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Longitudinal Emergence and Distribution of *Escherichia coli* O157 Genotypes in a Beef Feedlot▽

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The purpose of this study was to describe the prevalence and longitudinal distribution of *Escherichia coli* O157 in feedlot cattle and the feedlot environment. Pen floors, water tanks, other cattle in the feedlot, feed, and bird feces were sampled for 2 weeks prior to entry of the study cattle. Twelve pens of study cattle were sampled twice weekly. At each sample time cattle feces, water from tanks in each pen, bunk feed, feed components, bird feces, and houseflies were collected. Bunk feed samples were collected before and after cattle had access to the feed. Overall, 28% of cattle fecal samples, 3.9% of bird fecal samples, 25% of water samples, 3.4% of housefly samples, 1.25% of bunk feed before calf access, and 3.25% of bunk feed samples after cattle had access to the feed were positive for *E. coli* O157. Genetic analysis of *E. coli* O157 isolates was done using pulsed-field gel electrophoresis (PFGE). PFGE types identified in sampling of the feedlot prior to calf entry were different than the majority of types identified following calf entry. A single strain type predominated in the samples collected after entry of the cattle. It was first identified 5 days after entry of the first pen of cattle and was subsequently identified in all pens. Data support that the incoming cattle introduced a new strain that became the predominant strain in the feedlot.

Escherichia coli O157 is an important cause of hemorrhagic colitis and hemolytic uremic syndrome in humans (3, 7) and has been particularly associated with the consumption of ground beef. Research on *Escherichia coli* O157 has identified important aspects of preharvest ecology but has failed to identify validated critical control points for *E. coli* O157 in the feedlot. Individual cattle can be transiently colonized with *E. coli* O157 for 30 to 60 days (5, 12, 15, 16), but no persistent colonization has been shown. Prevalence in cattle has a seasonal distribution, being low in winter and higher in summer (12, 15, 16, 29). Further, individual genotypes of O157 have been shown to persist on individual farms over time despite population turnover (19, 22, 25). The source of the bacteria that colonize cattle is unknown, but feed and water are commonly contaminated with coliforms (26), suggesting fecal contamination. *E. coli* O157 can survive for an extended period in bovine feces (13, 17) and may serve as a source of contamination for feed or water. *E. coli* O157 is commonly found in water tanks and can persist for an extended period there (20), and it has also been commonly found in cattle feeds (9). Additionally, *E. coli* O157 has been identified in a wide range of other animals, including flies, birds, dogs, horses, and opossum (2, 6, 24, 30, 32), potentially providing opportunity for cattle exposure.

The complex nature of *E. coli* O157 ecology in feedlots suggests that a reservoir other than cattle may be important in maintenance of *E. coli* O157. The feedlot environment, feed, water, or other animals may be an important source of outside exposure. The role of the environment in the maintenance of *E. coli* O157 in cattle is an important question to be answered in understanding critical control points for *E. coli* O157 in feedlots. In order to assess the role of the feedlot environment in the ecology of *E. coli* O157 in the feedlot, we longitudinally followed pens of cattle, water tanks, feedbunk samples, feed components, houseflies, and bird feces to detect temporal patterns of appearance of specific genotypes of *E. coli* O157.

MATERIALS AND METHODS

Feedlot and cattle. A single feedlot in northeastern Kansas with a capacity of approximately 2,000 head was sampled from May to August in the study. Study calves were housed in 25-head drylot pens with concrete feeding pads and bunks. Pairs of pens were selected to assess contact between adjacent pens that shared a fence line and water tank. Six pairs of study pens were utilized. Nonadjacent pens were separated by two pens of calves not included in the study. Water tanks were demand-flow tanks with an approximately 5-gal reservoir. Tanks were positioned on pen fence lines with separate reservoirs and common water flow between adjacent pens. The calves included in the study were heifers from multiple origins but were purchased from a single salebarn over a 1-week period. Calves were trucked approximately 300 miles to the feedlot, arrived on four different days over a 6-day period in early June, and weighed 500 to 600 pounds on arrival. Cattle were part of a bovine respiratory disease trial at the feedlot, in which sick cattle and a healthy control from the same pen were pulled daily for treatment and returned to the home pen. On arrival, calves were established on a growing ration that included approximately 45% alfalfa hay, 35% dry rolled corn, 10% corn steep liquor, and a general mineral premix with rumensin/tylosin and melenogesterol acetate. The ration did not change significantly over the course of the study.

