

Results of a longitudinal study of the prevalence of *Escherichia coli* O157:H7 on cow-calf farms

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Objective—To describe the frequency and distribution of *Escherichia coli* O157:H7 in the feces and environment of cow-calf herds housed on pasture.

Sample Population—Fecal and water samples for 10 cow-calf farms in Kansas.

Procedure—Fecal and water samples were obtained monthly throughout a 1-year period (3,152 fecal samples from 2,058 cattle; 199 water samples). *Escherichia coli* O157:H7 in fecal and water samples was determined, using microbial culture.

Results—*Escherichia coli* O157:H7 was detected in 40 of 3,152 (1.3%) fecal samples, and 40 of 2,058 (1.9%) cattle had ≥ 1 sample with *E. coli*. Fecal shedding by specific cattle was transient; none of the cattle had *E. coli* in more than 1 sample. Significant differences were not detected in overall prevalence among farms. However, significant differences were detected in prevalence among sample collection dates. *Escherichia coli* O157:H7 was detected in 3 of 199 (1.5%) water samples.

Conclusions and Clinical Relevance—Implementing control strategies for *E. coli* O157:H7 at all levels of the cattle industry will decrease the risk of this organism entering the human food chain. Devising effective on-farm strategies to control *E. coli* O157:H7 in cow-calf herds will require an understanding of the epidemiologic characteristics of this pathogen. (*Am J Vet Res* 2000;61:1375–1379)

During the past decade, *Escherichia coli* O157:H7 has emerged as an important public health concern. Infection of humans with this pathogen is associated with a range of clinical syndromes, including mild to severe bloody diarrhea and hemolytic uremic syndrome, and it can result in death.¹ In numerous disease outbreaks, sources of bacteria have been traced to contaminated water and foods, including beef products.^{1,3} Estimates of the annual incidence of reported *E. coli* O157:H7 infections range from 2.1 cases/100,000 people in Washington in 1987⁴ to 8 cases/100,000 people in health management organizations in the United States.⁵ Most cases occur sporadically during the summer months.^{1,4,a}

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In addition to costs directly associated with disease in humans, reports of *E. coli* O157:H7 outbreaks may lead to a loss of consumer confidence in the safety of food products. Within the agricultural industry, the cost of recalling potentially contaminated foods can be high, and the perception that meat may contain *E. coli* O157:H7 could affect export markets.

Despite the disease-causing potential of *E. coli* O157:H7 in humans, the organism does not appear to cause disease in cattle.^{6,8} Therefore, healthy cattle harboring *E. coli* O157:H7 may enter the food chain. The USDA-Food Safety Inspection Service has recognized that many foodborne human pathogens are not associated with grossly visible lesions on carcasses or organs of animals. Therefore, to ensure the safety of meat products, Hazard Analysis and Critical Control Point (HACCP) programs have been instituted in the meat-processing industry. These programs involve identification of critical points for contamination or transmission of pathogens and the establishment of control programs at these critical points to decrease risk. There is considerable interest in extending HACCP or other pathogen-control programs to farms to further minimize the risk of pathogens entering the human food chain. However, current knowledge of the natural ecologic characteristics of *E. coli* O157:H7 in beef cattle is insufficient to enable design of effective control programs.

Studies to estimate the prevalence of *E. coli* O157:H7 have been conducted in dairy cattle,^{9,13} beef cattle in feedlots,^{9,14} cattle housed on pasture,⁹ and cattle at slaughter.^{13,15} Those studies in beef cattle involved estimates of prevalence at a single point in time. Longitudinal studies to provide information on shedding patterns of *E. coli* O157:H7 in beef cattle housed on pasture have not been performed. Therefore, the objective of the study reported here was to determine the frequency and distribution of *E. coli* O157:H7 throughout a 1-year period in 10 cow-calf herds in Kansas.

