Reduction of Carriage of Enterohemorrhagic *Escherichia coli* O157:H7 in Cattle by Inoculation with Probiotic Bacteria

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Bacteria inhibitory to *Escherichia coli* O157:H7 were isolated from cattle and evaluated for their potential for reducing carriage of *E. coli* O157:H7 in calves. Eighteen of 1,200 bacterial isolates from cattle feces and intestinal tissue samples were screened and determined to inhibit the growth of *E. coli* O157:H7 in vitro. Seventeen of the isolates were *E. coli* and one was *Proteus mirabilis*. None produced Shiga toxin. Genomic DNA fingerprinting by pulsed-field gel electrophoresis revealed 13 distinguishable profiles among the 18 isolates. Two calves inoculated perorally with a mixture of all 18 isolates (10^10 CFU) appeared to be normal and did not develop signs of clinical disease throughout a 25- to 27-day observation period. These bacteria colonized segments of the gastrointestinal tract and were in feces at the termination of the experiment (25 and 27 days postinoculation) at levels of 50 to 200 CFU/g. Fifteen cannulated calves were studied to determine the efficiency of the probiotic bacteria in reducing or eliminating the carriage of *E. coli* O157:H7. Nine calves served as controls, with each animal receiving perorally 10^10 CFU of *E. coli* O157:H7. *E. coli* O157:H7 was detected intermittently in the rumen samples from all control animals throughout 3 weeks postinoculation, whereas *E. coli* O157:H7 was shed at various levels in feces continuously throughout the experiment (mean, 28 days). *E. coli* O157:H7 was isolated from the rumens and colons of eight of nine and nine of nine calves, respectively, at the termination of the study. Six calves each received perorally 10^10 CFU of probiotic bacteria and then 2 days later received 10^10 CFU of *E. coli* O157:H7. *E. coli* O157:H7 was detected in the rumen for only 9 days postinoculation in two animals, for 16 days in one animal, for 17 days in two animals, and for 29 days in one animal. *E. coli* O157:H7 was detected in feces for only 11 days postinoculation in one animal, for 15 days in one animal, for 17 days in one animal, for 18 days in one animal, for 19 days in one animal, and for 29 days in one animal. At the end of the experiment (mean, 30 days), *E. coli* O157:H7 was not recovered from the rumen of any of the six animals treated with probiotic bacteria; however, *E. coli* O157:H7 was recovered from the feces of one of the animals. This animal was fasted twice postinoculation. These studies indicate that selected probiotic bacteria administered to cattle prior to exposure to *E. coli* O157:H7 can reduce the level of carriage of *E. coli* O157:H7 in most animals.

During the past decade, *Escherichia coli* O157:H7, an important human pathogen causing hemorrhagic colitis and hemolytic-uremic syndrome, has been reported as a cause of human illness with increased frequency (1, 10, 16). Cattle, especially young animals, have been implicated as a principal reservoir of *E. coli* O157:H7 in calves. Eighteen of 1,200 bacterial isolates from cattle feces and intestinal tissue samples were screened and determined to inhibit the growth of *E. coli* O157:H7 in vitro. Seventeen of the isolates were *E. coli* and one was *Proteus mirabilis*. None produced Shiga toxin. Genomic DNA fingerprinting by pulsed-field gel electrophoresis revealed 13 distinguishable profiles among the 18 isolates. Two calves inoculated perorally with a mixture of all 18 isolates (10^10 CFU) appeared to be normal and did not develop signs of clinical disease throughout a 25- to 27-day observation period. These bacteria colonized segments of the gastrointestinal tract and were in feces at the termination of the experiment (25 and 27 days postinoculation) at levels of 50 to 200 CFU/g. Fifteen cannulated calves were studied to determine the efficiency of the probiotic bacteria in reducing or eliminating the carriage of *E. coli* O157:H7. Nine calves served as controls, with each animal receiving perorally 10^10 CFU of *E. coli* O157:H7. *E. coli* O157:H7 was detected intermittently in the rumen samples from all control animals throughout 3 weeks postinoculation, whereas *E. coli* O157:H7 was shed at various levels in feces continuously throughout the experiment (mean, 28 days). *E. coli* O157:H7 was isolated from the rumens and colons of eight of nine and nine of nine calves, respectively, at the termination of the study. Six calves each received perorally 10^10 CFU of probiotic bacteria and then 2 days later received 10^10 CFU of *E. coli* O157:H7. *E. coli* O157:H7 was detected in the rumen for only 9 days postinoculation in two animals, for 16 days in one animal, for 17 days in two animals, and for 29 days in one animal. *E. coli* O157:H7 was detected in feces for only 11 days postinoculation in one animal, for 15 days in one animal, for 17 days in one animal, for 18 days in one animal, for 19 days in one animal, and for 29 days in one animal. At the end of the experiment (mean, 30 days), *E. coli* O157:H7 was not recovered from the rumen of any of the six animals treated with probiotic bacteria; however, *E. coli* O157:H7 was recovered from the feces of one of the animals. This animal was fasted twice postinoculation. These studies indicate that selected probiotic bacteria administered to cattle prior to exposure to *E. coli* O157:H7 can reduce the level of carriage of *E. coli* O157:H7 in most animals.