Sample collection. The study feedlot was sampled three times beginning 2 weeks prior to the arrival of the study cattle. During the prearrival period, feedlot occupancy was low (approximately 200 head) and pens designated to house the study cattle were empty. Prior to study cattle arrival, water tanks (62 samples) and the pen surface (75 samples) were sampled in designated study pens as well as in nearby pens. Additionally, fecal samples were collected from cattle in nearby pens (83 samples) along with bird feces (16 samples), mixed feed (30

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samples), steam-flaked corn (3 samples), ground hay (3 samples), and lagoon water (one sample). Once the study cattle arrived, 10 fecal samples, 1 water sample, and 2 mixed-feed samples (1 before cattle had access to the feed and 1 after) were collected twice weekly (Mondays and Wednesdays) from each of 12 pens of cattle for 11 weeks. A single sample was also collected twice weekly from component feeds (steam-flaked corn and ground hay, 100 to 200 m from pens) and from the feedlot lagoon. Available bird feces on environmental surfaces around the feedlot were also collected twice weekly (9 to 18 samples per week). Cattle feces were collected fresh from the pen floor by observing cattle defecate and collecting the sample immediately afterward to avoid collecting duplicate samples from an individual animal on a single day. Pen surface samples were collected using individual clean plastic spoons to scrape the surface of the pen. Water and sediment samples were collected together from the water tank in the pen into clean 60-ml tubes by scraping the side of the tank with the tube as the water was collected. Feed samples were collected before and after cattle had access to the feed. Preaccess feed samples were collected from the feed truck chute as the feed was delivered to the bunk. Postaccess feed samples were collected from the bunk after the cattle had access to the feed for 1 to 2 h. All feed samples were collected with a new, clean glove by taking three grab samples and combining them into one sample. Fly samples were collected four times weekly by use of a sweep net from the feed bunk and the storage area for steam-flaked corn.

Fecal culture method. The fecal culture method has been previously described (27) and was used for fecal and pen surface samples. Briefly, it consisted of enrichment of 1 g of feces in 9 ml of gram-negative broth supplemented with cefixime (0.05 mg/liter), cefsuludin (10 mg/liter), and vancomycin (8 g/liter). Samples were incubated for 6 h at 37°C followed by immunomagnetic separation (IMS) and spread plated on Sorbitol MacConkey agar plates supplemented with cefixime (50 ng/ml) and tellurite (2.5 μg/ml). Plates were incubated overnight at 37°C, and up to six sorbitol-negative colonies with characteristic *E. coli* O157 morphology were picked using sterile toothpicks onto blood agar plates. Blood agar plates were incubated overnight at 37°C followed by an indole test on each colony. Indole-positive colonies were checked for the O157 antigen using a latex agglutination assay (Rim *E. coli* O157; Remel, Lenexa, KS). Positive isolates were confirmed as *E. coli* by RapID API test (bioMerieux, Hazelwood, MO).

Feed culture method. The feed culture method has been previously described (9). Briefly, the feed sample was mixed by kneading and shaking the sample bag, and 10 g was placed in a sterile plastic bag with 90 ml of gram-negative broth with no antibiotics added. Samples were incubated for 6 h at 37°C, followed by immunomagnetic separation (IMS). The subsequent identification protocol was the same as that for fecal samples.

Water culture method. The water culture method has been previously described (27). Briefly, 5 ml of water was placed in a sterile tube containing 5 ml of double-strength tryptic soy broth with no antibiotics added. Samples were incubated at 44°C for 24 h, followed by immunomagnetic separation (IMS) and subsequent identification with the same protocol as that for fecal samples. For fecal feed and water samples, one isolate per positive sample was stored at −80°C on Protect beads for later genetic analysis by selecting the first isolate confirmed positive by RapiD API test (bioMerieux, Hazelwood, MO).