Materials and Methods

Study population—The study group consisted of 10 commercial cow-calf farms located in northeast Kansas. Farms were selected for the study on the basis of the willingness of the owners to participate in a year-long study and time of year of parturition for the herd (spring rather than fall or throughout the year). Five farms had < 100 cows, and 5 herds had > 300 cows. The study was initiated in December 1996 (prior to the start of the calving season) and continued until December 1997 (cows) or the period immediately prior to weaning in fall 1997 (calves). Each farm was visited approximately monthly for a 1-year period. Fresh fecal samples (approximately 50 g) were collected immediately after a cow was observed defecating. At each visit, fecal samples were collected from approximately 10% of the cows and 10% of the calves (after start of the calving season and before weaning) in

the herd as well as all bulls that were available. When necessary, farms were visited on consecutive days to obtain a sufficient number of samples for that month; therefore, feces from a specific animal could have been collected more than once in a monthly sample. Fecal samples were identified on the basis of animal. Most adult cattle were identified on the basis of ear tags. At some farms, calves were identified uniquely by ear tags with a similar number code as for the cows. On other farms, individual calves could not be identified uniquely. Whenever possible, water samples (approx 50 ml) were obtained from all water sources available to each herd.

Microbial culture—Samples were processed on the same day on which they were collected. Feces (10 g) or water (10 ml) was mixed with 90 ml of EHEC enrichment broth (EEB)¹⁶ in a stomacher for 30 seconds. Broth was incubated under static conditions at 37 C for 12 hours. After incubation, 1 ml of broth was removed and added to 20 µg of anti-O157-coated magnetic beads.^b The bead-broth mixture was mixed at room temperature (24 C) for 30 minutes, after which it was placed in a magnetic block for 5 minutes. Broth then was removed, using a pipette, and the beads were washed in 1 ml of PBS solution (pH 7.5) and vortexed. Beads were separated out again, using the magnetic block, and washed twice more. The final mixture was suspended in 200 µl of PBS solution.

Prior to March 22, 1997, 100 µl of bead suspension was plated directly onto sorbitol MacConkey agar^c supplemented with sodium tellurite and cefixime^d (CT-SMAC) and incubated overnight at 37 C. After that date, an additional enrichment phase was added; the resuspended beads (200 µl) were added to 9 ml of EEB and incubated for an additional 18 hours at 37 C. After incubation, a swab specimen was obtained from the broth, plated on CT-SMAC (streaking for isolation), and incubated overnight at 37 C. After incubation, nonsorbitol-fermenting (gray-white) colonies were transferred to blood agar plates and incubated overnight at 37 C. Isolates were tested for the O157 and H7 antigens, using latex agglutination.^e Isolates were confirmed as *E coli* by use of a biochemical test strip.^f

Statistical analysis—Prevalence of *E coli* O157:H7 was calculated as the number of fecal samples (or cattle) with positive results divided by total number of fecal samples (or cattle) tested. Proportions of positive results among herds, sample collection dates, and sample types were compared, using the χ^2 test for homogeneity of proportions.¹⁷ A χ^2 test was used to test for associations between fecal shedding of *E coli* O157:H7 in cow-calf pairs. Because the sample size was small, the associated *P*-value was calculated, using the Fisher exact test.¹⁷

Results

Overall, 40 of 3,152 (1.3%) fecal samples had positive results for *E coli* O157:H7. The number of cattle that had positive results on at least 1 fecal sample was 40 of 2,058 (1.9%). Number of fecal samples collected per animal ranged from 1 to 8, and the interval between sample collections ranged from 1 to 347 days. As the number of samples collected per animal increased, the likelihood of obtaining at least 1 sample with positive results also increased significantly ($P < 0.001$; Table 1). None of the cattle were shedding *E coli* O157:H7 at more than 1 sample collection. Of the 23 cattle with positive results that had samples collected more than once, 11 had positive results on the first collection date on which samples were obtained.

