Rectoanal Mucosal Swab Culture Is More Sensitive Than Fecal Culture and Distinguishes Escherichia coli O157:H7-Colonized Cattle and Those Transiently Shedding the Same Organism

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Enrichment and direct (nonenrichment) rectoanal mucosal swab (RAMS) culture techniques were developed and compared to traditional fecal culture for the detection of Escherichia coli O157:H7 in experimentally infected and naturally infected cattle. Holstein steers (n = 16) orally dosed with E. coli O157:H7 were sampled after bacterial colonization starting 15 days postinoculation. Enrichment RAMS cultures (70.31% positive) were more sensitive than enrichment fecal cultures with 10 g of feces (46.88% positive) at detecting E. coli O157:H7 (P < 0.01). Holstein bull calves (n = 15) were experimentally exposed to E. coli O157:H7 by penning them with E. coli O157:H7-positive calves. Prior to bacterial colonization (1 to 14 days postexposure), enriched fecal cultures were more sensitive at detecting E. coli O157:H7 than enriched RAMS cultures (P < 0.01). However, after colonization (40 or more days postexposure), the opposite was true and RAMS culture was more sensitive than fecal culture (P < 0.05). Among naturally infected heifers, enriched RAMS or fecal cultures were equally sensitive (P = 0.5), but direct RAMS cultures were more sensitive than either direct or enriched fecal cultures at detecting E. coli O157:H7 (P < 0.01), with 25 of 144, 4 of 144, and 10 of 108 samples, respectively, being culture positive. For both experimentally and naturally infected cattle, RAMS culture predicted the duration of infection. Cattle transiently shedding E. coli O157:H7 for <1 week were positive by fecal culture only and not by RAMS culture, whereas colonized animals (which were culture positive for an average of 26 days) were positive early on by RAMS culture. RAMS culture more directly measured the relationship between cattle and E. coli O157:H7 infection than fecal culture.

Since Escherichia coli O157:H7 was first identified as a human pathogen (27, 33), investigations have demonstrated that human disease outbreaks are often linked to a bovine food source or bovine waste-contaminated water (2, 7, 30, 39). Although most of the known outbreaks of E. coli O157:H7-associated disease in humans are food borne or waterborne, several recent studies indicate that a significant number of human infections are acquired from direct contact with cattle, the environment, or unknown sources (1, 12, 30, 34). An accepted premise is that the reduction of the number of cattle infected with E. coli O157:H7 or the elimination of E. coli O157:H7 from cattle will effect a reduction in the rate of disease in humans. To this end, a great deal of research has focused on describing the ecology and epidemiology of E. coli O157:H7 in cattle, with the hope of identifying interventions to reduce its prevalence in cattle (11, 16, 17, 19, 20, 23, 24, 28, 29). Within this body of research, numerous methods for detecting E. coli O157:H7 in bovine fecal samples have been developed and used (8, 9, 22, 36, 37, 40). The reported sensitivities of detection by these various methods vary greatly, and problems occur when data from studies that have used methods with disparate sensitivities are compared. Nonetheless, fecal cultures show that E. coli O157:H7 occurs on the majority of cattle farms and that prolonged carriage by individual animals appears to be rare (4, 13, 16, 17, 28).

E. coli O157:H7 localization within the gastrointestinal tract and potential mechanisms of colonization in cattle have been the topics of several studies (4, 6, 13, 25, 32, 35). Numerous studies (4, 6, 13, 20, 28) have reported that animals are culture positive for both long durations (≥30 days) and short durations (≤10 days). Presumably, E. coli O157:H7 attaches and replicates at a site or sites in the gastrointestinal tracts of colonized animals and results in bacteria in the feces for a long duration. In contrast, ingestion of E. coli O157:H7 from an environmental source without establishment of a colonization site within the animal results in transient shedding of the bacteria in feces for a few days. Whether cattle on farms are typically colonized with E. coli O157:H7 or are only transiently shedding the bacteria is not known; however, several studies have documented the persistence of infection in individual cattle, and others have provided epidemiological evidence that at least some cattle are colonized (4, 6, 13, 16, 28).

