Prevalence of shiga-toxigenic *Escherichia coli* O157:H7 in adult dairy cattle

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Objective—To describe shiga-toxigenic *Escherichia coli* O157:H7 (STEC O157:H7) fecal shedding prevalence, seasonal fecal shedding patterns, and site-specific prevalence from the oral cavity, skin, and feces of dairy cattle.

Design—Cross-sectional study.

Animals—Adult dairy cattle from 13 herds in Louisiana.

Procedure—Samples were cultured for STEC O157 by use of sensitive and specific techniques, including selective broth enrichment, immunomagnetic separation, monoclonal antibody-based O:H enzyme immunoassay serotyping, and polymerase chain reaction virulence gene characterization. Point estimates and 95% confidence intervals were calculated for fecal shedding prevalence as well as site-specific prevalence from the oral cavity, skin, and feces. Logistic regression was used to assess seasonal variation and differences at various stages of lactation with respect to fecal shedding of STEC O157 in cattle sampled longitudinally.

Results—Summer prevalence in herds (n = 13) was 38.5%, with a cow-level prevalence of 6.5%. Among positive herds, prevalence ranged from 3% to 34.6%. Samples from 3 of 5 herds sampled quarterly over 1 year yielded positive results for STEC O157. In herds with STEC O157, an increase in cow-level prevalence was detected during spring (13.3%) and summer (10.5%), compared with values for fall and winter. Site-specific prevalences of STEC O157:H7 from oral cavity, skin, and fecal samples were 0%, 0.7%, and 25.2%, respectively.

Conclusions and Clinical Relevance—Our data indicated that STEC O157:H7 was commonly isolated from dairy cows in Louisiana, seasonally shed, and isolated from the skin surface but not the oral cavity of cows. (*J Am Vet Med Assoc* 2004;224:1151–1158)

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Shiga-toxigenic *Escherichia coli* O157:H7 (STEC O157:H7) is an important but uncommon human enteric pathogen that can be transmitted by various routes.[1-3] Affected humans may present with nonbloody diarrhea or hemorrhagic colitis and may develop hemolytic uremic syndrome.[1] Ruminants, particularly cattle, have been described as the primary reservoir of zoonotic STEC O157:H7.[2,3] Dairy cattle have been implicated in foodborne, waterborne, and direct contact transmission to humans. Human disease has been associated with consumption of contaminated ground meat, milk, and milk by-products obtained from dairy cattle.[4-6] Environmental contamination of fields with fecal material[7] and effluent from dairy farms[8] has also been associated with outbreaks. Results of epidemiologic investigations involving dairy cattle have indicated that dairy-associated STEC O157:H7 field isolates possess characteristics similar to those of the common enterohemorrhagic *E coli* O157:H7 clone that is associated with human disease.[9,10]

Enigmas of the epidemiology of human STEC O157 infections in the United States include a marked increase in infections associated with the summer months (seasonal variation) in general and a greater number of infections in northern states, compared with that in southern states (latitudinal variation).[5,11] During the summer, fecal shedding of the organism among cattle, including dairy cattle, is greater than at other times of the year,[12-14] and this phenomenon appears to positively correlate with the increased number of STEC O157 infections in humans.[15,16] This suggests a biologically plausible hypothesis that increased prevalence in cattle provides greater exposure risk for humans during the summer months.

Reasons for the latitudinal or geographic variation in occurrence of human STEC O157 infections are unclear and have not been investigated in depth. Reporting bias, differential immunity in the human population, and differential prevalence in animal populations could contribute to this variability. Geographic variation in prevalence among dairy cattle nationwide has not been extensively studied, although information has been gathered via National Animal Health Monitoring System surveys.[17] Understandably, most studies in dairy cattle have been conducted in regions where human STEC O157 infections are more commonly reported. Similar to the hypothesis proposed for seasonal variation, geographic differences in the number of human infections might suggest that prevalence of STEC O157:H7 among cattle populations would vary between northern and southern states and correlate with geographic differences in human disease. Available data indicate that cattle in all regions of the United States are colonized with STEC O157:H7,[16]

although Garber et al[17] reported that dairy herds in southern regions shed STEC O157:H7 more frequently than herds in northern states.

