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Persistent Colonization of Sheep by *Escherichia coli* O157:H7 and Other *E. coli* Pathotypes

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Shiga toxin-producing *Escherichia coli* (STEC) is an important cause of food-borne illness in humans. Ruminants appear to be more frequently colonized by STEC than are other animals, but the reason(s) for this is unknown. We compared the frequency, magnitude, duration, and transmissibility of colonization of sheep by *E. coli* O157:H7 to that by other pathotypes of *E. coli*. Young adult sheep were simultaneously inoculated with a cocktail consisting of two strains of *E. coli* O157:H7, two strains of enterotoxigenic *E. coli* (ETEC), and one strain of enteropathogenic *E. coli*. Both STEC strains and ETEC 2041 were given at either 107 or 1010 CFU/strain/animal. The other strains were given only at 1010 CFU/strain. We found no consistent differences among pathotypes in the frequency, magnitude, and transmissibility of colonization. However, the STEC strains tended to persist to 2 weeks and 2 months postinoculation more frequently than did the other pathotypes. The tendency for persistence of the STEC strains was apparent following an inoculation dose of either 107 or 1010 CFU. One of the ETEC strains also persisted when inoculated at 1010 CFU. However, in contrast to the STEC strains, it did not persist when inoculated at 107 CFU. These results support the hypothesis that STEC is better adapted to persist in the alimentary tracts of sheep than are other pathotypes of *E. coli.*

*Escherichia coli* O157:H7 and other serotypes of Shiga toxin-producing *E. coli* (STEC) are important causes of food-borne illnesses in people. Most clusters of disease are traced to contaminated food or water, but person-to-person transmissions also occurs (24, 32, 56). Contaminated beef has been the source of several large outbreaks of disease, and cattle are considered to be a reservoir for *E. coli* O157:H7 and other STEC serotypes (24, 32, 48, 58). Healthy cattle shed *E. coli* O157:H7 intermittently. Among U.S. cattle the overall animal prevalence of *E. coli* O157:H7 is approximately 2 to 3%, while the herd prevalence is much higher (16, 20, 25, 46, 58, 59). Fecal shedding of *E. coli* O157:H7 is often seasonal, with increased shedding during the summer (7, 25, 27, 41), which corresponds to an increased incidence of human disease (27, 37). Frequently individual cattle are transiently colonized (<1 month) by one particular strain, but occasional animals excrete multiple strains of *E. coli* O157:H7 (1, 16, 53). Individual strains of *E. coli* O157:H7 can be isolated from some herds for as long as 2 years, whereas other herds remain culture negative for several years (53). *E. coli* O157:H7 has also been isolated from healthy sheep (7, 29, 36), sheep's milk (50), and wild deer (47, 51). Food products from other ruminants, such as venison jerky (33) and goat's milk (3), have also been implicated as sources of human STEC infection. Both cattle and sheep also harbor other serotypes of STEC, usually at a much higher prevalence than serotype O157:H7 (2, 18, 35, 46, 58).

In contrast to ruminants, *E. coli* O157:H7 is isolated much less frequently from other domestic and wild animals (7, 26, 53, 57). This may be partially due to a sampling bias toward cattle. However, in a survey of 4,229 market swine in the United States, the incidence of *E. coli* O157:H7 was less than 0.07% (5). *E. coli* O157:H7 was not isolated from 1,000 fecal samples from either swine or poultry in England (7). There are recent reports of *E. coli* O157:H7 being isolated from swine in Chile (49) and Japan (45). Although *E. coli* O157:H7 occurs in non-ruminant animals such as dogs, horses, birds, and flies, there is no evidence that the agent is as prevalent or as persistent in these animals as it is in ruminants (6, 26, 53, 57). Furthermore, the prevalence of all types of STEC appears to be greater in ruminants than in other types of domestic animals (2).

