Distribution of *Escherichia coli* O157:H7 within and among cattle operations in pasture-based agricultural areas

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Objective—To determine the distribution of *Escherichia coli* O157:H7 in pasture-based cattle production areas.

Sample Population—Two 100-km^2 agricultural areas consisting of 207 pasture, 14 beef-confinement, and 3 dairy locations within 24 cattle operations.

Procedure—13,726 samples from cattle, wildlife, and water sources were obtained during an 11-month period. *Escherichia coli* O157:H7 was identified by use of culture and polymerase chain reaction assays and characterized by pulsed-field gel electrophoresis (PFGE).

Results—Odds of recovering *E coli* O157:H7 from feeder-aged cattle were > 4 times the odds for cow-calf or dairy cattle. There was no difference in prevalence for pastured versus confined cattle after controlling for production age group. Number of samples collected (37 to 4,829), samples that yielded *E coli* O157:H7 (0 to 53), and PFGE subtypes (0 to 48) for each operation varied and were highly correlated. Although most PFGE subtypes were only detected once, 17 subtypes were detected on more than 1 operation. Ten of 12 operations at which *E coli* O157:H7 was detected had at least 1 subtype that also was detected on another operation. We did not detect differences in the probability of having the same subtype for adjacent operations, nonadjacent operations in the same study area, or operations in the other study area.

Conclusions and Clinical Relevance—Strategies aimed at controlling *E coli* O157:H7 and specific subtypes should account for the widespread distribution and higher prevalence in feeder-aged cattle regardless of production environment and the fact that adjacent and distant cattle operations can have similar subtypes. (*Am J Vet Res* 2004;65:1367–1376)

Escherichia coli O157:H7 is an important public health concern. Infections in humans can result in serious complications and even death.[1,2] Cattle feces are recognized as a major source of *E coli* O157:H7, and these bacteria are considered ubiquitous in cattle herds.[3,4] The fact that *E coli* O157:H7 is widespread in cattle may represent a serious public health risk as a result of contamination of meat, other foods, water, and the environment. In addition, reduced consumer confidence and recalls of contaminated product have proved costly to the beef industry. In an effort to control *E coli* O157:H7 on cattle farms or operations, tremendous resources have been devoted to determining the epidemiologic characteristics of this pathogen in cattle and cattle production environments.

Identification of factors that influence transmission and maintenance within and among cattle operations may provide points for control of *E coli* O157:H7 in cattle.[4,5] Epidemiologic and ecologic characteristics of *E coli* O157:H7 in cattle operations are not clear but are considered to be complex.[3,4] *Escherichia coli* O157:H7 are widely distributed on cattle operations, and individual-animal prevalence within a herd or pen is highly variable. Factors that may influence prevalence are often interrelated but include microbial, animal, herd, environmental, and production factors. The transitory nature of fecal shedding and age-, feed-, and time-related differences in prevalence among cattle have been documented, yet the importance or role of these factors in the epidemiologic and ecologic features of *E coli* O157:H7 remains unclear.[4] Genetically similar strains of *E coli* O157:H7 have been detected in the feces of cattle and other species as well as in water, feed, and other environmental sources on cattle operations.[6-8] Therefore, it has been suggested that instead of targeting specific cattle operations, an ecologic approach may be necessary for control of *E coli* O157:H7 in cattle production environments.[5,8,9]

Many observational studies of *E coli* O157:H7 in the United States have focused on confined feedlot or dairy cattle operations,[6,7,10-14] even though most of the US cattle are in industry segments that primarily rear

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cattle in range or pasture environments.[15] Mechanisms for maintenance, transmission, and distribution of *E coli* O157:H7 may differ in intensively managed confined cattle, compared with mechanisms for extensively managed cattle in pasture environments.[4,5] Although confined cattle share many common potential sources of exposure for *E coli* O157:H7, the scale of cattle contact, use of shared water sources, and movement of certain wildlife species among operations may differ for cattle in range or pasture environments.[4,5,8]

Knowledge of the frequency of disease (or disease agent) on the basis of location (ie, distribution) is a first and essential step in defining epidemiologic characteristics of a disease.[16] There is little information on the distribution of *E coli* O157:H7 strains within and among cattle in adjacent or nearby pasture-based operations that may have common exposures. A longitudinal study[8] of *E coli* O157:H7 in pasture-based environments was conducted by our study group, and in that report we described the genetic diversity, frequency, and persistence of *E coli* O157:H7 strains. The objective for the study reported here was to determine the distribution of *E coli* O157:H7 and *E coli* O157:H7 subtypes in cattle groups, water sources, and wildlife within and among cattle operations in pasture-based environments.

Materials and Methods

Study population—Two distinct geographic areas (1 in Kansas and 1 in Nebraska) separated by a distance of approximately 100 km were used in the study. Each of the study areas encompassed approximately 100 km^2. The areas were chosen on the basis of the predominance of grazing operations in areas with land use devoted exclusively to agriculture; both states typically are among the top 5 states for number of beef cattle.[15] Selected areas had numerous wildlife and sources of water within distinct watersheds as well as producers who were willing to participate.