Fly culture method. Houseflies were captured during the study beginning during the fourth week after the study cattle had arrived. Housefly capture was delayed, because populations were very low prior to this date. The fly culture method has been previously described (2). Briefly, houseflies from each collection were individually homogenized in 1 ml of phosphate-buffered saline, and serial dilutions were drop plated onto Sorbitol MacConkey agar containing cefixime (25 μg/liter) and tellurite (1.25 mg/liter). After overnight incubation at 37°C, sorbitol-negative colonies were tested for O157 antigen by latex agglutination (Oxoid Limited, Basingstroke, England) and counted. Up to five positive colonies per sample, depending on the concentration of *E. coli* O157, were subcultured to trypticase soy agar (Becton Dickinson) and confirmed as *E. coli* by RapiD API tests (bioMerieux, Hazelwood, MO). Multiple isolates were randomly selected for genetic analysis from individual flies with enumerated concentrations of *E. coli* O157 above 5×10^3.

PFGE typing. Pulsed-field gel electrophoresis (PFGE) was performed on all fecal isolates obtained from samples collected on Mondays ($n = 322$), with the exception of eight isolates that could not be found or for which *E. coli* O157 could not be isolated from the Protect beads. PFGE was also performed on isolates from samples collected on both days from water tanks ($n = 67$), lagoon water ($n = 13$), mixed feed ($n = 10$), component feeds ($n = 3$), bird feces ($n = 6$), pen surfaces ($n = 2$), and houseflies ($n = 52$), with the exception of two water isolates, one postfeed isolate, and one fly isolate that could not be found or for which *E. coli* O157 could not be isolated from the Protect beads. A single isolate was selected for PFGE from 9 of the individual housefly samples, and multiple isolates were selected for PFGE from 16 of the individual housefly samples. PFGE was performed according to PulseNet guidelines (http://www.cdc.gov/pulsenet/protocols/ecoli_salmonella_shigella_protocols.pdf) (14). *E. coli* O157 isolates were prepared for PFGE in agarose gel and digested using XbaI (Promega Corp., Madison, WI). Restriction fragments were separated by contour-clamped homogeneous field (CHEF) PFGE using a CHEF-DRII drive apparatus (electrophoresis cell, drive module, control module, pump, casting stand, combs, and CHEF-DR II Chiller system; Bio-Rad Laboratories, Richmond, CA). PFGE patterns were photographed and scanned using Bio-Rad's gel documentation systems (Gel Doc 2000) and TDS Quantity One software. BioNumerics software (Applied Maths, Belgium) was used to analyze the PFGE gel images and quantify the relationships between microorganisms. Isolates were categorized as subtypes (100% Dice similarity) and types (>95% Dice similarity).

Virulence gene detection. Water tank ($n = 66$), lagoon water ($n = 13$), mixed-feed ($n = 10$), component feed ($n = 3$), bird feces ($n = 6$), pen surface ($n = 2$), and fly ($n = 125$) isolates and a subset of the fecal isolates selected randomly ($n = 96$) were tested for virulence genes stx_1, stx_2, and *eaeA* by multiplex PCR with a PTC-200 thermal cycler, commercially available oligonucleotide primers (GenScript), and freshly boiled cells as previously described (11). Temperature conditions consisted of a 95°C denaturation for 3 min, followed by 30 cycles of 95°C for 20 s, 58°C for 40 s, and 72°C for 90 s. The final step was a 72°C incubation for 5 min. The PCR products were visualized on 3% (wt/vol) agarose 3:1 (Amresco) gel with ethidium bromide.

Analysis of data. Descriptive statistics and analysis were produced in STATA (version 8.2; College Station, TX). For assessment of PFGE type (95% Dice similarity) diversity between fecal and water isolates, a random-effect logistic regression model was fit to the data utilizing a dichotomous outcome (type 7 versus non-type 7) and a random effect for pen to account for potential non-independence due to repeated sample collections within pens. Subtype (100% Dice similarity) diversity over time within the most common type was assessed by collapsing the data into counts of the most common subtype versus all other subtypes (within the most common type) by week. A random-effect logistic regression model was fit to the data utilizing a dichotomous outcome of the proportion of the most common subtype to all other subtypes (within the most common type) and a random effect for pens to account for potential nonindependence due to repeated sample collections within pens.