A recent study provides compelling evidence that a primary site of E. coli O157:H7 colonization in cattle is the rectoanal junction of the gastrointestinal tract (32). This finding is supported by necropsy analyses of the gastrointestinal digesta and tissue of sheep colonized with E. coli O157:H7 (13). That study found the bacteria only in rectal tissue and feces (13). These findings are in contrast to those from previous studies that suggest that other sites in the gastrointestinal system are colonized, including the rumen and colon (6, 25, 35, 38). If the rectoanal junction is a site of colonization, the use of swab
samples from this mucosal surface should be superior to the use of traditional fecal samples for the detection of *E. coli* O157:H7 and may have the potential to differentiate cattle that have been colonized over the long term from cattle that are transiently culture positive.

The present study used previously published methods of detecting *E. coli* O157:H7 but applied these to a novel type of sample, obtained by swabbing the rectoanal mucosal surface, rather than fecal material. Rectoanal mucosal swab (RAMS) samples and fecal samples were obtained from (i) experimentally infected steers, (ii) experimentally exposed calves, and (iii) naturally infected dairy heifers; and cultures with the two types of samples were compared for their abilities to detect *E. coli* O157:H7. Both selective enrichment and direct culture techniques were used, and the results were correlated to the durations that individual animals were culture positive for *E. coli* O157:H7.

**MATERIALS AND METHODS**

*Animals.* Three groups of cattle were sampled in this study: artificially infected 8- to 10-month-old Holstein steers (n = 16), intentionally exposed 4- to 6-month-old Holstein bull calves (n = 15), and 4- to 14-month-old heifers (n = 40) located at the University of Idaho (UI) and the Washington State University (WSU) dairy farms. The artificially infected or exposed cattle were housed in large-animal isolation facilities and were demonstrated to be culture negative for *E. coli* O157:H7 at least three consecutive times over a 2-week period before being used in the experiment. The heifers at both dairies were not artificially inoculated or exposed to *E. coli* O157:H7. The UI Animal Care and Use Committee approved all experimental protocols with the animals.

*E. coli* O157:H7 experimental inoculation and experimental exposure. The Holstein steers were orally inoculated once with a four-strain mixture of *E. coli* O157:H7 (10^10^ CFU/strain) containing WSU isolates 180, 400, and 588 (14) and strain ATCC 43895 (American Type Culture Collection [ATCC], Manassas, Va.). This mixture is representative of human and cattle *E. coli* O157:H7 isolates. Two Holstein calves (referred to as Trojan calves) were orally inoculated with the same quantity and strains of *E. coli* O157:H7 as the steers, and the pen mates were exposed to *E. coli* O157:H7 by association with these dosed calves. In addition, a 200-liter tub of water was inoculated with 10 g of *E. coli* O157:H7-positive feces collected from the Trojan calves and was introduced into the pen 3 weeks after the Trojan calves were introduced. The water in this tub was in addition to the automatic water fountains that provided drinking water to the animals.

**Sample collection.** A fecal sample and a RAMS sample were collected from each animal. Fecal samples were collected aseptically by rectal palpation or aseptically during defection and placed into a Whirl-Pak bag (Nasco, Ft. Atkinson, Wis.). RAMS samples were obtained by inserting a sterile foam-tipped applicator (catalog no. 10812-022; VWR International, Buffalo Grove, Ill.) approximately 2 to 5 cm into the anus, and by using a rapid in-and-out motion, the entire mucosal surface of the rectoanal junction was swabbed. Each RAMS was placed into a culture tube containing 3 ml of Trypticase soy broth (TSB; Difco Laboratories, Detroit, Mich.) containing ceftaxime, potassium tellurite, vancomycin, and 4-methylumbelliferyl-beta-D-glucurononic acid dihydrate (100 μg/ml; Biosynth Ag, Staad, Switzerland) (SMac-CTVM), and the plates were incubated at 37°C for 18 h. The numbers of sorbitol- and beta-glucuronidase-negative colonies on plates with a total of 30 to 300 colonies were counted, and a subset of the colonies was assayed for the O157 antigen by a latex agglutination test (Pro Lab Diagnostics, Richmond Hill, Ontario, Canada).

**RESULTS**

All animals remained healthy during the study period. At most samplings, the RAMS was collected first. A fecal sample of 10 g or more was then collected by aseptic rectal palpation or freely if the animal defecated. Very little visible fecal material was collected on the RAMS, and the amount of material on each RAMS ranged from 0.1 to 0.4 g, with an average of 0.24 g. Direct and enrichment RAMS cultures contained few non-O157 colonies, used low volumes of media, required minimal technician hands-on time, and could be completed within 1 day of sample collection. Enrichment fecal cultures required 48 h or more from the time of sample collection to the time that results were available. The enrichment fecal culture protocol was used as sensitive as selective enrichment with immunomagnetic bead capture of *E. coli* O157:H7, detecting 1 CFU/10 g (23).