Collection of samples—Samples were collected between October 1999 and September 2000 from all cattle, wildlife, and water sources that were identified within the study areas. Total number of samples collected from cattle in each area was proportional to the overall population of cattle in the study area. Samples were obtained from all cattle operations within a study area and all cattle locations (ie, pens or pastures) within each operation. We did not collect samples from cattle when they were moved outside the boundaries of the study areas. Approximately every 30 to 60 days, we collected samples from 20% of the cattle at pasture locations and 10% of the cattle confined to pens (ie, feedlots, holding lots, drylots, and dairy pens). Freshly voided feces (up to 50 g) were collected from cattle observed defecating.

Fresh fecal droppings from wildlife also were collected into sterile bags during periods of sample collection from cattle. In addition, local hunters and trappers submitted fresh droppings as well as feces removed from carcasses of wildlife harvested within the defined boundaries of the study areas. At the time of collection of samples from cattle, samples (50 mL) of water and water sediment-biofilm were collected into sterile tubes from all water sources to which cattle had direct access. In addition, samples were also obtained from water sources to which cattle did not have direct access but that were within a study area; these samples were collected on a rotational basis every 30 to 60 days. Approximately 20% to 40% of the samples collected during each sample collection period were water samples, depending on the number of water sources at that location. Sites for sample collection were recorded by use of a series of maps (aerial photographs and parcel ownership maps)[a,b] that were generated with a geographic information system.[c]

Microbial culture and molecular testing—Samples were processed and analyzed as described elsewhere.[8] Briefly, 1 g of feces was placed in 9 mL of universal enrichment broth containing novobiocin; this solution was incubated for 16 to 18 hours at 37°C. A swab specimen was then transferred directly to **sorbitol-MacConkey (SMAC)** plates that contained cefixime and potassium tellurite. Plates were streaked for isolation and incubated for 16 to 18 hours at 37°C. Then, up to 10 morphologically typical colonies were transferred to SMAC and blood-agar plates. After incubation for 8 hours at 37°C, we identified sorbitol-fermenting colonies on SMAC plates and corresponding colonies on blood-agar plates, then continued the incubation for 16 additional hours. Colonies on SMAC plates that were sorbitol-negative and indole-positive were checked for O157:H7 by use of latex agglutination. Isolates that were morphologically typical, non-sorbitol fermenting, and indole-positive and that had positive results for O157:H7 by use of latex agglutination were presumptively considered to be *E coli* O157:H7 on the basis of **microbial culture and latex agglutination (C-LA)**.[8]

We used **polymerase chain reaction (PCR)** assays to confirm that C-LA isolates were *E coli* O157:H7.[8] Isolates possessing genes for intimin (*eae*) and 1 or both shiga toxins (*stx*1 and *stx*2) were considered *E coli* O157:H7. We recovered DNA by use of an extraction method described elsewhere.[17] Detection of *stx*1 or *stx*2 genes (or both) was determined in separate 5′ nuclease assays conducted by use of a commercially available detection kit.[d] Detection of the *eae* gene was determined by use of an *eaeA*-based *E coli* O157:H7-specific 5′ nuclease assay in accordance with conditions described elsewhere.[18]

Isolates were subtyped by use of **pulsed-field gel electrophoresis (PFGE)** to separate *Xba*I-digested genomic DNA; it was performed by use of methods standardized for a surveillance system[19,e] in accordance with conditions described elsewhere.[8,19] Digital images of PFGE banding patterns were visually examined, each unique banding pattern was assigned a PFGE pattern number, and confirmation gels were used to verify identical and unique patterns. We defined isolates with identical banding patterns as having the same PFGE subtype.[8]

Data analyses—We used a commercially available geographic information system[c] to help us manage collection of samples and data, cross-reference data recorded in the field with the digital aerial photographs and parcel ownership maps, and determine the boundaries and distances between sample locations. Locations of sample collections were classified as beef confinement or dairy confinement when the cattle were confined to pens; all other locations were classified as pasture. Beef-confinement locations included all feedlots, drylots, and temporary holding pens. Cattle were initially classified into 4 production segments (cow-calf, dairy, stocker [weaned calves and yearling cattle on pasture], and feedlot [weaned calves and yearling cattle in pens]). Age was not determined for each animal; however, cattle classified as cow-calf included adult breeding stock and unweaned calves (generally < 8 months old), whereas cattle classified as stocker and feedlot cattle (ie, feeder cattle) included weaned calves and yearling cattle up to approximately 24 months of age. Water sources were classified as tank, pond, or free-flowing water (ie, creeks, streams, and rivers).