Both calves and adult cattle have been experimentally inoculated with *E. coli* O157:H7 (4, 11, 28, 30). The magnitude and duration of shedding is greatest during the first 2 weeks postinoculation (p.i.) and decreases thereafter. In general, calves shed higher numbers of *E. coli* O157:H7 organisms for a longer duration than do mature animals (11). This parallels the results of on-farm studies that show a greater percentage of young animals colonized with *E. coli* O157:H7 than adults (25, 27, 41, 58). However, there is considerable animal-to-animal variability in both the numbers of bacteria shed and the duration of shedding (4, 11). The infection persists for several months in some cattle and calves (11). Sheep have also been experimentally inoculated with *E. coli* O157:H7 (34, 37). The quantity and duration of fecal shedding are similar to those for cattle. Diet appears to influence the colonization of *E. coli* O157:H7 in both cattle and sheep (10, 30, 34, 37). Serum antibody to O157 lipopolysaccharide or Shiga toxin 1 (Stx1) acquired from a prior infection does not protect calves from reinfection (31). Sheep and cattle that have cleared a previous colonization with *E. coli* O157:H7 can also be reinfeected (11, 37).

Despite the widespread epidemiological evidence that most ruminants are colonized by STEC, the reasons for this observation are not known. We hypothesized that STEC bacteria are better adapted to colonize and persist in the alimentary tracts of ruminants than are other pathotypes of *E. coli*. We tested this hypothesis by analyzing the frequency, magnitude, duration, and transmissibility of *E. coli* O157:H7 colonization compared to those for other pathotypes of *E. coli* when sheep were inoculated with a cocktail consisting of multiple strains representing three pathotypes of *E. coli*. We found that the inoculated STEC strains tended to persist longer than the

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colonization of sheep by e. coli O157:H7

TABLE 1. Strains of e. coli used in the inoculum cocktail

<table>
<thead>
<tr>
<th>Strain</th>
<th>Pathotype</th>
<th>Serogroup</th>
<th>Virulence determinant(s)</th>
<th>Source</th>
<th>Reference(s)</th>
<th>Selective medium</th>
<th>carbohydrate reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>86-24</td>
<td>STEC</td>
<td>O157:H7</td>
<td>eae, Stx2</td>
<td>Human</td>
<td>15, 23</td>
<td>SN dulcitol/positive</td>
<td></td>
</tr>
<tr>
<td>3081</td>
<td>STEC</td>
<td>O157:H7</td>
<td>eae, Stx1, Stx2</td>
<td>Bovine</td>
<td>11, 13</td>
<td>KA sorbitol/negative</td>
<td></td>
</tr>
<tr>
<td>2041</td>
<td>ETEC</td>
<td>O157:H43</td>
<td>F4, F41, LT, Stb</td>
<td>Porceine</td>
<td>52</td>
<td>TN sorbose/positive</td>
<td></td>
</tr>
<tr>
<td>637</td>
<td>ETEC</td>
<td>O64:NM</td>
<td>F5, STa</td>
<td>Porcine</td>
<td>43, 54</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>E2348/69</td>
<td>EPEC</td>
<td>O127:H6</td>
<td>eae</td>
<td>Human</td>
<td>39, 44</td>
<td>SN dulcitol/positive</td>
<td></td>
</tr>
</tbody>
</table>

Materials and methods

Inoculum cocktail. Strains of e. coli used in the inoculum cocktail are listed in Table 1. All of the strains used in the cocktail are animal or human pathogens. STEC strain 86-24 was isolated from an outbreak of human disease and causes attaching and effacing lesions in experimentally infected animals (15, 23). STEC strain 3081 was isolated from a healthy bovine, can persist asymptomatically as long as 6 months in the alimentary tract of experimentally infected cattle, and causes attaching and effacing lesions in the intestines of neonatal pigs and calves (11, 13). ETEC strains 2041 and 637 cause diarrhea in experimentally inoculated weaned and neonatal pigs, respectively (43, 52, 54). F4 fimbriae (K88) host adapt ETEC 2041 for swine, and F5 fimbriae (K99) host adapt ETEC 637 for neonatal calves, pigs, and lambs. EPEC strain E2348/69 causes diarrhea in experimentally inoculated humans and attaching and effacing lesions in animal models (39, 44). Generally, EPEC and ETEC strains colonize the lower small intestines. It is not known if STEC strains colonize a specific site in mature ruminants. In short-term experimental studies (2 to 30 days), STEC bacteria have been recovered from the rumen, ileum, and sites throughout the lower intestinal tract (4, 11, 13).

