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Diversity, Frequency, and Persistence of *Escherichia coli* O157 Strains from Range Cattle Environments†

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Genetic diversity, isolation frequency, and persistence were determined for *Escherichia coli* O157 strains from range cattle production environments. Over the 11-month study, analysis of 9,122 cattle fecal samples, 4,083 water source samples, and 521 wildlife fecal samples resulted in 263 isolates from 107 samples presumptively considered *E. coli* O157 as determined by culture and latex agglutination. Most isolates (90.1%) were confirmed to be *E. coli* O157 by PCR detection of intimin and Shiga toxin genes. Pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested preparations revealed 79 unique patterns (*Xba*I-PFGE subtypes) from 235 typeable isolates confirmed to be *E. coli* O157. By analyzing up to three isolates per positive sample, we detected an average of 1.80 *Xba*I-PFGE subtypes per sample. Most *Xba*I-PFGE subtypes (54 subtypes) were identified only once, yet the seven most frequently isolated subtypes represented over one-half of the *E. coli* O157 isolates (124 of 235 isolates). Recurring *Xba*I-PFGE subtypes were recovered from samples on up to 10 sampling occasions and up to 10 months apart. Seven *Xba*I-PFGE subtypes were isolated from both cattle feces and water sources, and one of these also was isolated from the feces of a wild opossum (*Didelphis* sp.). The number of *Xba*I-PFGE subtypes, the variable frequency and persistence of subtypes, and the presence of identical subtypes in cattle feces, free-flowing water sources, and wildlife feces indicate that the complex molecular epidemiology of *E. coli* O157 previously described for confined cattle operations is also evident in extensively managed range cattle environments.

Escherichia coli O157 has become a significant public health concern with a worldwide distribution (3, 9). Although the majority of *E. coli* O157-related human disease in the United States is estimated to be food borne (27), other forms of transmission (waterborne, animal-to-person, and person-to-person) can occur (3, 9). Cattle feces have been implicated as a main source of contamination in waterborne and food-borne *E. coli* O157 outbreaks and sporadic infections (3, 9). Therefore, significant resources have been devoted to determining the epidemiology and ecology of *E. coli* O157 in cattle production environments.

Molecular techniques for genotyping or subtyping *E. coli* O157 and other pathogens have been used to investigate the sources of the organisms in outbreaks of human disease (10). These techniques, particularly pulsed-field gel electrophoresis (PFGE), also have been used in investigations of *E. coli* O157 in cattle production environments (12, 20, 25, 31, 35, 39). The ecology and molecular epidemiology of *E. coli* O157 in cattle operations appear to be complex (21, 32). Several PFGE subtypes can be found in a single cattle operation, but some *E. coli* O157 subtypes seem to predominate (39). Subtypes can persist in bovine production environments for more than 1 year, and indistinguishable subtypes have been detected in the feces of bovine and nonbovine species, as well as in environmental niches, such as water and feed (35, 39).

Although there is evidence of diversity and persistence of *E. coli* O157 subtypes in cattle environments, subtype-specific studies of United States cattle operations thus far have been almost exclusively focused on the intensively managed beef feedlot and dairy industries (12, 16, 21, 25, 31, 35, 39). Feedlot and dairy cattle are important sources of beef, yet they represent less than one-half of the total live cattle in the United States (28). Beef cattle production in the United States generally consists of three industry segments: cow-calf, stocker, and feedlot. Cow-calf and stocker cattle, as well as some dairy cattle, are reared primarily in range- or pasture-based environments. Laegreid et al. (24) discussed differences between extensively managed range cattle operations and intensively managed and confined systems and the fact that there may be different exposure and transmission mechanisms for *E. coli* O157 in different systems. Feed sources, wildlife exposure, water sources, animal density, and other environmental and management factors, which can vary between cattle operations, may play a role in the epidemiology of *E. coli* O157 in cattle environments (21, 24, 32). Some fecal shedding patterns of *E. coli* O157 in feedlot cattle may be the result of cattle colonized before arrival (i.e., during the cow-calf or stocker phase) (16, 24). Furthermore, the vast majority of all cattle, including those from range and pasture environments, are eventually processed as beef and should therefore be included in preharvest food safety efforts. However, little is known about subtypes of *E. coli* O157 in United States range cattle environments. Therefore, the objective of this study was to determine the diversity, frequency, and persistence of *E. coli* O157 strains

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from cattle, wildlife, and water sources within range cattle production environments.