The proportion of individual dairy cattle (cow-level prevalence) colonized by STEC O157:H7 is reported to be low (1% to 5% for heifers and < 1% for adult dairy cattle). For example, in a review[18] of STEC O157:H7 in healthy cattle, cow-level fecal prevalence estimates in dairy cattle were commonly < 1% and none were > 5%. The proportion of herds (herd-level prevalence) shedding STEC O157:H7 ranged from 0% to 100%.

Insensitive detection methods were suggested as a factor contributing to the low prevalence estimates. Meyer-Broseta et al[18] proposed the use of **immunomagnetic separation (IMS)** isolation techniques to enhance diagnostic sensitivity. By use of more sensitive methods (selective enrichment and IMS isolation techniques), high-prevalence estimates in beef feedlot cattle have been reported[19]; results were often an order of magnitude higher than findings of previous studies that did not employ IMS isolation techniques. By use of selective enrichment and IMS isolation techniques, prevalence of STEC O157:H7 colonization of the oral cavity and various hide surfaces of beef feedlot cattle was higher than that associated with their feces.[20] In addition, results of studies[8,9,13,14] involving more sensitive detection methods that were conducted in Canada and Europe indicated that dairy cattle may also be colonized at higher prevalence rates than previously reported. Studies of dairy cattle in the United States have primarily used less sensitive detection methods.[18]

Epidemiologic studies have identified factors associated with STEC O157:H7 fecal shedding in cattle, but agreement between findings of these studies is lacking. The data indicated that there was no association between herd size and fecal shedding of STEC O157:H7. With the exception of the lack of a herd size effect, few significant associations have been consistently identified between STEC O157:H7 fecal shedding and types of feeds, feeding strategies, manure management, production indices, and cattle management or farm management systems.[21]

The dairy industry in Louisiana is small by national standards and was ranked 30th in the state livestock data rankings in 1998 with regard to the number of milk-producing cows.[22] However, concerns have been raised regarding surface water contamination by dairy cattle because the animals are concentrated in areas where human activities are common and water tables are high. Results of 1 study[23] suggested that dairy cattle might be a significant source of fecal coliforms, particularly *E coli*, in contaminated surface water from Louisiana watersheds associated with dairy farms.

Like most southeastern states, human STEC O157:H7 infections are less common in Louisiana, compared with the STEC O157:H7 infections reported in some northern states. The purpose of the study reported here was to describe STEC O157:H7 fecal shedding prevalence, seasonal fecal shedding patterns, and site-specific prevalence from the oral cavity, skin, and feces of adult dairy cattle in Louisiana.

Materials and Methods

Cross-sectional studies were designed to estimate cow-level and herd-level point prevalence of STEC O157:H7 fecal shedding in dairy cattle (point prevalence study), describe seasonal shedding patterns in a longitudinal dairy study of 5 herds sampled on 4 occasions during a 9-month period (longitudinal study), and estimate the site-specific point prevalence in the oral cavity, on skin of the dorsum, and in the feces of Louisiana dairy cattle (site study). From these samples, isolates and their putative virulence factors were characterized. **Pulsed-field gel electrophoresis (PFGE)** patterns were compared visually to differentiate subtypes within and among herds.

Sample population—Louisiana Cooperative Extension Services personnel identified a cross-sectional sample of farms enrolled in the Dairy Herd Improvement Association. Selection of these farms was nonrandom and made without influence of the authors of this report; dairy farms were not selected on the basis of farm practices or production indices. Farms were included in the study if they were willing to participate. None were excluded if willing to participate. Thirteen of 15 herds identified were willing to participate and enrolled. Fecal samples were obtained once during June, July, or August of 2001 for the point prevalence study. Herds were sampled in the summer to increase the probability of detecting cattle that were shedding STEC O157:H7. In the longitudinal study, fecal samples were obtained from 5 herds in February, May, July, and October of 2001. Fecal samples, skin swabs (from the dorsum of cows), and oral cavity swabs were obtained from cows in 2 herds that had high fecal prevalence in the point prevalence study to estimate 3 site prevalences of STEC O157:H7.

