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Incidence, Duration, and Prevalence of *Escherichia coli* O157:H7 Fecal Shedding by Feedlot Cattle during the Finishing Period

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ABSTRACT

The objective was to describe variability in prevalence, incidence, and duration of fecal shedding of naturally occurring *E. coli* O157:H7 by a group of feedlot cattle over time. One hundred steers, randomly assigned to 10 pens, were fed a high-concentrate finishing diet for 136 days (19 weeks). Rectal feces from each animal were tested for *E. coli* O157:H7 every week for 19 weeks. *E. coli* O157:H7 was recovered from each animal that completed the study and was detected from at least one animal every week. Average pen prevalence of cattle shedding *E. coli* O157:H7 varied significantly over time ($P < 0.0001$) and across pens ($P < 0.0001$), ranging from 1 to 80%. Pairwise comparisons of mean pen prevalence of *E. coli* O157:H7 between weeks and estimation of the predicted probability of an incident case of *E. coli* O157:H7 over time allowed the definition of three distinct phases—namely, the preepidemic, epidemic, and postepidemic periods. Average pen prevalence varied significantly over time ($P < 0.01$) and across pens ($P < 0.001$) for all time periods. The odds of an incident case were significantly greater during epidemic and postepidemic periods relative to the preepidemic period ($P = 0.0002$ and $P = 0.03$, respectively). Duration of infection was significantly longer for first or second infections that began during epidemic or postepidemic periods relative to the preepidemic period ($P < 0.001$). Both incidence and duration of shedding peaked during the epidemic period. Pen-level prevalence of cattle shedding *E. coli* O157:H7 was affected by both incidence and duration of shedding and could be explained by time- or pen-dependent risk factors, or both.

E. coli O157:H7 has been identified as a potential hazard associated with consumption of beef products *(1, 13, 28).* This organism is widely distributed among groups of feedlot cattle *(6, 10, 27).* Determining critical control points for *E. coli* O157:H7, however, requires a greater understanding of the ecology of this organism. Unfortunately, its epidemiology in cattle production systems is still poorly understood *(25).*

Fecal and hide prevalence of *E. coli* O157:H7 from cattle presented for slaughter at meat processing plants have been significantly correlated with carcass contamination *(6),* indicating a role for control of *E. coli* O157:H7 in live cattle. It is important that studies of the ecology of *E. coli* O157:H7 identify the factors that influence shedding of this organism in cattle, so as to minimize the level of shedding, particularly in those cattle going to slaughter.

In studies conducted in commercial feed yards, we found that the percentage of cattle shedding *E. coli* O157:H7 in a pen ranged from 1 to 80% *(27).* Also, the percentage of cattle shedding *E. coli* O157:H7 varied greatly within pens in a feedlot, but not between feedlots *(27).* Because each pen of cattle in that study was tested only once, we were unable to monitor changes in prevalence over time. The objective of this study was to describe the prevalence, incidence, and duration of fecal excretion of *E. coli* O157:H7 by feedlot cattle over the course of the feeding period and to determine whether factors that contributed to *E. coli* O157:H7 shedding were related to time or place.

MATERIALS AND METHODS

Study design. The design was a longitudinal observational study to monitor individual cattle for the presence of *E. coli* O157:H7 in rectal feces during the feedlot finishing period.

Animals. One hundred steers were randomly assigned to 10 pens (10 animals each) on arrival to the feed yard at the Agriculture Research and Development Center, University of Nebraska–Lincoln, Ithaca, Nebr. The pens were arranged in a row, with a water trough shared between adjacent pens and a separate feed bunk for each pen. The steers were fed a high-concentrate finishing diet for 136 days starting in June 2000. One animal was removed from the study during the seventh week because of its aggressive behavior. The cattle were tested once each week for 19 weeks. Previously, these calves had been purchased from Nebraska ranchers during October and November 1999. They were then fed cornstalks, corn gluten feed, and hay until the observational study started. The calves were turned onto harvested cornfields on arrival then raised in confinement from 10 March until the start of the study.