Descriptive and univariate statistics were calculated, and a value of $P \leq 0.05$ was used for all hypothesis testing. Univariate methods consisted of simple correlations for ranked data and exact tests for comparing homogeneity of

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proportions.[f,g] The Spearman **correlation coefficient (r)** for ranked data was used to investigate associations between variables with continuous or categoric (ie, count) data that were not normally distributed.[f]

Cattle prevalence was modeled as a binomial outcome by use of a mixed-effects generalized linear model that allowed for multiple random-effect variables.[h] Month and operation were included as random-effect variables because of the lack of independence between samples. Two independent variables associated with prevalence, as determined on the basis of univariate methods ($P < 0.15$), were investigated as fixed effects in the model; the 2 variables were production age groups (feeder cattle [ie, feedlot and stocker cattle] or brood stock [cow-calf and dairy]) and production environment (pasture [stocker cattle and cow-calf] or confinement [feedlot and dairy]). Initially, both of the selected variables were entered in the model and then were removed by use of a backward selection approach ($P \leq 0.05$). **Odds ratios (ORs)** and corresponding **95% confidence intervals (95% CIs)** were calculated from parameter estimates.[h] Poisson regression models were used to investigate the relationship between herd-level count data (number of samples with positive results and number of subtypes detected from cattle) and independent variables (number of samples collected and proportion of samples obtained from various types of cattle).[h]

To compare the distribution of PFGE subtypes among operations at which *E coli* O157:H7 isolates were detected, we calculated the number of shared PFGE subtypes for all such pairs of operations (66 operation pair comparisons for 12 operations at which *E coli* O157:H7 isolates were detected). A Fisher exact test was used to analyze a 3×2 contingency table that contained the number of operation pairs that were adjacent, within the same area, or in differing study areas that did or did not share at least 1 PFGE subtype.[g]

Results

Source of samples and isolates—Overall prevalence and molecular results for *E coli* O157:H7 isolates for the study have been described elsewhere.[8] In summary, 237 isolates from 93 samples were confirmed as *E coli* O157:H7, with an overall prevalence of 0.9% (82/9,122) for fecal samples obtained from cattle, 0.2% for water samples (10/4,083), and 0.2% (1/521) for fecal samples obtained from wildlife. We collected fecal samples from raccoons (n = 230), deer (141), coyotes (100), opossums (25), birds (9), and other species (16) but only detected *E coli* O157:H7 isolates in 1 fecal sample from an opossum. We detected 79 unique PFGE subtypes, with 54 isolated once, 15 isolated twice, and 10 isolated 3 or more times. Seventy subtypes were identified for 211 isolates from fecal samples of cattle, 16 subtypes for 21 isolates from water sources, and 2 subtypes for 3 isolates from 1 fecal sample obtained from wildlife. Number of PFGE subtypes per sample, given the number of isolates analyzed, was not different between fecal samples obtained from cattle and samples obtained from water sources.[8]

Study population—Samples were obtained from 5 cattle operations (131 locations) in the area in Kansas and 19 cattle operations (93 locations) in the area in Nebraska (**Table 1**). All cattle producers in both study areas agreed to participate. Cattle operations in the area in Kansas were predominately cow-calf ranches that ranged from approximately 75 to nearly 1,300 breeding cows. Up to 8,000 cattle (including cows, bulls, replacements heifers, suckling calves, stocker calves, and yearling calves) were in this area, depending on the season. The largest herd represented more than half of the cattle in this area. Four of 5 operations in the area in Kansas had at least 1 location at which cattle were in confinement pens (beef-confinement locations). The area in Nebraska had 13 operations that were predominately cow-calf operations; 3 that exclusively raised stocker calves, yearling calves, or feedlot cattle; and 3 dairies. Cow-calf operations ranged from 18 to approximately 300 breeding cows, each dairy milked between 75 and 100 cows, and the stocker-feedlot operations (confined or pasture, depending on season) ranged from 42 to approximately 1,500 cattle. Although equal in geographic size and containing a greater number of cattle operations, the area in Nebraska generally had half as many total cattle as the area in Kansas, with a maximum of approximately 4,000 cattle (depending on season). Most cattle in these 2 areas were beef cow-calf or stocker calves on pasture (up to 10,000 cattle). However, all cattle in the areas, including confined dairy cattle or beef cattle in small drylots or feedlots, were included in the study (approx 2,000 cattle). There were 207 locations classified as pasture, 14 as beef confinement, and 3 as dairy confinement. Although the addition of new cattle to operations during the study period varied between none and numerous, no cattle were exchanged directly among participating operations during the study.

Prevalence results—Overall prevalence values for all samples obtained from cattle, water, and wildlife were 1.8% (37/2,012) for beef-confinement locations, 0.5% (54/11,265) for pasture locations, and 0.4% (2/449) for dairy locations. However, univariate analysis indicated that the prevalence of *E coli* O157:H7 for samples obtained from cattle was significantly higher than for samples obtained from water sources. Prevalence for samples obtained from wildlife was not different than for samples obtained from cattle or water sources. Although samples from water sources in both areas had presumptive positive results (C-LA; 13/2,586 from the area in Kansas and 1/1,497 from the area in Nebraska), only isolates from the area in Kansas (10 samples) were confirmed as *E coli* O157:H7. Water samples with positive results for *E coli* O157:H7 were collected on 4 operations in the Kansas area (6/1,262, 1/533, 1/220, and 2/476, respectively) but not from the fifth operation (0/95). Univariate analysis indicated that the prevalence of *E coli* O157:H7 was not associated with type of water source (free flowing, 4/1,205; pond, 3/1,999; and tank, 3/782) or type of location (pasture, 7/3,493; beef confinement, 3/493; and dairy confinement, 0/97). In addition, the prevalence in fecal samples obtained from cattle was not significantly higher on days when water samples yielded positive results.

