

Longitudinal Study of *Escherichia coli* O157:H7 in a Beef Cattle Feedlot and Role of High-Level Shedders in Hide Contamination[∇]

Terrance M. Arthur,^{1*} James E. Keen,^{1,2} Joseph M. Bosilevac,¹ Dayna M. Brichta-Harhay,¹
Norasak Kalchayanand,¹ Steven D. Shackelford,¹ Tommy L. Wheeler,¹
Xiangwu Nou,^{1†} and Mohammad Koohmaraie^{1‡}

U.S. Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, Nebraska 68933-0166,¹ and University of Nebraska—Lincoln, Department of Veterinary and Biomedical Sciences, Great Plains Veterinary Educational Center, Clay Center, Nebraska 68933²

Received 13 January 2009/Accepted 8 August 2009

The objectives of the study described here were (i) to investigate the dynamics of *Escherichia coli* O157:H7 fecal and hide prevalence over a 9-month period in a feedlot setting and (ii) to determine how animals shedding *E. coli* O157:H7 at high levels affect the prevalence and levels of *E. coli* O157:H7 on the hides of other animals in the same pen. Cattle ($n = 319$) were distributed in 10 adjacent pens, and fecal and hide levels of *E. coli* O157:H7 were monitored. When the fecal pen prevalence exceeded 20%, the hide pen prevalence was usually (25 of 27 pens) greater than 80%. Sixteen of 19 (84.2%) supershedder ($>10^4$ CFU/g) pens had a fecal prevalence greater than 20%. Significant associations with hide and high-level hide (≥ 40 CFU/100 cm²) contamination were identified for (i) a fecal prevalence greater than 20%, (ii) the presence of one or more high-density shedders (≥ 200 CFU/g) in a pen, and (iii) the presence of one or more supershedders in a pen. The results presented here suggest that the *E. coli* O157:H7 fecal prevalence should be reduced below 20% and the levels of shedding should be kept below 200 CFU/g to minimize the contamination of cattle hides. Also, large and unpredictable fluctuations within and between pens in both fecal and hide prevalence of *E. coli* O157:H7 were detected and should be used as a guide when preharvest studies, particularly preharvest intervention studies, are designed.

It is now well established that at the time of harvest, hides are the major source of *Escherichia coli* O157:H7 contamination on beef carcasses (1, 4, 22). Thus, reducing the levels of food-borne pathogens on cattle hides has been the focus of many pre- and postharvest research efforts. For postharvest applications, hide interventions (i.e., washing of hide-on carcasses with various antimicrobial agents) are direct approaches and have been shown to be efficacious for reducing hide and carcass contamination rates (2, 4, 5, 22).

In the area of preharvest research, several approaches have been taken to reduce the prevalence of *E. coli* O157:H7 in feces of cattle presented for slaughter. These approaches include, among others, feeding cattle probiotics (dietary administration of beneficial bacteria to compete with *E. coli* O157:H7), vaccination, and bacteriophage treatment (8, 24, 30). These intervention approaches are indirect. By reducing the fecal pathogen load, the pathogen prevalence and the level on hides are reduced through lower cross-contamination at the feedlot, and subsequently, carcass contamination rates decrease. While the effectiveness of preharvest interventions var-

ies, no preharvest intervention is 100% effective in reducing the fecal prevalence of *E. coli* O157:H7. It is not known what level of pathogen reduction in feces would be necessary to significantly reduce hide and carcass contamination during processing. Key pieces of information needed to address this question are the number of shedding cattle in a pen needed to contaminate the hides of most of the cattle in the same pen and at what level the shedding cattle are contaminated.

Aside from the number of cattle shedding a pathogen, the concentration of the pathogen in feces plays a pivotal role in spreading the pathogen between animals. Recently, cattle shedding *E. coli* O157:H7 at levels of $>10^4$ CFU/g (“supershedders”) have been associated with high rates of transmission of the pathogen between cohort animals (18, 23). Matthews et al. reported that 20% of the *E. coli* O157:H7 infections in cattle on Scottish farms were responsible for 80% of the transmission of the organism between animals (18). Another study reported similar findings; 9% of the animals shedding *E. coli* O157:H7 produced over 96% of the total *E. coli* O157:H7 fecal load for the group (23). While a number of studies have indicated the importance of supershedders in fecal transmission dynamics, there is a general lack of information concerning the effects of high shedding rates on hide prevalence and load. Accordingly, the objectives of this study were (i) to investigate the dynamics of *E. coli* O157:H7 prevalence and levels in feces and on hides of feedlot cattle over time and (ii) to determine how pathogen prevalence and levels on hides in a pen are affected by individuals shedding *E. coli* O157:H7 at high levels.

In the analysis presented here, fecal shedding was analyzed

* Corresponding author. Mailing address: Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, NE 68933-0166. Phone: (402) 762-4227. Fax: (402) 762-4149. E-mail: terrance.arthur@ars.usda.gov.

† Present address: USDA, ARS, Animal & Natural Resource Institute, Building 201, BARC-East, 10300 Baltimore Ave., Beltsville, MD 20705-2350.

‡ Present address: IEH Laboratories and Consulting Group, 15300 Bothell Way NE, Lake Forest Park, WA 98155.

[∇] Published ahead of print on 14 August 2009.

TABLE 1. Feeding regimen

Dates (mo/day/yr)	Dry matter composition of diet (%)				
	Protein supplement ^a	Corn silage	Dry rolled corn	High-moisture corn	Alfalfa hay
9/13/2004 to 9/30/2004	2.4	20.0	34.0		43.6
10/1/2004 to 10/19/2004	2.6	43.0	31.5		22.9
10/20/2004 to 12/7/2004	4.5	66.0	29.5		
12/8/2004 to 12/17/2004	4.5	38.5		57.0	
12/18/2004 to 12/25/2004	4.5	25.5		70.0	
12/26/2004 to 4/30/2005	4.5	19.3		76.3	
5/1/2005 to 5/3/2005	4.5	12.8	82.8		

^a The protein supplement contained rumensin.

using the following three categories based on the level of *E. coli* O157:H7 being shed: shedding positive (presumed concentration, ≥ 1 CFU/g), high-density shedder (≥ 200 CFU/g), and supershedder ($\geq 10^4$ CFU/g). Several definitions of *E. coli* O157:H7 supershedders have been offered previously. One-time shedding levels of $>10^3$ or $>10^4$ CFU/g have been used in multiple studies (17, 23, 24), while other groups have required persistent colonization of the rectoanal junction, as well as high cell counts, for an animal to qualify as a supershedder (10). Recently, Chase-Topping et al. (9) reviewed the requirements for supershedder status and provided a working definition: an animal that excretes $>10^4$ CFU/g. In doing this, Chase-Topping et al. noted the high stringency of this definition and acknowledged that with such a definition some supershedders will be missed if they are sampled at times other than peak shedding times (9). In the current study, this was a concern. In an attempt to investigate the link between high-shedding-level animals and hide contamination, greater leeway was needed in the classification. When it is sampled on a monthly basis, an animal shedding at high levels can have a large impact on the hide status of pen cohorts between sampling intervals but not be shedding at peak levels on the day of sample collection. Hence, the categories described above were selected to analyze the relationship between fecal shedding and hide contamination.

