


Escherichia coli O157:H7 in the gallbladders of experimentally infected calves

William C. Stoffregen, Joachim F. L. Pohlenz, Evelyn A. Dean-Nystrom

Abstract. Fifteen weaned calves (age 89–141 days) were treated with dexamethasone (0.25 mg/kg, IV) for 3 days before, the day of, and the day after inoculation with 10 colony-forming units of either Escherichia coli O157:H7 (strain 86-24, which produces Shiga toxin 2 and intimin; n = 13) or nonpathogenic E. coli (strain 123, which does not produce Shiga toxin or intimin; n = 2). All calves were necropsied 4 days after inoculation. Histologic lesions of attaching and effacing bacteria were observed in the large intestine (12/13) and in the gallbladder mucosa (5/13) of calves inoculated with E. coli 86-24. Cholecystitis was present in 12 of 13 calves that received E. coli 86-24. Inoculum bacteria were recovered from the distal colons or feces (13/13) and gallbladders (3/4) of calves inoculated with 86-24.

Enterohemorrhagic Escherichia coli (EHEC), a subset of Shiga toxin–producing E. coli (STEC), are associated with a wide range of clinical manifestations, including asymptomatic carriage, nonhemorrhagic diarrhea, hemorrhagic colitis, hemolytic–uremic syndrome, and thromboembolic thrombocytopenic purpura in humans.15 Shiga toxin–producing E. coli O157: H7 is the most frequently reported serotype associated with human EHEC disease.13,14 Human EHEC infections can often be traced to the ingestion of improperly cooked, O157:H7–contaminated beef products, or produce or water contaminated with bovine manure. Cat-
Table 1. Results from 15 calves experimentally treated with dexamethasone, inoculated with *E. coli* O157:H7 strain 86-24 or control strain 123, and necropsied at 4 d postinoculation.

<table>
<thead>
<tr>
<th><em>E. coli</em> inoculum (strain)</th>
<th>Calf No.</th>
<th>Cholecystitis</th>
<th>A/E lesions*</th>
<th>Inoculum bacteria in distal colon/feces?</th>
</tr>
</thead>
<tbody>
<tr>
<td>86-24‡</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>yes/yes</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+</td>
<td>−</td>
<td>yes/yes</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>+</td>
<td>−</td>
<td>yes/yes</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>+</td>
<td>−</td>
<td>yes/yes</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>+</td>
<td>−</td>
<td>yes/yes</td>
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<tr>
<td></td>
<td>6</td>
<td>+</td>
<td>+</td>
<td>yes/yes</td>
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<tr>
<td></td>
<td>7</td>
<td>+</td>
<td>+</td>
<td>yes/yes</td>
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<td></td>
<td>8</td>
<td>+</td>
<td>−</td>
<td>no/yes</td>
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<td>+</td>
<td>+</td>
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<td></td>
<td>11</td>
<td>+</td>
<td>+</td>
<td>yes/yes</td>
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<td></td>
<td>12</td>
<td>+</td>
<td>+</td>
<td>yes/yes</td>
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<td></td>
<td>13</td>
<td>+</td>
<td>+</td>
<td>yes/yes</td>
</tr>
<tr>
<td>123§</td>
<td>C1</td>
<td>−</td>
<td>−</td>
<td>no/no</td>
</tr>
<tr>
<td></td>
<td>C2</td>
<td>−</td>
<td>−</td>
<td>no/no</td>
</tr>
</tbody>
</table>

* Attaching and effacing lesions.
† yes = $\geq10^6$ colony-forming units per gram of tissue or feces.
‡ O157:H7 strain.
§ Control strain.

tle have been shown to carry EHEC strains in their gastrointestinal tracts for varying periods of time. 1,4,9 Although cattle are generally regarded as asymptomatic carriers of human EHEC, STEC O157:H7 and other STEC can cause nonhemorrhagic or mucohemorrhagic diarrhea in experimentally inoculated calves. 3,4,6,16 Dysentery has also been reported in calves naturally infected with non-O157 STEC. 10 Many STEC, including STEC O157:H7, produce intimin, an outer-membrane protein that facilitates the intimate attachment of the EHEC bacterium to the host cell membrane. Loss of microvilli and effacement of host cell cytoplasm follows attachment. These characteristic lesions of bacterial attachment and cytoplasmic effacement (A/E) are associated with both natural and experimental STEC infections. 4,5,6,7,10,12,16