During the past decade, *Escherichia coli* O157:H7, an important human pathogen causing hemorrhagic colitis and hemolytic-uremic syndrome, has been reported as a cause of human illness with increased frequency (1, 10, 16). Cattle, especially young animals, have been implicated as a principal reservoir of *E. coli* O157:H7 (4, 7, 19, 21, 22, 24, 26), with undercooked ground beef being a major vehicle of food-borne outbreaks. A recent national survey performed by the National Animal Health Monitoring System of the U.S. Department of Agriculture revealed that 1.6% of feedlot cattle shed *E. coli* O157:H7 in their feces and that 0.4% shed *E. coli* O157:nonmotile (O157:NM) in their feces (6). A major study of calves on dairy farms revealed that 1.5 to 2.9% of animals between 24 h of age and weaning and 4.9 to 5.3% of animals between the age of weaning and 4 months shed *E. coli* O157:H7 in their feces (26). Experimental infection of calves and adult cattle with *E. coli* O157:H7 infection revealed that shedding of *E. coli* O157:H7 varies widely among animals of the same age group (14 to >20 weeks) but persists longer in calves than in adults, and previous infection does not prevent reinfection with the same strain of *E. coli* O157:H7 (5).

Many concerns have been raised regarding *E. coli* O157:H7 contamination of foods. Such concerns have been heightened by the tolerance of *E. coli* O157:H7 to acidic conditions. Proper cooking is an effective method of killing *E. coli* O157:H7 in foods. Insanitary practices in preparing foods often result in food-borne illness; hence, methods for reducing or eliminating the carriage of *E. coli* O157:H7 in cattle are needed to reduce the level of exposure to the pathogen in food and the environment (23). The purpose of this study was to isolate potential probiotic bacteria and to evaluate their efficacy at reducing the level of carriage of *E. coli* O157:H7 by cattle. Probiotic bacteria are those that beneficially affect the host by improving its microbial balance, including eliminating or reducing microorganisms that are carried by the host and that are harmful to humans.

**MATERIALS AND METHODS**

Source and isolation of potential probiotic bacteria. Bacteria to be screened for activity bactericidal for or inhibitory to *E. coli* O157:H7 were isolated from cattle feces or cattle gastrointestinal tissue (intestine and colon). Fecal samples were collected from cattle that were confirmed to be negative for *E. coli* O157:H7 by fecal testing (26). Fifty-five fecal samples were serially diluted (1:10) in 0.1
M phosphate buffer (phosphate-buffered saline [PBS]; pH 7.2), 0.1 ml of each dilution was plated onto sorbitol MacConkey agar (SMA), and the plates were incubated for 16 h at 37°C. Up to 10 colonies were randomly selected, and each one was transferred to a test tube containing 10 ml of Trypticase soy broth (TSB; BBL, Cockeysville, Md.). Cultures were incubated for 16 h at 37°C. Eighty-six tissue samples (1 g each in 9 ml of PBS) were homogenized individually (Ultra-Turrax T25 homogenizer; Janke & Kunkel IKA-Labortechnik, Staufen, Germany) at 9,500 rpm for 1 min, and 0.1-ml portions were plated onto the surfaces of SMA plates. The plates were incubated for 16 h at 37°C. Up to 10 colonies were each transferred to test tubes containing 10 ml of TSB, and the tubes were incubated for 16 h at 37°C.