MATERIALS AND METHODS

Animals. Charolais crossbred cattle (133 heifers and 186 steers) were sampled in this study. The cattle were weaned at pasture and brought to a feedlot. On the day of weaning, calves were separated from their mothers and transported to the feedlot. At the feedlot, the animals were sorted by gender into six steer pens and four heifer pens. Within each gender group animals were equally distributed between pens based on the herd of origin (two original herds) and date of birth. Animals were fed using the regimen described in Table 1.

Feedlot pens. Ten adjacent pens (50 ft by 250 ft) were used. All of the pens were adjacent, with the exception of pens 8 and 9, which were separated by an alley. There was a common water trough for every two pens. Water troughs were cleaned once a week. A continuous feedbunk was used to feed all of the pens. Two short-term holding pens were utilized for the treatment of sick animals. Nonstudy animals were not allowed in these pens during the study.

Environmental samples. Prior to the arrival of the weaning calves, the pens and water troughs were screened for *E. coli* O157:H7. The pens were cleaned and left idle for approximately 30 days before arrival of the calves. In the week before the calves arrived, each pen was sampled by dividing the pen into eight sections and compositing soil within each section to make a 10-g sample. During each sampling period, sponge samples were collected from the bottom of each of the water troughs.

Cattle sampling. Hide and fecal samples were collected once a month from September 2004 to May 2005. An additional sampling was done in April, result-

ing in sampling times that were separated by 2 weeks. In the September sampling period, cattle were sampled as they entered the feedlot from the pasture at weaning. Hide samples were collected by using a sterile sponge (Nasco, Fort Atkinson, WI) premoistened with buffered peptone water (Difco, Becton Dickinson, Sparks, MD) and swabbing an approximately 1,000-cm² area behind the left shoulder. The area was wetted with distilled water prior to sampling. Fecal samples (10 g) were collected by rectal palpation.

Enumeration. *E. coli* O157:H7 in hide, fecal, soil, and water samples was enumerated using a protocol previously described by Brichta-Harhay et al. (7). For each hide sample, the sponge was homogenized by hand massaging it prior to the addition of enrichment medium, and 250 μ l of solution was placed in a microcentrifuge tube. The tube was vortexed and then kept static for 3 min to allow the debris to settle. Following the settling period, 50 μ l of the sample was spiral plated onto ntCHROMAgar (CHROMAgar-O157 [DRG International, Mountainside, NJ] supplemented with novobiocin [20 mg/liter; Sigma, St. Louis, MO] and potassium tellurite [0.8 mg/liter; Sigma]) plates. When fecal samples were used for enumeration, enrichment medium (90 ml of tryptic soy broth [TSB] [Becton Dickinson] with phosphate buffer [30 g of TSB per liter, 2.31 g of KH₂PO₄ per liter, 12.54 g of K₂HPO₄ per liter] [TSB+PO4]) was added to a 10-g fecal sample, and the mixture was homogenized by hand massage. One milliliter of the sample mixture was placed in a microcentrifuge tube and vortexed. Enumeration was then carried out as described above for hide samples. Soil samples were examined using the method described above for fecal samples. Water trough samples were processed as described above for hide samples. The limits of detection for the enumeration assays were 200 CFU/g, 40 CFU/100 cm², 200 CFU/g, and 40 CFU/100 cm² for the fecal, hide, soil, and water trough samples, respectively (7).

Sample processing for prevalence analysis. Hide and water trough sponge samples were enriched with 80 ml of TSB, while fecal samples were enriched with 90 ml of TSB+PO4. Ten grams of composite soil was mixed with 90 ml of TSB+PO4 for enrichment. The hide and fecal sample bags were incubated at 25°C for 2 h and then at 42°C for 6 h before they were incubated at 4°C overnight. The soil and water trough sample bags were incubated at 42°C for 8 h before they were incubated at 4°C overnight. Following incubation, the samples were processed by performing immunomagnetic separation, in which 1 ml from each enrichment was subjected to anti-O157 immunomagnetic bead cell concentration (Invitrogen, Carlsbad, CA). Fifty microliters of the final bead-bacterium complexes were spread plated onto (i) ntChromagar and (ii) cSMAC (sorbitol MacConkey agar [Becton Dickinson] supplemented with cefixime [0.05 mg/liter] and potassium tellurite [2.5 mg/liter; Invitrogen]). All plates were incubated at 35 to 37°C for 18 to 20 h. After the plates were incubated, up to three suspect colonies were picked and tested using latex agglutination (DrySpot *E. coli* O157; Oxoid). PCR was used to confirm that each isolate harbored genes for the O157 antigen, H7 flagella, and at least one of the Shiga toxins (12).

The relationship between hide *E. coli* O157:H7 prevalence and density and fecal prevalence and density at the pen level was of primary interest. The underlying hypothesis (path model) was that fecal shedding leads directly or indirectly (via the environment) to hide contamination of penmates in close temporal proximity.

First, relationships between *E. coli* O157:H7 prevalence, fecal shedding, high-density fecal shedding ($\geq 2 \times 10^2$ CFU/g), fecal supershedding ($>10^4$ CFU/g), hide prevalence, and high-density hide contamination (≥ 40 CFU/100 cm²) were examined with bivariate plots using Excel. Fecal load and hide load variables were also created. Fecal load was defined as the geometric mean number of fecal *E. coli* O157:H7 CFU/g for all cattle in a specific pen and sampling time and was calculated. Cattle that were fecal positive only with enrichment were given a fecal value of 100 CFU/g, the midpoint between zero and the quantification detection limit, 200 CFU/g. Hide load was defined similarly as the geometric mean number of *E. coli* O157:H7 hide CFU/100 cm² for all cattle in a specific pen and sampling time and was calculated. Cattle that were hide positive only with enrichment were given a hide load value of 20 CFU/100 cm², the midpoint between zero and the quantification detection limit, 40 CFU/100 cm².

Specific fecal and hide relationships suggested by bivariate plots were explored further, and the results were quantified using multiple-logistic-regression modeling for correlated data (generalized estimating equations; Proc GenMod, v9.1; SAS, Cary, NC). Statistical adjustment for correlated (clustered) data was necessary because the same cattle were repeatedly sampled over time during the 8-month follow-up period and because the animals were housed in pens. For this analysis, a cluster was defined as the cattle that were housed together in the same feedlot pen during the study. Five outcomes were of interest: (i) hide prevalence (number of hide-positive cattle in a pen at each sampling time/number of cattle in the pen), (ii) high-density hide prevalence (number of high-density hide-contaminated cattle in a pen at each sampling time/number of cattle in the pen),

