiple trips to two beef processing plants. One of the processing plants was located in the southern United States, while the other was in the northern United States. A significant difference was seen in the overall hide prevalence of *E. coli* O157:H7, with the southern plant having a higher prevalence than the northern plant. However, when the data were analyzed by month, hide prevalence differed significantly for only one of the five months. In 1999, the U.S. Department of Agriculture's National Animal Health Monitoring System surveyed fecal samples from beef cattle feedlots for the presence of *E. coli* O157:H7 *(30).* The National Animal Health Monitoring System study sampled feedlots from 11 of the top cattle-feeding states. For the analysis of geographic differences, the 11 states were grouped into three regions: northern, middle, and southern. The report stated that there was no geographic trend identified in the percentage of pens with samples that were culture positive for *E. coli* O157:H7. With the day-to-day and seasonal variation of *E. coli* O157:H7 hide prevalence on beef cattle hides, large populations of cattle would need to be sampled over an extended time period to accurately assess regional variation.

In summary, *E. coli* O157:H7 was recovered from the hides of cattle in nine major beef production regions of North America. Several of the isolates collected were of indistinguishable RDPs, even though they came from cattle separated by hundreds of miles. This issue must be taken into account when the source tracking of isolates is based on PFGE analysis.

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