Prevalence estimates for samples obtained from cattle were 0.5% (34/6,762) for cows, bulls, heifers, and suckling calves on cow-calf pastures (ie, cow/calf), 2.1% (12/584) for samples obtained from weaned beef calves and yearlings on pasture (ie, stockers), 2.5% (34/1,349) for samples obtained from feeder cattle in

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pens (ie, feedlot), and 0.5% (2/427) for samples obtained from dairy cattle. Univariate analysis indicated significant differences in prevalence between cow-calf and stocker, cow-calf and feedlot, and feedlot and dairy operations. Prevalence values for stocker and dairy cattle ($P = 0.053$), cow-calf and dairy, or stocker and feedlot operations were not significantly different.

The regression model for prevalence in cattle investigated production age group (feeder cattle or brood stock cattle) and production environment (pasture or confinement) as fixed effects and included operation and month as random effects. Production age group was associated with prevalence, and feeder cattle were significantly ($P < 0.001$) more likely (OR, 4.47; 95% CI, 2.41 to 8.28) to yield *E coli* O157:H7 than were brood stock cattle. However, production environment was not associated with prevalence in fecal samples obtained from cattle, and confined cattle were no more likely to yield *E coli* O157:H7 than were pasture cattle (OR, 1.1; 95% CI, 0.6 to 2.0). Variance parameter estimates were 0.48 for month and 0.73 for operation with 0.84 for the residual.

At least 1 sample that yielded *E coli* O157:H7 was collected during each of the 11 months and on 45 of 216 (20.8%) days on which samples were collected. All 5 cattle operations in the Kansas area and 7 of 19 (37%) operations in the Nebraska area had at least 1 sample that yielded *E coli* O157:H7 during the study period. There was a wide range in the number of total samples collected from each operation during the study period (37 to 4,829) because of the large variation in herd size (Table 1). In addition, there was a wide range in the number of samples from each operation that yielded *E coli* O157:H7 (0 to 53). The num-

Table 1—Number of operations; locations and cattle within those operations; samples from cattle, wildlife, and water sources collected for detection of *Escherichia coli* O157:H7; and pulsed-field gel electrophoresis (PFGE) subtypes from two 100-km^2 agricultural areas.

Cattle operations (n = 24)	Locations (224)	Maximum No. of cattle*	Samples: No. collected	Samples: No. yielding O157:H7 (No. of isolates)	PFGE subtypes: No.	PFGE subtypes: Total No. for operation
Kansas						
1	43 pasture	3,200	3,754	24 (62)	23	48
	3 confined	1,650	1,075	29 (74)	28	—
2	33 pasture	1,300	1,548	3 (9)	8	10
	1 confined	200	196	1 (3)	2	—
3	15 pasture	550	642	6 (15)	7	8
	1 confined	40	13	1 (3)	2	—
4	29 pasture	1,400	1,687	6 (12)	7	8
	1 confined	160	74	1 (3)	3	—
5	5 pasture	95	198	1 (3)	1	1
Nebraska						
1	4 pasture	550	166	3 (9)	2	8
	3 confined	1,000	438	5 (12)	8	—
2	4 pasture	220	336	3 (9)	8	8
3	4 pasture	120	145	2 (6)	3	3
	1 confined	45	14	0	0	—
4	8 pasture	240	265	0	0	0
5	2 pasture	70	85	0	0	0
6	4 pasture	190	224	0	0	0
7	8 pasture	240	304	0	0	0
8	3 pasture	60	101	0	0	1
	1 dairy	140	192	1 (1)	1	—
9	3 pasture	90	117	0	0	2
	1 dairy	120	123	1 (3)	2	—
10	1 pasture	40	47	0	0	0
	1 dairy	120	134	0	0	—
11	1 pasture	50	47	0	0	0
	1 confined	50	37	0	0	—
12	3 pasture	220	253	4 (10)	5	5
	1 confined	40	11	0	0	—
13	4 pasture	160	149	2 (3)	2	2
14	8 pasture	270	344	0	0	0
	1 confined	40	73	0	0	—
15	1 pasture	35	75	0	0	0
16	13 pasture	320	426	0	0	0
	1 confined	140	75	0	0	—
17	1 pasture	25	37	0	0	0
18	4 pasture	90	136	0	0	0
19	6 pasture	110	161	0	0	0

*Represents the approximate 1-time capacity of all cattle in each type of location for that operation, depending on season; the overall 1-time capacity of an operation does not represent the sum of location types.
— = Not applicable.

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ber of samples that yielded *E coli* O157:H7 for each operation was significantly correlated with the total number of samples collected for all sample types (r, 0.62) and samples of feces from cattle (r, 0.62). Evaluation of results of the regression model revealed that the number of samples obtained from cattle that

Table 2—Distribution of *E coli* O157:H7 subtypes that were isolated 3 or more times.