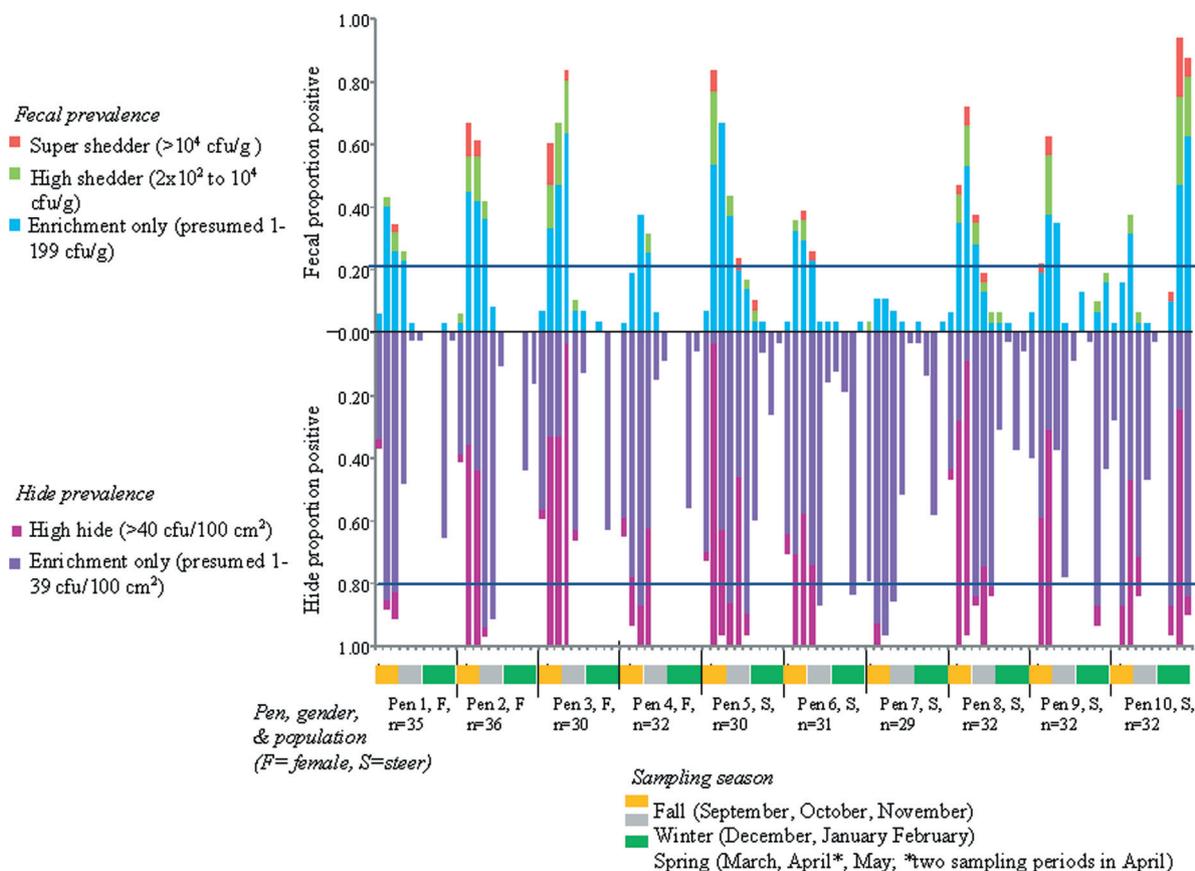


FIG. 1. Fecal prevalence and hide prevalence in 10 feedlot pens for 10 sequential sampling times, based on *E. coli* O157:H7 density. The monthly sampling prevalence and enumeration data for the different pens are shown. The horizontal lines indicate the 20% fecal prevalence and 80% hide prevalence values, which were defined as the threshold limits.

(iii) presence or absence of any high-density hide-contaminated cattle in a pen at a specific sampling time (dichotomy), (iv) hide prevalence greater than 80% (dichotomy), and (v) high-density hide prevalence greater than 20% (dichotomy).

For hide outcomes i to v, three separate dichotomous fecal explanatory variables were examined as explanatory variables: (i) fecal prevalence greater than 20% (dichotomy), (ii) presence or absence of one or more high-density-fecal-shedding animals in a pen, and (iii) presence or absence of one or more fecal “supershedding” animals in a pen. For outcome vi, pen fecal prevalence, explanatory variables ii and iii were examined. All models were adjusted to take into account the effects of season on *E. coli* O157:H7 occurrence by creating a categorical variable “season” that was included in all initial logistic regression models. Samples collected in September, October, and November were considered “fall” samples, samples collected in December, January, and February were considered “winter” samples, and the remaining specimens collected in March, April, and May were considered “spring” samples. Winter was defined as the reference condition.

For logistic regression, odds ratios (OR) with 95% confidence intervals were generated as measures of both the magnitude and direction of association between the six *E. coli* O157:H7 outcomes and the three explanatory variables. Explanatory variables with an OR greater than 1.0 have increased outcome likelihood relative to the reference condition, while explanatory variables with an OR less than 1.0 have decreased outcome likelihood relative to the reference condition. Both for bivariate plots and for logistic regression models, only data for samples collected between 15 October and 2 May were included (sampling events 2 through 10). The results from the first sampling were excluded because the cattle were coming off pasture and entering the feedlot at the time. Thus, their *E. coli* O157:H7 status at sampling time 1 represented pasture status, not feedlot pen status.

RESULTS AND DISCUSSION

This project was designed to monitor the fluctuations over time of *E. coli* O157:H7 hide and fecal prevalence for 319 feedlot cattle housed in neighboring pens. For 7 of the 10 pens and for both of the short-term holding pens at least one soil sample was positive for *E. coli* O157:H7 before the animals entered the feedlot (data not shown), and for one section of pen 5 the *E. coli* O157:H7 concentration was 200 CFU/g of soil. No water troughs were positive for *E. coli* O157:H7 at that time.

In September, the cattle were sampled as they entered the feedlot at weaning. Fecal samples from 16 (5%) of the 319 animals coming from the pasture were positive for *E. coli* O157:H7 (Fig. 1). Two animals were shedding *E. coli* O157:H7 at high levels, at concentrations of 1,400 and 9,000 CFU/g of feces (Table 2). Over one-half (54%) of the animals were positive for *E. coli* O157:H7 on their hides (Table 3), and nine animals harbored high levels of *E. coli* O157:H7 on their hides (≥ 40 CFU/100 cm²). The fecal prevalence at weaning (5%) was similar to that reported in other studies of range beef calves at weaning (11, 14, 25, 27). The hide prevalence (54%) of the animals coming off pasture was higher than expected, and to our knowledge this report is

TABLE 2. Numbers of animals shedding high levels of *E. coli* O157:H7

Date	No. of animals shedding ≥ 200 CFU of <i>E. coli</i> O157:H7/g of feces in pen ^a :										Total (n = 319)
	1 (n = 35) ^b	2 (n = 36)	3 (n = 30)	4 (n = 32)	5 (n = 30)	6 (n = 31)	7 (n = 29)	8 (n = 32)	9 (n = 32)	10 (n = 32)	
Sept. 14		1					1				2
Oct. 18	1	8 (4)	8 (4)		9 (2)	1		4 (1)	1 (1)		32 (12)
Nov. 15	3 (1)	7 (2)	6			3 (1)		6 (2)	8 (2)		35 (8)
Dec. 13	1	2	6 (1)	2	2	1 (1)		3 (1)		1	18 (3)
Jan. 10			1		1 (1)			2 (1)			4 (2)
Feb. 7					1			1			2
Mar. 7					2 (1)			1			3 (1)
Apr. 4										1 (1)	1 (1)
Apr. 18									1	15 (6)	16 (6)
May 2									1	8 (2)	9 (2)

^a The numbers in parentheses are the numbers of animals shedding $>10^4$ CFU of *E. coli* O157:H7/g of feces (i.e., supershedders). No data indicates that no animals were determined to be shedding *E. coli* O157:H7 at levels of ≥ 200 CFU/g of feces.
^b n is the number of animals per pen.

the only report of *E. coli* O157:H7 prevalence for beef calves from a range environment.