**Comparison of RAMS culture and fecal culture for detection of *E. coli* O157:H7 in experimentally colonized steers.** Steers were given a single oral dose of *E. coli* O157:H7, and during the first week postinoculation the highest *E. coli* O157:H7 titers in feces ranged from 2.1 × 10^4 to 9.5 × 10^6 CFU/g. During the second week postinoculation all steers remained fecal culture positive for *E. coli* O157:H7, but many samples contained less than 10 CFU/g and enrichment culture was required to detect the bacteria. Comparisons of RAMS and fecal cultures were started 2 weeks postinoculation to allow the bolus of inoculated bacteria to pass through the gastrointestinal tract and bacterial colonization to occur. Enrichment culture of RAMS samples (39 of 45; 70.31% positive) was more sensitive than enrichment culture of 10-g fecal samples (46.88% positive) (P < 0.01) at detecting *E. coli* O157:H7 2 or more weeks postinoculation (Table 1). The majority of these RAMS samples (39 of 45) were also positive by direct culture. The average duration that steers remained culture positive for *E. coli* O157:H7 was 48 h or more from the time of sample collection to the time that results were available. The enrichment fecal culture protocol was used as sensitive as selective enrichment with immunomagnetic bead capture of *E. coli* O157:H7, detecting 1 CFU/10 g (23).
positive was 40 days (range, 15 days [one animal] to 71 days [three animals]).

Longitudinal comparison of RAMS and fecal culture with calves experimentally exposed to *E. coli* O157:H7. To more closely simulate acquisition of a natural *E. coli* O157:H7 infection and avoid the large oral bolus of $10^{10}$ CFU of *E. coli* O157:H7 often used to generate experimental infections, Holstein bull calves were exposed to experimentally infected culture-positive calves by penning the animals together. The culture-positive calves, referred to as Trojan calves, carried the infection and spread it to the test animals. The exposed test calves were analyzed for *E. coli* O157:H7 by enrichment RAMS and fecal culture every 3 to 4 days for 54 days postexposure. The experimental exposure to the Trojan calves caused infections in almost all the test calves within the first 2 weeks postexposure, and both RAMS and fecal samples from 16 of 17 (94%) bull calves (including the Trojan calves) were culture positive at least once. When all culture results were analyzed together, the sensitivities of the enrichment cultures of RAMS and fecal samples appeared to be similar (Table 1). However, when the culture results were compared longitudinally over the course of the infections, the sensitivities differed. Early in the period of exposure (1 to 14 days) enrichment fecal cultures were more sensitive at detecting *E. coli* O157:H7 than enrichment RAMS culture (*P* < 0.01). Late in the period of exposure (40 days or longer), the opposite was true and enrichment RAMS cultures were more sensitive (*P* < 0.05) (Fig. 1). Presumably, early in the exposure period, the calves were ingesting *E. coli* O157:H7 from their environment and the bacteria were in the digesta and feces but may or may not have attached to the rectoanal junction mucosa (at this time, fecal cultures were the most sensitive); and late in the exposure period, the bacteria, if present, were colonizing the rectoanal junction (at this time, RAMS cultures were the most sensitive).

Comparison of RAMS culture and fecal culture for detection of *E. coli* O157:H7 in naturally infected dairy heifers. To show the sensitivity of RAMS culture for the detection of naturally infected cattle, heifers from two university dairies were sampled in the summer, a season that is associated with a high prevalence of culture positivity, 18 heifers from one university dairy were sampled weekly during the late fall and early winter. For these more frequently sampled animals, direct RAMS culture was compared to direct fecal and enrichment fecal cultures. Twenty-five of 44 RAMS samples tested by direct culture, 4 of 144 fecal samples tested by direct culture, and 10 of 108 fecal samples tested by enrichment culture were positive for *E. coli* O157:H7. Direct culture of RAMS samples was more sensitive at detecting *E. coli* O157:H7 than either enrichment or nonenrichment culture of fecal samples (*P* < 0.01) (Fig. 2). All positive samples with the exception of one sample tested by enrichment fecal culture.