Screening of cultures for anti-E. coli O157:H7 properties. A five-strain mixture of E. coli O157:H7 was cultured, including strains 932 (human isolate), C9727 (human isolate), E009 (meat isolate), E0018 (cattle isolate), and E0122 (cattle isolate), was used to screen culture supernatants for anti-E. coli O157:H7 activity. Approximately 10³ cells of E. coli O157:H7 of approximately equal populations of each strain in 0.1 ml were plated onto the surfaces of duplicate SMA and Trypticase soy agar (TSA) plates. Cultures were sedimented by centrifugation (4,000 x g for 20 min), and the supernatant from each culture was filter sterilized (0.2-μm-pore-size cellulose acetate membrane; Nalgene Co., Rochester, N.Y.) for determination of anti-E. coli O157:H7 properties. A disc (diameter, 12 mm; Dispens-O-Disc; Difo Laboratories, Detroit, Mich.) was placed on the surface of each SMA and TSA plate, and 0.1 ml of filter-sterilized supernatant from a single culture was applied to the surface of the disc. In addition, a disc containing 10 μl of L-5 (Trypticase soy broth; BBL, Salt Lake City, Utah) and another disc with 70% ethanol, which were used as positive controls, and a disc with PBS, which was used as a negative control, were applied to each plate. The cultures were incubated for 16 h at 37°C and were observed for zones of inhibition. Plate bacteria were selected from those which produced a clear zone of 1 mm or greater surrounding each disc.

Preparation of E. coli O157:H7 cultures for inoculation into calves. The same five-strain mixture of E. coli O157:H7 described above was used to inoculate calves. To facilitate enumeration of these bacterial isolates, the strains were selected for resistance to nalidixic acid (50 μg/ml) by exposure to serially increased (1:2; i.e., 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 25, and 50 μg/ml) concentrations of nalidixic acid in MacConkey agar every 24 h. Each strain of nalidixic acid-resistant E. coli O157:H7 was transferred into 10 ml of TSB containing nalidixic acid (50 μg/ml) and was incubated for 16 to 18 h at 37°C with agitation (150 rpm). A 2-ml suspension of each isolates was transferred to 300 ml of TSB. After incubating at 37°C for 16 to 18 h with agitation (150 rpm), the bacteria were sedimented by centrifugation (4,000 x g for 20 min) and were washed three times in PBS. PBS was added to sedimented bacteria in an amount needed to obtain an optical density (OD) at 630 nm of 0.5 (ca. 10⁶ CFU/ml). The five isolates (2 x 10⁶ CFU of each strain) of E. coli O157:H7 were mixed in 250 ml of 2% sterilized skim milk just prior to oral inoculation of the calves. Enumeration of the bacteria was confirmed by plate counts on TSA and SMA plates containing nalidixic acid at 50 μg/ml (SMA-NA plates).

Preparation of probiotic bacteria for inoculation into calves. All 18 probiotic bacterial isolates were grown in TSB containing nalidixic acid (50 μg/ml) by the procedure described above for ease of enumeration of the organisms in feces. The bacteria were grown individually in 10 ml of TSB containing nalidixic acid (50 μg/ml). A 1-ml portion of each isolate was transferred to 100 ml of TSB. After incubation at 37°C for 16 to 18 h, the bacteria were sedimented by centrifugation (4,000 x g for 20 min), washed, and adjusted to an OD at 630 nm of 0.5 by the method described above. The 18 strains of probiotic bacteria (10⁹ CFU), with approximately equal populations of each strain, were mixed into 250 ml of 2% sterilized skim milk just prior to oral inoculation of the calves. The bacterial population was confirmed by enumeration of serial dilutions on TSA and SMA-NA plates, in duplicate.