RESULTS

Prevalence in the feedlot from all samples during the 2 weeks prior to the arrival of the study cattle was 0.7% (2/273). Prevalence from all samples following arrival of study cattle was 17.1% (811/4,750). Individual and overall sample prevalence by week and sample type is reported in Table 1. Prevalence was low until week 5, when prevalence increased markedly, mostly due to fecal and water samples. Fecal and water prevalence remained high throughout the rest of the study. Isolates were sporadically found in the remaining samples taken (Table 1).

PFGE was performed on a total of 475 isolates from the study, including 52 isolates from houseflies. Of the isolates that had undergone PFGE, 197 were submitted for PCR analysis of the virulence genes *eaeA*, stx_1, and stx_2. One hundred eighty-eight (95.4%) had the *eaeA* gene and at least one Shiga toxin gene (188 isolates had the stx_2 gene and 184 had the stx_1 gene). All seven of the PFGE type 1 isolates were negative for all three virulence genes. The other two negative isolates were type seven (2/168 tested; 1.2%), one was negative for all three virulence genes, and one was positive for only stx_1.

Isolates that did not have the *eaeA* gene and at least one Shiga toxin were excluded from further analysis, leaving 466 *E. coli* O157 isolates in 34 unique PFGE subtypes (100% Dice similarity) and 11 PFGE types (95% Dice similarity) over the course of the 13 weeks. In the first 3 weeks of the study, PFGE type three was the predominant isolate, accounting for 75%

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TABLE 1. Samples positive/samples taken and prevalence of *E. coli* O157 by week and sample type[a]

Sample	No. positive/total no. of samples (%) at week:													Overall prevalence, no. positive/ total no. of samples (%)
	1	2	3	4	5	6	7	8	9	10	11	12	13	
Bovine feces	0/13 (0)	0/70 (0)	7/80 (8.8)	17/180 (9.4)	88/240 (36.7)	80/240 (33.3)	63/240 (26.3)	69/240 (28.8)	77/240 (32.1)	76/240 (31.7)	68/240 (28.3)	75/240 (31.3)	37/120 (30.8)	657/2,383 (27.6)
Water	0/21 (0)	1/41 (2.4)	1/21 (4.8)	2/17 (11.8)	9/24 (37.5)	7/24 (29.2)	13/24 (54.2)	8/24 (33.3)	12/24 (50.0)	2/24 (8.3)	9/24 (37.5)	3/24 (12.5)	2/12 (16.7)	69/304 (22.7)
Lagoon water		0/1 (0)	0/2 (0)	2/2 (100)	2/2 (100)	2/2 (100)	1/2 (50)	2/2 (100)	1/2 (50)	1/2 (50)	1/2 (50)	1/2 (50)	0/1 (0)	13/22 (59.1)
Bird feces	0/4 (0)	0/12 (0)	0/16 (0)	0/11 (0)	1/12 (8.3)	0/14 (0)	0/9 (0)	0/12 (0)	1/16 (6.3)	2/18 (11.1)	0/18 (0)	2/14 (14.3)	0/9 (0)	6/165 (3.6)
Flaked corn	0/2 (0)	0/2 (0)	0/2 (0)	0/2 (0)	1/2 (50)	0/2 (0)	0/2 (0)	1/2 (50)	0/2 (0)	0/2 (0)	0/2 (0)	0/2 (0)	0/1 (0)	2/24 (8.3)
Ground hay	0/2 (0)	0/2 (0)	1/2 (50)	0/2 (0)	0/2 (0)	0/2 (0)	0/2 (0)	0/2 (0)	0/2 (0)	0/2 (0)	0/2 (0)	0/2 (0)	0/1 (0)	1/24 (4.2)
Preaccess feed		0/12 (0)	0/6 (0)	0/18 (0)	1/24 (4.2)	0/24 (0)	0/24 (0)	0/24 (0)	0/24 (0)	2/24 (8.3)	0/24 (0)	0/24 (0)	0/12 (0)	3/240 (1.25)
Postaccess feed	0/6 (0)	0/12 (0)	2/6 (33.3)	1/18 (5.6)	4/24 (16.7)	0/24 (0)	0/24 (0)	1/24 (4.2)	0/24 (0)	0/24 (0)	0/24 (0)	0/24 (0)	0/12 (0)	8/246 (3.25)
Pen surface	0/40 (0)	1/35 (2.9)												1/75 (1.3)
Fly prevalence				1/30 (3.3)	0/40 (0)	4/70 (5.7)	18/200 (9)	14/200 (7)	4/200 (2)	4/200 (2)	4/200 (2)	3/200 (1.5)	1/200 (0.5)	53/1,540 (3.4)
Overall prevalence	0/86 (0)	2/187 (1.1)	11/135 (8.1)	23/280 (8.2)	106/370 (28.6)	93/402 (23.1)	95/527 (18.0)	95/530 (17.9)	95/534 (17.8)	87/536 (16.2)	82/536 (15.3)	84/532 (15.8)	40/368 (10.9)	813/5,023 (16.2)
Proportion of type 7	0%	0%	28.6%	87.5%	100%	96%	96.1%	94.9%	94.5%	94.4%	97.8%	100%	87.5%	