The results obtained with experimentally infected steers, enrichment RAMS culture and enrichment fecal cultures were equally sensitive (*P* = 0.50). Twenty-five of 80 RAMS samples and 26 of 80 fecal samples from heifers were culture positive (Table 1). To investigate natural infection in a season not usually associated with a high prevalence of culture positivity, 18 heifers from one university dairy were sampled weekly during the late fall and early winter. For these more frequently sampled animals, direct RAMS culture was compared to direct fecal and enrichment fecal cultures. Twenty-five of 144 RAMS samples tested by direct culture, 4 of 144 fecal samples tested by direct culture, and 10 of 108 fecal samples tested by enrichment culture were positive for *E. coli* O157:H7. Direct culture of RAMS samples was more sensitive at detecting *E. coli* O157:H7 than either enrichment or nonenrichment culture of fecal samples (*P* < 0.01) (Fig. 2). All positive samples with the exception of one sample tested by enrichment fecal culture.
isms in RAMS and fecal samples consistently decreased at
(P than that found in feces on all sampling dates (data not shown)
organisms collected on the RAMS was signi
ificant periods (average, 26 days) and presumably
E. coli with the durations of infection. Animals that were positive for
experimentally infected with
E. coli
with the fecal culture status of individual animals naturally infected or
infected or experimentally infected with
E. coli
O157:H7 by RAMS culture remained positive for sig
ificantly longer periods (average, 26 days) and presumably
represented colonized animals (Table 2). In contrast, the infec
tions were rapidly cleared from animals that were positive for
E. coli
O157:H7 by fecal culture only and not by RAMS
culture (all but one of the animals were positive on only one
sampling day; Table 2), and presumably, these represented
transiently shedding animals.

There were exceptions to the common pattern of RAMS
culture-positive status and a long-term duration of
E. coli
O157:H7 infection. Animal 711 was consistently culture posi
tive for 28 days and was then intermittently culture positive for
49 days through the last day of sampling. The animal was fecal
culture positive on eight sampling occasions but was RAMS
culture positive on only three sampling days. The highest
E. coli
O157:H7 titer shed by animal 711 was 1.0 \times 10^{10} CFU of
E. coli
O157:H7/g of feces. Also, animals 712 and 728 were
RAMS culture positive but shed
E. coli
O157:H7 only briefly on only one or two sampling days, and then they cleared the
infection (Table 2).

Variations to RAMS culture method. The surprising finding that the direct RAMS culture was often more sensitive than either direct or enrichment fecal culture led us to more care
fully analyze the RAMS culture protocol. Because there is so little fecal contamination on the RAMS samples, a subset of
RAMS samples was collected in TSB without cefixime, potas
sium tellurite, and vancomycin and was subsequently enriched
without cefixime, potassium tellurite, and vancomycin. The results obtained for four animals sampled on five different occasions over a 2-month period were compared. Direct and
enriched RAMS cultures in TSB were as sensitive or more sensitive than RAMS cultures in TSB-CTV. Approximately
80% of the positive RAMS samples collected in TSB were also
positive when the samples were cultured in TSB-CTV (data not shown). The colonies on enrichment RAMS plate cultures were \approx 90% \ E. coli
O157:H7, but enrichments in TSB had
about 10-fold higher titers of
E. coli
O157:H7 than enrichments in TSB-CTV (data not shown). In addition, we com
pared enrichment incubation times of 6 and 18 h and found
that 6 h was a suf
cient incubation time in the RAMS culture technique.

