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Isolation of *Escherichia coli* Serotype O157:H7 and Other Shiga-Like-Toxin-Producing *E. coli* from Dairy Cattle

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We examined 1,266 fecal specimens from healthy cattle during the investigations of two sporadic cases of hemolytic uremic syndrome associated with raw milk consumption and an outbreak of gastroenteritis and hemolytic uremic syndrome caused by *Escherichia coli* serotype O157:H7. We collected specimens from heifers, calves, and adult cows on 22 farms, in a stockyard, and in a packing house. We also collected 3 raw hamburger specimens from a restaurant and 23 raw milk samples from two farms. All specimens were examined for *E. coli* O157:H7 by using sorbitol-MacConkey agar, H immobilization, O157 agglutination, and tissue culture cytotoxicity. *E. coli* O157:H7 was isolated from 16 heifers or calves and 1 adult cow on 22 farms, 1 stockyard calf, 2 beef specimens, and 1 raw milk sample. Selected fecal specimens were also examined for the presence of other Shiga-like-toxin-producing *E. coli* (SLTEC) by testing polymyxin B extracts of colony sweeps and then testing individual colonies for toxin production. SLTEC other than O157 was isolated from 8 of 10 farms investigated and from the stockyard; 8% of adult cows and 19% of heifers and calves were positive for SLTEC. Several animals were positive for SLTEC by colony sweep only. This investigation demonstrates that dairy cattle are a reservoir of *E. coli* O157:H7 and other SLTEC.

Escherichia coli serotype O157:H7, which produces Shiga-like toxin (SLT), also known as verocytotoxin, is a known cause of hemorrhagic colitis and hemolytic uremic syndrome (HUS). It is capable of causing the full spectrum of disease, ranging from asymptomatic carriage to HUS and thrombotic thrombocytopenic purpura (11). Consumption of raw milk and ground beef has been linked epidemiologically with several outbreaks of disease caused by *E. coli* O157:H7 (3, 12, 25, 28), and the outbreak strain has been recovered from implicated beef products in two outbreaks (12, 28). In 1986, we isolated *E. coli* O157:H7 from the feces of young cattle on dairy farms associated with two cases of HUS associated with raw milk consumption (18). During a milk-borne outbreak of gastroenteritis and HUS caused by *E. coli* O157:H7 in Canada, the organism was recovered from the feces of patients and from the feces of young cattle in dairy herds associated with the outbreak (3). Other SLT-producing *E. coli* (SLTEC) isolates have also been associated with dairy cattle (5a, 22). Although *E. coli* O157:H7 has been cultured from a variety of retail meat products (7), dairy cattle, a source of both raw milk and beef products, are a likely reservoir. We explored the possible role of dairy cattle in SLTEC-associated disease during the epidemic investigation of an outbreak of hemorrhagic colitis, HUS, and thrombotic thrombocytopenic purpura associated with beef consumption in Washington State (25) and a more detailed investigation conducted of the dairy farms associated with the two sporadic cases of HUS associated with raw milk consumption in Wisconsin (18).

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MATERIALS AND METHODS

We examined 1,266 fecal specimens from healthy dairy cattle during the two investigations. Animals younger than 4 months were classified as calves, those from ages 4 to 24 months were classified as heifers, and those older than 24 months were classified as adults. In the Wisconsin investigation, fecal specimens were collected from 438 adult cows and 262 heifers and calves from the 2 farms with which the two HUS cases were associated, a dairy farm adjacent to those farms, 10 control dairy farms in the area, and a stockyard that received cattle from that region. Specimens obtained from the two case farms were collected during two visits, 3 weeks apart. Specimens obtained from the adjacent farm, control farms, and stockyard were collected during the second visit. Twenty-three raw milk specimens were also collected from the two case farms during the second visit. In the Washington State outbreak, specimens were collected from 224 adult cows and 342 heifers and calves on nine farms (eight in southwest Washington, one in Oregon) from which the implicated meat could have originated and from the packing house that processed the meat from those farms. Three suspect raw hamburger specimens were also examined. In both investigations, additional specimens were collected up to 50 days after the first sampling from animals that were initially positive for *E. coli* serotype O157 to determine the length of excretion. In Wisconsin, 108 heifers and calves from two of the farms and the stockyard were examined 1 year later; with the exception of one heifer that was initially positive and tested again, samples were not previously obtained from any of the animals.