PFGE subtype*	Samples: No. (No. of collection dates)	Operation†	Location (No. of pens)‡	Types of samples (No.)
9	18 (10)	K1	Weaning pens (4)	Cattle (6), water (2)§
			Main pens (2)	Cattle (3)II
			West pens	Cattle (1)
		K3	Ranch base	Wildlife (1)
		K4	Weaning pens (4)	Cattle (1)
			N pasture[a]	Water (1)
			H pasture[a]	Cattle (3)
53	13 (9)	K1	V pasture	Cattle (2)
			WX pasture	Cattle (4)§
			S pasture	Cattle (1)
		K3	Base pens[b]	Cattle (1)
			HA pasture[b]	Cattle (1)
			HS pasture[c]	Cattle (1)
			NE pasture	Cattle (1)
			LS pasture[c]	Cattle (1)
		N3	H pasture	Cattle (1)
41	11 (6)	K1	WX pasture	Cattle (1)
		K2	IB pasture	Cattle (1)
		N1	S pasture	Cattle (1)
			A pasture	Cattle (2)
			Base pens	Cattle (2)
		N3	H pasture	Cattle (1)
			N pasture	Cattle (1)
		N12	N pasture	Cattle (2)
45	9 (7)	N1	Weaning pens (3)	Cattle (3)¶
			Main pens	Cattle (1)
			WR pasture	Cattle (1)
			T pasture	Cattle (1)
		N1	A pasture	Cattle (1)
			Base pens	Cattle (1)
		N12	N pasture	Cattle (1)
51	8 (4)	K1	V pasture	Cattle (2), water (1)
			W pasture	Cattle (3)
			WR pasture	Cattle (1)
			WL pasture	Water (1)
58	6 (6)	K1	Main pens (4)	Cattle (4)#
			WR pasture	Cattle (1)
			U pasture	Cattle (1)
52	5 (3)	K1	West pens (1)	Cattle (1)
			Main pens (3)	Cattle (3)
		K5	N pasture	Cattle (1)
63	3 (3)	K4	Weaning pens	Cattle (1)
		N2	S pasture	Cattle (1)
		N12	N pasture	Cattle (1)
47	3 (3)	K1	Main pens	Cattle (1)
		K2	IB pasture	Cattle (1)
		N2	J pasture	Cattle (1)
107	3(3)	K1	WL pasture	Cattle (1)
			V pasture	Cattle (1)
		K3	NE pasture	Cattle (1)

*Numbers for PFGE subtypes are for identification purposes only and do not indicate a numeric relationship. †Letters represent study area (Kansas [K] or Nebraska [N]) and numbers represent cattle operation within area (there were 5 operations in Kansas and 19 operations in Nebraska). ‡Pen and pasture locations were uniquely identified for each operation. §Represents the same group of cattle at the same location on 2 collection dates. IIRepresents different groups of cattle at the same location on 3 collection dates. ¶Represents different groups of cattle at the same location on 2 collection dates. #Represents different groups of cattle at the same location on 4 collection dates.

[a,b,c]Locations with the same superscript letter held the same group of cattle on 2 collection dates.

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yielded *E coli* O157:H7 per herd was associated with number of samples collected from cattle and was associated but not significantly ($P = 0.08$) with the proportion of samples collected from feeder cattle.

Results of PFGE—Fifteen PFGE subtypes of *E coli* O157:H7 were isolated only from samples obtained in the Nebraska area (24 total subtypes), 55 were isolated only from samples obtained in the Kansas area (64 total subtypes), and 9 subtypes were isolated from samples from both study areas **(Table 2)**. Of the 211 PFGE-characterized isolates from cattle, 118 were from pastured cattle, 89 from beef-confinement cattle, and 4 from dairy cattle. In both areas, the number of PFGE subtypes detected per operation varied greatly (0 to 48). The number of subtypes detected per operation was significantly correlated with the total number of samples collected (*r*, 0.63) and number of samples that yielded *E coli* O157:H7 (*r*, 0.98). Similarly, the number of subtypes from cattle per operation was positively correlated with the number of samples collected from cattle (*r*, 0.63) and the number of samples obtained from cattle that yielded *E coli* O157:H7 (*r*, 0.98). Regression analysis of cattle data indicated that the number of subtypes per herd was associated with the number of samples collected from cattle but not the proportion of samples collected from feeder cattle.

Seventeen PFGE subtypes were detected on multiple operations. Ten of 24 operations and 10 of 12 operations in which *E coli* O157:H7 was detected had at least 1 PFGE subtype that also was detected on another operation. Eight of these 10 operations shared PFGE subtypes with operations in both study areas, whereas 2 shared subtypes only with other operations within the same study area. We did not detect significant differences in the probability of sharing a PFGE subtype for adjacent operations, nonadjacent operations in the same study area, or operations in the other study area.

Of the 16 PFGE subtypes detected in 10 water samples that yielded *E coli* O157:H7, 7 also were detected in samples obtained from cattle. One subtype was detected in samples obtained from water, cattle, and wildlife (Table 2). On 3 days of sample collection, the same PFGE subtype was isolated from water and fecal samples of cattle with access to that water source. On 1 day of sample collection, 3 PFGE subtypes were detected from a large pond and 4 subtypes were detected from cattle in that pasture, yet only 1 of these subtypes was isolated from both sources. Two creeks, which were potentially shared by cattle groups on more than 1 operation, had 2 and 4 PFGE subtypes, respectively, on the same day. No samples from cattle that had access to these creeks yielded *E coli* O157:H7 during the study period, even though 1 of the subtypes isolated from these water sources (subtype 9) was detected in cattle at multiple other locations (Table 2). Tanks in 2 pens in the same feedlot were found to contain the same PFGE subtype on subsequent sample collections; 1 tank was shared between 2 pens that both contained cattle shedding that subtype.