After a 5-week feedlot acclimation period, hide and fecal samples were collected from all animals, and it was determined that the *E. coli* O157:H7 prevalence had risen sharply. The overall fecal prevalence had gone up to 40% by the October sampling period, and for one pen, (pen 5) the overall fecal prevalence was as high as 83%. It should be noted that pen 5 also had the highest *E. coli* O157:H7 load in the soil before animals were placed in the pens. The high level of *E. coli* O157:H7 in the soil may have contributed to the increased colonization of the animals in the pen. A pulsed-field gel electrophoresis analysis was performed with all isolates collected in this study, but the diversity of genotypes was too low to allow tracking of particular strain types. The hide prevalence had also risen by the October sampling period, to an overall level of 98%, and 8 of the 10 pens were 100% positive for *E. coli* O157:H7. The prevalence remained high through the November sampling period, and the fecal and hide prevalence values were 49% and 98%, respectively. During the winter months, the hide and fecal prevalence values dropped each month (Fig. 1 and Table 3).

As the overall hide prevalence dropped, fluctuations in pen prevalence were seen (Table 3). In the February sampling period, pens 5 and 8 had hide prevalence rates of 97% and

84%, respectively, and the hide prevalence rates for the other pens did not exceed 16%. Pens 6 and 7, which shared water troughs with pens 5 and 8, respectively, had hide prevalence rates of 16% and 3%, respectively. The fecal prevalence of *E. coli* O157:H7 for pen 5 (17%) was the highest fecal prevalence for the sampling period, while pen 8 had a fecal prevalence rate of 6%, just under that for pen 3 (7%). While pen 3 had a fecal prevalence equivalent to that of pen 8, the hide prevalence rate for pen 3 was only 13%. One factor common to the two pens that had high hide prevalence rates (pens 5 and 8) was that both pens contained one animal shedding at a high level (Table 2).

The presence of animals shedding *E. coli* O157:H7 at high levels was likely to be the source of the high hide prevalence rates. In the March sampling period, the pens with one or more animals shedding high levels of *E. coli* O157:H7 in their feces again had the highest hide prevalence rates. The prevalence rates in adjacent pens remained low. *E. coli* O157:H7 was not detected in the water troughs associated with these pens during either the February or March sampling period (data not shown). Of particular interest was a change in pen prevalence that was detected in the first April sampling period. Pens 5 and 8 no longer had any animals shedding at high levels, and their hide prevalence rates dropped to 7% and 3%, respectively. In the same period, an animal in pen 10 was found to be shedding at high levels. The hide prevalence in this pen rose from 0% in March to 97% in April. A second sampling period was added in April, 14 days later. At this sampling time, the number of high-density shedders in pen 10 had increased to 15 and the hide prevalence was 100% (Tables 2 and 3). Also, one animal shedding *E. coli* O157:H7 at a high density was identified in pen 9, and the hide prevalence in this pen had risen from 3% in the previous sampling period to 94%. In the final sampling period, the pens containing animals shedding *E. coli* O157:H7 at high levels (pens 9 and 10) again had the highest hide prevalence rates.

It should be noted that the water trough common to pens 9 and 10 was found to harbor *E. coli* O157:H7 in both April sampling periods and could have been a cause of the increased prevalence in pen 9. Cattle water troughs have been reported to harbor *E. coli* O157:H7 (16, 29), but it is not known how this affects the transmission of this organism between animals. One

TABLE 3. Hide prevalence of *E. coli* O157:H7

Date	Hide prevalence (%) in pen ^a :										Total (319)
	1 (35)	2 (36)	3 (30)	4 (32)	5 (30)	6 (31)	7 (29)	8 (32)	9 (32)	10 (32)	
14 Sept.	37	42	60	66	73	71	79	47	41	28	54
18 Oct.	89	100	100	94	100	100	100	100	100	100	98
15 Nov.	91	100	100	100	97	100	97	97	100	100	98
13 Dec.	49	97	100	100	100	100	86	88	38	84	84
10 Jan.	3	92	67	16	100	87	52	100	78	47	64
7 Feb.	3	11	13	9	97	16	3	84	9	3	24
7 Mar.	0	0	0	0	60	13	3	31	0	0	10
5 Apr.	0	0	0	0	7	19	14	3	3	97	14
18 Apr.	66	44	63	56	27	84	59	38	94	100	63
2 May	3	17	0	6	3	0	0	6	44	91	17
Mean	34.1	50.3	50.3	44.7	66.4	59	49.3	59.4	50.7	65	52.6

^a Values in parentheses represent the number of animals per pen. Gray, 0; blue, 1–20; green, 21–40; yellow, 41–60; orange, 61–80; red, 81–100.