Swabs from stool specimens were placed in the medium described by Cary and Blair (4) and held at −70°C until examination. The stool swabs collected from 566 animals during the Washington State investigation and specimens

collected from 226 animals on case farms and 132 animals on control farms in Wisconsin were inoculated onto sorbitol-MacConkey agar (SMAC) (9, 17). Specimens from 342 animals collected from the Wisconsin control farms, a farm adjacent to the case farms, and a stockyard were inoculated onto SMAC and into modified Trypticase soy broth enrichment medium (7). The modified Trypticase soy broth was incubated at 37°C for 24 h before it was plated onto SMAC. SMAC plates were incubated at 37°C for 24 h. Up to 20 sorbitol-negative colonies were selected from each plate and tested for lactose fermentation. Isolates that fermented lactose within 24 h were screened in a semisolid medium containing *E. coli* serotype H7 antiserum for sorbitol fermentation and H-specific immobilization (9). Isolates that did not ferment sorbitol within 24 h and that were immobilized were screened with *E. coli* serotype O157 antiserum. Sorbitol-negative isolates which agglutinated O157 antiserum were biochemically identified and serotyped by using *E. coli* O and H antisera (8).

All *E. coli* serotype O157 isolates were tested for production of SLT-I and SLT-II by using DNA probes (23) and HeLa cell culture cytotoxicity determination (15). Cytotoxic activity was neutralized by using monoclonal antibodies against SLT-I (27) and polyclonal and monoclonal antibodies against SLT-II (6). All *E. coli* O157 isolates were tested for antimicrobial susceptibilities by using chloramphenicol, trimethoprim-sulfamethoxazole, cephalothin, tetracycline, sulfisoxazole, nalidixic acid, ampicillin, carbenicillin, kanamycin, streptomycin, gentamicin, and trimethoprim high-potency disks (1). A subset of *E. coli* O157 isolated from patients, food, and animals was selected on the basis of epidemiologic association with human illness for further analysis for plasmid DNA by the method of Birnboim and Doly (2) for plasmid isolation and the method of Meyers et al. (19) for gel electrophoresis.

Selected fecal specimens collected from cattle in the Wisconsin investigation were examined for non-O157 SLTEC. Specimens from 33 adult cows and 99 heifers and calves were examined by the colony sweep assay: polymyxin B extracts of growth from colony sweeps were diluted 1:5 and 1:50 before they were placed on HeLa cell monolayers (14). Individual colonies from specimens that had positive colony sweeps were confirmed for toxin production on HeLa cell monolayers. For specimens positive at the 1:5 dilution, 20 colonies were selected and tested. For specimens positive at the 1:50 dilution, 10 colonies were selected and tested. Cytotoxic activity was neutralized by using polyclonal antibodies against SLT-I and SLT-II. The remaining specimens from 121 adult cows and 69 heifers and calves were examined by testing up to 20 colonies selected from MacConkey agar for SLT-I and SLT-II by using a DNA probe.

Meat and milk samples were examined by Michael Doyle, Food Research Institute, Madison, Wis., for *E. coli* serotype O157:H7, using an immunoblot procedure (7).

RESULTS

E. coli serotype O157:H7 was recovered from bovine fecal specimens obtained from Wisconsin and Washington states. In Wisconsin, the organism was recovered from cattle on both case farms, the adjacent farm, 2 of 10 control farms in the same area, and a stockyard that received cattle from that region (Table 1). It was also recovered from a raw milk sample obtained from one of the case farms. In the Washington State investigation, *E. coli* O157:H7 was recovered from cattle on four of nine farms and from a raw beef specimen that could have originated from animals on those farms; *E. coli* O157:NM was isolated from one heifer on a fifth farm. *E. coli* O157:H7 was recovered from 5 of 85 heifers and calves (5.9%) from case farms and 3 of 149 heifers and calves (2.0%) from control farms in Wisconsin. *E. coli* O157:H7 or *E. coli* O157:NM was recovered from 7 of 315 heifers and calves (2.2%) in Washington State. In total, it was recovered from 5 of 210 calves (2.3%), 12 of 394 heifers (3.0%), but only 1 of 662 adult cows (0.15%) ($P < 0.001$).

In the Wisconsin investigation, *E. coli* serotype O157:H7 was isolated from a patient with HUS, a heifer on the implicated farm (farm 1) during the initial visit to the farm, and a calf and a milk specimen during a subsequent visit. The isolate obtained on the first visit to farm 1 had the same toxin type and plasmid profile as those of the isolate from the patient, but the isolates obtained on the second visit were different (Table 2). *E. coli* O157:H7 was not isolated from the other patient with HUS, but was recovered from three heifers on the implicated farm (farm 2) during two visits. All three isolates had the same toxin type and plasmid profile,

TABLE 1. Recovery of *E. coli* serotype O157:H7 from dairy cattle in two investigations

Source	No. positive/no. examined (%)		
	Farms	Adult cows	Heifers and calves
Wisconsin			
Case farms	2/2 (100)	0/141 (0)	5/85 (5.9)
Adjacent farm	1/1 (100)	0/28 (0)	1/9 (11.1)
Control farms	2/10 (20)	1/242 (0.4)	3/149 (2.0)
Stockyard	1/1 (100)	0/27 (0)	1/19 (5.3)
Washington			
Farms	5/9[a] (55.5)	0/224 (0)	7/315[a] (2.2)
Packing house	0/2 (0)	ND[b]	0/27 (0)
Total	11/25 (44)	1/662 (0.15)	17/604 (2.8)

[a] One isolate of *E. coli* O157:NM.
[b] ND, Not tested.