Both PFGE subtypes detected in a fecal sample from an opossum also were detected in fecal samples from cattle in the same study area. Although 1 of these subtypes was detected multiple times during the study (Table 2), the other subtype was detected only in the feces of an opossum and in the feces of heifers at that same pasture location during the next cattle sample collection (13 days later).

Specific subtypes of *E coli* O157:H7 were repeatedly isolated from water sources and samples obtained from wildlife and cattle within locations (pens or pastures), within cattle groups, within operations, or within study areas (Table 2). One PFGE subtype (subtype 9) was isolated from all 3 feedlot locations on 1 operation during a 10-month period, yet it was not isolated from any of the 33 pasture locations on that operation. This same subtype was isolated from an opossum (but not from cattle or water) on another operation that was more than 6.5 km away. At a third operation, this subtype was again isolated on consecutive days of sample collection from newly weaned heifers in a pen and from creek water in a pasture more than 5 km away; that pasture was used for a distinctly different group of cattle. Heifers that were shedding subtype 9 in a drylot were subsequently detected shedding the same subtype 2.5 months later in an adjacent pasture that had previously been devoid of cattle.

Subtype 41 was detected on an operation in 2 groups of stocker calves on 2 adjacent pastures and in feedlot cattle within 0.25 km of these pastures on the same sample collection day. Six months previously, this subtype had been isolated from a cow-calf pasture of another operation that was approximately 1.5 km away (Table 2).

Subtype 45 was detected in 4 pens in 2 adjacent lots on an operation in Kansas during a 6.25-month period. This subtype was subsequently detected in 2 groups of pastured cattle (a stocker calf in a nearby pasture and a suckling calf on a pasture adjacent to the lots).

Subtype 51 was isolated from 3 cattle groups (suckling calves, stockers, and heifers) and 2 ponds on a small group of summer pastures. Subtype 58 was detected in 4 pens during a 3.5-month period in the main feedlot of an operation in Kansas. It also was isolated during this time period from a stocker calf and a suckling calf that were in 2 adjacent pastures within 1.5 km of the main lot of that operation (Table 2).

Discussion

Escherichia coli O157:H7 was widely distributed in these areas that were predominately used for pasture-based cattle production because isolates were recovered from 40 locations on 12 cattle operations during all 11 months of the study. In addition, *E coli* O157:H7 isolates were detected in samples from several types of cattle (eg, beef calves, stockers, feedlot cattle, adult beef cows, and dairy calves), water sources (ie, ponds, tanks, and creeks), and wildlife (ie, opossum).

The geographic areas and cattle operations included in the study were not chosen randomly, but they were in areas with a large number of cow-calf operations and pasture-based cattle operations.[15] The emphasis of the study was on pasture environments, and most of the cattle in the study were reared in these environments. However, excluding confined cattle

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would not have given us a true assessment of a study area. Confined cattle were not managed as intensively as typical large dairy and feedlots. Confinement pens often were void of cattle, and producers often rotated cattle between pens and pasture, depending on the season. Samples from confined cattle were collected at a lower rate than samples from other cattle so that small geographic locations with high cattle density (ie, pens) were not overrepresented in the overview of the study area. However, the relatively high prevalence in beef-confinement locations resulted in a considerable number of *E coli* O157:H7 isolates recovered from relatively few locations. In addition, a large proportion of samples, isolates, and subtypes were from the largest operations (Table 1).

The observed prevalence of *E coli* O157:H7 was low but not dissimilar to other estimates of *E coli* O157:H7 prevalence for individual cattle, cattle herds, and environmental sources.[6,7,20,21] We almost certainly would have detected higher prevalence and distribution values by including immunomagnetic separation or other laboratory techniques that improve the detection of *E coli* O157:H7.[22-24] Differences in prevalence for various samples and types of cattle that we found by use of univariate analysis were similar to those reported in the literature,[3,4] although our power to detect significant differences was limited. Differences in prevalence could have resulted from differences in frequency or concentration of shedding.

Age, season, and factors such as diet and production environment have been associated with differences in prevalence.[3,4] However, these factors are often highly interrelated, and it can be difficult to determine the specific factor or factors responsible for differences in prevalence.[4] The model of prevalence in cattle used in the study reported here allowed us to compare prevalence estimates for various production environments and types of cattle while recognizing the nonindependent nature of the samples. Including month and operation as random effects allowed us to control for potential seasonal and herd effects. Analysis of our data suggested that a particular age cohort was associated with higher prevalence regardless of the production environment, season, and herd. Because samples obtained from feeder cattle were > 4 times more likely to yield *E coli* O157:H7 than samples obtained from cow-calf and dairy cattle, it appears that this age cohort, whether in pastures or feedlot pens, may represent the most important source of *E coli* O157:H7 in predominately pasture-based cattle areas. It has been reported in other studies[5,25] that many calves shed *E coli* O157:H7 at weaning and that weaning is associated with an increase in prevalence. Calves in our study that were weaned and placed in pastures or feedlot-drylot pens had a similar prevalence of *E coli* O157:H7. The management of calves during weaning and post-weaning placement in feedlots or pastures may be a critical period for control of *E coli* O157:H7 in cattle.