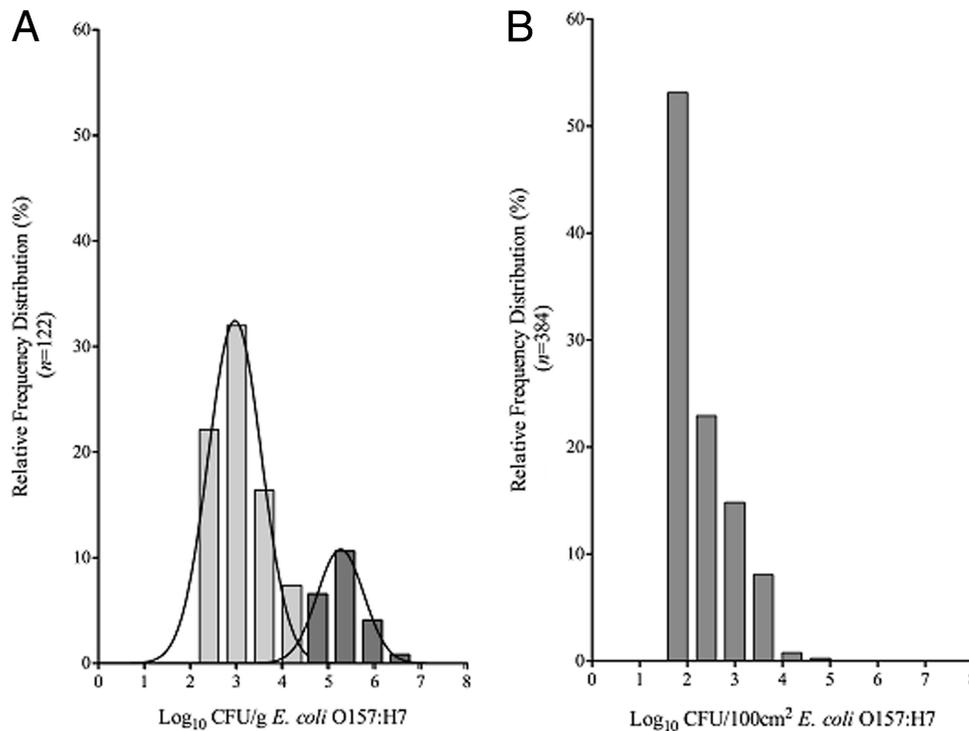


FIG. 2. Distribution of *E. coli* O157:H7 counts. Sample enumeration values (≥ 200 CFU/g for feces and ≥ 40 CFU/100 cm² for hides) were log transformed and binned based on total counts. (A) Plot with fecal bin widths of 1.6 log₁₀ CFU/g. The population had a bimodal distribution. The dark gray bars indicate the lower-density population (high shedders), and the light gray bars indicate the higher-density population (supershedders). (B) Plot with hide bin widths of 1.6 log₁₀ CFU/100 cm².

study attempted to calculate the transmission risk associated with *E. coli* O157:H7-contaminated water troughs by chlorinating the water in one-half of the study pens. However, the level of chlorination was not sufficient to reduce the prevalence in the water troughs; hence, the risk could not be determined (15).

At the individual level, 81% (256 of 319) of the animals shed *E. coli* O157:H7 at least once during this study, and 32% (104 of 319) of the animals shed at high levels at least once. Of the 3,190 fecal samples collected over the course of this study, 122 (3.8%) contained *E. coli* O157:H7 at a concentration of 200 CFU/g or higher. The high-level-shedding values ranged from 200 to 5.7×10^7 CFU/g of feces, and the distribution followed a bimodal pattern (Fig. 2A). While the duration of most of the high-level-shedding events did not extend beyond one sampling period, 13 of the 104 high-level-shedding animals shed *E. coli* O157:H7 at high levels in two consecutive sampling periods. These instances could represent continuous high-level-shedding events or distinct shedding events where the animals did not shed high levels at some time between sampling periods. Five animals were shown to have more than one distinct shedding event, i.e., high-level-shedding events that were separated by at least one sampling period in which an animal was not shedding. All animals harbored *E. coli* O157:H7 on their hides at some point in the study, and 70% (223 of 319) of the animals harbored *E. coli* O157:H7 at high levels. The range of values for the 384 high-level hide samples was 40 to 4.7×10^4 CFU/100 cm² (Fig. 2B).

Previous studies have reported large fluctuations in the fecal

prevalence both within a pen and between adjacent pens containing feedlot cattle. Khaitsa et al. (13) reported that the fecal prevalence of *E. coli* O157:H7 in a pen increased from 10% to 90% in 1 week. Also, in that study adjacent pens were shown to have disparities in fecal prevalence of as much as 100% (13). In another study (26), daily variations in the concentration of *E. coli* O157:H7 shed from an individual animal were observed to exceed 10^5 CFU/g of feces. To our knowledge, similar observations have not been reported for either the prevalence or the levels of *E. coli* O157:H7 on cattle hides.

The data for the large, rapid variations in the hide prevalence and load should be used to guide the design of preharvest research studies of the behavior of *E. coli* O157:H7 and particularly of preharvest interventions to control *E. coli* O157:H7. Studies designed to evaluate preharvest intervention effects on the hides of feedlot animals need to have sufficient pen replication to accommodate the inherent variation in the *E. coli* O157:H7 prevalence and load on the hides of feedlot cattle. In such studies, the pen is the experimental unit, and, as described here, there are large disparities in hide prevalence and load between adjacent pens even in the absence of treatment.

The second objective of the present study was to identify how animals shedding *E. coli* O157:H7 at high levels affect the prevalence and levels of *E. coli* O157:H7 on the hides of other animals in the same pen. Of particular interest was determination of the number of animals shedding sufficient *E. coli* O157:H7 to contaminate the hides of a majority of the animals in the pen.

Bivariate plots of pen hide and fecal density and prevalence

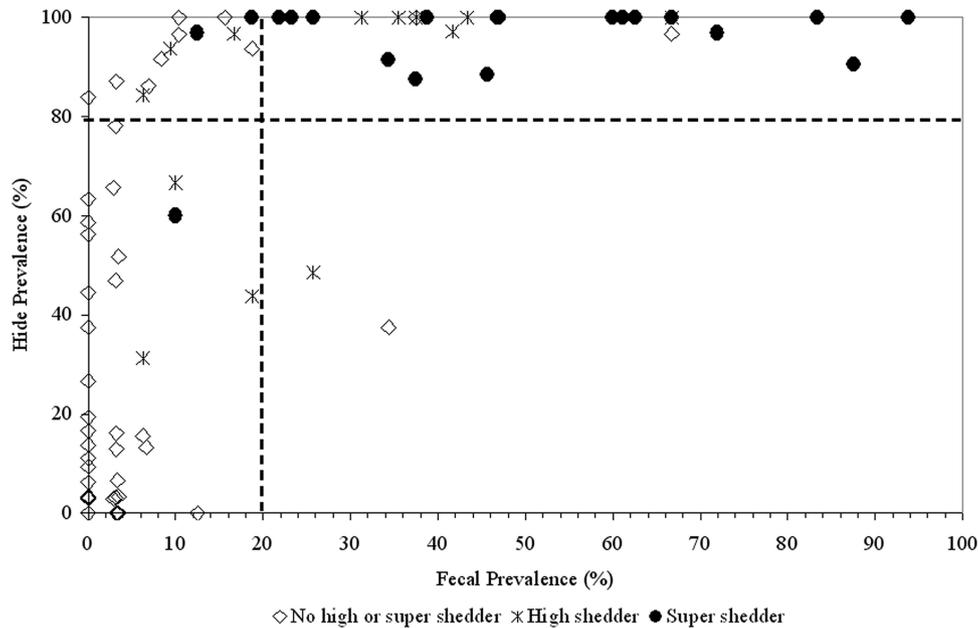


FIG. 3. Plot of fecal *E. coli* O157:H7 prevalence versus hide *E. coli* O157:H7 prevalence. The bivariate plot shows prevalence values for 90 feedlot cattle pens. A total of 319 cattle in 10 adjacent feedlot pens were sampled nine times from October through May. The dashed lines indicate 80% hide prevalence and 20% fecal prevalence.

were used to identify potential relationships. As shown in Fig. 3, there appears to be a threshold response relationship between fecal prevalence and hide prevalence; when the fecal pen prevalence exceeded 20%, the hide pen prevalence was usually (25/27 [92.6%] of the samples) greater than 80%. There was a highly variable (unstable) relationship between fecal prevalence and hide prevalence when the fecal prevalence was <20%. Hide prevalence could be high, moderate, or low with low fecal prevalence. Interestingly, the fecal prevalence for most pens containing one or more supershedders exceeded 20%. Seventeen of 19 supershedder pens (85%) also were pens with a fecal prevalence greater than 20%. These 17 pens contained 32 of the 35 (91.4%) “supershedder” cattle. Thus, 20% fecal prevalence was a functional threshold marker of supershedder pens and supershedding cattle. It is not clear which factor is responsible for this scenario. One hypothesis is that as fecal prevalence increases, the probability of supershedders increases. In contrast, increasing numbers of supershedders would lead to more environmental contamination and more potential for colonization, leading to increased fecal prevalence.

