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Escherichia coli O157:H7 Requires Intimin for Enteropathogenicity in Calves

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Enterohemorrhagic Escherichia coli (EHEC) strains require intimin to induce attaching and effacing (A/E) lesions in newborn piglets. Infection of newborn calves with intimin-positive or intimin-negative EHEC O157:H7 demonstrated that intimin is needed for colonization, A/E lesions, and disease in cattle. These results suggest that experiments to determine if intimin-based vaccines reduce O157:H7 levels in cattle are warranted.

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| Strain | Serotype | 
|---|---|---|---|---|
| O157:H7 | + | Stx2 | Phil Tarr | 15 |
| O157:H7 | – | Stx2 | 15 |
| O157:H7 | + | Stx2 | 15 |
| O91:H21 | – | Stx2d | Mohamed Karmali | 18 |

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Three of five calves inoculated with wild-type EHEC strain 86-24 developed watery diarrhea by 18 h postinoculation (Table 2). Two of three calves had blood-tinged diarrhea at 42 h postinoculation, and one of these calves died on day 2, about 2 h prior to the scheduled necropsy. Postmortem observations were compatible with enteritis as the cause of death. Similarly, two of seven calves inoculated with the complemented mutant strain 86-24Δ10(pEB310) developed diarrhea by 18 h, and three of four had diarrhea (blood tinged in two calves) at 42 h postinoculation. One such infected calf died on day 2, about 2 h prior to the scheduled necropsy. Again, postmortem observations were compatible with enteritis as the cause of death. In contrast, all calves inoculated with mutant strain 86-24Δ10, with B2F1, or with nonpathogenic control strain 123 remained healthy throughout the experiment.

Hyperemia, focal petechiae, and fibrinous exudates in the intestines were common postmortem observations in calves inoculated with strain 86-24 or 86-24Δ10(pEB310) but were not noted in any of the calves inoculated with mutant strain 86-24Δ10, strain B2F1, or control strain 123. A/E lesions containing O157:H7* bacteria were identified by immunostaining in the ileum and large intestines of five of five calves inoculated with strain 86-24 and six of seven calves inoculated with strain 86-24Δ10(pEB310). In addition to A/E lesions, a diffuse mucosal neutrophil infiltration with accompanying hemorrhage, edema, atrophy of ileal villi, and fibrinous to fibrinohemorrhagic exudates in the intestinal lumen were noted in histologic sections from some of these calves. Neutrophil infiltrates also occurred in the one calf that had no A/E lesions. Examination of sections of ileum from two calves [18 and 42 h postinoculation with strain 86-24Δ10(pEB310)] by electron microscopy confirmed the in vivo A/E activity of the complemented mutant (Fig. 1). No A/E lesions or histopathological abnormalities were detected in any calf inoculated with mutant strain 86-24Δ10, strain B2F1, or control strain 123. However, there were patchy layers of O157:H7* bacteria on the epithelium in the cecum, colon, and ileum of one calf necropsied 18 h after inoculation with mutant strain 86-24Δ10, but these bacteria were not associated with A/E lesions.

The numbers of the inoculated organisms (expressed as CFU per gram) recovered from tissues and feces of calves at 18 or 42 h postinoculation with eae* or eae strains of E. coli are shown in Fig. 2. Sorbitol-negative EHEC O157:H7 bacteria were quantitated on sorbitol MacConkey agar containing 100 μg of streptomycin per ml (strain 86-24), 100 μg of streptomycin and 20 μg of nalidixic acid per ml (strain 86-24Δ10), or 100 μg of ampicillin and 34 μg of chloramphenicol per ml [strain 86-24Δ10(pEB310)]. Samples from which the inoculated strain were not recovered were recorded as having <10<sup>3</sup> CFU/g. Selected sorbitol-negative isolates were tested for O157: H7 antigen by a latex agglutination assay (5). Strain B2F1 (O91: H21) and strain 123 (O43:H28) bacteria were quantitated on MacConkey agar containing 100 μg of streptomycin or 20 μg of nalidixic acid per ml, respectively. Colonies were tested for O91 and O43 antigens to identify strains B2F1 and 123, respectively, by filter blot immunoperoxidase assay (4), using anti-O91 and anti-O43 sera (E. coli Reference Center, Pennsylvania State University, University Park) and peroxidase-conjugated anti-rabbit immunoglobulin G (heavy and light

<table>
<thead>
<tr>
<th>Strain</th>
<th>Time of determination (h postinoculation)</th>
<th>n</th>
<th>No. positive for:</th>
<th>Diarrhea</th>
<th>Death</th>
<th>A/E bacteria in*:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Rectum</td>
<td>Colon</td>
<td>Cecum</td>
<td>Ileum</td>
</tr>
<tr>
<td>86-24</td>
<td>18</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>86-24Δ10</td>
<td>18</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>86-24Δ10(pEB310)</td>
<td>18</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>4</td>
<td>3</td>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>B2F1</td>
<td>42</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>123</td>
<td>42</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> A/E bacteria stained with E. coli O157:H7 antibody by immunoperoxidase technique.

<sup>b</sup> Calf found dead at ca. 40 h postinoculation; samples were collected for histopathological studies.