Virulence genes from all of the strains were confirmed using multiplex PCR (19; B. T. Bosworth and T. A. Casey, Abstr. 97th Gen. Meet. Am. Soc. Microbiol., 1997, abstr. D/B 309); at the annual meeting of the Food Research Institute, University of Wisconsin—Madison, 1999; and at the Verocytotoxigenic e. coli in Europe: Pathogenicity and Virulence meeting, Liège, Belgium, 1999.)

RESULTS

Transmission experiments. Sheep were acclimated for ≥2 weeks prior to the experiment as described above. Donor sheep (6 sheep total in 3 replicates) were orally inoculated with the cocktail shown in Table 1. The STEC strains, 86-24 and 3081, were given at 10⁷ CFU/strain, and the other three strains were given at 10⁶ CFU/strain. Three days p.i., each donor was moved into a clean room with a NAIVE animal of the same age and remained there for the duration of the experiment. Fecal samples were collected from each donor prior to moving. Fecal samples were collected on days 2, 3, 4, 15, 16, 58, 59, and 60 postexposure from both recipient and donor sheep. Samples were cultured as described above. Sheep were necropsied 2 months postexposure.

Antibody titers. Serum was heat inactivated for 1 h at 56°C. Neutralizing antibody titers to Stx1 and Stx2 were determined using monolayers of Vero cells (22). Antibody to O157 lipopolysaccharide was detected using a blocking enzyme-linked immunosorbent assay (ELISA) as described previously except that 1% skim milk (rather than fetal calf serum) was used to block nonspecific binding (38).

Fecal toxin titters. Assays for fecal Shiga toxin were performed on Vero cells as previously described except that the sheep feces were diluted 1:5 with PBS and mixed in a Stomacher blender prior to centrifugation (9, 21).

Statistics. Bacterial counts (CFU per gram of feces) from individual sheep were converted to log10 units and averaged over days 2, 3, and 4 (initial period), days 14, 15, and 16 (2 weeks), and days 58, 59, and 60 (2 months). Differences in the magnitude of shedding between strains were compared using a paired t test using Bonferroni’s method to correct for type 1 error (55). Differences in the magnitude of shedding due to treatment were compared using repeated-measures analysis of variance (ANOVA). Differences in the duration of shedding between strains were compared using a sign test (55).

RESULTS

Treatment 1. Sheep (n = 6) were simultaneously inoculated with all five strains in the cocktail at a dose of 10⁸⁰ CFU/strain. During the initial period p.i., all of the strains were recovered

Enterotoxigenic (ETEC) and enteropathogenic (EPEC) strains included in the same inoculum. We also found that all of the strains were transmitted to naive animals from infected donors and again that the STEC strains tended to persist longer in the recipient animals than did two of the other strains.

Evolution by e. coli O157:H7

On days when fecal samples were to be collected, the floor was cleaned, the sheep were individually penned, and fecal samples were collected from the floor within 2 h of passage. Individual fecal samples were collected on days 2, 3, 4, 14, 15, 16, 58, 59, and 60 p.i. Previous work with cattle demonstrated that the decrease in the magnitude of STEC shedding is comparatively rapid between 2 days and 2 weeks and is gradual between 2 weeks and 2 months; therefore, samples were not collected between the latter two time points (11). Two months p.i. was selected as the final period because shedding terminates in some, but not all, cattle, by this time (11).

