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Prevalence of *Escherichia coli* O157:H7 in Gallbladders of Beef Cattle▽†

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Gallbladders and rectal contents were collected from cattle ($n = 933$) at slaughter to determine whether the gallbladder harbors *Escherichia coli* O157:H7. Both gallbladder mucosal swabs and homogenized mucosal tissues were used for isolation. Only five gallbladders (0.54%) were positive for *E. coli* O157:H7. Fecal prevalence averaged 7.1%; however, none of the cattle that had *E. coli* O157:H7 in the gallbladder was positive for *E. coli* O157:H7 in feces. Therefore, the gallbladder does not appear to be a common site of colonization for *E. coli* O157:H7 in beef cattle.

Escherichia coli O157:H7 is a major food-borne pathogen in the United States. Cattle are the main reservoir for *E. coli* O157:H7, which colonizes the gastrointestinal tract and is shed in the feces. Previous studies have documented that prevalence and shedding patterns of *E. coli* O157:H7 in the feces of beef cattle are highly variable (1, 4, 5). The prevalence ranges from 2 to 3% to as high as 80% of cattle sampled (1, 4). The shedding patterns in cattle vary from transient, in which animals shed the organism only for a few days, to persistent, in which shedding may last for an extended period of time (1, 4, 12). The persistent shedding is most likely due to the organism colonizing a part of the gastrointestinal tract, such as the mucosa of the rectoanal junction (11). Previous studies have shown that the gallbladder may be a site of persistence and a source for fecal shedding of certain enteric food-borne pathogens, such as *Salmonella* or *Campylobacter* spp. (3, 6). Stoffregen et al. (13) demonstrated that when calves were immunosuppressed and experimentally inoculated, *E. coli* O157:H7 localized in the gallbladder. They speculated that the gallbladder may be a site and source of gastrointestinal *E. coli* O157:H7 and contamination of meat at slaughter. Recently, Jeong et al. (10) reported isolation of *E. coli* O157:H7 in gallbladders of cattle and suggested that the organism can reside at a low level in gallbladders of cattle. The purpose of this study was to determine whether the gallbladders of cattle harbor *E. coli* O157:H7.

The cattle sampled in this study were delivered to two commercial abbatoirs in the Midwest for slaughter. A total of 933 cattle were sampled on 11 different dates in the summer of 2005 (Table 1). Gallbladders were cut from the liver after evisceration of the carcass. The gallbladders were cut open, and bile was allowed to drain out. The tissue was inverted over a gloved hand of the collector, rinsed with tap water until free of visible bile, and then swabbed vigorously with a foam-tipped applicator (VWR International, Buffalo Grove, IL). The swab was then placed into a test tube containing 3 ml of gram-negative broth (BD, Franklin Lakes, NJ) containing cefixime, cefsulodin, and vancomycin (GNccv) and placed on ice (9). The entire gallbladder was then placed into a Whirl-Pak bag (Nasco, Ft. Atkinson, WI), and the bags were immediately placed on ice. Feces were accessed by making a full-thickness, longitudinal incision through the distal 15 cm of the rectum and anus and laying the tissue open. A fecal sample of approximately 5 g was obtained as distally as possible and placed in a Whirl-Pak bag, and the bags were immediately placed on ice. The samples were transported to the laboratory, where a gallbladder tissue sample was collected by cutting four mucosal samples approximately 1 cm wide and 2 to 4 cm long using standard tissue scissors. The four cut sections were then placed into a test tube containing 20 ml of GNccv, and the sample was homogenized (Polytron homogenizer; Brinkmann Instruments, Westbury, NY) for 1 min. Approximately 1 g of the fecal sample was placed into a test tube containing 9 ml GNccv.

The GNccv tubes containing feces, swabs, or the homogenized tissue were vortexed for 1 min and incubated at 37°C for 6 h. After the enrichment, the tubes were vortexed for 1 min and 1 ml of each sample was subjected to immunomagnetic separation (Dynal, Inc., New Hyde Park, NY). A 50-μl sample from the immunomagnetic separation tube was plated onto sorbitol MacConkey agar (BD, Franklin Lakes, NJ) containing cefixime (50 ng/ml) and tellurite (2.5 μg/ml). The plates were incubated at 37°C for 16 to 18 h. The detection limit of the technique was 30 CFU per swab sample or 10^2 CFU per gallbladder tissue sample. The plates were examined for the presence of sorbitol-negative colonies, which were then streaked onto blood agar plates (Remel, Lenexa, KS) and incubated at 37°C for 12 to 18 h. The colonies (up to six) were tested for indole production, latex agglutination for the O157 antigen (Oxoid Limited, Basingstoke, Hampshire, England), and species confirmation by API kit (Rapid 20E; bioMerieux, Inc., Hazelwood, MO). The positive gallbladder isolates were tested by PCR for stx_1, stx_2, *eaeA*, *hlyA*, and *fliC* genes (7, 8).