Preparation of calves before receipt of bacterial inoculation. Fifteen single-source male dairy calves were reared on milk replacer and were weaned at 6 weeks of age prior to transfer to the University of Georgia. The calves were housed individually in climate-controlled biolevel-2 concrete rooms. Each room had an individual floor drain and was cleaned once daily. Calves were fed a mixture of alfalfa pellets and sweet feed twice daily and had free access to water. The barn was kept at 5°C, and transported to the Center for Food Safety and Quality Enhancement for analysis. A volume containing 1 g of feces or rumen fluid was diluted serially (1:10) in 0.5 N NaCl to 10⁻¹, and 0.1 ml of each dilution was plated onto SMA-NA plates. Tissue samples for determination of the entire gastrointestinal tract collected at necropsy (see below) were held at 5°C until analysis. Uminal contents from each segment were separated and weighed, and the tissue was rinsed with 100 ml of PBS. The rinsed tissue was digested with proteinase K (2 mg of proteinase K, 50 mM Tris, 50 mM NaCl, 1 mM EDTA [pH 8.0]) at 56°C overnight. The samples were washed in 10 ml of 0.5 N NaCl and was homogenized for 1 min 9,500 rpm with an Ultra-Turrax homogenizer. A 0.1-ml sample of tissue or content suspension was inoculated onto SMA-NA plates in quadruplicate, and the plates were incubated at 37°C for 24 h for enumeration of E. coli O157:H7 or probiotic bacteria. If these bacteria were not detected by the direct plating method, a selective enrichment method (17) (modified TSB containing 50 μg of nalidixic acid/ml) was performed. The samples were each placed in 100 ml of selective enrichment medium and were incubated at 37°C for 24 h with agitation at 150 rpm. Dilutions of cultures were plated onto SMA-NA plates, and isolates were selected and further tested. Colonies typical of E. coli O157:H7 (sorbitol negative) were replated onto SMA-NA plates and were confirmed to be E. coli by biochemical methods and as O157 (26) and H7 (8) by serological methods. Colonies typical of probiotic bacteria (sorbitol positive) were randomly selected from plates with the highest dilution having colonies and were confirmed to be the inoculated probiotic bacteria by genomic DNA fingerprinting by a pulsed-field gel electrophoresis (PFGE) procedure (14).

Genomic fingerprinting of bacterial isolates. PFGE procedures similar to those described previously were followed (14). Bacteria were grown in 10 ml of TSB at 37°C for 24 h with agitation at 200 rpm. The bacteria were sedimented by centrifugation (4,000 x g for 20 min), washed three times in 75 mM NaCl containing 25 mM EDTA at pH 7.4 (SE), and resuspended in 0.5 ml of SE. The bacterial suspension was mixed with 0.5 ml of a 1% sodium lauroylsarcosine/ml [sodium lauroylsarcosine/ml (pH 8.0)] at 56°C overnight. The samples were washed in 10 mM Tris-5 mM EDTA (pH 7.5) and digested with 50 U of XbaI. After incubation at 37°C overnight, the reaction was stopped by the addition of 20 μl of 0.5 M EDTA. The DNA samples were electrophoresed on a 1.2% agarose gel in 0.5 x TBE buffer (10 x TBE buffer is 0.89 M Tris, 0.025 M EDTA, and 0.89 M boric acid) with a contour-clamped homogeneous electric field device (CHEF MAPPER; Bio-Rad). After electrophoresis for 24 h at 200 V with pulse times of 5 to 50 s and linear ramping and an electric field angle of 120° at 14°C, the gels were stained with ethidium bromide and the bands were visualized and photographed with UV transillumination.

Necropsy of calves. The calves were killed with intravenous sodium pentobarbital. The gastrointestinal tract was clamped at the esophagus and rectum and was removed in toto. Lengths (4 to 6 cm) of duodenum, proximal, middle, and distal jejunum, proximal and distal ileum, proximal and distal cecum, proximal and distal colon, and the area between the cannula and the rumen wall of the ascending colon, centripetal turn and centrifugal turn of the spiral colon, transverse colon, and descending colon were double tied to allow sampling of all sections for the enumeration of E. coli O157:H7 and probiotic bacteria in both the tissues and their contents with minimal cross-contamination. Sections and contents of the rumen, reticulum, omasum, and abomasum and sections of the kidney, spleen, liver, gall bladder, jejunal lymph node, ileal lymph node, cecal lymph node, and tonsil also were collected for culture and examination of E. coli O157:H7 and/or probiotic bacteria. Sections from all of these sites, as well as sections of prescapular lymph node, skeletal muscle, skin, tarsol, thyroid, thymus,
RESULTS