[a] Time line: weeks 1 and 2, prearrival sampling; week 3, calf arrival; weeks 3 to 13, postarrival sampling.

(6/8) of isolates during this period. It was identified during week 2 before the study cattle arrived at the lot but was not identified after week 4. Overall, 95% (443/466) of the tested isolates were PFGE type seven (95% similarity). The type seven isolate included 18 different subtypes, but 87% (386/443) of the type seven isolates were subtype 16 (100% similarity). All isolates from bird feces, steam-flaked corn, and feed samples taken prior to calf access were type seven. The type seven pattern accounted for 96% of bovine feces and 97% of water tank isolates, 86% of isolates from feed samples collected after calves had access to feed, and 92% of lagoon water isolates. Two PFGE types were found only in isolates from bovine feces (three isolates). A single isolate was selected for PFGE from 9 of the individual housefly samples, and multiple isolates were selected for PFGE from 16 of the individual housefly samples. In these 16 fly samples, two PFGE types were identified in three houseflies and three types in one housefly. The type seven pattern accounted for 90% of all housefly isolates, and type seven was isolated from all individual flies. Four PFGE types (four isolates) were found only in isolates from flies. The PFGE type seven isolate was first identified during week 3 in the feces and postaccess feed of the first group of study calves 5 days after their arrival. It became the predominant type in week 4 and subsequently accounted for greater than 95% of the isolates. By week 4 the type seven isolate was detected in 7 of 12 study pens, and by week 5 it was detected in all 12 study pens. The distribution of genetic types was not different between the water isolates and the bovine fecal isolates (Wald chi-square, $P = 0.7$). Between week 3, when the PFGE type seven first appeared, and the final sampling in week 13, there was increasing diversity of subtypes within the type seven pattern (Wald chi-square, $P = 0.03$) (Table 2).

DISCUSSION

The prevalence of *E. coli* O157 in calves and environmental samples in this study was consistent with those reported in recent studies (10, 27). The prevalence in the calves was low on arrival but increased approximately 2 weeks after the arrival of the first calves. It remained elevated throughout the study period in all pens. The pens included in this study were relatively close together, which may have facilitated transmission and persistence of *E. coli* O157 within the study group. The calves were also part of a study on bovine respiratory disease at the feedlot. Sick cattle from the group along with a healthy control from the same pen were pulled daily for treatment and returned to the home pen. While the pulling of sick cattle from the pen for treatment is standard practice at feedlots in the United States, the inclusion of a healthy control is not. This may have increased mixing of cattle within the hospital pens and facilitated transmission of *E. coli* O157 within the group, especially early in the study period when pull rates were highest.