To determine point prevalence estimates, fecal samples were obtained from adult lactating cattle. The number of animals necessary to classify the group of lactating cattle as STEC O157:H7-negative at the 95% confidence level was determined, assuming randomization and 3% prevalence, and this was used as the sample size. In the longitudinal study, we attempted to sample 8 adult cattle from 4 stages of lactation within each of 5 herds; lactation strata included nonlactating cows and cows in early lactation (< 100 days), mid-lactation (100 to 200 days), and late lactation (> 200 days). For our study, we assumed a 305-day standard lactation period. Cattle were chosen to obtain a cross-sectional estimate of prevalence among all adult cattle on the farm and assess differences in fecal shedding among the groups. Samples were collected in February, May, July, and October. Depending on the dairy workers' preference and facilities for restraining cattle, samples were collected prior to or shortly after milking.

Sampling protocol—Approximately 20 g of feces was collected per rectum from each cow. A new palpation sleeve was used to collect each sample; samples were maintained at ambient temperature during transport to the laboratory. Sampling of the oral cavity and skin for the site study was performed, as described.[20] Cows were restrained to facilitate sample collection. For sampling of each skin and oral cavity site, 2 gauze swabs were placed together and moistened with 5 mL of sterile water. The skin or hair surface area was swabbed using consistent pressure across the dorsal midline at the base of the neck. Oral cavity samples were obtained by use of long-handled obstetric forceps that were flame-sterilized with alcohol. The buccal surfaces, buccal and lingual gingival surfaces, and the tongue were swabbed in a uniform manner to adequately and consistently sample the surface area within the oral cavity; swab samples were immediately placed in culture medium.

Bacteriologic culture of specimens for *E coli*—Culture techniques were selected that had higher sensitivity than con-

ventional enrichment and direct plating techniques.[18,19,24-26] The culture techniques have been described.[20,27] Briefly, 10 g of fresh bovine feces was incubated at 37°C for 6 hours in 90 mL of gram-negative broth[a] supplemented with cefsulodin[b] (10 mg/L), vancomycin[b] (8 mg/mL), and cefixime[c] (0.05 mg/mL). Swab samples were placed in 20 mL of 1.5X (60 g/L) brilliant green 2% bile broth[d] and incubated at 37°C for 6 hours. Immunomagnetic separation was performed on a 1-mL aliquot of the culture suspension by use of anti-*E coli* O157 beads.[e] The microscopic paramagnetic beads are coated with antibodies that selectively bind O157 lipopolysaccharide; the bead-bacteria complex is captured with a magnetic device after a series of wash steps. An aliquot (50 µL) of the bead-bacteria complex was spread plated on sorbitol MacConkey agar containing cefixime (0.05 mg/L) and potassium tellurite (2.5 mg/L) and incubated at 37°C for 18 to 24 hours. A maximum of 3 colonies with typical STEC O157:H7 phenotypic characteristics were selected for characterization and placed into 5 mL of MacConkey broth and 2 mL of trypticase soy broth for 18 to 24 hours at 37°C.

Serotype confirmation by ELISA—Aliquots of broth culture were heat-killed at 100°C for 5 minutes and evaluated by indirect immunoassay for reactivity with anti-H7 MAb 2B7[28] and anti-O157 MAb 13B3,[29] as described.[20,27] Isolates that were reactive with MAb 13B3 (ie, that had O157-positive results via ELISA) were inoculated in trypticase soy broth and evaluated for motility via phase-contrast microscopy. Isolates that grew on sorbitol MacConkey agar (containing cefixime and potassium tellurite) and had appropriate phenotypic characteristics (ie, sorbitol-negative colonies that were 1 to 2 mm in diameter), fermented lactose in MacConkey broth (producing a yellow color change), and reacted with anti-O157 MAb 13B3 were considered to be *E coli* O157. Further classification was made by interpreting the result of the anti-H7 MAb 2B7 ELISA in conjunction with findings of phase-contrast microscopy; isolates reactive with MAb 2B7 that were also motile were considered to be *E coli* O157:H7.

Additionally, a subset of isolates was evaluated by use of gram-negative (AP80) autoidentification plates[f]; from results of reactions to 32 substrates, isolates were confirmed to be *E coli*. A single isolate from each *E coli* O157:H7-positive sample was archived in a brain heart infusion broth-glycerol suspension at –80°C for future characterization.