Laboratory methods. Feces were collected from the rectum of each animal in each pen each week of the study. The fecal

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samples were transported to the laboratory, and within 3 to 4 h after collection from the animal, subsamples were put into enrichment culture. Culture methods optimized for the detection of *E. coli* O157:H7 in fecal specimens were used *(27).* A multiplex PCR assay was modified from existing protocols using primers to detect genes for O157 antigen synthesis (*rfbE (22)*), intimin (eae_{O157} *(9)*), Shigatoxin-1 (*stx1 (21)*), Shigatoxin-2 (*stx2 (23)*), and H7 (*fliC (8)*). Isolates were initially identified as *E. coli* by biochemical tests and API 20E (bioMerieux, Hazelwood, Mo.) as previously described *(27).* Isolates that we finally designated as *E. coli* O157:H7 were positive for the O157 antigen by latex agglutination (Remel, Lenexa, Kans.) and further tested by multiplex PCR. Isolates with a positive PCR reaction for both $rfbE_{O157:H7}$ and $fliC_{H7}$ and at least one of the virulence factor genes (i.e., *stx1, stx2,* or eae_{O157}) were designated enterohemorrhagic *E. coli* O157:H7 *(8).* These criteria allow for detection of *E. coli* O157:H7 strains that have lost *stx* or *eae* genes and also circumvent the ambiguity associated with the nonmotile designation *(8).*

Data imputation. During weeks 11, 12, and 17, a portion of the samples were cultured in selective enrichment media that contained a tenfold increase in the concentration of cefixime, resulting in no growth on the respective isolation plates. The presence of *E. coli* O157:H7 was imputed for all no-growth observations (84 of 1,888) during these weeks, as well as for four additional observations in which a sample was not collected. Logistic regression (SAS: Proc Logistic) was used to model the probability of growth given the animal's culture results prior to and after the week in question. There was one missing observation each in weeks 6, 8, 13, and 19. There were 59, 17, and 9 missing observations in weeks 11, 12, and 17, respectively. We generated a response for the observations that were in question with the predicted probabilities of *E. coli* O157:H7 presence for periods that were similar to the weeks with missing values. Information from weeks 1 through 8 was used to impute *E. coli* O157:H7 values for the missing observations between weeks 1 and 8; information from weeks 9 through 15 was used to impute *E. coli* O157:H7 values for the missing observations between weeks 9 and 15; and information from weeks 1 through 18 was used to impute *E. coli* O157:H7 values for the missing observations between weeks 16 through 19. Missing observations from the animal removed from the study were not imputed because of insufficient follow-up information.

Statistical methods. *E. coli* O157:H7 pen prevalence was computed from the *E. coli* O157:H7 status of individual animals within the pen and was defined as the proportion of cattle in a pen shedding a detectable level of *E. coli* O157:H7 on any given sampling date. To describe the sources of variability in the pen-level prevalence of *E. coli* O157:H7, a mixed model analysis of variance (SAS: Proc Mixed) was used, with an AR(1) correlation structure and the variable pen as the repeated measure. Place (pen), time (week), and water tank sharing were investigated as sources of variability in the *E. coli* O157:H7 prevalence data. Pens and the sharing of water tanks were modeled as random effects, and week was modeled as a fixed effect. Each week corresponded to a particular time point along the feeding period of the cattle, so week-specific average responses were of interest. Pairwise comparisons of the mean pen prevalence of *E. coli* O157:H7 between weeks were made using Tukey's honestly significant difference test. As an exploratory analysis, the effect of time was investigated both as a continuous and categorical effect on the basis of the *E. coli* O157:H7 shedding pattern observed, and the mixed model was fit within each time period to determine whether pen and time were sources of variability in these time periods.

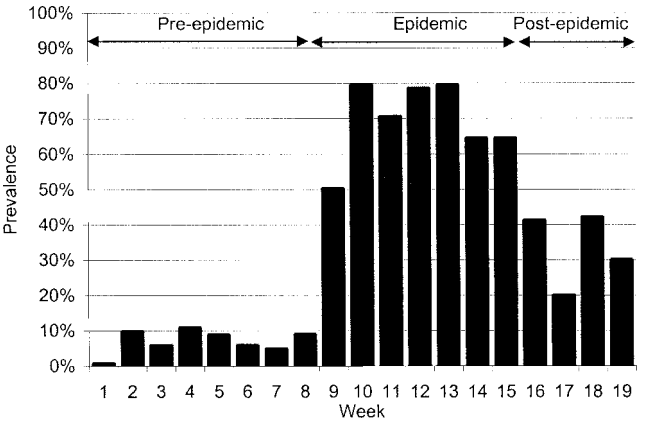

FIGURE 1. *The proportion of cattle detected shedding* Escherichia coli *O157:H7 each week of the 19-week feeding period (June to October 2000). Statistical analysis revealed three distinct prevalence periods, termed preepidemic, epidemic, and postepidemic (double arrows).*

This analysis enabled us to informally test for an interaction between time and pen effects on *E. coli* O157:H7 prevalence.