All 10 farms had at least 1 fecal sample with posi-

Table 1—Number and proportion of cattle with positive results for *Escherichia coli* O157:H7 in fecal samples, determined on the basis of the number of times samples were collected from a specific animal

Samples per animal	No. of cattle	No. of positive cattle (%)
1	1,413	17 (1.2)
2	386	8 (2.1)
3	149	6 (4.0)
4	59	3 (5.1)
≥ 5	51	6 (11.8)
Total	2,058	40 (1.9)

Table 2—Detection of *E coli* O157:H7 in fecal samples obtained from 10 cow-calf farms in Kansas, by herd

Herd	No. of cattle / No. of samples	No. positive for <i>E coli</i> O157:H7*
Small herds (< 100 cows)		
1	83/128	3
2	96/141	1
3	154/289	6
4	66/124	2
5	145/242	5
Large herds (> 300 cows)		
6	440/671	6
7	303/485	4
8	186/276	4
9	333/452	5
10	252/344	4

*None of the cattle had positive results on more than 1 sample. Therefore, No. of fecal samples with positive results is equivalent to No. of cattle with positive results.

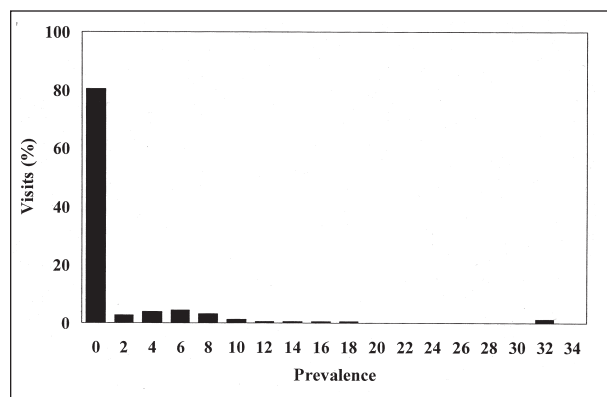


Figure 1—Frequency distribution for the prevalence of *Escherichia coli* O157:H7 in cattle among 154 sample collection dates for samples of feces and water obtained from 10 cow-calf farms in Kansas (1 farm/sample collection date).

tive results during the study period (Table 2). The interval between sample collections for farms ranged from 1 to 77 days. Overall prevalence of positive fecal samples for each herd ranged from 0.7 to 2.3%, and these values did not differ significantly ($P = 0.73$). However, the 5 herds with a small number of cattle (< 100 cows/herd) had a higher prevalence (1.75%), but not significantly so ($P = 0.07$), than the 5 herds with > 300 cows/herd (1.09%).

Prevalence of samples with positive results collected at each farm visit varied significantly ($P < 0.001$) from 0 to 33% (Fig 1). Significant differences were not detected in the prevalence of fecal shedding of *E coli* O157:H7 for each month between April and December

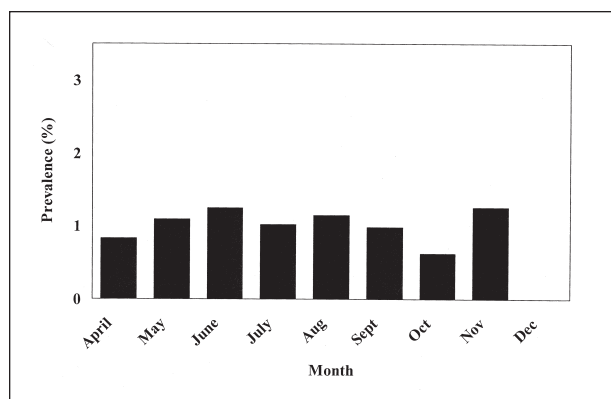


Figure 2—Prevalence of *E coli* O157:H7 in 3,152 fecal samples and 199 water samples obtained from 10 cow-calf farms in Kansas, by month.

Table 3—Detection of *E coli* O157:H7 in samples obtained from 10 cow-calf farms in Kansas, by type of sample

Type of sample	No. of samples	No. positive (%)
Feces		
Cow	2,313	28 (1.2)
Calf	804	11 (1.4)
Bull	35	1 (2.9)
Total	3,152	40 (1.3)
Water	199	3 (1.5)

1997 ($P = 0.98$; Fig 2). Collection dates before Mar 22, 1997 were excluded because of the change in methods for sample processing. The final week of March was excluded because of the small number of samples and the fact that many cows were giving birth during this month.

