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Prevalence of *Escherichia coli* O157 Shedding in Preweaned Calves on Colorado Dairies

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Abstract

To gain insight into a potential age-related predisposition for *Escherichia coli* pathogen shedding on dairies, this pilot study measured the prevalence of *E. coli* O157 (ECO157) in the feces of preweaned dairy calves. An aim of this study was to link these outcomes with the concurrent environmental presence of ECO157 and dam ECO157 shedding elucidated in a parallel study. Recto-anal mucosal swabs and a subset of fecal grab samples were collected from calves (2 to 8 weeks of age; $n = 399$) monthly between December 2013 and June 2014 on three dairies in northern Colorado. A subset of calf dams ($n = 111$) were also sampled via fecal grab. Concurrently, environmental samples were collected from locations within the vicinity of the calves: farm tractor tires, steering wheels, hutches, buckets, and gloves from the research technicians and the employees involved in calf rearing. The presence of ECO157 and virulence genes was measured in the samples and confirmed via PCR. Of the calves, only 1 (0.25%) of 399 individuals shed during the time period, and the ECO157 strain detected carried no measured virulence genes (*eaeA, stx*$_1$, and *stx*$_2$). No difference was seen in detection between the recto-anal mucosal swabs and the fecal grab technique. In contrast, 32% (35 of 111) of the dams shed ECO157, with 1.8% (2 of 111) of the shed isolates containing virulence genes. No ECO157 was detected in the environmental samples. These outcomes demonstrate a disparity between dam and calf ECO157 shedding and indicate that preweaned calves, managed similarly to those of this study, probably have a minor influence on dairy contamination and the transmission of ECO157.

Keywords

Calves; Dairy; O157; Shedding

Escherichia coli O157 (ECO157) is a gram-negative bacterium capable of causing human infection when ingested at a very low dose (1). It is well understood that cattle are a primary ECO157 reservoir, and human infections can result from the ingestion of dairy, meat, and produce products originally contaminated by these ruminants (6). Recently, several studies (20–22) have contributed to a better understanding of ECO157 shedding and transmission dynamics in dairy cattle. These include a study (17) that was conducted alongside the current study: a year-long risk factor analysis of ECO157 shedding in adult cattle from

three dry-lot dairies in northern Colorado. Although the concurrent study elucidated the risk factors for shedding in an early-lactation cohort of adult cows, it did not address the importance of the calves recently born to these dams.

ECO157 shedding in dairy calves may be of particular importance to the farm transmission of the ECO157 pathogen. Previous studies (3, 5) have shown a greater magnitude of shedding in calves (1 to 14 weeks of age) than in adults, a longer period of shedding postinoculation, and a likelihood that weaning animals will shed. The type of housing and management of calves used has been shown to modulate their rates of ECO157 shedding (2, 18). Although not specifically measured, the survival of ECO157 in the environmental vicinity of these calves probably plays a role in their increased shedding rates because ECO157 persists for varying periods in diverse environments (19). On large conventional dairies, milk-fed heifer calves (preweaned calves) are removed from their dams immediately postpartum, fed a milk replacer, and often housed individually until weaning. To our knowledge, no previous analysis of the shedding similarities between naturally infected calves and dams postseparation has been performed, and the impact of parturition and the immediate postpartum period on ECO157 transmission is unknown.

The goal of the current pilot study was to determine the prevalence of ECO157 shedding in preweaned dairy calves and to link the results to maternal shedding and the distribution of ECO157 in the surrounding dairy calf environments. Information of this nature may improve our understanding of ECO157 dairy ecology by designating the routes of ECO157 survival and infection within farm environments and herd animals.