MATERIALS AND METHODS

Study sites. Two distinct range cattle production environments (approximately 100 km^2 each) were chosen as study areas based on the presence of extensive range cattle populations, as well as numerous wildlife and water sources within distinct watershed zones. The two study areas were in Kansas and Nebraska, states annually among the top five in beef production (28). Although the majority of cattle in these areas were either beef cow-calf or stocker-yearling beef calves on range or pasture (approximately 10,000 cattle), all cattle sources in the areas, including three small dairies and several small drylots and feedyards (approximately 2,000 cattle), were studied. Generally, the confined cattle were not managed as intensively as cattle in typical large dairies and feedlots. Confinement lots often were void of cattle, and/or cattle were rotated between lots and range or pasture depending on the season.

Fecal and environmental samples. We sampled cattle feces, wildlife feces, and water sources in both study areas from October 1999 to September 2000. The total number of cattle samples collected in each area was proportional to the overall cattle population in the area. All cattle-holding locations within each area were sampled every 45 to 60 days. Feces from cattle observed defecating were collected from 10 to 20% of the cattle in a group on each visit. Up to 50 g of feces per fecal pat was collected with a spoon and placed into a sterile whirl pack bag. Fresh wildlife fecal samples were collected from the ground (scat) during cattle-sampling visits and also were submitted by local hunters and trappers who collected scat and/or directly removed feces from animals harvested within the areas. The entire available volume of wildlife feces (up to 50 g) was collected in a whirl pack bag. Water samples, including sediment and biofilm (50 ml), were collected directly in sterile tubes from water sources (such as ponds, tanks, creeks, and rivers) to which cattle had access during each cattle-sampling visit and from water sources to which cattle did not have direct access but which were within the areas on the 45- to 60-day rotational basis.

Recovery of *E. coli* O157. Samples collected in the field were stored in a cooler with frozen cool packs and immediately transported by ground to the laboratory at Kansas State University for processing and culture. Briefly, 1 g of feces was removed from a well-mixed sample bag, placed in 9 ml of universal pre-enrichment broth (Difco, Inc., Detroit, Mich.) containing 15 μg of novobiocin (Sigma, St. Louis, Mo.) per ml, and vortexed. Five milliliters of water and sediment from a water source sample was added to 5 ml of double-strength universal enrichment broth with novobiocin (15 μg/ml) and vortexed. After incubation for 16 to 18 h at 37°C, all samples were vortexed again, and a swab was plated onto sorbitol-MacConkey (SMAC) plates (Difco) supplemented with cefixime (50 μg/liter) and potassium tellurite (2.5 mg/liter) (Dynal, Inc., New Hyde Park, N.Y.). The plate was streaked for isolation and incubated for 16 to 18 h at 37°C.

Following incubation, up to 10 colonies exhibiting morphology typical of *E. coli* O157 colonies (gray or pale with a darker center) were replica plated onto SMAC and blood agar plates (Remel, Lenexa, Kans.) and incubated at 37°C for 8 h. Then, the sorbitol-fermenting colonies on SMAC and corresponding colonies on blood agar were noted, and incubation was continued (total time, 24 h). All SMAC colonies that were previously found to be sorbitol negative and indole positive were checked for O157 latex agglutination by using the manufacturer's recommendations (Remel). Morphologically typical, indole-positive, non-sorbitol-fermenting colonies which were positive for O157 latex agglutination were considered presumptively to be *E. coli* O157 positive based on culture and latex agglutination (C/LA). From each C/LA-positive sample, a maximum of three isolated colonies (if that many were present) that met the C/LA criteria (each colony from the maximum of 10 colonies chosen on the basis of initial morphology) were stored on Protect beads according to the recommendations of the manufacturer (Key Scientific Products, Round Rock, Tex.) and frozen at −80°C. Isolates presumptively considered to be *E. coli* O157 based on C/LA were checked once for H7 agglutination (Remel) by using corresponding blood agar plate colonies.