In vitro screening of potential probiotic bacteria that secrete a metabolite(s) inhibitory to E. coli O157:H7. A total of 1,200 bacterial colonies were isolated from the gastrointestinal tissues and feces of cattle determined not to excrete E. coli O157:H7 in their feces. These bacteria were screened for their ability to inhibit the growth of or kill E. coli O157:H7 in vitro, and 18 were determined to secrete antimicrobial metabolites. Among them, five colonies were isolated from feces, five were isolated from the small intestine, and eight were isolated from the colon. Seventeen of the 18 colonies were identified as E. coli and the other was identified as Proteus mirabilis (Table 1). All colonies were assayed for Shiga toxin production (26), and none produced Shiga toxin. Genomic DNA fingerprinting by PFGE revealed 13 different profiles among the 18 isolates.

Colonization of calves by probiotic bacteria. One calf was initially fed one strain of probiotic bacteria (E. coli 271 at $10^{10}$ CFU). The calf appeared to be clinically normal, and this E. coli strain was recovered by the enrichment procedure only from the contents of the ileum and the cecum at the termination of the experiment (12 days). Two calves were then fed all 18 strains (at approximately equal concentrations of each strain; $5 \times 10^8$ CFU each) of probiotic bacteria ($1 \times 10^{10}$ CFU per calf) as a mixture. The calves' feces were of normal consistency, and the bacteria were isolated from the gastrointestinal tract (both tissue and the contents of the rumen and colon) for up to 27 days (at the termination of the study, the counts were 50 to 200 CFU/g of feces).

Twenty-one colonies were isolated by direct plating methods from SMA-NA and TSA-nalidixic acid plates with the highest dilutions of tonsil, omasum, reticulum, rumen, proximal ileum, distal cecum, proximal loop of ascending colon, transverse colon, and feces of two calves at 26 days postinoculation with the probiotic bacteria. These calves were not challenged with E. coli O157:H7. The 21 colonies were analyzed by PFGE and were determined to have only four distinguishable DNA profiles. These four dominant isolates were all E. coli. Among the 21 colonies, 9 were strain 797, 7 were strain 786, 3 were strain 271, and 2 were strain 1019. Strains 786 and 797 were isolated from both calves, whereas strains 271 and 1019 were isolated from only one of the two calves.

Although some strains of the inoculated bacteria were recovered at necropsy from tissue specimens from different parts of the gastrointestinal tract, there were no pathological changes in any of the tissue samples assayed.

### TABLE 1. Selected characteristics of potential probiotic bacteria with antimicrobial activity against E. coli O157:H7 and isolated from cattle

<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>Source</th>
<th>DNA fingerprint*</th>
<th>Dominant strain*</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli 34</td>
<td>Feces</td>
<td>Unique</td>
<td>No</td>
</tr>
<tr>
<td>E. coli 71</td>
<td>Feces</td>
<td>Unique</td>
<td>No</td>
</tr>
<tr>
<td>P. mirabilis 208</td>
<td>Feces</td>
<td>Unique</td>
<td>No</td>
</tr>
<tr>
<td>E. coli 271</td>
<td>Intestine</td>
<td>Unique</td>
<td>Yes</td>
</tr>
<tr>
<td>E. coli 276</td>
<td>Intestine</td>
<td>Same as 271</td>
<td></td>
</tr>
<tr>
<td>E. coli 277</td>
<td>Intestine</td>
<td>Same as 271</td>
<td></td>
</tr>
<tr>
<td>E. coli 278</td>
<td>Intestine</td>
<td>Same as 271</td>
<td></td>
</tr>
<tr>
<td>E. coli 282</td>
<td>Intestine</td>
<td>Unique</td>
<td>No</td>
</tr>
<tr>
<td>E. coli 368</td>
<td>Colon</td>
<td>Unique</td>
<td>No</td>
</tr>
<tr>
<td>E. coli 457</td>
<td>Colon</td>
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<td></td>
</tr>
<tr>
<td>E. coli 460</td>
<td>Colon</td>
<td>Unique</td>
<td>No</td>
</tr>
<tr>
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<td>Colon</td>
<td>Same as 769</td>
<td>No</td>
</tr>
<tr>
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<td>No</td>
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<td>Unique</td>
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</tr>
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</tr>
<tr>
<td>E. coli 1019</td>
<td>Feces</td>
<td>Unique</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* DNA fingerprinting as determined by PFGE; unique indicates that the PFGE profile is different from those of the other strains in this study.