Incidence of *E. coli* O157:H7 shedding for a particular week was defined as the proportion of cattle whose feces were culture-positive that week but were negative the previous week (at risk). Each animal could have more than one incidence of shedding over the course of the feeding period, each with its own duration. A logistic regression model was used to model the probability of an incident case of *E. coli* O157:H7 as a function of time. Time (the feeding period) was split into three periods (weeks 1 to 8, 9 to 15, and 16 to 19) according to the observed patterns of *E. coli* O157:H7 shedding. The logistic regression models were fit by Generalized Estimating Equations to account for the likely correlation of responses within animals over time and within pens *(14, 16).* Computations were made using GenMod and GEEPLUS SAS Macro *(15).*

The duration of *E. coli* O157:H7 shedding was defined as the number of consecutive weeks an animal shed a detectable level of the organism. Data on duration of *E. coli* O157:H7 shedding was complicated because, at week 19, some of the infection durations were censored so that only the minimum length of the infections that were still present at week 19 could be determined. Therefore, a summary of the median time to infection, estimated from survival curves, for the animals in each pen was analyzed. A survival regression modeling procedure was used to determine whether the duration distribution differed depending on when the infection started or the infection number (first infection, second infection, etc.) among recurrent infections. The method, which was proposed and described earlier *(24),* is appropriate for analyzing the duration of recurrent infections within the same animal.

RESULTS

Prevalence of *E. coli* O157:H7 shedding. During the course of the study, 697 *E. coli* O157:H7 isolates were recovered. PCR detected *stx* genes in all isolates and the eae_{O157} gene in 696 of 697 isolates. The proportion of cattle detected shedding *E. coli* O157:H7 each week of the feeding period is shown in Figure 1. *E. coli* O157:H7 was recovered at least once during the feeding period from each animal that completed the study, and the organism was detected from at least one animal every week.