### Table 2. RAMS and fecal culture status and duration of
E. coli
O157:H7 infections in cattle

<table>
<thead>
<tr>
<th>Type of infection and animal no.</th>
<th>Status of first ( E. coli ) ( O157:H7 )-positive culture</th>
<th>Time (days) to first ( E. coli ) ( O157:H7 )-negative culture</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experimental</strong>( ^a )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>704</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>707</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>711</td>
<td>-</td>
<td>( &gt;28^c )</td>
</tr>
<tr>
<td>712</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>717</td>
<td>-</td>
<td>4</td>
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<tr>
<td>718</td>
<td>-</td>
<td>4</td>
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<tr>
<td>725</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>703</td>
<td>+</td>
<td>42</td>
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<td>+</td>
<td>14</td>
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<tr>
<td>712</td>
<td>+</td>
<td>4</td>
</tr>
<tr>
<td>718</td>
<td>+</td>
<td>( &gt;39^c )</td>
</tr>
<tr>
<td>725</td>
<td>+</td>
<td>( &gt;18^c )</td>
</tr>
<tr>
<td>728</td>
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<td>7</td>
</tr>
<tr>
<td>705</td>
<td>+</td>
<td>14</td>
</tr>
<tr>
<td>707</td>
<td>+</td>
<td>( &gt;25^c )</td>
</tr>
<tr>
<td>714</td>
<td>+</td>
<td>18</td>
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<td>717</td>
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<td>720</td>
<td>+</td>
<td>( &gt;43^c )</td>
</tr>
<tr>
<td>721</td>
<td>+</td>
<td>31</td>
</tr>
<tr>
<td><strong>Natural</strong>( ^b )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2167</td>
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<td>2</td>
</tr>
<tr>
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<tr>
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<tr>
<td>2171</td>
<td>+</td>
<td>49</td>
</tr>
<tr>
<td>2172</td>
<td>+</td>
<td>21</td>
</tr>
</tbody>
</table>

\( ^a \) Calves were exposed to culture-positive Trojan calves that were orally inoc
ulated with
E. coli
O157:H7 to create infections; RAMS and fecal samples were obtained from the exposed calves every 3 to 4 days.

\( ^b \) Naturally infected heifers on a dairy; RAMS and fecal samples were obtained every 7 days.

\( ^c \) Animals were culture positive on the last sampling day, so the exact number of days to culture-negative status could not be determined.

### Discussion

An important finding in this work was that culture of swabs of the rectoanal junction mucosa was, in almost all cases, as
sensitive as and usually more sensitive than culture of feces at
detecting
E. coli
O157:H7 in cattle. This is likely because the
RAMS sampled the site of
E. coli
O157:H7 colonization, sup
porting the findings of Naylor et al. (32), and the RAMS
samples were minimally contaminated with fecal material,
which contains high titers of other bacteria. Also, the ability of
RAMS culture to detect
E. coli
O157:H7 among naturally
infected heifers whose fecal samples were not culture positive suggested that some animals harbor this pathogen much longer than previous estimates made on the basis of fecal culture data.

These animals that are colonized for the long term are not shedding detectable levels of \textit{E. coli} O157:H7 in their feces and may represent an on-farm reservoir not previously identified. Fecal cultures were more sensitive than RAMS cultures at detecting \textit{E. coli} O157:H7 only initially following artificial exposure to \textit{E. coli} O157:H7. This may indicate that upon exposure \textit{E. coli} O157:H7 is present in digesta and feces and is only subsequently attached and colonizes the rectoanal junction mucosa, or it may indicate that bacterial division at that mucosal site took several days to create detectable numbers of the pathogens.

Recognition of which cattle have the greatest potential to negatively affect the safety of our food or contaminate the environment and successful intervention remain unrealized goals. One animal shedding $10^6$ CFU of \textit{E. coli} O157:H7/g of feces releases the same number of pathogens into the environment as 100,000 cattle shedding 10 CFU/g of feces. Therefore, techniques that only improve the sensitivity of \textit{E. coli} O157:H7 detection may not be helpful in addressing the epidemiology of \textit{E. coli} O157:H7. The technique with RAMS samples had several benefits, in addition to improved sensitivity. The technique required small volumes of media, minimal hands-on technician time, no expensive equipment, and a small number of reagents; and it produced easily interpreted plate cultures with few colonies other than \textit{E. coli} O157:H7 within 1 day from sample collection. In addition, among both experimentally and naturally infected cattle, RAMS culture predicted the duration that the cattle remained culture positive for \textit{E. coli} O157:H7. This is the first report of a technique with this ability. With few exceptions, animals that were RAMS culture positive remained positive for many weeks and were considered colonized. In contrast, cattle that were fecal culture positive only and RAM culture negative were most often culture positive on only one sampling day and were considered to be transiently shedding the pathogen. Among the cattle in this study we found only a few animals that did not follow this pattern. The finding of one experimentally infected animal that shed fecal \textit{E. coli} O157:H7 over the long term without consistently being RAMS culture positive may indicate that a minority of animals may be colonized at some other gastrointestinal tract site or are particularly sensitive to ingesting \textit{E. coli} O157:H7 from environmental sources and disseminating the bacteria in feces, or some combination of these attributes.