Samples (5 g) were processed immediately by fivefold dilution with phosphate-buffered saline (PBS) and mixing in a Stomacher blender for 1 min; then serial 10-fold dilutions were made in PBS. Aliquots were plated directly onto selective media in triplicate and incubated for 24 h at 37°C. The sensitivity of the direct plating was ≥50 CFU/g. Samples (10 g in 100 ml of medium) were also cultured at 37°C in enrichment broth (TSB with 0.15% bile salts) with agitation for 24 h and then plated onto selective media (11). Samples from water buckets (10 ml) were cultured in enrichment broth. The identities of the isolates recovered were confirmed by serology (slide agglutination in antisera or a commercial kit for O157) for O antigens. Sheep were necropsied at the end of the experiment (2 months p.i.). The contents of the rumen, ileum, cecum, colon, and rectum as well as tissues from the tonsil and lymph nodes were cultured in enrichment broth.

When the enrichment cultures were positive, the original contents (which were held at 4°C overnight) were plated directly the next day. Preliminary experiments comparing the bacterial counts of tissue contents cultured immediately versus after holding for 24 h at 4°C did not show quantitative differences between the treatments (data not shown).

Transmission experiments. Sheep were acclimated for ≥2 weeks prior to the experiment as described above. Donor sheep (6 sheep total in 3 replicates) were orally inoculated with the cocktail shown in Table 1. The STEC strains, 86-24 and 3081, were given at 10⁷ CFU/strain, and the other three strains were given at 10⁶ CFU/strain. Three days p.i., each donor was moved into a clean room with a NAIVE animal of the same age and remained there for the duration of the experiment. Fecal samples were collected from each donor prior to moving. Fecal samples were collected on days 2, 3, 4, 15, 16, 58, 59, and 60 postexposure from both recipient and donor sheep. Samples were cultured as described above. Sheep were necropsied 2 months postexposure.

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RESULTS

Treatment 1. Sheep (n = 6) were simultaneously inoculated with all five strains in the cocktail at a dose of 10⁸⁰ CFU/strain. During the initial period p.i., all of the strains were recovered
from all of the sheep (Fig. 1). The magnitude of colonization by all strains varied considerably for individual animals. In general, both STEC strains and ETEC 2041 were shed in greater numbers than the other two strains. These differences were significant between STEC 86-24 and EPEC E2348/69 and between STEC 86-24 and ETEC 637 ($P \leq 0.002$).

At 2 weeks p.i., STEC 86-24 was recovered from 6 of 6 sheep, while STEC 3081 and ETEC 2041 were recovered from 5 of 6 sheep. Again, the magnitude of shedding of both STEC strains and ETEC 2041 tended to be greater than those of the other two strains. Only STEC 86-24 and ETEC 637 were significantly different at this time ($P \leq 0.002$).

At 2 months p.i., STEC 86-24 was recovered from 2 of 6 sheep, STEC 3081 from 4 of 6 sheep, and ETEC 2041 from 3 of 6 sheep. Neither EPEC E2348/69 nor ETEC 637 was recovered at 2 months p.i. These six sheep were not necropsied.

**Treatment 2.** The objective of treatment 2 was to determine if strains that persisted for 2 months in treatment 1 would still persist if they were inoculated at a lower dose. Since the magnitude and duration of colonization by ETEC 2041 were similar to those for the STEC strains, and these three strains persisted longer than the other two strains in sheep given treatment 1, the dose of STEC 86-24, STEC 3081, and ETEC 2041 was lowered to $10^7$ CFU/strain in treatment 2. The dose

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**FIG. 1.** Fecal shedding of *E. coli* strains from six sheep inoculated with a cocktail containing five strains given at a dose of $10^{10}$ CFU/strain/animal (treatment 1). Lines represent the means for each strain. (A) Strains STEC 86-24 and STEC 3081; (B) strains ETEC 2041, ETEC 637, and EPEC E2348/69.
of ETEC 637 and EPEC E2348/69 was left at $10^{10}$ CFU/strain. Six sheep were given this treatment regimen. Again, all of the strains were recovered from all of the sheep during the initial period p.i. (Fig. 2). As was seen with treatment 1, there was a wide variation in the magnitude of shedding for individual sheep.