Characterization of isolates via polymerase chain reaction assay—Isolates were characterized by **polymerase chain reaction (PCR)** assay for rfb_{O157} and $fliC_{H7}$ and the putative virulence factors *stx*1, *stx*2, *eae*A, and *hly*A. Somatic (O157), flagellar (H7), and virulence factor gene sequences were amplified by use of previously published primer pair sequences.[30,31] Duplex PCR reactions for *stx*1 and *stx*2 and for *eae*A and rfb_{O157} were performed. Uniplex PCR reactions were run for *hly*A and $fliC_{H7}$. The PCR cycling conditions that were used have been described.[30,31]

Amplified gene products were electrophoresed on 2% agarose gels that were subsequently stained with ethidium bromide. Gel images were captured digitally, photographed, and scored. Discrete bands of the correct size for rfb_{O157} (259 bp), $fliC_{H7}$ (625 bp), *stx*1 (180 bp), *stx*2 (255 bp), *eae*A (384 bp), and *hly*A (534 bp) that were similar to the bands of the positive controls were considered positive PCR reactions. *Escherichia coli* O157:H7 isolates were considered to be STEC O157:H7 if the PCR reactions were positive for *stx*1 or *stx*2 (or both).

Molecular subtyping of isolates—All STEC O157:H7 isolates were subtyped by enzyme restriction and PFGE, as described.[32] For restriction enzyme digestion, the enzyme *Xba*I was selected initially. However, after each of 3 attempts to use this enzyme, electrophoresis revealed that enzyme digestion did not occur or was incomplete. Another enzyme, *Spe*I,[g] was used to digest DNA of isolates. After PFGE separation of *Spe*I-digested DNA, gels were stained with ethidium bromide. For epidemiologically related isolates obtained from a single herd, genetic relatedness was determined according to the criteria of Tenover et al.[33] For isolates from separate herds, a difference of > 2 electrophoretic bands was considered an indication that isolates were separate subtypes. Strict criteria are suggested for determining subtypes of epidemiologically unlinked isolates because STEC O157:H7 is considered a highly conserved clone.[26,34-36]

Statistical analyses—Prevalence estimates were calculated by dividing the number of samples with positive results by the total number of samples. The prevalence proportions and associated **confidence intervals** (Fisher's exact 95% **CIs**) were calculated by use of a statistical software calculator.[h] The longitudinal data were analyzed by use of a multivariable logistic regression model[i] to estimate the effect of sample date (season) and lactation status on the probability of STEC O157:H7 fecal shedding. We assumed that for cattle from the same herd sampled at different time points, results would be correlated, compared with results for cattle from different herds. Therefore, we used **generalized estimating equations (GEEs)** to adjust for correlation in fecal shedding between cattle within specific herds.[37,38] The repeated option was used in the logistic regression model; models were calculated with independent and exchangeable correlation matrices.[j] Wald statistics were evaluated to estimate the effect of lactation and season in the model. On the basis of the results of the initial models that included lactation strata and sample month as 4-level variables, a simpler GEE model with lactation and season collapsed into dichotomized variables (lactation, yes or no; season, warm or cold) was developed. For the 2 categories of lactation, data from nonlactating cows and cows in lactation (ie, all other cows) were grouped. For the 2 categories of season, data from May and June were grouped and data from February and October were grouped.

Results

Point prevalence study—Of the 13 herds that were included in the point prevalence study during 2001, STEC O157:H7 was isolated in 5. Cow- and herd-level point prevalence estimates and exact 95% CIs for STEC O157:H7 fecal shedding in the point prevalence study were calculated (**Table 1**). Bivariate analysis indicated that STEC O157:H7 fecal shedding status differed significantly ($P < 0.05$) by herd.

Site study—Samples from the oral cavity, skin of the dorsum, and feces were obtained from cattle in 2 herds (58 and 77 cows in herds A and B, respectively). Site-specific prevalence estimates and 95% CIs were calculated. No STEC O157:H7 isolates were obtained from the oral cavity swabs (site estimate, 0%; 95% CI, 0.0 to 2.7). In herd A, STEC O157:H7 was identified from the skin of 1 cow (site estimate, 0.7%; 95% CI, 0.0 to 4.1); the PFGE pattern obtained from this skin isolate could not be distinguished from the pattern of the STEC O157:H7 isolate detected in that cow's feces or other isolates recovered from feces of other cattle in the same herd. Shiga-toxigenic *E coli* O157:H7 was detected in feces from 12 of 58 cows in herd A and 22 of 77 cows in herd B (20.7% and 28.6%, respectively); overall, fecal prevalence was 25.2% (95% CI, 18.1 to 33.4).