Fecal prevalence of *E. coli* O157:H7 ranged from 0 to 22.9%,

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TABLE 1. Prevalence of *Escherichia coli* O157:H7 in feces, gallbladder tissue, and gallbladder swabs of beef cattle at slaughter

Sample date (day-mo-yr)	No. of cattle sampled	No. (%) of cattle positive		
		Feces	Gallbladder mucosal swab	Gallbladder mucosal tissue
23-May-05	79	0	0	0
24-May-05	97	0	0	0
31-May-05	83	4 (4.8)	0	0
1-Jun-05	40	5 (12.5)	0	0
7-Jun-05	95	14 (14.7)	1 (1.1)	2 (2.1)
14-Jun-05	114	2 (1.8)	0	1 (0.9)
28-Jun-05	125	1 (0.8)	0	0
5-Jul-05	55	9 (16.4)	0	0
7-Jul-05	97	15 (15.5)	0	0
21-Jul-05	100	5 (5.0)	0	0
25-Jul-05	48	11 (22.9)	0	1 (2.08)
Total	933	66 (7.1)	1 (0.1)	4 (0.4)

TABLE 2. Virulence genes in *E. coli* O157:H7 isolates from gallbladders of beef cattle

Sample type	Presence (+) or absence (−) of gene				
	eaeA	stx_2	stx_1	*hlyA*	*fliC*
Gallbladder mucosal tissue (n = 4)	+	+	−	+	+
Gallbladder mucosal swab (n = 1)	+	+	+	+	+

with an average prevalence of 7.1% (Table 1). A total of four *E. coli* O157:H7 isolates were obtained from 933 gallbladder tissue samples (0.43%), and only one gallbladder swab sample was positive (0.13%). Two of the gallbladder tissue isolates and the one swab isolate were obtained on the same day of sampling and all from different animals (Table 1). No animal sampled had both a positive gallbladder tissue and a positive gallbladder swab. In the same manner, none of the cattle that had *E. coli* O157:H7 isolated from the gallbladder had a positive fecal sample. Possibly, the gallbladder could be a rare site of occurrence of *E. coli* O157:H7 in cattle that do not shed the bacteria in the feces. The four gallbladder tissue samples had identical virulence gene profiles, being negative only for stx_1. The isolate from the gallbladder swab was positive for all five genes (Table 2).

E. coli O157:H7 is an important pathogen from a food safety standpoint. It is critical to attempt to identify sites of colonization in the gastrointestinal tract and all points of possible meat contamination during the slaughter process. Previous studies have shown that enteric organisms colonize the gallbladders of both humans and animals and that, in the case of food animals, these sites could serve as a source for contamination of meat. Ertas et al. (6) recovered *Campylobacter* spp. from 66 of 100 (66.0%) sheep gallbladders sampled at slaughter. Buchwald and Blaser (3) showed that foci of *Salmonella enterica* serovar Typhi remained in gallbladders of humans after the bacteremic phase of infection with *S. enterica* serovar Typhi. Additionally, up to 40% of *Salmonella* carriers showed cholelithiasis (3). Cholelithiasis has also been reported to occur in children with *E. coli* O157-associated hemolytic-uremic syndrome, suggesting that the gallbladder may play a role in human infections as well (2). Stoffregen et al. (13) experimentally inoculated immunosuppressed calves with *E. coli* O157:H7 and upon necropsy found that 12 of 13 gallbladders had cholecystitis and 5 of 13 gallbladders showed histologic evidence of attachment and effacement lesions. They were then able to isolate *E. coli* O157:H7 from three of four gallbladders (13). Jeong et al. (10) have reported on the isolation of *E. coli* O157:H7 from the gallbladders of experimentally inoculated and naturally infected cattle. Calves inoculated orally with one strain or a four-strain cocktail of *E. coli* O157:H7 were positive in gallbladder. Additional evidence was provided by testing the bile from 150 gallbladders collected at an abattoir, and four of those samples (2.7%) were positive for *E. coli* O157:H7; the authors concluded that *E. coli* O157:H7 can reside transiently or permanently at a low level in the gallbladders of cattle (10). The results of our study show that *E. coli* O157:H7 in the gallbladders of naturally shedding cattle is a rare occurrence. We conclude that the gallbladder of cattle is not a common site of prevalence of *E. coli* O157:H7 and therefore is not likely to serve as a source for fecal shedding or contamination of meat at slaughter.

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