At 2 weeks p.i., STEC 86-24 was recovered from 6 of 6 sheep and STEC 3081 was detected in 5 of 6 sheep. In contrast to treatment 1, ETEC 2041 was recovered only from 1 of 6 sheep. At this time the magnitudes of shedding of both STEC strains were significantly greater than those of the other three strains ($P \leq 0.002$).

At 2 months p.i., STEC 86-24 and STEC 3081 were each recovered from 2 of 6 sheep and the other three strains were not recovered. At necropsy, only STEC 86-24 and STEC 3081 were recovered from the rectal contents (feces) of two sheep. None of the cocktail strains were recovered from the other necropsy samples.

**Treatment 3.** The primary purpose of treatment 3 was to produce donor animals for the transmission experiment. Treat-
ment 2 also demonstrated that both STEC strains, but not ETEC 2041, persisted when the inoculum dose was lowered to $10^7$ CFU. In treatment 3 the dose of ETEC 2041 was raised back to $10^{10}$ CFU and the STEC dose remained at $10^7$ CFU/strain. ETEC 637 and EPEC E2348/69 were given at $10^{10}$ CFU/strain. Six sheep were given this treatment regimen. As was seen in the previous experiments, all of the strains were recovered from all of the sheep during the initial period p.i. (Fig. 3). The initial magnitudes of shedding of STEC 86-24 and STEC 3081 tended to be greater than those of the other strains, but the differences were not significant.

At 2 weeks p.i., STEC 86-24 was recovered from 4 of 6 sheep, STEC 3081 from 2 of 6 sheep, and ETEC 2041 from 3 of 6 sheep. The magnitudes of shedding of STEC 86-24 and ETEC 2041 at this time also tended to be greater than those of the other strains, but the differences were not significant. At 2 months p.i., none of the inoculum strains were recovered from any of the sheep given treatment 3.

**Dose effect.** The magnitudes of shedding of both STEC 86-24 and STEC 3081 were significantly different ($P \leq 0.05$) between treatment 1 ($10^{10}$ CFU inoculum) and treatment 2 or 3 ($10^7$ CFU inoculum) during the initial period (Fig. 1A, 2A, and 3A). By 2 weeks p.i., the magnitude of STEC shed was not significantly different between sheep given treatment 1 and those given treatment 2. The magnitude of shedding of ETEC 2041 was significantly different ($P \leq 0.05$) between all three treat-
ments during the initial period (Fig. 1B, 2B, and 3B). The magnitude of shedding of ETEC 2041 continued to be significantly different (P ≤ 0.05) between treatment 1 and treatment 2 at 2 weeks and 2 months p.i. This is in contrast to both STEC strains.

**Infectious dose of in vitro-grown STEC.** In subsequent experiments, the doses of both STEC strains in the cocktail was lowered to 10⁵ CFU/strain and the other strains remained at 10⁴ CFU/strain. STEC 86-24 was recovered from 4 of 6 sheep during the initial period p.i. (Table 2; range, <50 to 10⁶ CFU/g). It persisted in two sheep at 2 weeks p.i. (10³ to 10⁶ CFU/g) and one of these sheep at 2 months p.i. (<50 CFU/g). STEC 3081 was recovered from 2 of 6 sheep during the initial period (<50 CFU/g) but was not recovered at 2 weeks or 2 months p.i. The dose of both STEC strains was further lowered to 10⁴ CFU/strain, and the other strains remained at 10⁵ CFU/strain. In this experiment STEC 86-24 was recovered from 2 of 6 sheep during the initial period (<50 CFU/g) but was not recovered at 2 weeks and 2 months p.i. The other three strains in the cocktail were recovered at similar magnitudes and times as those described previously (treatments 1 to 3). At necropsy ETEC 2041 was recovered from the rumina, ilea, ceca, and rectums, (range, <50 to 10⁶ CFU/g) of 2 of 12 sheep. These two sheep were sharing a room, and one of them shed ETEC 2041 in moderate numbers (range, 10⁶ to 10⁹ CFU/g) throughout the study. ETEC 2041 was recovered from the second animal only at enrichment levels (<50 CFU/g) at 2 weeks and 2 months p.i.

