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Escherichia coli O157:H7 in Microbial Flora of Sheep

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We found naturally occurring, potentially virulent *Escherichia coli* O157:H7 strains in sheep. The incidence of *E. coli* O157:H7 was transient and ranged from 31% of sheep in June to none in November. The use of a sensitive culture technique and the choice of the proper sampling season were both essential for detecting this bacterium in sheep. DNA hybridizations showed that 80% of the *E. coli* O157:H7 isolates had at least two of the Shiga-like toxin types I or II or the attaching-effacing lesion genes.

Escherichia coli O157:H7 is the most common enterohemorrhagic *E. coli* serotype implicated in human cases of infectious bloody diarrhea and the hemolytic-uremic syndrome (6). It is a widely held assumption that cattle are the only source of *E. coli* O157:H7 from food (5). Epidemiological investigations have clearly linked *E. coli* O157:H7 infection to the consumption of contaminated and improperly cooked beef (8). Asymptomatic cattle are a natural reservoir for this bacterium (7). Previous studies of sheep, goats, pigs, poultry, dogs, and cats have reported only non-O157 *E. coli* serotypes (1). However, after chickens or sheep are infected with *E. coli* O157:H7, this bacterium persists in the microbial flora of these alternate hosts (12, 16). Although *E. coli* O157:H7 has been cultured from non-beef grocery store meats including lamb, pork, and poultry, investigators have not excluded cross-contamination from beef as the source of adulteration (3). Therefore, we tested whether sheep might be a natural reservoir for *E. coli* O157:H7.

To test for the presence of *E. coli* O157:H7 in sheep, 10-g fecal samples were taken from 35 free-ranging sheep maintained by the United States Sheep Experiment Station, Agricultural Research Service, U.S. Department of Agriculture, near Dubois, Idaho. Samples were obtained from healthy ewes over a 6-month period (Table 1). The samples were cultured by a selective enrichment protocol that detects *E. coli* O157:H7 in ovine feces at levels of 1 CFU/10 g of feces (12). Briefly, feces were transported to the laboratory for culture in ice-cold, sterile Trypticase soy broth (BBL/Becton Dickinson, Detroit, Mich.) supplemented with cefixime (50 μg/liter; Lederle Laboratories, Pearle River, N.Y.; provided by D. D. Hancock, Washington State University), potassium tellurite (2.5 mg/liter; Sigma Chemical Co., St. Louis, Mo.), and vancomycin (40 mg/liter; Sigma) (TSB-CTV). Upon receipt in the laboratory, the total culture volume was increased with fresh TSB-CTV (20%; wt/vol) and the specimens were incubated, with aeration, at 37°C for 18 to 20 h. Serial dilutions of the cultures in sterile saline (0.15 M NaCl) were plated onto sorbitol MacConkey agar (Difco Laboratories, Detroit, Mich.) supplemented with cefixime (50 μg/liter), potassium tellurite (2.5 mg/liter), and 4-methylumbelliferyl-β-D-glucuronide (MUG; 100 mg/liter; Biosynth Ag Biochemica and Synthetica, Skokie, Ill.). Ten to 30 sorbitol-negative, MUG-negative colonies per animal (160 total) were analyzed for the *E. coli* O157:H7 serotype and their biochemical profiles. The isolates were tested for reactions on indole, methyl red, Voges-Proskauer, and citrate media and for cellobiose utilization and growth in potassium cyanide broth to confirm that they were *E. coli* (4, 14) and were tested for agglutination with O157 and H7 antisera (Prolab Diagnostics, Richmond Hill, Ontario, Canada, and Difco Laboratories). Of the 160 colonies, 114 were confirmed to be *E. coli* O157:H7. *E. coli* O157:H7 ATCC 43890, ATCC 43889, and ATCC 43894 from the American Type Culture Collection (ATCC), which express the Shiga-like toxin (SLT) genes *slt*-I, *slt*-II, and both *slt*-I and *slt*-II, respectively, and the attaching-and-effacing lesion gene (*eae*) were used as positive controls. *E. coli* HB101, which lacks the O157 and H7 antigens and the *slt*-I, *slt*-II, and *eae* genes was used as the negative control.