Initial attempts to establish a reproducible weaned calf STEC O157:H7 infection model were hampered by large variations in the susceptibility of cattle to experimental STEC infections. 3,5 It was hypothesized that high-dose corticosteroid administration would simulate the physiologic response of cattle during the pre-slaughter period and increase their susceptibility to STEC infection. Administration of corticosteroids in high doses mimics the effects of physiologic stress, and corticosteroids have been widely used in the experimental reproduction of numerous viral, protozoal, and bacterial diseases in various species of animals. Stress due to feed and water restriction, assemblage of large groups, and transportation during the pre-slaughter period has been proposed as a factor in the high incidence of shedding of STEC bacteria by slaughter cattle. 9 The addition of high-dose corticosteroid treatment during the peri-inoculation period enhanced the susceptibility of weaned calves 8 and resulted in the observations reported in this study.

Fifteen weaned calves, ranging in age from 89 to 141 days and fecal culture–negative for STEC O157:H7, were used in this study. The calves were of the following breeds: Jersey (n = 6), Hereford (n = 5), and Holstein Friesian × Black Angus cross (n = 4). All calves were housed in an environmentally controlled facility in individual pens for at least 2 weeks before inoculation and for the duration of the study and fed a standard beef grower–finisher ration with hay cubes.

All calves were treated with dexamethasone (0.25 mg/kg) by IV administration for 5 consecutive days: 3 days before inoculation, on the day of inoculation, and on the day after inoculation. Beginning 3 days before the first dexamethasone treatment and for the duration of the experiment, peripheral venous blood samples were collected daily for complete and differential blood counts. Feed was withheld from all animals for 2 days before inoculation. Calves were inoculated, through an oral–rumen tube, with 200 ml trypticase soy broth (TSB) containing $10^{10}$ colony-forming units of either *E. coli* O157:H7 (strain 86-24, which produces Shiga toxin 2 and intimin; n = 13) or a nonpathogenic *E. coli* control strain (strain 123, 4 which does not produce Shiga toxin or intimin; n = 2). Fecal samples for bacterial counts were collected...
from the rectum daily, from the day of inoculation until necropsy. Rectal temperatures and clinical observations, including fecal characteristics, were noted daily on all calves. All calves were euthanized 4 days after inoculation, and complete necropsies were performed. Samples of distal colon and feces were collected using aseptic techniques and processed for bacterial counts as previously described. Samples of gallbladder for bacterial counts were also collected from 4 of the calves inoculated with strain 86-24. Tis-
sues including liver, gallbladder, ileum, ileocecal valve, cecum, ascending colon, spiral colon, and distal colon were fixed in 10% neutral buffered formalin and processed by routine methods. Five-micrometer-thick tissue sections were stained with hematoxylin and eosin for histological examination and stained by an avidin–biotin–horseradish peroxidase immunohistochemical technique for E. coli O157:H7 as previously described. Sections of gallbladder were fixed in 1% glutaraldehyde for 4 hours and processed by routine methods for transmission and scanning electron microscopy.

All calves exhibited leukograms consistent with physiologic stress/corticosteroid administration beginning 1 day after the first dexamethasone administration and persisting for the duration of the study. The stress leukograms were characterized by leucocytosis, neutrophilia without left shift, lymphopenia, eosinopenia, and occasionally monocytosis. No calves experienced diarrhea or any significant change in fecal consistency, and no blood was observed in any fecal samples collected after inoculation.