* Dominant strain indicates that the strain was recovered at 26 days postinoculation (termination of study) from the contents of the gastrointestinal tracts of two calves experimentally administered all 18 potential probiotic bacterial isolates.

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FIG. 1. Detection of E. coli O157:H7 in rumen fluid of nine control calves administered only E. coli O157:H7. Bacterial enumeration was performed by surface plating on SMA-NA plates, in duplicate. The arrow indicates that detection of E. coli O157:H7 was by an enrichment procedure in which 9 ml of rumen fluid was positive for E. coli O157:H7.
Efficiency of probiotic bacteria in reducing carriage of \textit{E. coli} O157:H7 in calves. Of the nine control calves administered only \textit{E. coli} O157:H7, all remained clinically healthy, with no evidence of fever or diarrhea. \textit{E. coli} O157:H7 was isolated intermittently from the rumen fluid of all animals during the first 3 weeks postinoculation (Fig. 1). Shedding of \textit{E. coli} O157:H7 in the feces at various levels was generally detected continuously for the duration of the experiment (mean, 28 days) (Fig. 2). At necropsy, \textit{E. coli} O157:H7 was isolated from all nine calves, as follows: rumen contents of five of nine calves, reticulum contents of three of nine calves, omasum contents of one of nine calves, and the colons of nine of nine calves (Table 2). No pathological changes were observed in any of the tissue samples examined microscopically.

All six calves that were administered probiotic bacteria 2 days before treatment with \textit{E. coli} O157:H7 remained healthy, with no evidence of fever or diarrhea during the entire experiment. \textit{E. coli} O157:H7 was detected in rumen samples, collected through a rumen cannula, for up to 9 days after challenge in two animals, 16 days in one animal, 17 days in two animals, and 29 days in one animal (Fig. 3). The number of \textit{E. coli} O157:H7 in the rumens of calves treated with probiotic bacteria was significantly less ($P < 0.05$) at 18 days posttreatment and thereafter than in the control group treated only with \textit{E. coli} O157:H7. \textit{E. coli} O157:H7 was detected in the feces of probiotic-treated calves for up to 11, 15, 17, 18, 19, and 29 days (at the termination of the experiment) in one animal each (Fig. 4). The number of \textit{E. coli} O157:H7 in the feces of calves treated with probiotic bacteria was significantly less ($P < 0.05$) at 15 and 18 days posttreatment and thereafter than in the control group treated only with \textit{E. coli} O157:H7. At necropsy (mean, 30 days), \textit{E. coli} O157:H7 was not recovered from rumen samples from any of these six animals (Table 3); however, \textit{E. coli} O157:H7 was recovered from the colon of one of the six animals (Table 3). Probiotic bacteria were not detected in any segments of the gastrointestinal tract of the \textit{E. coli} O157:H7-positive animal which was twice fasted for 2-day periods (days 16 and 17 and days 23 and 24) postinoculation during the study.

Sites of localization of probiotic bacteria. All calves treated with probiotics were necropsied between 29 and 30 days after the administration of \textit{E. coli} O157:H7. Of the six calves administered probiotic bacteria 2 days before treatment with \textit{E. coli} O157:H7, probiotic bacteria were recovered at necropsy from the contents of the rumens, reticulums, omasas, and colons of five of six calves. However, probiotic bacteria were not isolated at necropsy from the one animal from which \textit{E. coli} O157:H7 was isolated from cecal and colonic contents at populations of $2.5 \times 10^3$ to $5.0 \times 10^3$ CFU/g (Tables 3 and 4).


discussion

Ruminants including cattle (3, 5), deer (12), and sheep (13) have been identified as carriers of \textit{E. coli} O157:H7. The primary sites of \textit{E. coli} O157:H7 localization in calves are the rumen and colon (3). The rumen appears to be the most important site for long-term carriage of \textit{E. coli} O157:H7 because it may serve as the source of the bacteria found in the colon (3). Histologic examination of colonic tissue revealed no evidence of attachment of \textit{E. coli} O157:H7 to colonic tissue. Hence, the presence of \textit{E. coli} O157:H7 in the colon may be a transient state whereby the bacteria are passing through rather