Smith et al. (29) reported increased prevalence in pens of cattle associated with dry dusty conditions or wet muddy conditions compared to an intermediate state between dusty and muddy. Weather factors could impact transmission of *E. coli* O157; however, weather was mild over the course of the study. There were 12 total days with measurable precipitation, including 4 days in the first 3 weeks after cattle arrived (weeks 3 to 5).

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TABLE 2. Genetic diversity of PFGE type 7: number of isolates of each subtype by week

PFGE subtype	No. of isolates at week:										
	3	4	5	6	7	8	9	10	11	12	13
11					3		1				
12					1						
13					1						
14			2	1							
15			3		1	1					
16	2	15	82	43	63	35	55	28	36	26	3
17							1		1	1	3
18											1
19						1			1		
20			1	1	2			1	4		
21								1			
22								1			
23			1				1				
24				2			10	2			
25					1				2		
26					1						
27				1							
28								1			
Proportion of subtype 16	100%	100%	92.1%	89.6%	86.3%	94.6%	79.7%	82.4%	81.8%	96.3%	42.9%

The pen floors remained dry or had minimal mud throughout the study, corresponding to the intermediate pen condition designation and baseline prevalence in Smith et al. (29).

The role of hypothesized "super shedders" (23) in the rapid transmission of the type seven strain in this feedyard is unknown. We did not monitor individual cattle or assess shedding levels of individual samples. If some cattle were shedding at very high levels, they may have contributed to the rapid spread of the type seven strain throughout the feedyard.

E. coli O157 can persist for an extended period of time on a farm (19, 21, 28). It is less clear whether the cattle on the farm or the feedlot environment are the reservoir of persistent strains. Lahti et al. (18) reported that the farm was the source of *E. coli* O157 for cattle in a Finnish finishing unit. They reported that *E. coli* O157 persisted well on barn surfaces and, based on failing to find *E. coli* O157 in three of four lots of incoming cattle, suggested that the cattle were colonized following arrival. The fourth lot of incoming cattle were not sampled prior to entry into the finishing facility but were found to be shedding a genotype of *E. coli* O157 that was closely related to genotypes previously isolated from the farm surface samples 1 day after arrival. Available experimental data support extended survival of *E. coli* O157 on farm surfaces (31) which could serve as an exposure source; however, the management practices of Finnish finishing units are substantially different from midwestern United States feedlots. LeJeune et al. (19) found that a group of four highly related genetic types of *E. coli* O157 persisted on a feedlot for 4 months. The authors did not collect any environmental samples but, based on the persistence of the predominant strains in the face of a large population turnover, suggested that feedlot environment was the reservoir of *E. coli* O157 on the farm.

In our study population, the incoming calves appear to have introduced the most common strain of *E. coli* O157 into the feedlot. The feedlot had few cattle (approximately 200 head) on site during sampling prior to study calf arrival. No cattle were present in the study pens, and only a few cattle (approximately 50 head) nearing the end of their feeding period were present in nearby pens. The prevalence of *E. coli* O157 at the feedlot was low, and PFGE type seven was not detected from a total of 273 samples taken from the feedlot prior to arrival of the study calves. PFGE type seven was first detected (during week 3) in cattle feces and feed collected from the bunk after cattle access in a pen that had arrived 5 days previously. It subsequently accounted for the vast majority of isolates. While we cannot categorically rule out its presence prior to the arrival of the study calves, its detection, contemporaneous with the arrival of the calves, suggests it was the source.