Number of samples collected, number of samples with positive results, and percentage of samples with positive results for cows, calves, bulls, and water sources were calculated (Table 3). Significant differences were not detected in the prevalence of *E coli* O157:H7 among various types of samples ($P = 0.82$). The pathogen was found in contained water (1 sample obtained from a pond) and free-flowing water (2 samples obtained from streams). Water samples with positive results were found at least once on 2 of 10 farms. However, cattle with positive results were not identified on the date on which the water sample had positive results for *E coli* O157:H7.

Feces collected from a calf could be matched to feces collected from its dam for 139 cow-calf pairs. A calf whose dam shed *E coli* O157:H7 was not more likely to shed bacteria than a calf from a dam never identified as shedding (Fisher exact test; $P = 0.14$). However, this sample included only 7 cows and 3 calves with positive results, with 1 cow-calf pair having *E coli* O157:H7 identified in the calf and its dam.

Discussion

The observed prevalence of *E coli* O157:H7 for specific cattle and herds was slightly higher in the study reported here for cattle in Kansas than in a study of cattle housed on pasture in Washington.⁹ These results may reflect an actual geographic difference or may have been attributable to differences in diagnostic

testing methods or sample collection strategies used. Diagnostic methods are evolving to increase the sensitivity for detection of *E coli* O157:H7, and differences in laboratory methods will influence determination of prevalence.^{18,19} In our study, the method was modified after the first 3 months to include an enrichment phase, which increases the sensitivity for detection of *E coli* O157:H7 in meat samples.²⁰

Samples of fresh feces were collected only after cattle were observed defecating, as opposed to per-rectal sample collection used in numerous other studies. Our method could have biased the results if cattle shedding *E coli* O157:H7 defecated more often than nonshedding cattle. Cattle in the region are housed on large pastures and handled infrequently, and there is not a convenient way to restrain them for sample collection. Researchers previously suggested that most animals shedding *E coli* O157:H7 are not ill,⁶⁻⁸ and none of the cattle from which we collected samples in this study were identified as ill by the producer or investigators. Therefore, we do not believe that the sample collection method preferentially selected cattle that were shedding *E coli* O157:H7.

The study reported here was longitudinal in nature, with samples collected approximately monthly throughout a 1-year period. Surveys in dairy cattle involving collection of a single sample tend to find a lower prevalence of *E coli* O157:H7, compared with results for longitudinal studies. The 100% herd-level prevalence found in this study supports the view that *E coli* O157:H7 is widespread in cattle herds. The probability of identifying specific cattle shedding *E coli* O157:H7 in feces for at least 1 sample collection increased as the number of sample collections increased; however, despite multiple samples from 645 cattle during the study period, none of the cattle shed bacteria for more than 1 sample collection. This suggests that fecal shedding of *E coli* O157:H7 in specific cattle is transient or intermittent, which agrees with studies on fecal shedding patterns in dairy cattle.^{7,21-24} Therefore, collection of samples of cattle at a single point in time for prevalence estimates of *E coli* O157:H7 generally would be expected to underestimate herd-level prevalence.

In other studies, investigators have reported an increased prevalence during the summer months,^{9,11,22} herd-level clustering,^{14,25} and peaks in within-herd prevalence over time.^{22,25} These differences in prevalence have implications for devising control programs. Because the culturing methods changed during our study, ascertaining the extent to which season influenced the prevalence of *E coli* O157:H7 was not possible. However, an increase was not apparent in the warmer months, compared with cooler months in which the same diagnostic methods were used. Clustering of herd-level prevalence suggests that risk factors may influence shedding patterns. Studies in dairy^{7,26} and feedlot²⁷ cattle have identified associations between feeding and management factors and detection of *E coli* O157:H7. In our study, we did not detect significant differences in prevalence among farms. This may have been attributable to differing management and environment for cattle housed on pastures, com-

pared with other segments of the cattle industry. However, only a few farms in a small geographic area were surveyed. Therefore, further studies will be necessary to confirm or dispute the lack of farm-level clustering of the prevalence of *E coli* O157:H7 in cattle housed on pasture.