At the pen level, the percentage of cattle shedding a

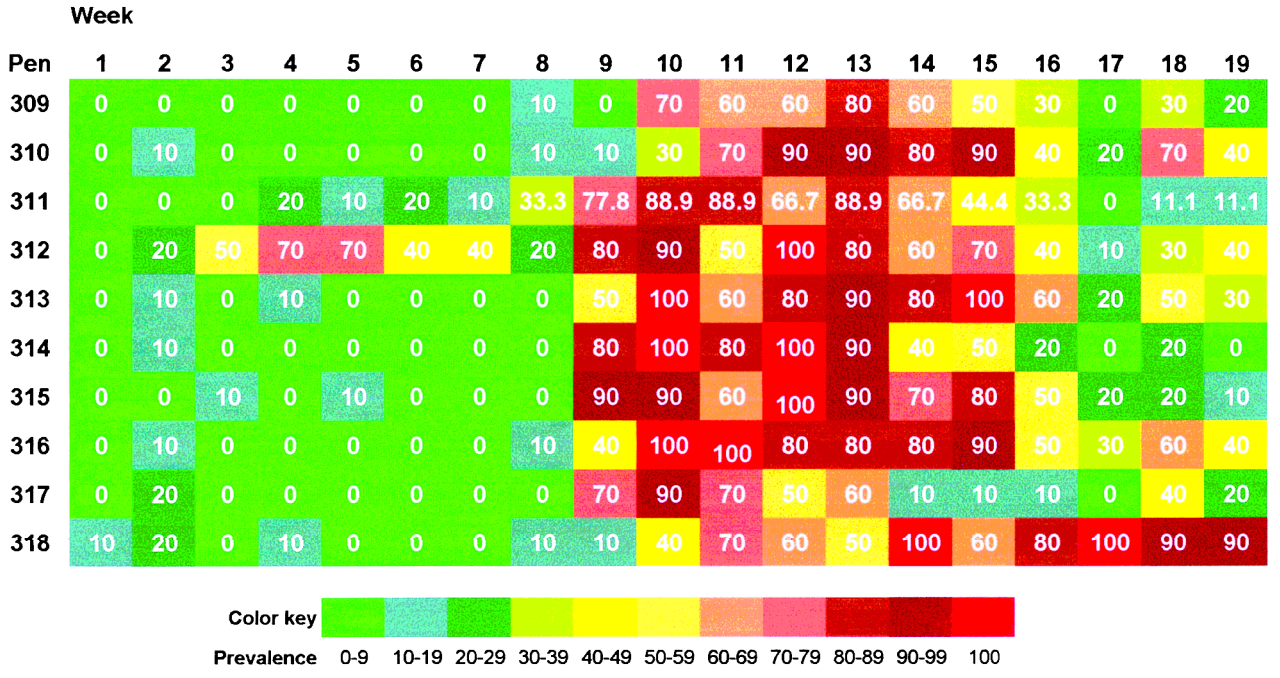

FIGURE 2. *The prevalence of* Escherichia coli *O157:H7 fecal shedding by pen and week for the 19 weeks of the feeding period (June to October 2000) illustrates the distribution of the agent by time and place.*

detectable level of *E. coli* O157:H7 within a given pen of steers in the feed yard over the feeding period ranged from 0 to 100% (Fig. 2). A pattern of *E. coli* O157:H7 shedding could be seen, whereby the first 8 weeks were characterized by low pen prevalence (range, 0 to 70%; median, 0%), followed by 7 weeks of high pen prevalence (range, 0 to 100%; median, 79%), and finally, 4 weeks of medium pen prevalence (range, 0 to 100%; median, 30%). On the last sampling date, 30 of 99 steers that completed the study (30.3%) were excreting a detectable level of *E. coli* O157:H7 in their feces, and 9 of 10 pens had at least one steer shedding *E. coli* O157:H7 in its feces. Ninety percent of the animals in one pen, 40% of the animals in three pens, 30% of the animals in one pen, 20% of the animals in two pens, and 10 to 11% of the animals in two pens were culture-positive for *E. coli* O157:H7 on the last sampling date.

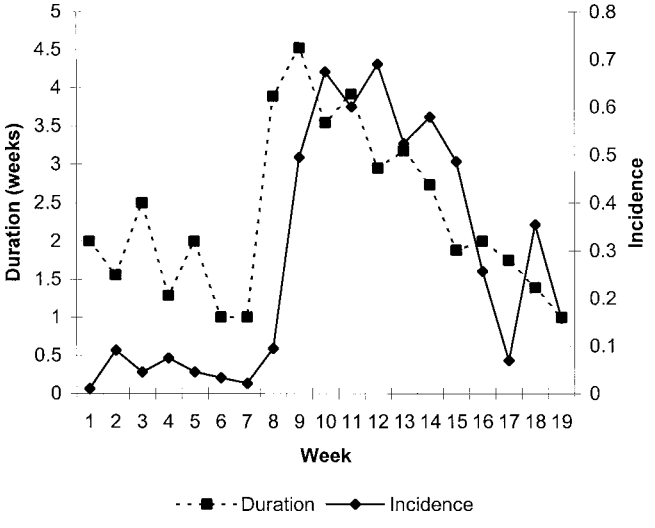

FIGURE 3. *Incidence density and duration of* Escherichia coli *O157:H7 shedding by cattle for the 19 weeks of the feeding period (June to October 2000).*

Both pen ($P < 0.0001$) and week ($P < 0.0001$) were significant sources of variability in *E. coli* O157:H7 prevalence. Pairwise comparisons of mean pen prevalence of *E. coli* O157:H7 between weeks allowed the definition of three distinct phases within the feeding period. We thereafter named the three periods preepidemic (weeks 1 to 8), epidemic (weeks 9 to 15), and postepidemic (weeks 16 to 19) (Fig. 1). When the mixed model analysis of variance was fit onto data for the three prevalence periods observed, *E. coli* O157:H7 prevalence differed significantly over time ($P < 0.01$) and across pens ($P < 0.001$) for the three periods. Results of a mixed model analysis of variance indicated no evidence of the water tank acting as a significant source of variability in *E. coli* O157:H7 prevalence ($P = 0.7$).