Virulence genes. PCR-based methods were used to confirm that the C/LA-positive isolates were *E. coli* O157 by determining the presence of virulence genes for Shiga toxins (stx_1 and/or stx_2) and intimin (*eae*) (29). The C/LA-positive isolates were inoculated from Protect beads into 5 ml of GN broth (Difco) containing cefsulodin (10.0 mg/liter), vancomycin (8.0 mg/liter), and cefeximine (0.05 mg/liter). Cefsulodin and vancomycin were obtained from Sigma, and cefeximine was obtained from Dynal, Inc. After 12 h of incubation at 37°C, DNA was recovered from 25 μl of the GN broth by using a guanidinium thiocyanate extraction method described previously (33). The resulting eluted samples were stored at −20°C or used directly in PCR assays. The presence of the stx_1 and/or stx_2 gene was determined by separate 5′ nuclease assays by using commercially available detection kits (TaqMan *E. coli* STX1 and STX2) according to the recommendations of the manufacturer (PE Applied Biosystems, Foster City, Calif.). The ABI Prism 7700 sequence detection system (PE Applied Biosystems) was used for sample and data analysis. The presence of the *eae* gene was determined by using an *eaeA*-based *E. coli* O157:H7-specific 5′ nuclease assay under amplification conditions described previously (29). Upon completion of the PCR, the MicroAmp optical 96-well reaction plate was placed in an LS-50B PCR detection system with a 96-well microplate reader upgrade (PE Biosystems), and the presence of DNA was calculated to reflect the 99% confidence value by using the methods described previously (29). We considered C/LA-positive isolates possessing the *eae* gene and one or both of the Shiga toxin genes to be confirmed *E. coli* O157 isolates.

PFGE. All *E. coli* isolates that were confirmed to be O157 isolates were subtyped by PFGE separation of *Xba*I-digested genomic DNA by using standardized methods (7). These methods are the methods used by the PulseNet National Molecular Subtyping Network (http://www.cdc.gov/pulsenet/) for subtyping food-borne bacterial pathogens. Briefly, PFGE plugs were made by mixing cell suspensions having the appropriate optical density and equilibrated at 55°C with an agarose (Bio-Rad, Hercules, Calif.) solution that was cooled to the same temperature. For each isolate, a plug was placed in a 1.5-ml tube containing lysis buffer (7) with TE (10 mM Tris, 1 mM EDTA), sodium dodecyl sulfate, and proteinase K (Gibco BRL, Rockville, Md.). After incubation each plug was washed four times (20 min each) in 50 ml of TE. Restriction enzyme digestion was performed with *Xba*I (American Allied Biochemical, Aurora, Colo.) used according to the manufacturer's specifications. Electrophoresis was performed in a 1% agarose gel with 0.5× TBE (Bio-Rad) by using a Chef Mapper under identical electrophoresis conditions for all gels, as follows: 14°C, 6 V/cm, 5S-50S, linear 120°, 22 h. DNA bands were visualized under UV light after ethidium bromide staining. Digital images of each gel were obtained by using a Gel Doc 1000 (Bio-Rad). The PFGE banding patterns were visually examined, and each unique banding pattern was assigned a PFGE pattern number. Confirmation gels were used to verify all unique PFGE patterns and to verify all indistinguishable patterns. Isolates with indistinguishable PFGE banding patterns were considered the same subtype.

Data analysis. We entered data directly into spreadsheets and generated descriptive statistics, data tables, and figures using commercially available software (Microsoft Excel and Microsoft Access; Microsoft, Bellevue, Wash.). A Fisher's exact test was used to determine if the numbers of *Xba*I-PFGE subtypes detected per sample, given the number of isolates recovered, were different for water and cattle samples (1). The *P* value used for significance of comparisons was 0.05.

RESULTS

A total of 13,726 samples were collected from cattle feces (9,122 samples), water sources (4,083 samples), and wildlife feces (521 samples) from the two study areas over the 11-month sampling period. Ninety-two cattle samples (1.01%), 14 water samples (0.34%), and one wildlife sample (0.2%) were presumptively positive for *E. coli* O157 as determined by C/LA (Table 1). We recovered 263 isolates by collecting a maximum of three isolates per C/LA-positive sample (Table 1). Fecal samples from raccoons (230 samples), deer (141 samples), coyotes (100 samples), opossums (25 samples), birds (9 samples), and other species (16 samples) were also collected, yet the only wildlife isolates were from the feces of one wild opossum (*Didelphis virginianus*). Seven water source isolates came from cattle tanks (four samples; 0.51% prevalence), 11 water source isolates came from ponds or lakes (five samples; 0.25% prevalence), and 12 water source isolates came from free-flowing creeks or streams (five samples; 0.41% prevalence). A total of 130 of the C/LA-positive cattle isolates were from cows, bulls, heifers, and calves on cow-calf pastures (40 of 6,762 samples; 0.59% prevalence), 127 isolates were from weaned beef calves and yearlings on pasture and/or in drylots (48 of 1,933 samples; 2.48% prevalence), and 8 isolates were