Bivariate plot analysis also led to the observation that when >80% of cattle in a pen were hide positive, the high-density hide prevalence increased rapidly (Fig. 4). Conversely, the high-density hide prevalence was very low (<10%) if the hide prevalence was <80%. Thus, 80% hide prevalence is a functional threshold marker of increased high-density hide contamination. Most pens containing one or more “supershedder” cattle were pens with a hide prevalence greater than 80% (18 of 19 pens [94.7%]). Fourteen “supershedder” pens were found to be pens where the high-density hide prevalence exceeded 20%. These pens contained 29 of 35 the supershedding cattle (82.9%). Both Fig. 3 and 4 show that as the density of

shedding increases, the impact on hide contamination also increases.

Based on the outcomes of the plot analysis, multiple-logistic-regression modeling was used to quantify the risk of fecal shedding for hide contamination. The results are shown in Table 4. Significant associations with hide and high-level hide contamination (Table 4, models I to V) were identified for the variables (i) fecal prevalence greater than 20%, (ii) one or more high-density shedders (≥ 200 CFU/g) in a pen, and (iii) one or more supershedders ($>10^4$ CFU/g) in a pen. Increases in hide prevalence and high-level hide prevalence were at least nine times more likely in the presence of any of these explanatory variables. While this analysis demonstrates how dramatically the risk factors influence hide contamination, the inter-relatedness of these three variables leads to an inability to discern if increasing fecal prevalence or shedding at high levels is the cause of increased hide prevalence and load.

Previous studies have implicated high-level-shedding individuals as the driving force for increased fecal prevalence. Matthews et al. (19) used cattle from multiple Scottish farms as the basis for development of a mathematical model of *E. coli* O157:H7 transmission. From their work it was determined that the observed distribution of shedding animals was not adequately described by the initial theoretical model used (19). In order to correct the model, Matthews and Woolhouse incorporated the concept that a small proportion of animals (supershedders) are highly infective for the rest of the population (20). With the revised model, it was shown that in the Scottish cattle population studied, approximately 20% of the infections could account for 80% of the transmission (18). Cobbold et al. (10) also concluded that the shedding of *E. coli* O157:H7 in a pen is influenced by supershedders, noting that cattle that did not shed *E. coli* O157:H7 over the course of the study were five

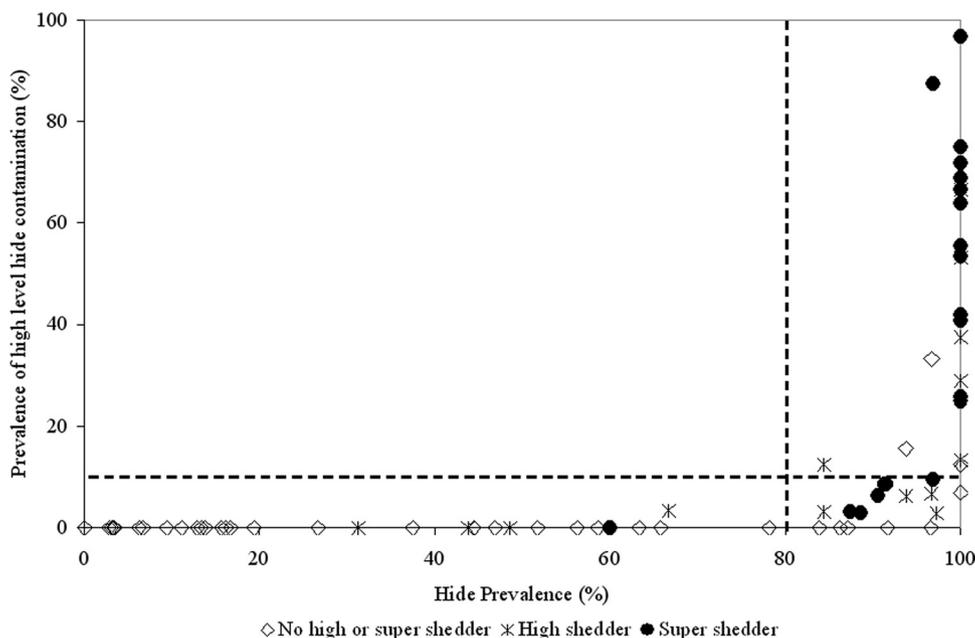


FIG. 4. Plot of hide *E. coli* O157:H7 prevalence versus high-density hide *E. coli* O157:H7 prevalence. The bivariate plot shows prevalence values for 90 feedlot cattle pens. A total of 319 cattle in 10 adjacent feedlot pens were sampled nine times from October through May. The dashed lines indicate 80% hide prevalence and 10% high-level hide prevalence (≥ 40 CFU/100 cm²).

times more likely to be housed in a pen that did not contain a supershedder. Incorporation of these results into the present study suggests that high-level-shedding events lead to fecal prevalence greater than 20%, hide prevalence greater than 80%, and the spike in high-level hide contamination seen when the hide prevalence is greater than 80%.

The effect of supershedders on hide contamination was recently simulated by placing inoculated fecal pats in pens containing naïve cattle (28). The inoculated strains were detected in hide samples from the high-level-inoculum group 1 day after deposition of the fecal pats. Overall, the pens receiving the high-level-inoculum fecal pats had the highest hide prevalence

(3%); for the low-level-inoculum pens there was only one positive hide sample (0.45%), and for the control pens there was no positive hide sample (28). Similar findings were obtained by McGee et al. (21) when inoculated steers, each shedding *E. coli* O157:H7 at levels greater than 500 CFU/g of feces, were placed in pens with five uninoculated, noncolonized cohorts. Hide samples from 66% of the cohort animals were found to be positive for the marked strains after 48 h of exposure to the high-level-shedding animals (21). In one pen all of the occupants (the inoculated animal and the five cohorts) harbored *E. coli* O157:H7 on their hides within 24 h of comingling (21). In the current study, pulsed-field gel electrophoresis was per-

TABLE 4. Logistic regression models of associations between the presence of one or more high-level-fecal-shedding cattle in a feedlot pen and the outcomes of hide prevalence and high-level-hide-contamination prevalence analyses