As indicated in Table 2, 11 of the 35 sheep (31%) from which samples were obtained in June were positive for *E. coli* O157:H7. Among these ewes, seasonal variation in the incidence of *E. coli* O157:H7-positive animals was seen. Two animals (5.7%) remained positive in August (Table 2), and none (0%) were positive in November. The sheep showed no signs of disease and were healthy throughout the study. There was no correlation between animal breed and shedding of *E. coli* O157:H7. To compare the protocols of selective enrichment of a 10-g sample and nonenrichment of a 0.1-g sample (swab), samples obtained in August were cultured for *E. coli* O157:H7 by both techniques (12). Although sheep were *E. coli* O157:H7 positive by the selective enrichment of a 10-g fecal sample, they were *E. coli* O157:H7 negative by nonenrichment culture of a fecal swab sample.

Multiple virulence factors contribute to the pathogenicity of *E. coli* O157:H7. These include two major types of SLTs (SLT type I [SLT-I] and SLT-II) and the ability to cause attaching-and-effacing lesions that disrupt the microvilli on the intestinal wall of the host (6, 13, 20). DNA hybridization was used to determine if the genes encoding these three main virulence determinants are present (Table 2). Three previously described plasmids, pSC25 (9), pMJ331 (18), and pCVD434 (10), were used as probes for the hybridization experiments. The 656-bp *Pst*I-*Hin*dIII fragment of pSC25, the 1,750-bp *Sph*I-*Pst*I fragment of pMJ331, and the 1,000-bp *Sal*I-*Kpn*I fragment of pCVD434 were used to detect *slt*-I, *slt* -II, and *eae*, respectively. Colony blots were prepared with maximum-strength Nytran membranes (pore size, 0.45 μm; Schleicher & Schuell, Keene,

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TABLE 1. Physical, dietary, and environmental conditions during the study

Mo[a]	Age (mo)[b]	Wt (kg)[c]	Range	Location[d]	Temp (°C)[e]		Total precipitation (cm/mo)
					Minimum	Maximum	
June	14.5	59	Sagebrush and bunchgrass	Dubois, Idaho	10.8 ± 4	26 ± 5	3.4
August	16.5	68	Sagebrush and bunchgrass	Dubois, Idaho	10.9 ± 2	31 ± 2	2.4
November	19.5	64	Alfalfa and barley aftermath	Aberdeen, Idaho	−7.2 ± 7	2.2 ± 5	1.1

[a] Month when sheep fecal samples were cultured for *E. coli* O157:H7.
[b] Average sheep age; standard deviation, ±1.4 months.
[c] Average sheep weight; standard deviation, ±7 kg.
[d] Location of the grazing range.
[e] Average ± standard deviation minimum and maximum temperatures.

N.H.) and were hybridized with labelled probe DNA by the protocol in the Rad-Free Probe Labelling and Detection System (Schleicher & Schuell), except that the duration of the posthybridization washes was increased to 10 min. The probe used to detect the attaching-and-effacing lesion gene has been described previously and hybridizes with both *eae* and the eae_{O157} genes (19). The *slt*-I gene was specific for *slt*-I and did not cross-hybridize with *slt*-II or its variants. The *slt*-II gene probe cross-hybridizes with *slt*-II variants. The majority of ovine *E. coli* O157:H7 isolates (90 of 114) had SLT and attaching-and-effacing lesion genes and therefore are potential human pathogens (Fig. 1). *E. coli* O157:H7 isolates from a single sheep often had more than one gene hybridization pattern (Table 2). Also, the *E. coli* O157:H7 isolates shed by animals A and G in both June and August had different gene hybridization patterns.

The frequency of cattle positive for *E. coli* O157:H7 by fecal swab tests has ranged from 0 to 4% (2, 11). These low numbers stand in contrast to the higher incidence of *E. coli* O157:H7 in sheep from which samples were obtained in June and tested by our selective enrichment procedure. The incidence of *E. coli* O157:H7 in cattle may be lower than that in sheep or it may have been underestimated by less sensitive sampling methods. The dramatic variation in the incidence of *E. coli* O157:H7-positive sheep over 6 months shows that sheep, like cattle, are transiently, and possibly seasonally, colonized with this organism. Dramatic seasonal variation also has been observed in a study of cattle, in which *E. coli* O157:H7-positive animals were identified only in June, July, and September but not in any of the 9 other months tested (7, 11). This seasonal variation coincides with the seasonal variation in reported human cases of infection with *E. coli* O157:H7 (8).