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TABLE 1. Frequency and characterization of *E. coli* O157 isolates obtained from cattle feces, water sources, and wildlife feces in midwest range cattle production environments in 1999 and 2000

Samples collected		C/LA		PCR characterization: no. of isolates positive (% of C/LA-positive isolates)				Confirmed *E. coli* O157[b]	
Type	No.	No. of presumptively positive samples (%)	No. of isolates[a]	*eae*	stx_1 only	stx_2 only	Both *stx* genes	No. of isolates (% of C/LA-positive isolates)	No. of samples (% of samples collected)
Cattle	9,122	92 (1.01)	230	215 (93.5)	11 (4.78)	47 (20.3)	154 (67.0)	212 (92.2)	82 (0.90)
Water	4,083	14 (0.34)	30	23 (76.7)	0	11 (36.7)	11 (36.7)	22 (73.3)	10 (0.24)
Wildlife	521	1 (0.20)	3	3 (100)	0	0	3 (100)	3 (100)	1 (0.20)
Total	13,726	107 (0.78)	263	241 (91.6)	11 (4.18)	58 (22.1)	168 (63.9)	237 (90.1)	93 (0.68)

[a] A maximum of three isolates were recovered from each positive sample.
[b] Confirmed to be *E. coli* O157 are those identified by C/LA and positive for intimin (*eae*) and one or more Shiga toxin genes (*stx*) by PCR.

from dairy cattle on pasture and/or in pens (4 of 427 samples; 0.94% prevalence).

The majority of the 263 isolates recovered by the C/LA method were confirmed to be *E. coli* O157 isolates by PCR detection of the *eae* gene and at least one *stx* gene. The *eae* gene was present in 241 (91.6%) of the 263 isolates (Table 1), and at least one of the *stx* genes was present in 237 (90.1%) of the isolates. We detected 11 isolates with only the stx_1 gene (4.78%), 58 isolates with only the stx_2 gene (22.1%), and 168 isolates with both *stx* genes (63.9%). Therefore, 237 isolates (90.1% of the C/LA-positive isolates) and 93 samples (0.68% of the samples collected) were confirmed to be *E. coli* O157 isolates and *E. coli* O157-positive samples, respectively (Table 1). The overall prevalence of confirmed *E. coli* O157 (0.68%) and the prevalence estimates for cattle (0.90%), water (0.24%), and wildlife (0.20%) were similar to the prevalence estimates for C/LA-positive isolates. A total of 211 of 237 isolates confirmed to be *E. coli* O157 isolates (89.0%) and 228 of 263 C/LA-positive isolates (86.7%) were positive for the H7 antigen as determined by latex agglutination.

We identified 79 unique *Xba*I-PFGE subtype patterns from 235 isolates confirmed to be *E. coli* O157 isolates from 92 samples (two isolates were not typeable despite five attempts). The majority of *Xba*I-PFGE subtypes (54 subtypes) were identified only from samples collected on one sampling occasion (Fig. 1). Most isolates of the same *Xba*I-PFGE subtype had the same Shiga toxin genes (Table 2). Eleven isolates confirmed to be *E. coli* O157 isolates (4.6%) and three *Xba*I-PFGE subtypes (3.8%) possessed only the stx_1 gene, 58 isolates (24.5%) and 19 subtypes (24.1%) possessed only the stx_2 gene, and 168 isolates (70.9%) and 57 subtypes (72.2%) possessed both genes.

For the 93 samples confirmed to be *E. coli* O157-positive samples, a single *Xba*I-PFGE subtype was recovered from 35 samples, two different subtypes were recovered from 40 samples, and in 17 samples all three isolates analyzed had unique patterns (one positive sample contained one nontypeable isolate). We identified 70 different *Xba*I-PFGE subtypes from 211 cattle isolates (82 samples), 16 subtypes from the 21 water isolates (nine samples), and two subtypes from the three wildlife isolates (one sample). We detected an average of 1.77 *Xba*I-PFGE subtypes per cattle sample (range, 1 to 3 subtypes), 2.11 subtypes per water sample (range, 1 to 3 subtypes), and two subtypes in the wildlife sample. The numbers of *Xba*I-PFGE subtypes recovered per sample (given the number of isolates recovered) were not significantly different for water and cattle samples ($P = 0.15$).