Model	Pen-level outcome	Explanatory variable (risk factor)	OR	OR 95% confidence interval	P value
Ia	Hide prevalence	Fecal prevalence greater than 20%	9	2.8–29.2	0.0002
Ib		Any high-density fecal shedder in pen ^a	14.4	6.5–31.8	<0.0001
Ic		Any fecal supershedder in pen ^b	27.1	5.5–134.8	<0.0001
IIa	High-level hide prevalence ^c	Fecal prevalence greater than 20%	19.6	6.9–55.5	<0.0001
IIb		Any high-density fecal shedder in pen	17.6	6.5–47.8	<0.0001
IIc		Any fecal supershedder in pen	10.2	5.4–19.3	<0.0001
IIIa	Any high-level hide cattle in pen	Fecal prevalence greater than 20%	22.9	4.4–119.4	0.0002
IIIb		Any high-density fecal shedder in pen	72.9	18.7–284.8	<0.0001
IIIc		Any fecal supershedder in pen	176.8	9.5–3,309	0.0005
IVa	Hide prevalence of >80%	Fecal prevalence greater than 20%	44.3	9.3–211.3	<0.0001
IVb		Any high-density fecal shedder in pen	46.4	6.3–343.7	0.0003
IVc		Any fecal supershedder in pen	26.2	8.2–84.1	<0.0001
Va	High-level hide prevalence of >20%	Fecal prevalence greater than 20%	53.6	5.5–519.9	0.0006
Vb		Any high-density fecal shedder in pen	50.2	4.9–520.7	0.001
Vc		Any fecal supershedder in pen	44.8	6.7–302.2	<0.0001

^a A high-density fecal shedder is an animal shedding ≥ 200 CFU of *E. coli* O157:H7/g of feces.

^b A fecal supershedder is an animal shedding $>10^4$ CFU of *E. coli* O157:H7/g of feces.

^c Animals harboring *E. coli* O157:H7 on the hide at levels of ≥ 40 CFU/100 cm².

formed with all *E. coli* O157:H7 isolates in an attempt to attribute hide contamination to the animals shedding high levels of bacteria. Unfortunately, the diversity of *E. coli* O157:H7 genotypes in the feedlot was too low (97% of the isolates were classified in two pulsed-field gel electrophoresis patterns) to reach any conclusions regarding attribution (data not shown).

High-level shedding also can play a role in hide contamination outside the feedlot. Recent work has shown that 80% of the *E. coli* O157:H7 strains isolated from beef carcasses at processing did not come from the feedlot where the cattle originated (1). The sources of these isolates were determined to be the processing plant lairage environment and the trailers used to transport the animals to the processing plant (1). In these areas, where there is high cattle density and rapid turnover, a few animals shedding high levels of bacteria can contaminate the hides of many animals just prior to harvest. Omisakin et al. (23) determined that for one period of time, 9% of cattle presented for slaughter at a British abattoir were responsible for 96% of the total *E. coli* O157 shed by all animals entering the abattoir during that time.

The relationships involving animals harboring *E. coli* O157:H7 on their hides, especially high concentrations of *E. coli* O157:H7, are important as it has been determined that at harvest the hide is the main source of contamination for the carcass (1, 4, 22) and previous studies have shown that as hide prevalence increases, carcass prevalence also increases (3, 6, 22). It is clear that interventions focusing on reducing or eliminating high-level-shedding events would be very beneficial for reducing the *E. coli* O157 burden in the production and processing environments. It has been suggested that spread of *E. coli* O157:H7 between cattle could be controlled if one could prevent high-level shedding in the 5% of the individuals that are the main source of contamination (18). The data presented here suggest that preventing high-level-shedding events should be a main goal of preharvest intervention research. It is not realistic to expect or required for an intervention to completely eliminate *E. coli* O157:H7 from cattle presented for harvest. The function of preharvest intervention is to reduce the load on the incoming cattle to bring the load in line with the capacity of the postharvest interventions used. Thus, removing the high-level-shedding events and reducing fecal prevalence to less than 20% should go a long way toward meeting this objective.

In summary, this study showed that there are large variations in the hide and fecal carriage of *E. coli* O157:H7 among feedlot pens. Also, large fluctuations in hide and fecal prevalence and levels can occur within a pen in a short span of time, as shown by the increase in hide prevalence from 3% to 94% in only a 2-week period. We provide evidence here of strong associations between high-level fecal shedding and hide (including high-level hide) contamination in live cattle. The results presented here suggest that the *E. coli* O157:H7 fecal prevalence should be reduced to less than 20% and the levels of shedding should be kept below 200 CFU/g to minimize the contamination of cattle hides.

ACKNOWLEDGMENTS

This project was funded in part by The Beef Checkoff.

We thank Julie Dyer, Sue Hauver, Bruce Jasch, Kim Kucera, Frank Reno, and Greg Smith for technical support and Marilyn Bierman for secretarial support.

Names of products are necessary to report available data factually; however, the USDA neither guarantees nor warrants the standard of a product, and the use of a name by the USDA implies no approval of the product to the exclusion of other products that may also be suitable.