MATERIALS AND METHODS

Preweaned calves (2 to 8 weeks of age; $n = 399$) and dairy environmental locations ($n = 129$) were sampled every 2 to 6 weeks (approximately once per month) between December 2013 and July 2014. A cohort of dams within 21 days of calving ($n = 111$) was also sampled during the study period as part of a larger study (17) of ECO157 risk factors in early-lactation cows. Calves and dams that met the specified criteria were convenience sampled during each sampling period in an effort to obtain samples from as many eligible pairs as possible. Each individual was sampled only once during the study. The three freestall and dry-lot dairies sampled were contracted with Colorado State University herd health management, were within a 20-mi (32.2-km) radius of Fort Collins, Colorado, and represented a combined population of 2,750 lactating cattle. Herds 1 and 3 were sampled nine times during the study period, and herd 2 was sampled eight times. The calves were reared individually in hutches and bedded at the beginning of the preweaning period with either fresh straw or shavings. Additional bedding was provided on an as-needed basis but was not changed prior to weaning. The individual calves did not suckle but were removed from their dams postcalving and given 4 L of either previously frozen or fresh unpasteurized individual colostrum via orogastric intubation in the first 12 h of life. Each day, the calves were fed 6 to 8 L of milk replacer, pasteurized or unpasteurized whole milk, along with free access to either a mixed grain or grain and pellet calf starter. Although calf feeding strategies were generically similar among the herds, there were characteristic differences. The managers of herd 3 supplemented the calves' milk replacer with *Lactobacillus* (Superior

Milk Products, Keenesburg, CO), those of herd 2 added trimethoprim-sulfadiazine (Uniprim, 3.5 g per calf; Neogen, Lansing, MI) to the milk for 12 days, and those of herd 1 supplemented the milk replacer with decoquinate (Decoxx, 0.5 mg/kg; Zoetis, Parsipanny, NJ) and used a medicated dairy calf grain containing 50 g per U.S. ton (907 kg) of chlortetracycline and 50 g per U.S. ton of lasolocid (Ranch Way Feeds, Fort Collins, CO).

At each visit, a foam-tipped recto-anal mucosal swab (RAMS; foam-tipped applicators, VWR International, Radnor, PA) was used to sample the recto-anal-mucosal junction of each calf using light pressure. Swiffer (Proctor and Gamble, Cincinnati, OH) swabs were used separately to sample the inside of the calf hutches, the inside of the calf feed buckets, the steering wheel and tires of tractors used for calf feeding, and the boots of personnel via wiping with moderate pressure. The gloves (VWR International) worn by the research technicians and employees involved in calf rearing were also collected on the day of sampling. The farm employees were not made aware of the purpose or goals of this study. The dams were sampled by obtaining more than 10 g of feces via rectal palpation. To verify that the detection results were not affected by the sensitivity of the RAMS collection technique, 10% ($n = 39$) of study calves were also convenience sampled via fecal collection (following digital stimulation) during the last two sampling periods.

ECO157 isolation was performed following blind standard laboratory procedures, as previously described (17). Briefly, the Swiffer swabs and the gloves were placed in 90 mL of buffered peptone water (HiMedia Laboratories, Mumbai, India) the RAMS were placed in 5 mL of buffered peptone water, and the fecal samples were diluted (1:10) in buffered peptone water for enrichment. We direct plated 100 μL of the solutions onto sorbitol MacConkey agar with 5-bromo-4-chloro-3-indolyl-β-d-glucuronide (Oxoid Diagnostic Reagents, Basingstoke, Hampshire, England) containing 1.25 mg of potassium tellurite and 0.025 mg of cefixime (HiMedia Laboratories). The fecal enrichment solution remaining after direct plating was incubated for 6 h at 37°C, followed by storage overnight at 4°C. The plates were incubated at 37°C for 24 h, and those containing ≥100 suspect colonies were chosen for ECO157 latex agglutination, following manufacturer's instructions (Oxoid Diagnostic Reagents). Latex-positive colonies were grown in tryptic soy broth (BD, Sparks, MD) for 6 h and stored at −80°C in 10% sterile glycerol (Sigma Aldrich, St. Louis, MO). The enriched samples stored at 4°C and not confirmed as ECO157 positive through direct plating were subjected to immunomagnetic separation using Dynabeads anti-*E. coli* O157 and a BeadRetriever System (Life Technologies, Oslo, Norway). The immunomagnetic separation sample extracts were plated onto 5-bromo-4-chloro-3-indolyl-β-d-glucuronide containing 1.25 mg of potassium tellurite and 0.025 mg of cefixime and incubated for 24 h at 37°C. Suspect colonies were confirmed using latex agglutination and archived as previously described.