Table 1—Point prevalence of fecal shedding of shiga-toxigenic *Escherichia coli* O157:H7 (STEC 0157:H7) in adult dairy cattle in 13 Louisiana herds during summer, 2001

Herd	Sample date	Herd size	Target No.	Prevalence*	95% CI
1	6/4/01	80–90	55	0/55 (0%)	0.0–6.5
2	6/4/01	80–90	55	0/54 (0%)	0.0–6.6
3	6/13/01	80–90	55	18/52 (34.6%)	22.0–49.1
4	6/25/01	240–250	90	11/100 (11%)	5.6–18.8
5	6/25/01	27	Census	0/26 (0%)	0.0–13.2
6	6/25/01	130–140	60	0/63 (0%)	0.0–5.7
7	7/3/01	130–140	60	10/63 (15.8%)	7.9–27.3
8	7/9/01	130–140	60	0/60 (0%)	0.0–6.0
9	7/9/01	130–140	60	11/61 (18.0%)	9.4–30.0
10	7/17/01	130–140	60	2/66 (3.0%)	0.4–10.5
11	7/23/01	80–90	55	0/55 (0%)	0.0–6.5
12	7/23/01	130–140	60	0/61 (0%)	0.0–5.9
13	8/20/01	130–140	60	0/75 (0%)	0.0–4.8
Cow-level prevalence estimate				52/791 (6.6%)	4.9–8.5
Herd-level prevalence estimate				5/13 (38.5%)	13.9–68.4
Cow-level prevalence (among positive herds)				52/342 (15.2%)	11.6–19.5

*Prevalence = Number of cattle with STEC O157:H7–positive fecal samples divided by the total number of cattle from which samples were collected.
CI= Confidence interval.

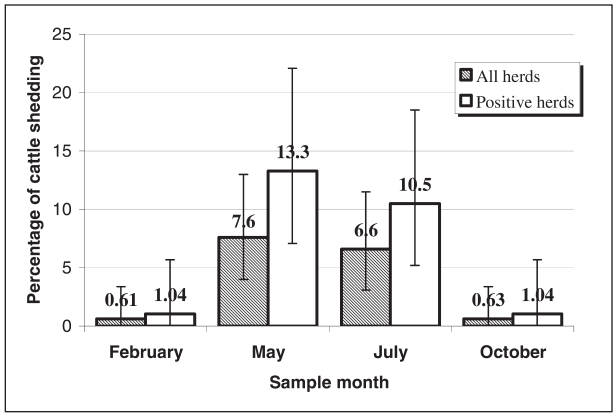

Figure 1—Prevalence of fecal shedding of shiga-toxigenic *Escherichia coli* O157:H7 (STEC O157:H7) in adult dairy cattle in 5 Louisiana herds (sampled quarterly) during summer, 2001. Error bars indicate the exact 95% confidence intervals for the proportion of cattle shedding STEC O157:H7 in their feces. Point estimates for cattle fecal shedding in all herds and the estimate from STEC O157-positive herds are indicated.

Table 2—Generalized estimating equations logistic regression (exchangeable correlation) for estimation of sampling month effect and lactation status effect on the dichotomous outcome of STEC O157:H7 fecal shedding in 5 herds of adult dairy cattle in Louisiana

Variable and category	Odds ratio	Standard error	95% CI	*P* value*
Month				
October	1.00	NA†	NA	NA
February	1.00	1.27	0.1–12.7	0.974
May	12.10	1.16	1.2–117.2	0.032
July	10.70	1.20	1.0–113.1	0.049
Lactation status				
Nonlactating	1.00	NA†	NA	NA
< 100 d	3.80	0.82	0.7–18.8	0.108
100 to 200 d	2.60	0.17	1.9–3.7	< 0.001
> 200 d	2.90	0.28	1.7–5.0	< 0.001

A model covariance structure was used to account for collection of multiple (n = 4) fecal samples from the same 5 herds over time; the exchangeable matrix was estimated with constant correlation between any 2 observation times.
*Wald statistic *P* value. †Reference category.
NA = Not applicable.