Although most isolates recovered from a single sampling visit were the same subtype, on 12 sampling dates four or more *Xba*I-PFGE subtypes were detected, and on two of these dates eight and nine different subtypes were isolated. Seven *Xba*I-PFGE subtypes were detected in both cattle fecal and water source samples, and one of these also was isolated from the wildlife sample. Two recurring subtypes were isolated from samples collected 10 months apart, and several less frequently found subtypes were isolated from samples collected more than 6 months apart (Table 2). The two most frequently isolated *Xba*I-PFGE subtypes were recovered on 10 and 9 different sampling dates, and 10 subtypes were recovered from samples collected on three or more different dates (Fig. 1). The seven most frequently isolated *Xba*I-PFGE subtypes represented over one-half of the total number of typeable isolates confirmed to be *E. coli* O157 isolates (124 of 235 isolates) (Table 2).

DISCUSSION

The observed period-prevalence estimates for *E. coli* O157 in cattle feces were similar to previous estimates for range beef

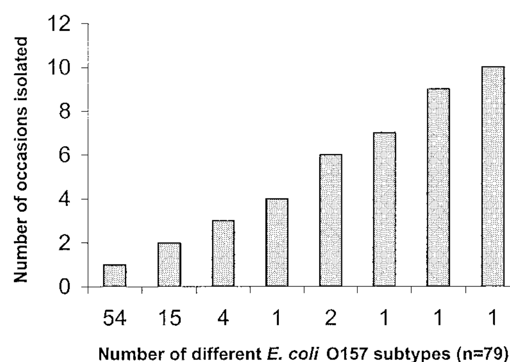

FIG. 1. Frequency distribution of different *E. coli* O157 subtypes recovered from range cattle environments in 1999 and 2000. Subtypes are *E. coli* O157 types with unique patterns after PFGE separation of *Xba*I-cleaved chromosomal DNA. The number of occasions is the number of unique sampling visits (date and location) on which a subtype was isolated.

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TABLE 2. Toxin characteristics, sources, and persistence of the most frequently isolated *E. coli* O157 *Xba*I-PFGE subtypes recovered from range cattle environments in 1999 and 2000

PFGE pattern[a]	Shiga toxin profile[b]	No. of samples (no. of isolates)	No. of samples from the following sources:			Dates on which isolates were detected		
			Cattle	Water	Wildlife	First (mo/day/yr)	Last (mo/day/yr)	No.[c]
9	stx_1 and stx_2	18 (30)	14	3	1	10/11/99	8/14/00	10
53	stx_1 and stx_2	13 (23)	13	0	0	10/13/99	8/08/00	9
41	stx_1 and stx_2	11 (22)	11	0	0	11/04/99	7/10/00	6
45	stx_1 and stx_2	10 (15)	10	0	0	10/26/99	7/17/00	7
51	stx_2 only	8 (16)	6	2	0	5/30/00	8/02/00	4
58	stx_1 and stx_2	6 (9)	6	0	0	4/27/00	8/14/00	6
52	stx_2 only	5 (9)	5	0	0	2/24/00	8/14/00	3

[a] PFGE pattern numbers are identifiers only and have no numerical relationship to each other.
[b] The predominant profile of Shiga toxin genes for each subtype.
[c] Number of different sampling dates on which isolates were recovered.

cattle obtained by using culture techniques (18, 38). A variety of culture methods, many including immunomagnetic separation (IMS) techniques, have been used to improve the sensitivity and/or detection threshold for *E. coli* O157 cultures (8, 24, 26, 36). By using improved techniques, fecal prevalence estimates higher than those traditionally reported have been described (11, 23, 41); however, in these studies the researchers investigated confined (not range or pasture) cattle. Laegreid et al. (24) reported a higher prevalence in range beef calves (6.9%) than the prevalence observed in calves of similar ages in the present study (2.48%). However, two techniques (one that included IMS) were used in parallel to increase the overall sensitivity in the previous study (24). Given the variability in cattle shedding and our choice of detection methods, we likely underestimated prevalence. However, our overall cattle C/LA prevalence value was similar to that of a previous study of range cattle in the same geographic region (1.27%), in which IMS techniques were used (38).