**Transmission between sheep.** The sheep in treatment 3 were used as donors for the transmission experiment. The infected donor sheep transmitted all five of the strains in the cocktail to naive sheep (Table 3). STEC 86-24 was transmitted to naive sheep on 4 of 5 occasions when the donor was shedding <10⁴ CFU/g at the time it was placed in the room with the naive animal and to 1 of 1 naive sheep when the donor was shedding 10⁵ CFU/g. STEC 3081 was transmitted to 2 of 6 naive sheep when the donor was shedding ≤10⁴ CFU/g. ETEC 2041 was transmitted to 3 of 3 naive sheep when the donor was shedding <10⁴ CFU/g and to 3 of 3 naive sheep when the donor was shedding ≥10⁵ CFU/g. EPEC strain E2348/69 was transmitted to 1 of 6 naive sheep when the donor was shedding ≥10⁴ CFU/g and to 1 of 1 naive sheep when the donor was shedding 10⁵ CFU/g. Although both STEC strains and ETEC 2041 were transmitted more frequently to naive sheep, EPEC E2348/69 was transmitted to one sheep when the donor was shedding ≥10⁴ CFU/g of that strain.

**Antibody titers.** Preexisting titers of antibody to Stx1 (geometric mean, 1:142; range, 1:32 to 1:1,000) but not to Stx2 were found in 8 of 18 sheep tested in this study. Two months p.i., 5 of 18 sheep had ≥4-fold increases in Stx1 antibody titers. None of the sheep had antibody to Stx2 after challenge. Preexisting antibody to the O157 lipopolysaccharide was detected in 1 of 16 sheep. Titers of antibody to O157 increased ≥2-fold at 2 months p.i. in 6 of 16 sheep tested.

None of the sheep were clinically ill during the study, and no free fecal toxin was detected during the initial period p.i. in the 14 sheep tested (magnitude of fecal STEC shedding, nondetectable to 10³ CFU/g). Whenever water buckets were culture positive for one of the cocktail strains (14 of 62), at least one of the sheep in the room was shedding that strain in its feces. The cocktail strains were not recovered from the four uninoculated control sheep.

**DISCUSSION**

Regarding our hypothesis that STEC strains are better adapted than other pathotypes of *E. coli* for colonization and persistence in sheep, we found no consistent differences between STEC strains and the other strains in the frequency or intensity of colonization during the initial period, nor were we able to demonstrate that STEC strains were transmitted between sheep more readily than strains representing other pathotypes. However, the STEC strains tended to persist longer than did the other pathotypes. This tendency for longer persistence of

**TABLE 2. Infectious dose of in vitro-grown *E. coli* O157:H7 in sheep**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Time p.i.</th>
<th>No. of animals positive/no. inoculated at a STEC dosea of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10⁵ CFUa</td>
</tr>
<tr>
<td>86-24</td>
<td>Initial</td>
<td>4/6</td>
</tr>
<tr>
<td></td>
<td>2 wk</td>
<td>2/6</td>
</tr>
<tr>
<td></td>
<td>2 mo</td>
<td>1/6</td>
</tr>
<tr>
<td>3081</td>
<td>Initial</td>
<td>2/6</td>
</tr>
<tr>
<td></td>
<td>2 wk</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td>2 mo</td>
<td>0/6</td>
</tr>
<tr>
<td>2041</td>
<td>Initial</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>2 wk</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>2 mo</td>
<td>2/6</td>
</tr>
<tr>
<td>637</td>
<td>Initial</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>2 wk</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>2 mo</td>
<td>0/6</td>
</tr>
<tr>
<td>E2348/69</td>
<td>Initial</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>2 wk</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td>2 mo</td>
<td>0/6</td>
</tr>
</tbody>
</table>

* STEC 86-24 and 3081 were inoculated at 10⁵ or 10⁶ CFU/strain/animal, and ETEC 2041, ETEC 637, and EPEC E2348/69 were inoculated at 10⁶ CFU/strain/animal.