PCR targeting the ECO157 *rfb* gene was conducted on all latex-positive isolates, and all *rfb*-positive (and thus ECO157-positive) isolates were subsequently PCR tested for the enterohemorrhagic *E. coli* virulence genes stx_1, stx_2, and *eaeA* using the PCR protocol outlined next. For PCR, 10 μL of thawed tryptic soy broth was centrifuged at 5,000 × *g* for 5 min and resuspended in 30 μL of molecular-grade water. Once it was resuspended, 5 μL of the culture template was placed in Qiagen Multiplex PCR Plus Kit reaction master mixes

(Qiagen, Venlo, Limburg, The Netherlands), according to the manufacturer instructions, with minor modifications. Briefly, each 25-μL PCR reaction consisted of 12.5 μL of master mix, 2.5 μL of primer mix containing 0.2 μM each primer, 5 μL of molecular-grade water, and 5 μL of culture template. The thermal cycling conditions consisted of an initial incubation at 95°C for 5 min, followed by 40 cycles of amplification with denaturation at 95°C for 30 s, annealing at 57°C for 1.5 min, and extension at 72°C for 30 s, ending with a final extension at 68°C for 10 min. Thermocycling was performed using an MJ Research PTC-100 thermal cycler (Bio-Rad, Hercules, CA). The PCR products were analyzed visually using agarose gel electrophoresis with a 2% agarose gel (Lonza Group Ltd., Basel, Switzerland).

RESULTS

During the period of the study, ECO157 was detected in one calf sample (1 of 399; 0.25%) from herd 3 (Table 1). The sample was collected via RAMS in December, contained the *rfb* gene, and lacked all the measured virulence genes (stx_1, stx_2, and *eaeA*). Although every potential *E. coli* virulence gene was not assessed, this isolate was probably nonvirulent owing to the lack of major virulence genes found during in-depth characterization of isolates containing only the *rfb* gene from the early-lactation cows (17). The collection and enrichment of the calf feces collected via digital stimulation showed no difference in ECO157 outcome from the sampling using RAMS. ECO157 was not detected in any samples collected from the calves' environment (gloves, boots, tractor tires, tractor steering wheel, hutches, and feed buckets).

Of the dams, ECO157 isolates with only the *rfb* gene were detected in 35 samples (32%). In addition, one dam sample (0.9%) from herd 3 contained an ECO157 isolate with the *rfb* and *eaeA* genes, and one (0.9%) from herd 1 contained an isolate with the *rfb* and stx_2 genes (Table 1). The dam of the calf that produced the nonvirulent ECO157 isolate did not shed any variant of ECO157 when sampled. Additional statistical analyses were not performed on the gathered data because of the lack of positive outcomes. The larger study (17) performed in early-lactation cattle showed that dams shed ECO157 at low rates between the months of August and November, prior to the December onset of this calf study. Because this pilot project did not sample the calves during these fall and late summer months, it is unknown whether the prevalence of calf shedding would have been different during these seasons.