Although *E coli* O157:H7 is considered ubiquitous in cattle herds, studies[3,4] have revealed that *E coli* O157:H7 may not be detected in all herds. Lack of detection may be attributable to transient shedding in cattle.[4,26] We found that the probability of detecting *E coli* O157:H7 and multiple subtypes was highly dependent on the number of samples collected. Transient shedding combined with longitudinal collection of samples, relatively poor diagnostic sensitivity, and an effective increase in sample size could explain these results. Our subtyping results contradict those reported in another study[13] in which investigators found no correlation between the number of subtypes detected and the number of samples collected or the duration of sample collection.

Pulsed-field gel electrophoresis techniques have been widely used in investigations of the molecular epidemiologic characteristics of *E coli* O157:H7 on cattle operations.[6,7,10,13] The limitations of PFGE and potential for misclassification are recognized.[13,27-32] However, the number of PFGE subtypes identified in the study reported here reveals the genetic diversity of *E coli* O157:H7 in pasture-based environments. Multiple subtypes for each cattle operation, multiple subtypes for each date of sample collection, infrequent detection of most subtypes, and repeated isolation of relatively few subtypes are consistent with results of other studies.[4,6,7,10,13] Similar genetic diversity of *E coli* O157:H7 has been reported[6,7,10,13] for confined cattle operations. In 1 study,[13] investigators found a greater number of subtypes per herd in feedlots than in dairies. Although it is difficult to compare studies of *E coli* O157:H7 because of differences in diagnostic methods, cattle populations, and strategies for sample collection, analysis of our data revealed that collection of samples from feeder-age cattle appeared to increase the number of samples that yielded *E coli* O157:H7 but not the genetic diversity of *E coli* O157:H7 for each herd. Feedlot or stocker cattle in our study were often retained within a herd, rather than being purchased from several source herds as is typical for many commercial feedlots. Additional studies are needed to determine the microbial or herd factors that explain the reason that many subtypes can be detected briefly within herds but fail to be maintained within those herds, whereas a much smaller number of subtypes appear to persist or perpetuate within a herd.

Isolation of common *E coli* O157:H7 subtypes from cattle and noncattle sources in cattle environments is consistent with other reports[6,7,10] for confined cattle operations. Detection of the same PFGE subtypes in water tanks and fecal samples obtained from cattle suggest that water may be involved in the epidemiologic characteristics of *E coli* O157:H7 in cattle production environments. *Escherichia coli* O157:H7 can disseminate through groups of cattle that use a water source that yields *E coli* O157:H7.[7] We documented that feedlot cattle in adjacent pens were shedding the same subtype that was detected in a shared water tank. Water troughs can serve as a long-term reservoir and recurrent source of *E coli* O157:H7 for cattle.[33] Although *E coli* O157:H7 isolates were rarely recovered from water sources, the recurrent isolation of subtypes in feedlots throughout several months could have been a result of water tanks serving as a long-term reservoir and source of repeated exposure. Certain subtypes also were isolated from cattle and natural water sources (ponds and creeks). Because *E coli* O157:H7 can survive in flowing water (river models) for up to

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27 days,[34] the potential exists for transmission of these organisms between operations via natural water systems. For 5 of the 9 times that water sources yielded *E coli* O157:H7, samples obtained from cattle at that location did not yield *E coli* O157:H7, perhaps indicating contamination of water was from sources other than those cattle.

Detection of PFGE subtypes in opossum feces, which also were isolated from fecal samples of cattle in the same study area, indicates that some *E coli* O157:H7 strains are not specific to cattle and that wildlife could be involved in the maintenance or transmission of *E coli* O157:H7. Detection of subtype 69 in feces obtained from cattle < 2 weeks after it was isolated from the opossum at the same location could have been attributable to common exposures or interspecies transmission. It was interesting that the opossum was also shedding subtype 9 that previously and subsequently was detected on 2 adjacent operations but was never detected on the operation at which the fecal sample from the opossum yielded *E coli* O157:H7. We did not isolate *E coli* O157:H7 from fecal samples of other wildlife in this study; however, fecal samples were obtained from wildlife species that are documented to shed *E coli* O157:H7, including deer,[35] raccoons,[7] and birds.[36]

Escherichia coli O157:H7 subtypes indistinguishable by use of PFGE have been isolated from regionally distinct cattle in confinement.[7,13,37] Distinct and identical PFGE patterns have been found among herds when samples were collected from range beef calves at weaning.[5] Although most PFGE subtypes in the study reported here were detected only once (54), more than two-thirds of recurring subtypes were detected on more than 1 cattle operation (17/25). Furthermore, 10 of 12 operations at which *E coli* O157:H7 was detected had at least 1 subtype that also was detected on another operation. Local and regional transmission mechanisms or common exposures may have existed because 2 operations shared subtypes only with other operations in the same study area, whereas 8 operations shared PFGE subtypes with operations in both study areas. Local mechanisms could include shared water sources, local wildlife movement, human or vehicle movements, and fence-line contact among cattle. Contaminated common feed sources have been suggested as a possible means for regional transmission of *E coli* O157:H7 in confined cattle.[3,13] Although this would seem less likely in these predominately pasture-based areas in which cattle were typically fed little if any commercial feeds, nearly half of the isolates from cattle were from confinement locations.