Little is known about the factors that influence the transient colonization and shedding of *E. coli* O157:H7 from ruminants. The sheep used in the present study were healthy ewes of similar age and breed, so that it is unlikely that these factors influenced differences in the shedding of *E. coli* O157:H7. However, from early summer through midautumn, several measurable changes in environment and diet occurred. Animals grazed on sagebrush and bunchgrass throughout the summer months until their diet was changed in the autumn to alfalfa and barley aftermath. We (12) and others (15, 17) have proposed that diet may influence colonization by *E. coli* O157:H7 and the multiplication, shedding, and clearance of *E. coli* O157:H7 from the guts of ruminants. In addition, the survival of *E. coli* O157:H7 in the environment may contribute to the incidence of *E. coli* O157:H7-positive animals. Thus, the warmer and more moist conditions of June compared with the

TABLE 2. Characteristics of ovine *E. coli* O157:H7 isolates

Mo[a]	*E. coli* O157:H7-positive animal	No. of *E. coli* O157:H7 isolates	No. of isolates hybridizing with the following DNA probes[b]:							
			eae$^+$				*eae*$^-$			
			I$^+$	II$^+$	I$^+$/II$^+$	I$^-$/II$^-$	I$^+$	II$^+$	I$^+$/II$^+$	I$^-$/II$^-$
June	A	2				2				
	B	1			1					
	C	11	1	3	6	1				
	D	5		2		3				
	E	11		7	4					
	F	7				3				4
	G	23		11	7		3	2		
	H	8	2		5			1		
	I	9	1		6			2		
	J	2			2					
	K	5			5					
August	A	26	2	12	12					
	G	4		1						3

[a] Month during which *E. coli* O157:H7-positive sheep were detected.
[b] *eae*, attaching-and-effacing lesion gene probe; I, SLT-I gene probe; II, SLT-II gene probe; $^+$, hybridization observed; $^-$, no hybridization observed.

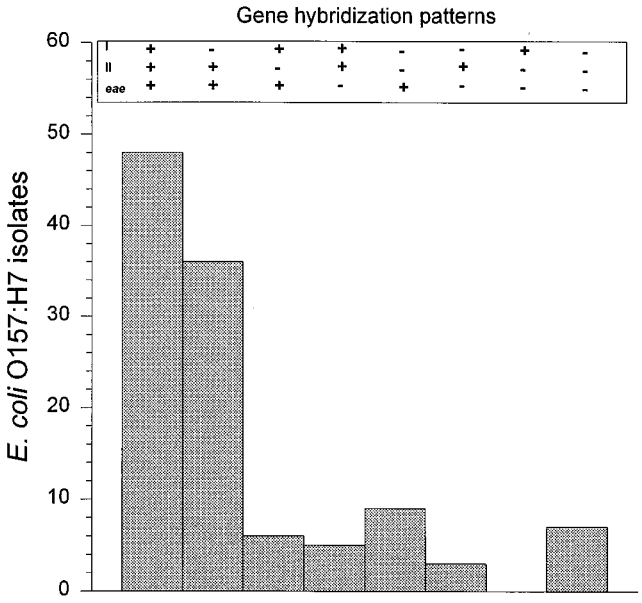

FIG. 1. Hybridization patterns for SLT and the attaching-and-effacing lesion genes among ovine *E. coli* O157:H7 isolates. Colony blot DNA hybridization was used to probe for *slt*-I (I), *slt*-II (II), and *eae* (*eae*) gene sequences. The isolates are grouped by hybridization category. *eae*, attaching-and-effacing lesion gene; I, SLT-I gene probe; II, SLT-II gene probe; +, hybridization observed; −, no hybridization observed.

conditions in August and November may have contributed to the higher incidence of animals that tested positive in June.

The most significant finding in the present investigation is that we detected naturally occurring *E. coli* O157:H7 in sheep. The use of a sensitive culture technique and the choice of the proper sampling season were both essential for detecting this pathogenic *E. coli* serotype in sheep. These results show that sheep, like cattle, are hosts for this human pathogen. Thus, grocery lamb meat may not be cross-contaminated by beef but, rather, may be contaminated by its preharvest source.

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