**TABLE 3. Transmission of *E. coli* cocktail strains to six naive sheep**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Pathotype</th>
<th>Time</th>
<th>No. of naive animals positive/no. exposed to inoculated donors sheddinga <em>E. coli</em> at the following CFU/g:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;10²</td>
</tr>
<tr>
<td>86-24</td>
<td>STEC</td>
<td>Initial</td>
<td>1/2</td>
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<tr>
<td></td>
<td></td>
<td>2 wk</td>
<td>0/2</td>
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<td></td>
<td>2 mo</td>
<td>0/2</td>
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<td>E2348/69</td>
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<tr>
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<td>2 mo</td>
<td>0/4</td>
</tr>
<tr>
<td>637</td>
<td>ETEC</td>
<td>Initial</td>
<td>0/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 wk</td>
<td>0/2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 mo</td>
<td>0/2</td>
</tr>
</tbody>
</table>

* At the time of exposure to a naive animal.
STEC was evident at 2 weeks but more apparent at 2 months p.i. Furthermore, greater persistence could be demonstrated when STEC strains were inoculated at a dose 1,000-fold lower than the doses of two of the other three strains. The inoculum doses of STEC used in this study were varied from quite high ($10^{10}$ CFU) down to a dosage that might be expected to occur naturally ($10^4$ to $10^5$ CFU). The highest doses were used to compare colonization potentials among strains rather than to reflect precisely what is thought to occur in nature. Previous studies with cattle naturally infected with E. coli O157:H7 indicate that some animals shed as much as $10^5$ CFU/g of feces (53, 59). At necropsy (2 months p.i.) both STEC 86-24 and STEC 3081 were recovered only from the rectal feces and not from any other tissue or intestinal contents. It is not known where STEC strains colonize the alimentary tracts of mature ruminants, although this study and others (11, 14) suggest that the lower intestinal tract may be a likely site. Brown et al., however, recovered E. coli O157:H7 primarily from the rumina of calves necropsied 2 to 4 weeks p.i. (4). There are differences in the magnitude and duration of colonization by E. coli O157:H7 between calves and mature cattle (11). Perhaps the site(s) colonized also differs with age. We assume that the STEC persisted in the sheep. However, there is no evidence in this study to rule out the possibility that the STEC strains were continuously reintroduced from the environment by ingestion.

The 50% infectious dose of in vitro-grown STEC 86-24 and 3081 inoculated as a cocktail was approximately $10^5$ CFU (Table 2). However, data from the transmission study indicated that an animal may be infectious when it is shedding considerably less than $10^5$ CFU/g (Table 3). Sheep shedding as little as $10^2$ to $10^5$ CFU/g of STEC transmitted these strains to some naïve sheep. ETEC 2041 and EPEC 2348/69 shed at comparable levels also resulted in the transmission of those strains to some naïve sheep. In contrast, ETEC 637 was transmitted only when an animal was shedding at least $10^5$ CFU/g. In any case, we do not know the route of transmission to the naïve sheep or the actual dose of a particular strain that the naïve sheep received. Penmates were in contact with one another throughout the study and shared both water and feed sources. Water troughs have been proposed as an environmental reservoir and a source of E. coli O157:H7 contamination for cattle (16, 26, 53). Both STEC strains and ETEC 2041 were transmitted to a greater percentage of naïve sheep than were EPEC E2348/69 or ETEC 637. Transmission of E. coli O157:H7 from experimentally inoculated donors to naïve sheep has been documented previously (34, 37). In contrast to the findings of the prior study (37), our data indicated that the transmitted strains could be recovered for up to 2 weeks from some sheep. These differences could be due to the STEC strains that were used or to differences between sheep.