FIG. 1. Electron micrograph of absorptive cells from the ileum of a calf 18 h after inoculation with EHEC O157:H7 strain 86-24Δ10(pEB310). This strain is an eae mutant which has been complemented with the eae gene. The intestinal lumen is to the left. Bacteria are intimately attached to absorptive cell membranes with subjacent electron-dense filaments in absorptive-cell cytoplasm. Most of the absorptive-cell microvilli have been effaced. There are pedestals beneath bacteria to the upper left.
Greater numbers of the inoculated organisms were recovered from the intestines of calves inoculated with strain 86-24 or strain 86-24 eaeΔ10(pEB310) than from those of calves inoculated with strain 86-24 eaeΔ10, strain B2F1, or control strain 123 at 42 h postinoculation (Fig. 2). Because of the large degree of variation among animals and the small number of animals, there was no significant difference among the numbers of bacteria at the individual tissue level. However, when we took the group average for each tissue and treated the tissues as a block, the mean CFU per gram for all samples obtained at 42 h postinoculation from the group of calves inoculated with eae mutant strain 86-24 eaeΔ10 was lower ($P < 0.05$; analysis of variance and least significant difference test) than the means for calves inoculated with eae+ strain 86-24 or 86-24 eaeΔ10(pEB310). The only exception was that the one calf that did not develop clinical signs or have A/E lesions after inoculation with strain 86-24 eaeΔ10(pEB310) had bacterial levels comparable to those in calves inoculated with strains that lacked the eae gene. The numbers for strains 86-24 eaeΔ10 and B2F1 were similar to those for control strain 123. The number of strain 86-24 eaeΔ10 organisms recovered from feces was similar to that of the eae+ strains. The inoculum strain accounted for a larger percentage of the total number of coliforms isolated from calves inoculated with eae+ EHEC than from calves inoculated with eae mutant EHEC, strain B2F1, or the control E. coli strain (Fig. 3). The presence and severity of A/E lesions in tissues from calves inoculated with eae+ strains correlated with the number of inoculated bacteria recovered.
TABLE 3. Findings in CDCD piglets at 18 h after inoculation with an eae-variant 86-24 or 86-24eaeΔ10(pEB310) or an eae mutant 86-24eaeΔ10 EHEC strain or nonpathogenic E. coli 123

<table>
<thead>
<tr>
<th>Inoculated strain</th>
<th>No. of piglets tested</th>
<th>No. with colonic edema</th>
<th>No. with A/E bacteria</th>
<th>Mean log$_{10}$ CFU ± SD of E. coli O157:H7/g of tissue in:</th>
</tr>
</thead>
<tbody>
<tr>
<td>86-24</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>6.9 ± 0.5, 5.9 ± 0.3</td>
</tr>
<tr>
<td>86-24eaeΔ10</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>7.1 ± 0.8, 5.5 ± 0.6</td>
</tr>
<tr>
<td>86-24eaeΔ10(pEB310)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>5.6 ± 1.4, 5.5 ± 1.0</td>
</tr>
<tr>
<td>123</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>ND, ND</td>
</tr>
</tbody>
</table>

* A/E bacteria found only in the cecum.
* A/E bacteria found in the cecum (three of three) and the ileum (one of three).
* ND, not determined.

(data not shown). A/E lesions were only seen in tissues containing ≥10^6 CFU of eae-variant EHEC/g of tissue.

In earlier studies we showed that the histopathology of EHEC O157:H7 infection in neonatal calves is similar to that in CDCD piglets, but O157:H7 bacteria do not cause diarrhea in CDCD piglets by 18 h postinoculation (5). In this study, we compared the pathogenicity of isogenic eae-variant and eae mutant derivatives of EHEC O157:H7 strain 86-24 in <8-h-old CDCD piglets (8). As shown in Table 3, CDCD piglets developed colonic edema and A/E lesions by 18 h after inoculation with the eae-variant strain 86-24 or 86-24eaeΔ10(pEB310) but not with the eae mutant. In contrast to calves, the A/E lesions occurred mainly in the ceca of the piglets. The numbers of inoculated bacteria recovered from the cecum or ileum at 18 h postinoculation were similar in all experimental groups (Table 3), and bacterial counts did not correlate with the presence or absence of A/E lesions. These results indicate that intimin plays a critical role in EHEC O157:H7 pathogenesis in CDCD piglets and extend the findings of earlier studies with these strains in gnotobiotic piglets (15).

In this study we have clearly demonstrated that the eae gene locus is required for E. coli O157:H7 strain 86-24 to intensively colonize the intestines and cause diarrhea and A/E lesions in neonatal calves and to cause colonic edema and A/E lesions in CDCD piglets. The eae mutant and B2F1 data indicate that eae-mediated adherence to the intestinal mucosa is critical for EHEC to cause fibrinohemorrhagic enterocolitis and diarrhea in calves. Similarly, the results confirm that eae-mediated colonization is necessary for intestinal lesion formation in CDCD piglets. These results suggest that anti-intimin vaccines might interfere with EHEC infections. Such vaccines could help reduce the levels of EHEC in cattle and thus reduce the number of EHEC infections in humans. The CDCD piglet EHEC infection model will be useful for preliminary experiments to test the efficacy of anti-intimin vaccines.

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REFERENCES