

Escherichia coli O157:H7 Colonization at the Rectoanal Junction of Long-Duration Culture-Positive Cattle[∇]

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Long-duration consistently *Escherichia coli* O157:H7 culture-positive cattle were euthanized and necropsied. Tissue and digesta from along the gastrointestinal tract (GIT) were cultured for the bacteria and examined histologically for lymphoid character. *E. coli* O157:H7 was detected only at the rectoanal junction mucosa and not at any other GIT location.

Escherichia coli O157:H7 causes hemorrhagic colitis and the life-threatening sequela hemolytic-uremic syndrome in humans (6). Healthy cattle are the major reservoir for *E. coli* O157:H7, and contaminated ground beef, direct animal contact, and bovine manure contamination of other foods or water account for the majority of human infections (2, 3). The predominant colonization site of this microorganism in the bovine gastrointestinal tract (GIT) has been identified as the rectoanal junction (RAJ) mucosa (7). Localization to this site is in contrast with other *E. coli* serotypes that are present in consistent numbers throughout the large intestine without an increase at the RAJ mucosa. This tissue area comprises a clear junction between the stratified squamous epithelium of the anal canal and the columnar epithelial mucosa of the rectum and is a lymphoid follicle-dense mucosa. Although the mechanisms underlying the tropism of this serotype for this lymphoid follicle-rich site are not known, many studies of experimentally and naturally colonized cattle conclude that the RAJ mucosa plays an important role in *E. coli* O157:H7 bovine carriage of this human pathogen (5, 7, 8, 10, 11). Also, application of *E. coli* O157:H7 at the RAJ mucosa results in carriage of the bacteria similar to that by naturally infected animals (10).

Three distinct patterns of *E. coli* O157:H7 carriage in cattle have been described previously (1, 9, 12). First, animals can be transiently culture positive for short durations of a few days and are considered passive shedders and are likely not colonized at the RAJ mucosa; second, cattle can be colonized and shed the bacteria for an average of 1 month and typically not longer than 2 months; and third, a few rare animals are colonized for a long duration and shed the bacteria from 3 to 12 months or longer. This unique situation in which a few animals develop long-duration colonization (>2 months) with *E. coli* O157 is likely due to bacterial association at the RAJ mucosa; however, it may be due to unique colonization by the bacteria at a site(s) in addition to the RAJ mucosa. For example, the gall bladder can harbor *Salmonella* spp. in carrier animals and a recent investigation reports *E. coli* O157:H7 in the gall blad-

ders of some cattle at slaughter; however, this report does not provide information about the *E. coli* O157:H7 culture history of the animals tested, so the duration for which each animal was culture positive is unknown (4). For the first time, the current study investigated the colonization site of *E. coli* O157:H7 along the GIT of animals for which a documented history of long-duration carriage of *E. coli* O157:H7 was available. To identify animals that fit the criteria for a long-duration-colonized animal, hundreds of cattle were cultured over several years and the rare long-duration, culture-positive animals were used in this case study (data not shown).

Three long-duration *E. coli* O157:H7 culture-positive Holstein steers, designated no. 711, no. 135, and no. 407, that had been enrolled in different previous trials were naturally or experimentally carrying the bacteria when they were identified for this study. Each animal was 4 to 5 months old when the first samples were cultured. All animal procedures were approved by the Institutional Animal Care and Use Committee. Cattle were penned separately and housed in a large quarantined animal isolation facility at the University of Idaho. Animal no. 135 was carrying *E. coli* O157 naturally. Dosed animals were given a single challenge of 10¹⁰ CFU of *E. coli* O157:H7 strain ATCC 43894, a known human pathogen isolated from

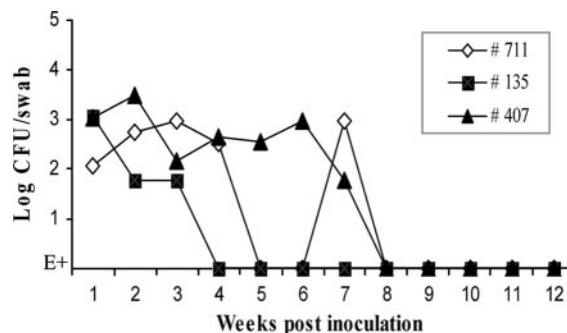


FIG. 1. Detection of *E. coli* O157:H7 by RAMS cultures from cattle. Three 5-month-old Holstein steers were penned separately. RAMS samples were cultured for *E. coli* O157 as indicated. Animal no. 135 was carrying a natural bovine *E. coli* O157 and was not challenged with a bacterial dose. Animal no. 711 received a single oral dose and animal no. 407 received a single rectal dose of 10¹⁰ CFU *E. coli* O157:H7 ATCC 43894 at day zero. E+, culture positive by enrichment culture only.

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TABLE 1. RAMS culture of long-duration *E. coli* O157:H7 culture-positive cattle

Month ^a	Result ^b for animal no.		
	711	135	407
1	D+	D+	D+
2	D+	E+	D+
3	E+	E+	E+
4	E+	E+	
5	E+	E+	
6	E+	E+	
7	E+	E+	
8		E+	
9		E+	
10		E+	
11		E+	

^a Weekly or biweekly RAMS cultures were analyzed each month.

^b D+, positive by direct RAMS culture; E+, positive by enrichment RAMS culture only.

ground beef during an outbreak (10). The *E. coli* O157 carried by animal no. 135 was unique from ATCC 43894 as determined by pulsed-field gel electrophoresis analysis of digested chromosomal DNA (data not shown). Animal no. 407 was orally dosed, and animal no. 711 was rectally dosed. Both applications lead to bacterial carriage that mimics natural infections (10).