The finding of periodic increases in within-herd prevalence of *E coli* O157:H7, with periods of non-shedding between those peaks, is in agreement with studies in dairy^{11,21,22} and feedlot²⁵ cattle. It suggests time-dependent risk factors for fecal shedding of *E coli* O157:H7. These peaks in shedding may represent exposure of cattle to a common, but time-limited, source of infection. Determining the reasons for these peaks, as well as determining specific herd-management risk factors, is important for devising effective on-farm control programs.

Studies in dairy cattle have documented differences in the prevalence of *E coli* O157:H7 among age groups,^{7,9,11,22} but such differences were not found in the study of beef cattle reported here. This may have been a result of differences in management of dairy cattle and cow-calf operations. Dairy cattle have differing types of housing and are raised in separate age groups that receive specific diets. Shere et al²¹ found that dairy calves first had positive test results following a change from individual to group housing. Cows and calves housed on pasture remain together prior to weaning (ie, for the duration of our study). As the calves develop functional rumens, they have access to the same food and water sources as their dams. In this study, calves did not appear to be at a greater risk for shedding *E coli* O157:H7 when their dam had positive test results (ie, were shedding *E coli* O157:H7). However, because the number of matched cow-calf pairs was small, the power to detect a difference was low, and the comparison was made for shedding status during the entire course of the study rather than at specific sample collection dates.

Identification of *E coli* O157:H7 in water samples in our study agrees with results of other studies.^{12,21,28} Shere et al²¹ reported that detection of *E coli* O157:H7 in drinking water was associated temporally with fecal shedding of the same strain of the bacteria. Genetic typing has revealed identical strains of *E coli* O157 in water troughs and samples obtained from cattle on 2 farms²⁸ and *E coli* O157:H7 in water and cattle with access to that water on another farm.¹² Thus, detection of *E coli* O157:H7 in water in tanks and ponds may serve as a means of transmission of the pathogen within a group of cattle that have access to that water. The finding of *E coli* O157:H7 in free-flowing water suggests that contamination between herds within a watershed may be possible. More studies are needed to determine sources of contamination, survivability of *E coli* O157:H7 in water, and distribution of these bacteria within watersheds. Contamination of water also may be a source of infection for humans. An outbreak of hemorrhagic colitis resulting from infection with *E coli* O157:H7 was associated with swimming in a lake in New York,²⁹ although there were not any livestock within the park's watershed. Contaminated water from a well was implicated as the cause of bloody diarrhea in

a child on a farm where *E coli* O157:H7 was isolated from 63% of dairy cattle.³⁰ Thus, management of contained and free-flowing water must be a component of control programs for *E coli* O157:H7.

Escherichia coli O157:H7 was identified in the feces of a small percentage of cattle on 10 cow-calf farms in Kansas and in samples of standing and free-flowing water obtained from 2 of the farms. Significant differences in prevalence were not found among farms. Fecal shedding was transient, and there were significant differences in the farm-level prevalence of *E coli* O157:H7 among sample collection dates. Analysis of this information suggests that identification of temporal risk factors, in addition to farm-level risk factors, will be important in designing on-farm control strategies for this pathogen.

^aMichel P. An epidemiologic study of human cases of verocytotoxinogenic *Escherichia coli* infection reported in Ontario. Dissertation, Department of Population Medicine, University of Guelph, Ontario, Canada: 1997.

^bDynabeads Anti-*Escherichia coli* O157, Dynal Inc, Lake Success, NY. ^dDifco, Detroit, Mich.

^cCT, Dynal Inc, Lake Success, NY.

^eRim *Escherichia coli* O157:H7 Latex Test, Remel, Lenexa, Kan.

^fAPI 20E biochemical test strips, bioMerieux, Vitek Inc, Hazelwood, Mo.

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