DISCUSSION

Similar to the results of this study, a low prevalence of ECO157 shedding has been reported (13, 15) in calves younger than 2 months. In the current literature (3, 7, 11, 13, 15, 18), there seems to be a consensus that calves at weaning, as opposed to prior to weaning, are an age group more at risk for both shedding and shedding higher levels of ECO157. Studies (2, 18) that looked at factors associated with shedding demonstrated that group housing and the nonuse of the coccidiostat decoquinate increased the risk of Shiga toxin bacterial shedding in preweaned calves. In the current study, decoquinate was used only in herd 1 during the study period. However, changes in nutrition and nutritional additives did not seem to increase or decrease the shedding rates among the groups because ECO157 was detected in only a single calf sample. The management strategy on all three farms was to

house the calves in individual hutches during the preweaning phase and to move them to group housing only after the weaning period. Not initiating the group housing of calves until postweaning is a common strategy employed on dairies, and this may help explain both the higher levels of ECO157 excretion seen in previous studies of weaned calves and the low level of ECO157 excretion seen in the preweaned calves of this study.

In the seasons of our study, ECO157 was detected at a higher frequency in the dams than in their calves. Pearce et al. (16) saw no association between Shiga toxin *E. coli* shedding in beef calves and their dams within 1 week of birth. In contrast, Cobbold and Desmarchelier (3) showed that dairy calves were twice as likely to shed Shiga toxin *E. coli* when their dams shed Shiga toxin *E. coli*. As previously mentioned, the housing management and immediate removal of the calves from their dams on the farms in the current study probably played a role in the lack of shedding association, and this seems to be an advantageous managerial approach for low-pathogen calf rearing. It is important to note that these pilot results represent observations from naturally infected dairy herds in a defined location; controlled studies over a longer time frame need to be conducted to confirm that the differences in ECO157 prevalence in calves are due to specific managerial and farm-level factors.

The two most common methods of sample collection for ECO157 detection are RAMS and collection of feces. RAMS have been cited previously as being a more sensitive detection method, especially for colonized animals, because the recto-anal-mucosal junction tends to contain ECO157 to a greater extent than the rest of the gastrointestinal tract (4, 9, 10, 12). However, conflicting evidence from other studies (8, 14) showed that the recto-anal-mucosal junction plays a small role in an animal's shedding status; these studies determined that collecting fecal material is a more sensitive method for detecting ECO157 shedding. When it became apparent during the current study that we were not detecting ECO157 via RAMS in the calves, yet were detecting it via fecal retrieval in their dams, we questioned the detection sensitivity of RAMS and chose to use both methods in parallel on a subset of the calves. However, the same ECO157 outcome was achieved regardless of the method we used. A previous study (22) that used both methods in parallel to sample heifers reported that fecal sampling showed a slight increase in sensitivity compared with RAMS. In the current study, not all the calves were sampled using both methods, so it is unknown whether our results would change if we had a greater number of fecal collections. Given low prevalence of shedding (1 of 399), there was not enough power in the current study to detect a difference in sensitivity between the two methods.

Although these results may be specific to geographical location, season, and the type of dairies (dry-lot and freestall), the current study supports the notion that preweaned calves that have been immediately removed from their dams and reared in individual hutches have a small impact on dairy contamination and the dissemination of ECO157. Nevertheless, this does not mean that management strategies to reduce ECO157 in dairies should ignore the peripartum period. Previous studies show a high variability in calf shedding rates from farm to farm, probably because of the different management strategies employed for calf rearing.

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TABLE 1.

Total calf and dam samples tested and ECO157 isolates detected, by herd

Dairy herd	Total calves sampled, *n*	Calves with isolates containing only *rfb*, *n* (%)[a]	Total dams sampled, *n*	Dams with isolates containing only *rfb*, *n* (%)	Dams with isolates containing *rfb* and stx_2, *n* (%)[b]	Dams with isolates containing *rfb* and *eaeA*, *n* (%)[c]
1	179	0	53	11 (20.8)	1 (1.8)	0
2	70	0	15	2 (2.8)	0	0
3	150	1 (0.7)	43	22 (51.2)	0	1 (2.3)
Total	399	1 (0.3)	111	35 (31.5)	1 (0.9)	1 (0.9)

[a] *E. coli* O157.

[b] *E. coli* Shiga toxin gene 2.

[c] *E. coli* attaching and effacing gene.