The low overall prevalence of *E. coli* O157 in water and wildlife samples reported here also was similar to previous results from bovine production environments (20, 38, 39). Sargeant et al. (39) detected *E. coli* O157 in 1.5% (3 of 199 samples) of water sources in similar range cattle environments. The presence of *E. coli* O157 in the feces of a wild opossum has not been reported previously. Other wildlife species known to shed *E. coli* O157 in their feces, including deer (15, 34, 37), raccoons (39), and birds (20, 39, 43), were not identified as *E. coli* O157 fecal positive in this study. However, the relatively small sample size for any one species limited our ability to detect a low prevalence of *E. coli* O157 in these species. Although our observed prevalence estimates were not unexpected, more sensitive culture methods may have resulted in higher estimates.

The similarities between C/LA and PCR-confirmed estimates of prevalence for cattle, water, and wildlife were not surprising given that overall, most C/LA-positive isolates were confirmed to be *E. coli* O157 isolates by PCR (Table 1). The observed confirmation rate was much higher than the rate recently reported in a study of *E. coli* O157 isolates from beef feedlot cattle, in which less than one-half of C/LA-positive isolates (26 of 54 isolates) were found to be *E. coli* O157 isolates by PCR (16). Other workers have reported a very high level of agreement between culture and PCR data (41). A lack of virulence genes in some isolates indicates that some C/LA techniques may result in false-positive results and in overreporting of *E. coli* O157 prevalence. The presence of both Shiga toxin genes in the majority of the *E. coli* O157 isolates, the presence of only stx_2 in a smaller percentage of the isolates, and the presence of only stx_1 in very few isolates were similar to the frequency distributions described in other studies of cattle isolates (4, 25, 35). Similar percentages of isolates that were positive after one H7 latex agglutination attempt for the C/LA-positive isolates (86.7%) and the isolates confirmed by PCR (89.0%) confirmed that the phenotypic expression of the antigen may not correlate consistently with the presence of virulence genes (13, 14, 19, 22).

PFGE of *Xba*I-cleaved DNA has been used extensively for studying the epidemiology of *E. coli* O157 in cattle environments (4, 12, 20, 35, 39). The merits and limitations of various typing methods for *E. coli* O157 have been discussed previously (6, 17, 35, 42). We chose a standardized method and classified isolates based on identical gel patterns, recognizing that further analyses of band differences may or may not indicate similarities among isolates with unique patterns (42; R. V. Goering and F. C. Tenover, Letter, J. Clin. Microbiol. **35:**2432-2433, 1997) and that including additional enzymes and/or typing methods can improve the discriminatory power (17, 30, 35). Unfortunately, because isolates were strictly categorized as either different or the same based on unique patterns, the *Xba*I-PFGE subtypes detected only once provide no information for comparing subtypes.

The number of *Xba*I-PFGE subtypes observed here (79 subtypes) is similar to the number found by an *Xba*I-PFGE comparison of 376 isolates from dairy and feedlot cattle (81 subtypes) (35) and to the number of subtypes for 343 fecal, hide, and carcass isolates from feedlot cattle (77 subtypes) (4). However, the number of subtypes observed was influenced by our decision to analyze up to three isolates per sample, as over one-half of the samples with typeable isolates contained more than one *Xba*I-PFGE subtype. Although the presence of multiple *E. coli* O157 subtypes in a cattle fecal sample was consistent with previous findings obtained in both challenge and observational cattle studies (2, 5, 12, 24), such diversity in a large number of field isolates from range cattle and water sources has not been reported previously. Furthermore, the relatively high average number of subtypes per sample ob-

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served in cattle and water source samples was not expected given that we analyzed no more than three isolates per sample. The heterogeneity within samples suggests that the number of isolates analyzed per sample may affect the precision of subtype comparisons if the number of positive samples is limited. When low prevalence and test sensitivity limit the number of positive samples that can be recovered from a source (e.g., water), it may be necessary to analyze more than one isolate per sample to minimize potential misclassification when subtypes from different sources are compared. For situations in which the number of bacterial strains is variable or unknown, there is a need for a quantitative method to determine the number of samples and the number of colonies per sample to be analyzed, similar to the model proposed by Singer et al. for avian cellulitis (40).