Rectoanal mucosal swab (RAMS) cultures (a reproducible and reliable method) were performed once or twice a week for the first 12 weeks, as previously described (8), to establish the nature of the bacterial colonization. Briefly, RAMS samples were collected, placed in cold Trypticase soy broth, and processed within 2 h. RAMS were cultured directly (quantitative data), and colonies were confirmed to be *E. coli* O157 colonies by latex agglutination (Pro-Lab Diagnostics, Toronto, Ontario, Canada). Samples negative by direct culture were enriched (qualitative data) at 37°C with aeration for 18 h and similarly

analyzed. After the first 3 months, samples were cultured once or twice per month until the animal was euthanized and necropsied.

The results of RAMS culture showed that animals no. 135, no. 407, and no. 711 were consistently *E. coli* O157:H7 culture positive until they entered this study at 12 months, 8 months, and 3 months, respectively. Figure 1 shows the culture results for the first 3 months. During this period, the animals were not shedding unusual levels of *E. coli* O157:H7 and had CFU/swab typical of the concentration of *E. coli* O157:H7 carried by naturally and experimentally infected cattle in general, ranging from 10³ CFU/swab to levels detectable only by enrichment culture (10). Table 1 shows monthly culture results until the animals were euthanized and necropsied. Interestingly, animal no. 135 carried *E. coli* O157:H7 naturally and remained culture positive for 12 months although the bacteria were detectable only by enrichment culture of RAMS samples after the first 4 weeks, indicating the samples had ≤30 CFU/swab (the limit of the direct plate technique) (10). It is not known if or for how long the animal was culture positive before day zero. This is one of the few descriptions of a naturally infected animal consistently shedding low numbers of *E. coli* O157 for 12 months. Digested chromosomal DNA obtained from select *E. coli* O157:H7 isolates each month was analyzed by pulsed-field gel electrophoresis, which confirmed that each animal was carrying the original strain of *E. coli* O157:H7 and no other strain throughout the study (data not shown).

Animals were euthanized and the necropsy performed. The contents of the digesta or swab or tissue samples were collected from various sections throughout the GIT from the rumen to the rectum. Three grams of digesta/tissue and a tissue swab sample were collected from each site and added to 27 ml or 3 ml Trypticase soy broth, respectively. Samples were held on ice and cultured within 2 h of collection. Tissue samples for histology were fixed in 10% neutral-buffered formalin, and rep-

TABLE 2. Culture and lymphoid characteristics of gastrointestinal tract samples from long-duration *E. coli* O157:H7 culture-positive cattle

Sample source	Animal no. 711		Sample types	Animal no. 135		Animal no. 407	
	Sample types ^a	No. of positive samples ^b /total no. of samples		No. of positive samples/total no. of samples	No. of GALT samples/total no. of samples	Sample types	No. of positive samples/total no. of samples
Rumen	C, S, T	0/18	C, T	0/4	0/2	C, T	0/4
Reticulum			C, T	0/2	0/1	C, T	0/2
Omasum			C, T	0/2	0/1	C, T	0/2
Abomasum			C, T	0/2	0/1	C, T	0/2
Duodenum			C, T	0/4	0/2	C, T	0/4
Proximal jejunum			C, T	0/6	0/3	C, T	0/6
Middle jejunum			C, T	0/6	1/3	C, T	0/6
Distal jejunum			C, T	0/6	1/3	C, T	0/6
Ileum			C, T	0/4	2/2	C, T	0/4
Cecum	C, S, T	0/9	C, T	0/4	2/2	C, T	0/4
Gall bladder	C, S, T	0/9	C, T	0/4		C, T	0/4
Proximal colon	C, S, T	0/9	C, T	0/10	5/5	C, T	0/10
Middle colon	S, T	0/6	C, T	0/10	4/5	C, T	0/10
Distal colon	S, T	0/12	C, T	0/10	5/5	C, T	0/10
RAJ	S, T	3/5	S, C, T	3/5	3/5	S, C, T	4/6
Feces		0/3		0/4			1/3
Total		3/71		3/83	23/40		5/83

^a C, samples from contents; S, samples from swab; T, samples from tissues.

^b Positive direct or enrichment culture.

representative sections from each level of GIT were trimmed into cassettes, dehydrated through a series of graded alcohols, embedded in paraffin, sectioned at 6 μm , and stained with hematoxylin and eosin. Sections were considered positive for gut-associated lymphoid tissue (GALT) if the mucosa contained either solitary lymphatic nodules or aggregated lymphatic nodules (Peyer's patches). The results are shown in Table 2. The total samples for cultures were 71, 83, and 83 throughout the GIT of animals no. 711, no. 135, and no. 407, respectively. Among them, *E. coli* O157:H7 was found only at RAJ sites even though many other GIT locations possessed lymphoid tissue, indicating this criterion alone did not suffice for *E. coli* O157:H7 colonization (Table 2). Interestingly, two of three animals were culture negative in fecal samples, although they were culture positive by RAMS culture, indicating the superior sensitivity of this technique. Studies by Naylor et al. found the RAJ mucosa to be the principal site of colonization of *E. coli* O157:H7 by necropsy after 14 to 28 days postinoculation (7). However, they also cultured *E. coli* O157:H7 in the contents and tissue samples from rumen, ileum, cecum, and colon, albeit at low levels (7). Also, Cray et al. showed the recovery of *E. coli* O157:H7 all along the GIT in the rumen, reticulum, abomasum, small intestine, ileum, cecum, and colon from cattle inoculated with this organism 2 to 18 days previously (1). These previous reports used cattle that had been culture positive for *E. coli* O157:H7 for less than 1 month.

This study showed that among animals with documented histories of long-duration *E. coli* O157:H7 colonization, the bacteria were cultured only from the RAJ mucosa and not from the gall bladder or other GIT locations even though these tissues were rich in GALT.

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