Incidence of *E. coli* O157:H7 shedding. The first 8 weeks of the feeding period were characterized by low incidence (0.01 to 0.10 new cases per animal per week) of fecal excretion of *E. coli* O157:H7. The incidence increased dramatically in week 9 (0.5 new cases per animal per week), reached the highest rate in week 12 (0.7 new cases per animal per week), and decreased during the last 5 weeks of the study (Fig. 3). Because new cases of *E. coli* O157:H7 for the proportion of samples affected by the delay in culture in weeks 11, 12, and 17, as well as the four time points for which no sample was drawn, were based on imputed data, incidence values for those weeks could have been under- or even overestimated.

The logistic model investigating the time period effect on the odds of an incident case included an intercept and two terms representing the time period of the observation during either the epidemic period (weeks 9 to 15) or the

TABLE 1. *Median duration in weeks that* E. coli *O157:H7 was recovered from individual cattle according to pen and infection number (the rank of each sequential new infection)*

	Infection number[a]				
Pen	1	2	3	4	5
309	2	1	1		
310	3	3	3	–	
311	1	2	2	2	
312	3	3	2	4	–
313	3	3	4		
314	5	1	1	1	
315	2	2	2		
316	4	3	3	–	
317	2	1	1	1	
318	2	5	–	–	–

[a] An empty cell implies that no animals experienced the higher order infection; a dash implies that the median could not be estimated from the data.

postepidemic period (weeks 16 to 19). The odds of an incident case were significantly greater during the epidemic period than the preepidemic period ($P = 0.0002$). Specifically, the odds of an incident case during the epidemic period were 21.1 times the odds of an incident case during the preepidemic period (95% CI: 7.5, 59.7). The odds of an incident case were significantly greater during the postepidemic period than the preepidemic period ($P = 0.03$)—specifically, 4.2 times the odds of an incident case during the preepidemic period (95% CI: 1.2, 15.3). The predicted probabilities of an incident case during the preepidemic, epidemic, and postepidemic periods were 0.06, 0.59, and 0.22 respectively.

Duration of *E. coli* O157:H7 shedding. The first 7 weeks of the feeding period were characterized by estimated short mean duration (≤2.5 weeks) of shedding of the organism. The duration of shedding increased dramatically in week 8, peaked in week 9, and gradually decreased during the last 10 weeks of the study (Fig. 3). Note that the estimate of duration could have been underestimated because some animals were still shedding at week 19 when the study ended, and the duration of their infections were censored. The mean duration of fecal shedding was longest during the epidemic period, with cases starting in weeks 8 and 9 and lasting on average 3.9 and 4.5 weeks, respectively. On the last sampling date (week 19), 30% of the steers were excreting a detectable level of *E. coli* O157:H7 in their feces, and these animals had been shedding the organism for a mean duration of 3.1 weeks (range, 1 to 10; median, 2). The average number of times each steer shed *E. coli* O157:H7 in the feces during the course of the study was 2.5 times (range, 1 to 5; median, 2).

The median duration (weeks) of *E. coli* O157:H7 infection is summarized for cattle within each pen according to infection number (Table 1). From the descriptive summary of the median duration times, it did not appear that the duration differed depending on infection sequence number (first infection, second infection, etc.). Also from the regression model, the duration of infection did not appear to differ significantly between first and second infections or first and third infections ($P > 0.4$ for both comparisons, relative risk for infection ending was 1.1 and 1.2 for second and third relative to first infections, respectively).

The results of survival regression modeling procedures to determine whether the duration distribution differed depending on when the infection started were based only on information from the first and second infections experienced by each animal. The first and second infections comprised about 75% of the observed infections. When looking for preepidemic/epidemic/postepidemic time period effects, we did not see third infections in the preepidemic period and we did not see first infections in the postepidemic period. The analysis compared the duration of infections that began in the preepidemic period to the duration of infections that began during either the epidemic period or the postepidemic period. The regression model results suggested that the duration of infection was significantly longer for first or second infections that began during the epidemic or postepidemic periods relative to the preepidemic period ($P < 0.001$). Specifically, the risk of the first or second infection ending when it began in the epidemic or postepidemic period was 0.36 relative to the risk of the infection ending when it began in the preepidemic period (95% CI: 0.20, 0.66).