Lack of an association between geographic proximity and the probability of sharing PFGE subtypes among herds could indicate that local and regional exposure or transmission mechanisms are similar. Low apparent prevalence and potential misclassification may limit the detection of important patterns or geographic clustering in studies of *E coli* O157:H7 distribution.[35] Classifying *E coli* O157:H7 strains into distinct categories on the basis of unique PFGE patterns also reduces the statistical power to investigate distributional patterns because no data are available on the relatedness among strains. Lack of data was especially evident for those subtypes detected only once, which provided no information for distribution analyses. The recurrent detection of certain PFGE subtypes (eg, 51, 45, and 58) from only 1 operation or from few locations within an operation may have been an indication of small-scale spatial clustering, such as a point-source exposure or continuing low-level circulation. Perhaps unmeasured management or environment factors would explain the reason that PFGE subtype 9 was detected only at confinement locations within 1 cattle operation, whereas subtype 51 was repeatedly isolated from a group of pastures but not pens at that same operation.

Evaluations of the geographic distribution of *E coli* O157:H7 have not generated uniform conclusions.[4] In 1 study,[37] investigators found that genetically related *E coli* O157:H7 recovered from US dairy operations were not associated with geographic region. Detection of common subtypes of *E coli* O157:H7 from cattle in various areas of the United States may be attributable to regional dissemination.[13] However, detection of indistinguishable subtypes from cattle environments with no known epidemiologic connections may or may not be indicative of epidemiologic relationships. In another study,[38] investigators determined that genetically similar isolates of *Mycobacterium bovis* from cattle could not be grouped on the basis of geographic location because of the genetic diversity of isolates, and they concluded that genetic typing without epidemiologic data would not be an effective method for trace-back studies. It has been suggested[32] that ≥ 6 restriction enzymes should be used when PFGE data of *E coli* O157:H7 are to be used to infer epidemiologic relationships. However, it also has been suggested[13] that isolating identical subtypes from epidemiologically unrelated sources by chance alone would be unlikely given the overall diversity of *E coli* O157:H7 subtypes.

Common transmission mechanisms, common exposures, or genetic relatedness of strains may explain the simultaneous detection of PFGE subtypes (eg, 45 and 41) in the same or different samples on multiple dates. Several associations may not have been recognized had only 1 isolate been analyzed per sample. This was especially evident when the number of samples that yielded *E coli* O157:H7 was limited (eg, those from water and wildlife sources) and indicates the need for quantitative methods for determining microbial sample collection strategies.

Detection of identical *E coli* O157:H7 subtypes from a cattle operation during a period of several months indicates that cattle operations can be a reservoir. Isolation of the same PFGE subtypes multiple times from the same cattle groups as they moved to various locations could indicate that subtypes are maintained in the cattle (Table 2). Some subtypes (eg, 9 and 58) were isolated from confinement locations throughout a period of several months even though there were differing groups of cattle, which could indicate environmental maintenance or recurring exposure. Lack of host specificity, reported intermittent shedding by cattle, and detection of *E coli* O157:H7 in water and other environmental sources suggest that

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cattle may not be the only reservoir on cattle operations.[4,6,39]

In the study reported here, we found that *E coli* O157:H7 can be isolated from various cattle, water, and wildlife sources, yet feeder-age cattle may represent a primary source of *E coli* O157:H7 even in predominately pasture-based production areas. Isolation of identical PFGE subtypes in natural water sources and wildlife that may move among cattle operations could explain, at least in part, the reason that various operations contained the same subtypes and some subtypes were detected only in relatively small areas within an operation. Mechanisms for distribution of *E coli* O157:H7 may be unrelated to distance because we found no difference in the probability of sharing a subtype among operations with differing spatial relationships, although our statistical power was limited. Given the distribution of *E coli* O157:H7 (and PFGE subtypes) in cattle environments, we believe that efforts aimed at controlling or tracing these bacteria should not be restricted to 1 or a few cattle operations in a local area. Furthermore, genetic subtyping by use of single-enzyme PFGE without epidemiologic information may not be a plausible method for *E coli* O157:H7 trace-back in these cattle environments, given the genetic diversity and widespread distribution.

[a]Data Access and Support Center, Kansas Geological Survey, University of Kansas, Lawrence, Kan.
[b]Nebraska Conservation and Survey Division, School of Natural Resource Sciences, Institute of Agriculture and Natural Resources, University of Nebraska, Lincoln, Neb.
[c]ArcView GIS, version 3.2, Environmental Systems Research Institute Inc, Redlands, Calif.
[d]Taqman *E coli* STX1 and STX2 Detection Kit, Applied Biosystems, Foster City, Calif.
[e]CDC-PulseNet: The National Molecular Subtyping Network for Foodborne Disease Surveillance. Available at: www.cdc.gov/pulsenet/. Accessed May 22, 2004.
[f]PROC CORR, SAS, version 8.1, SAS Institute Inc, Cary, NC.
[g]PROC FREQ, SAS, version 8.1, SAS Institute Inc, Cary, NC.
[h]PROC GLIMMIX, SAS, version 8.1, SAS Institute Inc, Cary, NC.

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