Although clonal turnover of *E. coli* O157 has been reported in experimentally infected cattle (2), field studies have suggested that the diversity of *E. coli* O157 strains seen in cattle operations cannot be explained entirely by mutation events (35). Given the observed overall diversity of subtypes, Rice et al. (35) suggested that the probability of detecting identical subtypes in samples from epidemiologically unrelated sources by chance alone seems to be low. This suggests that sources in cattle production environments with identical subtypes likely are linked in terms of the ecology and epidemiology of *E. coli* O157 in those settings.

The observed frequency distribution of *E. coli* O157 *Xba*I-PFGE subtypes (Fig. 1) was consistent with reports of the isolation frequency of subtypes in other cattle studies (4, 12, 35, 39). The high percentage of *E. coli* O157 *Xba*I-PFGE subtypes that were isolated on only one or two occasions during the study period (Fig. 1) could have resulted from minor alterations in the genetic material of *E. coli* O157 strains, which may or may not be maintained in a population (2, 4). It has also been suggested that rarely isolated *Xba*I-PFGE subtypes could result from rare exposures or introductions of subtypes which then fail to persist (35). In addition, some strains may be recovered at a different frequency due to differential performance of sampling and isolation techniques. The much higher isolation frequencies of relatively few *Xba*I-PFGE subtypes are consistent with the description of predominating strains on dairy farms (39), as well as in beef cattle and carcasses (4). *Xba*I-PFGE subtypes that are more frequently isolated may represent frequent common exposures, or these subtypes may be more apt to survive, be maintained, and/or propagate in either bovine or nonbovine sources. These mechanisms could explain the repeated isolation or persistence of subtypes during the study period. Previous studies have shown that subtypes are persistently isolated from confined cattle environments for up to 2 years (35, 39). Further characterization and comparisons of isolates from this and other studies may be necessary to determine if predominant strains from different environments are similar and which bacterial characteristics are associated with detection frequency and maintenance.

Despite distinctly different management and environment conditions, the diversity, frequency distribution, and persistence of *E. coli* O157 strains observed in range cattle environments in this study were not unlike those reported for confined cattle operations (12, 20, 35, 39). Direct comparisons are not possible, but it is interesting that 70 subtypes were obtained from 82 cattle samples (212 isolates) in this study, yet 81 *Xba*I-PFGE subtypes were obtained from 376 cattle samples or isolates from feedlot and dairy cattle (35). The potential mechanisms for maintenance, transmission, and distribution of *E. coli* O157 strains may be quite different in these production environments (24). Most cattle in this study were reared almost exclusively on range or pasture and were rarely confined to areas with high animal density. Cattle in range environments often have fence line contact with neighboring herds, and although uncommon, comingling may occur. In addition, wildlife and water sources that were found in this study to contain *Xba*I-PFGE subtypes indistinguishable from those found in cattle may represent common sources of exposure in neighboring range cattle herds. However, other suggested sources of *E. coli* O157 transmission between cattle herds, such as human contact, vehicle movement, cattle movement, and commercial feeds (21, 24, 35), generally occur less often in these range environments than in confined cattle settings. Recovery of indistinguishable subtypes from cattle, water, and wildlife on multiple occasions and locations may indicate mechanisms of dissemination and/or maintenance. Factors such as persistent recurring exposure, multiple sources of exposure, or subtype-specific survival and propagation mechanisms may explain the diversity and persistence of *E. coli* O157 subtypes in specific cattle environments and may be crucial for evaluating control measures.

The results of this study provide a unique description of *E. coli* O157 isolates recovered from multiple contiguous cattle sources, water sources, and wildlife in range cattle production environments. The observed number of *E. coli* O157 *Xba*I-PFGE subtypes, the frequency and persistence of specific subtypes, and the presence of indistinguishable subtypes in cattle, water, and wildlife indicate that the molecular epidemiology of *E. coli* O157 in range cattle production environments is complex. A clear description of the complex molecular epidemiology requires explicit definition of factors related to the molecular biology and micro- and macroecology of the organism. Determining the molecular epidemiology of *E. coli* O157 in segments of the cattle industry that are extensively managed and range or pasture based, as well as in cattle that are intensively managed and confined, may be crucial for evaluating strategies aimed at controlling *E. coli* O157 throughout all segments of the cattle industries.

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