Longitudinal dairy study—Longitudinal study data were obtained from 4 groups of adult dairy cows in 5 study herds that were sampled in February, May, July, and October. The number of cattle sampled during the study was 635 (cows in early lactation [n = 147], mid-lactation [171], and late lactation [175] and nonlactating cows [142]). Three of the 5 herds had at least 1 fecal sample that was STEC O157:H7-positive on bacteriologic culture at multiple time points, whereas cattle in 2 herds had negative results throughout. The number of cattle sampled in February, May, July, and October was 163, 157, 156, and 159, respectively. The percentage of cattle shedding STEC O157:H7 in feces was greater during May and July (the warmer months), compared with findings for February and October (**Fig 1**).

In the multivariable regression model, lactation status and month significantly influenced fecal shedding of STEC O157 in adult dairy cattle (**Table 2**). When season and lactation status were dichotomized in a simplified model, the odds of STEC O157:H7 fecal shedding among cattle from which samples were obtained in the warmer months (May and July) was 10.9 times (95% CI, 2.0 to 60.2) that of cattle from which samples were obtained in colder months (October and February). Similarly, the odds of fecal shedding among lactating cows were 3 times (95% CI, 1.6 to 6.1) that of nonlactating cows. Results of a GEE-based multiple logistic regression model incorporating the independent correlation structure were similar to those derived for the exchangeable model.

Isolate characterization and subtyping—Polymerase chain reaction characterization of isolates within STEC O157-positive herds participating in the point prevalence, longitudinal, and site investigations revealed consistent virulence factor complements (**Table 3**). All isolates identified as *E coli* O157:H7 yielded positive results for *rfb*$_{O157}$, *fliC*$_{H7}$, *eae*A, *hly*A, and *stx*2 via PCR assay. None of the isolates yielded

Table 3—Composite results of pulsed-field gel electrophoresis (PFGE) subtyping of STEC O157:H7 isolates from 6 herds of adult dairy cattle in Louisiana

Herd	Data collection (composite results)	Positive*	Total† (n)	PFGE subtype	Time Interval‡
A	Point prevalence, longitudinal, and site studies	42	326	a§	12
B	Point prevalence and site studies	33	331	b‖	6
C	Point prevalence and longitudinal studies	12	159	c§	17
D	Point prevalence study	10	63	d§	NA
E	Point prevalence and longitudinal studies	3	184	e‖	22
F	Point prevalence study	2	66	f, g¶	NA
Totals		**102**	**1,129**		

*Positive = Number of STEC O157:H7–positive samples for all studies. †Total = Total number of samples tested for all studies. ‡Interval represents the greatest number of weeks between isolation of STEC O157:H7 within herds. The isolates from herds D and F were obtained on the same sampling date during the point prevalence study. §Isolates considered indistinguishable within a herd. ‖Isolates considered indistinguishable or closely related within herd. ¶Isolates from herd F considered different.
NA = Not applicable.

positive PCR assay results for *stx*1. Results of PFGE indicated that subtypes within herds were indistinguishable or closely related (clones), except in herd F from which 2 isolates were obtained that had distinct PFGE patterns and were classified as different subtypes, according to the criterion of Tenover et al.[33]

Discussion

Our data indicated that fecal shedding of STEC O157:H7 was common and varied seasonally among adult dairy cattle in a subtropical southern region of the United States, where reported STEC O157:H7 infections in humans are relatively uncommon. These findings did not support the hypothesis that STEC O157 infections in humans are rare in southern states because of low prevalence in cattle. Seasonal variation in fecal shedding of STEC O157:H7 in dairy cattle in Louisiana was also intriguing; this region is subtropical with relatively small seasonal climatic changes. This finding suggests that fecal shedding in cattle might be subject to factors other than environmental conditions (eg, temperature) alone.

Because peak fecal shedding of STEC O157:H7 has been reported during the summer months, sampling in the point prevalence study was performed during June, July, and August to increase the probability of detection of the organism and estimate maximal STEC O157:H7 fecal shedding. Results from the point prevalence study indicated that STEC O157:H7 commonly colonized adult lactating dairy cattle in Louisiana and that fecal shedding occurred at high prevalence proportions in some herds, compared with those of other herds in US studies.[18] Prevalence proportion point estimates in STEC O157:H7-positive herds ranged from 3% to 34.6%.