The magnitude and duration of colonization of STEC 3081 in sheep given $10^{10}$ CFU were similar to those obtained with cattle, where persistence of this strain was documented for 7 weeks in 2 of 9 mature cattle given the same dose (11). When the inoculum dose was lowered to $10^7$ CFU, strain 3081 was recovered from 2 of 5 adult cattle 1 to 2 days p.i. and was not recovered after that. This is in contrast to our data with sheep, wherein 6 of 12 sheep given $10^7$ CFU of STEC 3081 were shedding at 2 weeks p.i. and 2 of 12 remained culture positive for at least 2 months. Sheep were used as a ruminant model because they are less expensive and easier to handle than cattle. In addition, sheep are naturally infected with E. coli O157:H7 and other STEC strains (7, 18, 29, 36).

Some of the differences between strains in the magnitude and duration of colonization may be due to host specificity, since neither EPEC E2348/69, ETEC 637, nor ETEC 2041 was originally isolated from a ruminant. However, pathogenic E. coli strains with virulence traits similar to those of EPEC E2348/69 and ETEC 637 (eae or F5 and STa) infect and cause disease in both lambs and calves (40, 42). The persistent colonization of ETEC 2041 in mature ruminants was unexpected. In contrast to F5 + ETEC strains (such as ETEC 637), F4 + ETEC strains are generally regarded as pig pathogens and are not commonly isolated from other species (42). The magnitude and duration of colonization by ETEC 2041 in sheep were significantly more dose dependent than those for the STEC strains (Fig. 1 and 2). Our data also suggest that there may have been exclusionary or suppressive competition for some niche between the STEC strains and ETEC 2041. When ETEC 2041 and the STEC strains were given at $10^6$ CFU, all three strains persisted for 2 months in some sheep (Fig. 1). When all three strains were given at $10^7$ CFU, only the STEC strains persisted (Fig. 2). When ETEC 2041 was given at a 1,000-fold-higher dose than the STEC strains, neither the STEC strains nor ETEC 2041 persisted past 2 weeks p.i. (Fig. 3). As part of another study, we inoculated several sheep with $10^{10}$ CFU of STEC 86-24 only (8). Fecal shedding during the initial period and 2 weeks p.i. was somewhat greater (1 to 2 log$_{10}$ CFU/g) than when the strain was given as part of a cocktail. At 2 months p.i., the magnitude of shedding and the percentage of culture-positive sheep that were inoculated with STEC 86-24 only were similar to those found when the strain was inoculated as part of a cocktail. This also suggests that there was competition between the strains in the cocktail and that this competition may have influenced the magnitude of STEC shedding during the initial and 2-week periods of the study reported here. Regardless of the competition between the strains in the cocktail, all of the inoculum strains were subjected to competition with the endogenous flora. Our data suggest that the STEC strains were able to establish a population and perhaps exploit a niche more consistently than the other pathotypes of E. coli.

If the E. coli O157:H7 strains selected for this study are representative of STEC in general, the tendency for STEC to persist in some animals may explain how the ruminant reservoir is maintained. Only a few persistent shedders in a herd would be enough to transmit STEC to naïve animals. Our data suggest that the infectious dose of in vivo-grown STEC may be lower than the $10^5$ CFU reported here for in vitro-grown STEC. Our initial focus was on transmission by sheep shedding low numbers (at or below the 50% infectious dose for in vitro-grown organisms) of STEC bacteria, because such transmission is relevant to natural transmission even though such an experimental design does not allow determination of the infectious dose for the in vivo-grown organisms. However, studies to determine the infectious dose of in vivo-grown STEC are now in progress. Studies with additional strains of E. coli will be required to determine if an enhanced ability to persist is a general attribute of STEC or occurred merely by chance in the two strains selected for this study. It would also be useful to determine if the persistence attribute in the STEC strains studied here would be apparent in nonruminant animals and if any of the known putative virulence factors of STEC strains ( intimin, Shiga toxin, and enterohemolysin) contribute to their persistence in ruminants.

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REFERENCES


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