DISCUSSION

The range of *E. coli* O157:H7 prevalence in pens of feedlot cattle in this longitudinal study was nearly identical to that we previously reported in a cross-sectional study of commercial feedlot cattle *(27).* Other researchers have reported similar *(3, 6, 18, 29)* or lower *(4, 7, 11, 12)* prevalence values than reported herein. The lower *E. coli* O157:H7 prevalence estimates were supposedly attributed to lower sensitivity methods and differences in methodology *(17).*

In an earlier study *(27), E. coli* O157:H7 prevalence varied between pens within a feedlot but not between feedlots, and we were unable to test the role of time on variability. In this study, we describe the variation in *E. coli* O157:H7 shedding between pens and across time during the feeding period for this group of cattle. Other longitudinal studies of feedlot cattle have been reported *(18, 19).* This pattern of change over time might suggest the existence of time-dependent risk factors for *E. coli* O157:H7 shedding, which underscores the importance of longitudinal study designs in our effort to understand the epidemiology of this organism in feedlot cattle.

The pattern of *E. coli* O157:H7 prevalence observed in this feedlot allowed us to define three phases within the feeding period. Further investigation showed a difference in the odds of *E. coli* O157:H7 incidence among the three phases, and this was the basis of defining the three periods as preepidemic, epidemic, and postepidemic. The limitations in our estimation of the odds of an incident case of *E. coli* O157:H7 stemmed from some imputed values and from the assumption that the odds of *E. coli* O157:H7 incidence were similar across all pens. It is possible this was

not the case. Also, because of sample size limitation (10 pens only), we were unable to test for space-time interaction on these data. Nevertheless, it was possible to discern an epidemic curve-like pattern of *E. coli* O157:H7 shedding in this feedlot.

In this study, the mean duration of *E. coli* O157:H7 fecal shedding, according to the week in which the infection started, was longest (4.5 weeks) during the epidemic period. Another study reported a typical duration of fecal shedding in cattle of approximately 1 month *(2).* Also, duration of fecal shedding of *E. coli* O157:H7 did not differ significantly between first and second infections or first and third infections. This might be an indication that first infections of cattle with *E. coli* O157:H7 neither confer significant long-term immunity to the cattle to further infections nor reduce duration of shedding of the organism. Another possible explanation of this observation is that cattle are exposed to different challenge levels or clonal types of *E. coli* O157:H7 during the different phases of an epidemic. There is a report *(5)* that a relatively high challenge dose ($>10^4$ and probably $\geq 10^7$ CFU) of *E. coli* O157:H7 grown in vitro is required to infect adult cattle compared to preweaned calves. Information concerning the immune responses and their potential roles in protection of cattle against *E. coli* O157:H7 infection is minimal. Experimental infection studies have shown that 1-week-old calves *(26),* 8- to 10-month-old ruminating calves *(5),* and adult (>1 year old) cattle *(20)* have reduced shedding of *E. coli* O157:H7 following repeat challenge with the homologous strain compared to the first challenge, suggesting some level of protective immunity results from infection. A limitation of this study was not knowing whether the infections at the different time sequences were caused by the same or different *E. coli* O157:H7 strains. This analysis is currently in progress. Furthermore, no information is currently available to suggest whether strain homology or heterology plays a significant role in increased susceptibility to repeated infection.

Prevalence of a disease is a function of both its incidence and duration. In this study, we investigated whether changes in *E. coli* O157:H7 prevalence were caused by changes in incidence or duration. Our results suggest that variation in *E. coli* O157:H7 pen-level prevalence was a function of changes in both incidence and duration, indicating a need to identify factors and subsequently control strategies that will reduce both incidence and duration of *E. coli* O157:H7 fecal shedding. Also, pens and time (weeks) were significant sources of variability in *E. coli* O157:H7 prevalence, indicating a need to study both pen-level and time-dependent factors. Therefore, longitudinal study designs are important for understanding the epidemiology and methods needed to control this organism.

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