Fecal shedding of STEC O157:H7 has been described as episodic and endemically unstable. One of the herds included in our study was STEC O157-negative at the time that samples were obtained for the point prevalence study but was identified as STEC O157-positive during the longitudinal study; this finding illustrates that sampling of herds at 1 time point only will lead to underestimation of the herd-level prevalence.[21,39] In the point prevalence study, cow-level prevalence (6.6%) in adult lactating dairy cattle was higher than reported (approx 1%) in most US studies.[18] We attributed the higher prevalence to the increased sensitivity of the detection methods used (selective enrichment and IMS isolation techniques) in our study.

Results of the site study indicated that viable STEC O157:H7 organisms may be detected via bacteriologic culture of samples obtained from the skin of the dorsum of adult lactating dairy cattle. At the time that the swab specimens of the oral cavity and skin were obtained, fecal prevalence was 20.6% and 28.6% in herds A and B, respectively. The finding that none of the oral cavity swabs and only 1 of the skin swabs was STEC O157-positive was not consistent with data reported by Keen and Elder[20] for feedlot cattle. Variation in the bacterial populations of the oral cavity and skin surfaces among groups of cattle may account for this difference. For example, compared with feedlot cattle, bacteria within the oral cavity or on the skin of dairy cattle may competitively exclude STEC O157:H7 organisms from those sites. Also, differences in management strategies used in dairies and feedlots (eg, feeding methods and extent of exposure to manure and fecal coliforms) may promote skin and oral cavity contamination or colonization in feedlot cattle, compared with dairy cattle, and may contribute to these differences.

In the site study, STEC O157:H7 was isolated from the skin swab obtained from 1 cow; results of PFGE indicated that the skin isolate and the isolate detected in feces from the same animal were indistinguishable from the herd subtype. Detection of viable STEC O157:H7 on the skin is an important finding because of recent outbreaks associated with direct contact with livestock at farms, fairs, and petting zoos. From a public health perspective, our data suggest that skin contamination or colonization with STEC O157:H7 can occur, and proper hygiene should be emphasized when people, particularly children, have direct contact with dairy cattle (through petting or feeding, for example).[40]

The longitudinal study results indicated that there is seasonal variation in fecal shedding of STEC O157:H7 by Louisiana dairy cattle, with highest prevalence during May and July. This finding is consistent with results of other studies that indicated high prevalence of fecal shedding of STEC O157:H7 during warm summer months that tapered to a nadir during winter.

The GEE approach to logistic regression account-

ed for clustering of the observations by herd and was used to control for correlation within the 5 herds that were repeatedly assessed over time. In our study, data (n = 635) regarding each cow were categorized binomially (ie, fecal shedding, yes or no) for use in the model. Variables of interest included month of sampling (February, May, July, or October), lactation stratum (nonlactating, < 100 days of lactation, 100 to 200 days of lactation, or > 200 days of lactation), season (cold [February and October] vs warm [May and July]), and lactation status (nonlactating vs lactating). Independent and exchangeable correlation structures were modeled. The conclusions derived from the original model (in which 4-level variables for lactation and sample month were used) and the subsequent model (dichotomized variables) were consistent with respect to month and the dichotomized season variable. In our study, warm season increased the odds of fecal shedding of STEC O157:H7 in adult dairy cattle relative to the cold season. On the basis of evaluation of the CIs and management differences, data were dichotomized with regard to lactation status (designated nonlactating vs lactating categories); in the 2-level variable model, lactating cows appeared to have a significant increase in the odds of fecal shedding of STEC O157:H7, compared with that of nonlactating cows.

Although the number of clusters required to obtain consistent results in GEE-based multiple logistic regression was not determined, the number of data clusters in our study (5 clusters or herds) might not have been sufficiently large to provide asymptotic normality.[41] Parameter estimates from ordinary logistic regression and GEE-based multiple logistic regression are typically similar or robust. Standard errors in the GEE-based multiple logistic regression are adjusted on the basis of the data dependence structure to give more appropriate (robust) SEs that affect inference.[42]

Differences in fecal shedding between nonlactating and lactating cows might be attributed to the effects of physiologic stress caused by lactation or different management practices used in those 2 groups of cows. Because of dietary differences, fecal pH might differ between nonlactating and lactating cows; furthermore, the 2 groups of cows might receive different proportions of long-stemmed fiber in the diet, be fed different amounts of grain per animal, and be housed under different conditions and at different animal density. The influence of such factors on fecal shedding remains to be elucidated.