If \textit{E. coli} O157:H7 primarily colonizes the mucosa of the rectoanal junction, RAMS samples should differentiate colonized from transiently positive cattle. Present fecal culture methods detect low levels of \textit{E. coli} O157:H7, and thus, many animals deemed culture positive shed \textit{E. coli} O157:H7 at low titers (<$10$ CFU/g of feces). Studies have demonstrated that most cattle are only transiently positive for \textit{E. coli} O157:H7, and persistence in an animal appears to be rare (4, 6, 13, 16, 28). The existence of persistently colonized cattle on farms, even if rare, could be an important part of the maintenance of this organism on a farm and a source of contamination of otherwise culture-negative cattle at slaughter. The conventional idea that most of the \textit{E. coli} O157:H7 contamination of beef carcasses at slaughter originates directly from the gastrointestinal contents of cattle being slaughtered is challenged by recent studies that show that the primary source of \textit{E. coli} O157:H7 isolates that contaminate carcasses are the hides of cattle being slaughtered (3, 21). The existence of colonized cattle in pens of cattle awaiting slaughter could be important sources of contamination of the pen environment and, consequently, the hides of pen mates. It is possible that the majority of hide contamination results from a minority of cattle that are shedding \textit{E. coli} O157:H7. Identification of colonized animals could allow elimination of carrier animals and promote pen environments with greatly reduced loads of this organism; consequently, this would reduce the levels of hide contamination.

Studies of fecal \textit{E. coli} O157:H7 in cattle have led to a variety of conclusions regarding bacterial colonization of cattle. These range from uncertainty as to whether \textit{E. coli} O157:H7 colonizes cattle to the identification of potential colonization sites, including the rectoanal junction and the rumen (10, 25, 32). Localization of \textit{E. coli} O157:H7 on the rumen walls of mature cattle has been reported, and the occurrence of the organisms at this location appears to be associated with concurrent fecal shedding (25). \textit{E. coli} O157:H7 persists in the rumens of 6- to 8-week-old calves that are not fully ruminant, but this persistence is not comparable to the situation for fully ruminant cattle going to slaughter (5, 10, 18, 35, 38). Other studies suggest that \textit{E. coli} O157:H7 colonizes various sites in the intestinal tract, including the cecum and colon; however, those studies did not provide conclusive evidence that colonization occurs at these sites (6, 10, 38). A previous study (13) that evaluated the gastrointestinal location of \textit{E. coli} O157:H7 in cattle and sheep rarely found \textit{E. coli} O157:H7 in the rumen beyond 7 days postinoculation and often found the bacteria in fecal samples from long-term carriers. In addition, upon necropsy \textit{E. coli} O157:H7 was found only in tissue from the distal rectum of sheep that were long-term carriers (colonized) (13). Recent evidence suggests that \textit{E. coli} O157:H7 colonizes the rectoanal junction of cattle (32). The results reported here support the finding that the rectoanal mucosal junction is a site of \textit{E. coli} O157:H7 colonization in cattle.

The association between season and RAMS culture sensitivity may be explained by changes in the environmental reservoirs of \textit{E. coli} O157:H7. In samples from dairy heifers, enrichment RAMS and fecal cultures were equally sensitive at detecting \textit{E. coli} O157:H7 during the summer months; however, RAMS cultures were much more sensitive than fecal cultures at detecting \textit{E. coli} O157:H7 in the same group of cattle during the winter months. This observation may be due to increased \textit{E. coli} O157:H7 titers in environmental reservoirs during the summer months that resulted in the ingestion of increased amounts of the organism and increased levels of transient shedding. The prevalence of \textit{E. coli} O157:H7 in the feces of cattle and the environment has been demonstrated to be higher during the warm months of summer to fall (16, 26, 31). These \textit{E. coli} O157:H7 reservoirs would likely be reduced or absent during the winter months. Environmental reservoirs likely exist in dairy heifer pens, although this has not been determined (15, 26, 29), and consequently, detection of \textit{E. coli} O157:H7 only by enrichment fecal cultures (a method that detects as little as 1 CFU/g of feces [23]) and not by RAMS culture may reflect the passage of ingested \textit{E. coli} O157:H7 rather than colonization.
In conclusion, the RAMS culture technique provides a new, easy method for the detection of E. coli O157:H7 in cattle and appears to delineate colonized from transiently shedding cattle. Large-scale epidemiological studies by the RAMS method are under way. These studies will potentially generate data on the prevalence of E. coli O157:H7 in cattle that differ from those from existing studies that used selectively enriched fecal cultures to detect the pathogen.

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