This study is consistent with both Lahti et al. (18) and LeJeune et al. (19) in finding a predominant genotype within the population of cattle. The inference regarding source is not necessarily in conflict. It seems likely that *E. coli* O157 may persist in a feedlot environment and serve as a reservoir for exposure and colonization of incoming animals. Substantial data exist to support the ability of *E. coli* O157 to persist in the environment on farms (13, 17, 18, 20, 25). This does not preclude the periodic introduction of new strains through cattle, feeds, or another source. The balance of the two factors, persistence and strain turnover, may depend on the prevalence of the new strain in incoming cattle and the fitness of the incoming strain versus the resident strain to compete in the feedlot environment and bovine gastrointestinal tract. The most common strain in this study appears to have been able to compete well in the feedlot over the course of this study. Once the new strain was introduced to the feedlot, we were able to detect it in the environment regularly in feed, water, birds, and houseflies, suggesting an important role of the environment in spread of the strain. If cattle commonly bring new strains of *E. coli* O157 into the feedlot, then routine cleaning between lots of calves is not likely to affect prevalence. Options to intervene with incoming cattle prior to arrival with products such as vaccines or probiotics may need to be examined.

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Use of a single restriction enzyme in this study may not have captured all the diversity present in the *E. coli* O157 isolates identified, and as such it is possible that not all the type seven isolates identified are genetically related (8). While this may have had some effect on our assessment, the common epidemiologic source and preponderance of the most common isolate are consistent with a common strain throughout the feedyard. We found some evidence in the data of increasing diversity over time within the type seven isolate in this study. The type seven isolate exhibited 95% similarity and was made up of 18 different subtypes (100% similarity). Initially, only subtype 16, within the type seven PFGE pattern, was identified, and the rest of the subtypes were recognized over time (Table 2). None of the other subtypes within type seven were isolated regularly or became dominant. Akiba et al. (1) showed that *E. coli* O157 undergoes genetic change following inoculation in experimental calves. The study involved two calves inoculated with a single strain of *E. coli* O157 and PFGE analysis of up to 10 isolates from each calf on each sampling day. The PFGE pattern of the inoculated strain changed by 1 day after inoculation in one calf and 2 days after inoculation in a second calf, indicating genetic diversification may account for emergence of new strains. Our data are consistent with the development of genetic change over time within individual strains of *E. coli* O157 in feedlot cattle. In our study, there was no difference in the diversity of isolates between cattle feces and water. There were too few isolates to assess differences between cattle feces and feed; however, all but one feed isolate was type seven, and the one exception was detected in week 3 and not subsequently. This suggests that feces, water, and probably feed share a common source and cycle of exposure and contamination.

There were four PFGE types identified in the housefly isolates from this study that were not identified in any other sample. This may suggest that houseflies had different exposure sources or may just be a function of testing multiple isolates from some housefly samples. However, given the large number of isolates typed from feces and water, it seems interesting that four unique types would show up in houseflies. A small dairy of approximately 200 milking cows was located approximately 400 m from the study feedlot. It is possible that some houseflies may have picked up *E. coli* O157 strains at the dairy and subsequently been captured and cultured at the feedlot. Alternately, the environment of the housefly gastrointestinal tract may provide a competitive advantage to different strains of *E. coli* O157 than water, feed, and bovine feces, allowing more common detection of minor strains. At least one type seven isolate was recovered from all houseflies tested. The commonality of isolates between houseflies, cattle feces, and water suggests that houseflies may play an important role in contamination of feed and transmission of *E. coli* O157 within the feedlot. Alam and Zurek (2) reported *E. coli* O157 counts from individual houseflies collected in their study of up to 10^5 CFU. In inoculation experiments, small doses of *E. coli* O157 have been shown to result in colonization of some calves. Besser et al. (4) were able to induce shedding in three of four calves exposed to 10^4 CFU of *E. coli* O157. As such, individual houseflies may carry a sufficient colonization dose, and fly control programs may be valuable in controlling the spread of *E. coli* O157 within the feedlot.

The data presented here support the body of evidence indicating that the ecology of *E. coli* O157 in the feedlot is complex, involving not only cattle but birds, feed, water, and houseflies. Although maintaining clean feedlot facilities and cleaning between lots of cattle may be an overall good management practice, it is unlikely to control *E. coli* O157 prevalence when new strains are introduced. Fly control could be a useful intervention; however, further research is necessary to establish the role and importance of flies in maintenance and transmission of *E. coli* O157 on the feedlot. Given the complex interplay of these factors in introduction, persistence, and transmission of *E. coli* O157, control measures will likely need to be multifaceted.

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