![FIG. 2. Detection of \textit{E. coli} O157:H7 in feces of nine control calves administered only \textit{E. coli} O157:H7. Bacterial enumeration was performed by surface plating on SMA-NA plates, in duplicate. The arrow indicates that detection of \textit{E. coli} O157:H7 was by an enrichment procedure in which 9 g of feces was positive for \textit{E. coli} O157:H7.](http://jcm.asm.org/)
than colonizing the colon. In addition, changes that affect the conditions of the rumen, such as fasting, may influence the presence of \textit{E. coli} O157:H7. Studies by Rasmussen et al. (18) revealed that \textit{E. coli} O157:H7 could grow unrestricted in rumen fluid collected from fasted animals. Factors influencing the conditions in the rumen include nutrition, feeding regimens, and animal handling at the farm (9).

The presence of bacteria that produce metabolites inhibitory to \textit{E. coli} O157:H7 at sites where O157:H7 strains localize is another factor that may influence the localization of O157:H7 in the gastrointestinal tract. Some strains of \textit{E. coli} can produce colicins that are inhibitory in vitro to diarrheagenic \textit{E. coli} strains, including strains of serotype O157:H7 (2, 15). Murinda et al. (15) assayed 24 \textit{E. coli} colicin-producing strains and determined that all \textit{E. coli} O157:H7 strains evaluated were sensitive to ColE1 to ColE8, K, and N on mitomycin C-containing agar and to ColG, ColH, and Mcc B17 on Luria agar. Colicins could be one of many metabolites produced by the probiotic bacteria in the rumen and other sections of the gastrointestinal tract. The findings from this study indicate that the probiotic bacteria localize at the same sites of calves as \textit{E. coli} O157:H7 (Tables 2 and 4); hence, the anti-\textit{E. coli} O157:H7 metabolites that the probiotic bacteria produce would be in the same proximity as the target bacteria.

The administration of probiotic bacteria, used in this study to treat calves prior to exposure to \textit{E. coli} O157:H7, decreases the duration of ruminal carriage of \textit{E. coli} O157:H7. Our studies with probiotic bacteria revealed that \textit{E. coli} O157:H7 was detectable in rumen fluid an average of 14 days (range, 9 to 17 days) postinoculation in five of six animals given probiotic
bacteria, whereas O157:H7 was detected in rumen fluid for an average of 26 days (range, 22 to 32 days) in control calves not receiving probiotic bacteria. At necropsy, E. coli O157:H7 was no longer detected in the rumens of six of six calves receiving probiotic bacteria. In contrast, E. coli O157:H7 was detected at necropsy in the contents of the rumen, reticulum, or omasum of seven of nine control animals.

Cray and Moon (5) reported that experimentally treated calves shed E. coli O157:H7 in their feces at least 7 weeks postinoculation. In our study, fecal shedding of E. coli O157: H7 was reduced from 25 to 32 days, when the calves were necropsied (control group), to 14 to 19 days in five of the six calves treated with probiotic bacteria. At necropsy, E. coli O157:H7 was recovered from the feces of only one of the six probiotic-treated animals, whereas it was recovered from all nine of the control group from which E. coli O157:H7 was recovered at 25 to 32 days postinoculation. The persistence of E. coli O157:H7 in one animal in the group treated with probiotic bacteria may be due to the apparent failure of probiotic bacteria to colonize this animal. It is possible that greater protection or clearance can be conferred by multiple treatments with probiotic bacteria.

Although the mechanism(s) by which our selected probiotic bacteria reduced the level of carriage of E. coli O157:H7 remains to be elucidated, these studies indicate that microbial interactions could be an important factor that contributes to the homeostasis of the bacterial flora in the gastrointestinal tract (11, 25). Hence, treatment of cattle with probiotic bacteria may reduce the level of carriage and fecal shedding of E. coli O157:H7 and may thereby reduce environmental contamination with this pathogen. Reducing E. coli O157:H7 carriage in cattle should decrease the likelihood of meat, vegetable, fruit, and water contamination, thereby decreasing the potential for food-, water-, and environment-associated E. coli O157:H7 illness in humans.

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REFERENCES