Results are summarized in Table 1. The only significant gross lesion observed was edema of the gallbladder serosa, which was seen in 12/13 calves inoculated with strain 86-24. Histologically, A/E lesions were observed in various segments of the large intestine in 12/13 calves inoculated with strain 86-24. These lesions were characterized by numerous punctate bacteria, identified as E. coli O157:H7 by immunohistochemistry, attached to segmental areas of surface and crypt epithelial cells. Effacement of apical cyttoplasm was also prominent. Some areas contained segmental erosions of the mucosa, which were accompanied by infiltrates of neutrophils. Similar A/E lesions were seen in the gallbladder mucosa in 5/13 calves inoculated with strain 86-24. The cholecystitis was characterized by transmural edema, multifocal infiltrates of lymphocytes, plasma cells, and neutrophils in the submucosa and lamina propria, and occasional segmental erosion of the mucosa with neutrophils in the lamina propria adjacent to the erosive area. Edema has not been noted, and A/E lesions have not been seen, in any of the gallbladders of O157:H7–infected calves not treated with dexamethasone (unpublished data). No gross or histopathologic lesions were seen in either of the 2 calves that received the nonpathogenic control strain 123. Inoculum bacteria were recovered from the feces or distal colon (13/13 calves) and gallbladder (3/4 calves) of calves inoculated with strain 86-24 but not from the distal colon or feces (gallbladder of controls was not cultured) of 2 calves inoculated with strain 123.

This is the first report of the localization of STEC bacteria and A/E lesions in the gallbladder of cattle. This finding identifies the gallbladder as a possible niche for STEC in cattle, as it is for other enteric pathogens, notably Salmonella. The gallbladder may be a site of and a source of gastrointestinal STEC, which can contaminate beef products. This observation warrants further investigation into the gallbladder as a site of infection in naturally and experimentally infected animals and the possibility of the gallbladder as a site of persistent infection in cattle. Reports of cholelithiasis following STEC O157:H7–associated hemolytic uremic syndrome in humans indicate that the gallbladder may also be involved in human STEC infections.

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References

Localized cutaneous infection with *Francisella tularensis* resembling ulceroglandular tularemia in a cat

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**Abstract.** A chronically draining subcutaneous mass was removed from the ventral cervical region of a 6-year-old spayed female Domestic Shorthair cat. The histopathologic diagnosis was severe locally extensive pyogranulomatous and necrotizing cellulitis. Bacterial culture yielded *Francisella tularensis* subsp. *tularensis* as the causative agent. Immunohistochemical evaluation of sections for *F. tularensis* was negative. One year later, the cat was euthanized because of progressive lethargy found to be due to hypertrophic cardiomyopathy with pulmonary thromboembolism. No evidence of cutaneous or systemic infection by *F. tularensis* was found at necropsy. This case appears to be a localized form of tularemia resembling the ulceroglandular form of tularemia in humans and suggests that bacterial culture may be more sensitive than immunohistochemistry in detecting organisms in cases of localized *F. tularensis* infection.

Tularemia is caused by the fastidious gram-negative coccobacillus *Francisella tularensis*. The organism occurs throughout the Northern hemisphere and is enzootic in wild rodents and rabbits in the United States. Transmission is through ticks and biting insects, direct contact with infected animals, or contaminated water or food. In humans, infection by *F. tularensis* can cause a variety of clinical syndromes, but the vast majority of patients present either with a localized infection of the skin and draining lymph nodes (ulceroglandular tularemia) or with a systemic infection (typhoidal tularemia). Ulceroglandular tularemia is the most common form of the disease in humans.

Tularemia has been reported sporadically in domestic animals, most often in cats, and outbreaks have been reported in farm-raised mink. Sporadic cases of tularemia have also occurred in dogs, sheep, and horses. All previous reports of tularemia in animals are of the systemic form. The systemic form of tularemia in animals is typically fatal, although treatment of early cases in cats with doxycycline or with enrofloxacin and amoxicillin–clavulanic acid can be curative. This report describes a cat with what appears to be the first confirmed localized infection by *F. tularensis* in an animal, resembling the ulceroglandular form of tularemia that occurs in humans.

A 6-year-old spayed female Domestic Shorthair cat from eastern Oregon was presented for treatment of a chronic draining cutaneous lesion of approximately 1-year duration. The lesion was associated with a firm swelling in the subcutis of the ventral cervical region in the area of the right submandibular salivary gland and mandibular lymph nodes. Systemic signs of illness were not present. The lesion persisted after treatment with amoxicillin–clavulanic acid and cephalosporin. The draining tract and associated nodular subcutaneous mass were widely excised. An unfixed portion was...