REFERENCES

- Arthur, T. M., J. M. Bosilevac, D. M. Brichta-Harhay, M. N. Guerini, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2007. Transportation and lairage environment effects on prevalence, numbers, and diversity of *Escherichia coli* O157:H7 on hides and carcasses of beef cattle at processing. *J. Food Prot.* **70**:280–286.
- Arthur, T. M., J. M. Bosilevac, D. M. Brichta-Harhay, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2007. Effects of a minimal hide wash cabinet on the levels and prevalence of *Escherichia coli* O157:H7 and *Salmonella* on the hides of beef cattle at slaughter. *J. Food Prot.* **70**:1076–1079.
- Arthur, T. M., J. M. Bosilevac, X. Nou, S. D. Shackelford, T. L. Wheeler, M. P. Kent, D. Jaroni, B. Pauling, D. M. Allen, and M. Koohmaraie. 2004. *Escherichia coli* O157 prevalence and enumeration of aerobic bacteria, *Enterobacteriaceae*, and *Escherichia coli* O157 at various steps in commercial beef processing plants. *J. Food Prot.* **67**:658–665.
- Bosilevac, J. M., T. M. Arthur, T. L. Wheeler, S. D. Shackelford, M. Rossman, J. O. Reagan, and M. Koohmaraie. 2004. Prevalence of *Escherichia coli* O157 and levels of aerobic bacteria and *Enterobacteriaceae* are reduced when hides are washed and treated with cetylpyridinium chloride at a commercial beef processing plant. *J. Food Prot.* **67**:646–650.
- Bosilevac, J. M., X. Nou, M. S. Osborn, D. M. Allen, and M. Koohmaraie. 2005. Development and evaluation of an on-line hide decontamination procedure for use in a commercial beef processing plant. *J. Food Prot.* **68**:265–272.
- Brichta-Harhay, D. M., M. N. Guerini, T. M. Arthur, J. M. Bosilevac, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2008. *Salmonella* and *Escherichia coli* O157:H7 contamination on hides and carcasses of cull cattle presented for slaughter in the United States: an evaluation of prevalence and loads by immunomagnetic separation and direct plating methods. *Appl. Environ. Microbiol.* **74**:6289–6297.
- Brichta-Harhay, D. M., T. M. Arthur, J. M. Bosilevac, M. N. Guerini, N. Kalchayanand, and M. Koohmaraie. 2007. Enumeration of *Salmonella* and *Escherichia coli* O157:H7 in ground beef, cattle carcass, hide and fecal samples using direct plating methods. *J. Appl. Microbiol.* **103**:1657–1668.
- Callaway, T. R., T. S. Edrington, A. D. Brabban, R. C. Anderson, M. L. Rossman, M. J. Engler, M. A. Carr, K. J. Genovese, J. E. Keen, M. L. Looper, E. M. Kutter, and D. J. Nisbet. 2008. Bacteriophage isolated from feedlot cattle can reduce *Escherichia coli* O157:H7 populations in ruminant gastrointestinal tracts. *Foodborne Pathog. Dis.* **5**:183–191.
- Chase-Topping, M., D. Gally, C. Low, L. Matthews, and M. Woolhouse. 2008. Super-shedding and the link between human infection and livestock carriage of *Escherichia coli* O157. *Nat. Rev. Microbiol.* **6**:904–912.
- Cobbold, R. N., D. D. Hancock, D. H. Rice, J. Berg, R. Stilborn, C. J. Hovde, and T. E. Besser. 2007. Rectoanal junction colonization of feedlot cattle by *Escherichia coli* O157:H7 and its association with supershedders and excretion dynamics. *Appl. Environ. Microbiol.* **73**:1563–1568.
- Dunn, J. R., J. E. Keen, R. Del Vecchio, T. E. Wittum, and R. A. Thompson. 2004. *Escherichia coli* O157:H7 in a cohort of weaned, preconditioned range beef calves. *J. Food Prot.* **67**:2391–2396.
- Hu, Y., Q. Zhang, and J. C. Meitzler. 1999. Rapid and sensitive detection of *Escherichia coli* O157:H7 in bovine faeces by multiplex PCR. *J. Appl. Microbiol.* **87**:867–876.
- Khaitsa, M. L., D. R. Smith, J. A. Stoner, A. M. Parkhurst, S. Hinkley, T. J. Klopfenstein, and R. A. Moxley. 2003. Incidence, duration, and prevalence of *Escherichia coli* O157:H7 fecal shedding by feedlot cattle during the finishing period. *J. Food Prot.* **66**:1972–1977.
- Laegreid, W. W., and J. E. Keen. 2004. Estimation of the basic reproduction ratio (R0) for Shiga toxin-producing *Escherichia coli* O157:H7 (STEC O157) in beef calves. *Epidemiol. Infect.* **132**:291–295.
- LeJeune, J. T., T. E. Besser, D. H. Rice, J. L. Berg, R. P. Stilborn, and D. D. Hancock. 2004. Longitudinal study of fecal shedding of *Escherichia coli* O157:H7 in feedlot cattle: predominance and persistence of specific clonal types despite massive cattle population turnover. *Appl. Environ. Microbiol.* **70**:377–384.
- LeJeune, J. T., T. E. Besser, N. L. Merrill, D. H. Rice, and D. D. Hancock. 2001. Livestock drinking water microbiology and the factors influencing the quality of drinking water offered to cattle. *J. Dairy Sci.* **84**:1856–1862.
- Low, J. C., I. J. McKendrick, C. McKechnie, D. Fenlon, S. W. Naylor, C. Currie, D. G. Smith, L. Allison, and D. L. Gally. 2005. Rectal carriage of enterohemorrhagic *Escherichia coli* O157 in slaughtered cattle. *Appl. Environ. Microbiol.* **71**:93–97.

18. **Matthews, L., J. C. Low, D. L. Gally, M. C. Pearce, D. J. Mellor, J. A. Heesterbeek, M. Chase-Topping, S. W. Naylor, D. J. Shaw, S. W. Reid, G. J. Gunn, and W. E. Woolhouse.** 2006. Heterogeneous shedding of *Escherichia coli* O157 in cattle and its implications for control. *Proc. Natl. Acad. Sci. USA* **103**:547–552.
19. **Matthews, L., I. J. McKendrick, H. Terner, G. J. Gunn, B. Synge, and M. E. Woolhouse.** 2006. Super-shedding cattle and the transmission dynamics of *Escherichia coli* O157. *Epidemiol. Infect.* **134**:131–142.
20. **Matthews, L., and M. Woolhouse.** 2005. New approaches to quantifying the spread of infection. *Nat. Rev. Microbiol.* **3**:529–536.
21. **McGee, P., L. Scott, J. J. Sheridan, B. Earley, and N. Leonard.** 2004. Horizontal transmission of *Escherichia coli* O157:H7 during cattle housing. *J. Food Prot.* **67**:2651–2656.
22. **Nou, X., M. Rivera-Betancourt, J. M. Bosilevac, T. L. Wheeler, S. D. Shackelford, B. L. Gwartney, J. O. Reagan, and M. Koohmaraie.** 2003. Effect of chemical dehairing on the prevalence of *Escherichia coli* O157:H7 and the levels of aerobic bacteria and *Enterobacteriaceae* on carcasses in a commercial beef processing plant. *J. Food Prot.* **66**:2005–2009.
23. **Omisakin, F., M. MacRae, I. D. Ogden, and N. J. Strachan.** 2003. Concentration and prevalence of *Escherichia coli* O157 in cattle feces at slaughter. *Appl. Environ. Microbiol.* **69**:2444–2447.
24. **Peterson, R. E., T. J. Klopfenstein, R. A. Moxley, G. E. Erickson, S. Hinkley, G. Bretschneider, E. M. Berberov, D. Rogan, and D. R. Smith.** 2007. Effect of a vaccine product containing type III secreted proteins on the probability of *Escherichia coli* O157:H7 fecal shedding and mucosal colonization in feedlot cattle. *J. Food Prot.* **70**:2568–2577.
25. **Renter, D. G., J. M. Sargeant, R. D. Oberst, and M. Samadpour.** 2003. Diversity, frequency, and persistence of *Escherichia coli* O157 strains from range cattle environments. *Appl. Environ. Microbiol.* **69**:542–547.
26. **Robinson, S. E., E. J. Wright, C. A. Hart, M. Bennett, and N. P. French.** 2004. Intermittent and persistent shedding of *Escherichia coli* O157 in cohorts of naturally infected calves. *J. Appl. Microbiol.* **97**:1045–1053.
27. **Sargeant, J. M., J. R. Gillespie, R. D. Oberst, R. K. Phebus, D. R. Hyatt, L. K. Bohra, and J. C. Galland.** 2000. Results of a longitudinal study of the prevalence of *Escherichia coli* O157:H7 on cow-calf farms. *Am. J. Vet. Res.* **61**:1375–1379.
28. **Stephens, T. P., T. A. McAllister, and K. Stanford.** 2008. Development of an experimental model to assess the ability of *Escherichia coli* O157:H7-inoculated fecal pats to mimic a super shedder within a feedlot environment. *J. Food Prot.* **71**:648–652.
29. **Van Donkersgoed, J., J. Berg, A. Potter, D. Hancock, T. Besser, D. Rice, J. LeJeune, and S. Klashinsky.** 2001. Environmental sources and transmission of *Escherichia coli* O157 in feedlot cattle. *Can. Vet. J.* **42**:714–720.
30. **Younts-Dahl, S. M., G. D. Osborn, M. L. Galyean, J. D. Rivera, G. H. Loneragan, and M. M. Brashears.** 2005. Reduction of *Escherichia coli* O157 in finishing beef cattle by various doses of *Lactobacillus acidophilus* in direct-fed microbials. *J. Food Prot.* **68**:6–10.