The characterization data of STEC O157:H7 isolates obtained in the study reported here indicated that the isolates possessed identical virulence factor complements. Shiga-toxin 2 was detected in each isolate; compared with *stx*1, *stx*2 is considered more important in human disease.[5,43] Assuming that the PCR product represented a functional gene that would enable *E coli* O157:H7 to produce *stx*2, the isolates obtained in our study appeared to have the virulence factors necessary to cause disease (diarrhea, hemorrhagic colitis, or hemolytic uremic syndrome) in susceptible humans and were therefore potential enterohemorrhagic *E coli* O157:H7.

Although *Xba*I is considered the most discriminatory enzyme used for PFGE of STEC O157:H7, digestion of chromosomal DNA with *Xba*I failed in our study, which prompted the use of *Spe*I for macrorestriction of the isolate DNA. On the basis of PFGE of *Spe*I-macrorestricted DNA and visual interpretation, 7 isolate subtypes were identified. In 1 herd that was enrolled in the point prevalence study and underwent sampling only once, 2 isolates were obtained; each isolate was a distinct PFGE subtype (subtypes f and g) and differed in their motility characteristics (data not shown). With the exception of the herd with subtypes f and g, isolates from within each of the remaining 5 herds had indistinguishable or closely related PFGE patterns and were classified accordingly (as subtypes a, b, c, d, and e). Four of these herds were used in more than 1 of our investigations reported here, and thus isolates were obtained at various sampling dates; nevertheless, PFGE subtypes remained indistinguishable or closely related across sampling dates. Across all of our investigations, the shortest interval between obtaining the first and last isolate from a given herd was 6 weeks and the longest interval was 22 weeks.

Our PFGE findings were consistent with those of other studies[4,9,13,44] that indicate that STEC O157:H7 are often clonal within a herd and that particular subtypes can persist for months or years. Rice et al[45] suggested that dairy herds have fewer subtypes than feedlot cattle and that the number of subtypes isolated in each herd is influenced by the movement of animals into and out of the herd. In our study, a factor that may have influenced the number of subtypes identified within a herd was the number of confirmed isolates that we selected and tested from any 1 sample.[46,47] We archived 1 isolate per STEC O157:H7-positive sample and may not have detected other PFGE subtypes present in the sample.

In conclusion, our data have implications for understanding and controlling foodborne, waterborne, and direct contact transmission of STEC O157:H7. Sensitive detection methods should be used to evaluate STEC O157:H7 in dairy cattle; enhanced detection techniques may reveal important cow-level and herd-level associations with fecal shedding of STEC O157:H7. Our findings indicated that adult cattle from dairy herds in a subtropical southern state with relatively few reported STEC O157:H7 human infections shed STEC O157:H7 frequently and seasonally. Further studies to evaluate factors that influence seasonal fecal shedding in cattle and geographic variation of infections in humans are needed to better define the epidemiology of STEC O157:H7 in cattle and human populations.

[a]Gram-negative broth, Difco, Sparks, Md.
[b]Cefsulodin, Vancomycin, Sigma Chemical Co, St Louis, Mo.
[c]Cefixime, Lederle, Pearl River, NY.
[d]Brilliant green 2% bile broth, Becton Dickenson, Cockeysville, Md.
[e]Dynabeads anti-*E coli*$_{O157}$ (product listings 710.03/710.04), Dynal Biotech Inc, Lake Success, NY.
[f]Sensititre gram-negative (AP80) autoidentification plates, Accumed International, Westlake, Ohio.
[g]*Spe*I (product R0133L), New England Biolabs Inc, Beverly, Mass.
[h]PEPI 4.0, Abramson and Gahlinger, Sagebrush, Salt Lake City, Utah.
[i]PROC GENMOD, SAS release 8.2, SAS Institute Inc, Cary, NC.
[j]REPEATED option, GENMOD, SAS release 8.2, SAS Institute Inc, Cary, NC.

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