TABLE 2. Plasmid profile and type of SLT produced by *E. coli* serotype O157:H7 recovered from patients, dairy cows, meat, and raw milk

Source	Type (no.) of specimens	Farm visit[a]	SLT	Plasmid profile (molecular mass [mDa])
Wisconsin				
Case farm 1	Human	1	II	65, 5.6, 4.4
	Heifer	1	II	65, 5.6, 4.4
	Calf	2	I	65, 4.9, 4.4
	Milk	2	I, II	65, 4.9, 4.4
Case farm 2	Heifers (3)	1, 2	II	65, 22
Washington				
Epidemic	Human	1	II	65, 22, 2.4
	Meat	1	I	65, 2.4
Farm 1	Heifers (2)	1	I, II	65, 2.4
	Heifer	1	I, II	65, 3.7, 2.4
Farm 2	Heifer	1	I, II	65, 2.4
Farm 3	Heifer	1	I, II	65, 2.4
Farm 4	Calf	1	I, II	65, 2.4

[a] Specimens were obtained in Wisconsin during two visits, 3 weeks apart.

which were different from those of all isolates associated with the other patient and farm. In the Washington State outbreak, *E. coli* O157:H7 recovered from humans had a different toxin type and plasmid profile from those of the meat sample and dairy animal isolates (Table 2). Isolates from five of the six heifers and calves were indistinguishable by toxin type and plasmid profile.

To determine the length of excretion of *E. coli* serotype O157:H7, we obtained additional specimens from nine heifers or calves that were initially culture positive. *E. coli* O157:H7 strains with the same plasmid profile were recovered from three of the nine animals when tested the second time. Of six animals from which samples were obtained once 43 to 50 days after the positive culture, two were still positive (on days 45 and 46). A heifer from which a sample was obtained on day 8 was positive, but was negative on days 9 and 10. A heifer from which samples were obtained four times between days 24 and 33 was always negative, as was a calf from which samples were obtained three times between days 7 and 9. On return visits to two farms and the stockyard in Wisconsin 1 year later, one animal that was initially positive was negative, but an additional animal was positive. The isolate obtained from the later trip had a different plasmid profile than those of the isolates obtained during the initial trips.

In the subset of 132 specimens from control farms examined by colony sweep for SLTEC, 43 (32.6%) were positive. Thirty-two (24.2%) were confirmed by culture. Four of the 32 isolates recovered were *E. coli* serotype O157:H7. In one instance, both *E. coli* O157:H7 and SLTEC O145:NM were recovered from the same animal.

Non-O157 SLTEC isolates were recovered from cattle on 8 of 10 farms and the stockyard during the Wisconsin investigation. Specimens from 32 of 168 heifers and calves (19.0%) but only 13 of 154 adult cows (8.4%) contained non-O157 SLTEC ($P = 0.004$).

Twenty-eight serotypes of SLTEC were recovered from dairy cattle (Table 3). Of the 17 *E. coli* serotype O157:H7 isolates, 9 produced only SLT-II, 7 produced both SLT-I and SLT-II, and 1 produced only SLT-I. The one *E. coli* O157:NM isolate produced both SLT-I and SLT-II. Most non-O157 *E. coli* isolates produced SLT-I or SLT-II alone; only two serotypes produced both SLT-I and SLT-II.

All of the *E. coli* O157:H7 isolates were susceptible to the antimicrobial agents tested, and the one *E. coli* O157:NM isolate was resistant to tetracycline only.

TABLE 3. Serotype and SLT produced by *E. coli* recovered from dairy cattle

No. of animals	*E. coli* serotype[a]	SLT
1	O10:NM	I
2	O15:H27	I, II
1	O22:H8[b]	I, II
4	O22:H8[b]	II
1	O22:H40	II
9	Related to O25 and O84:NM	II
1	O26:H11[b]	I
2	O45:H2[b]	I
2	O45:NM	I
1	O76:H21	I
1	O84:H2[b]	I
1	O103:NM	I
1	O103:H2[b]	I
4	O111:NM[b]	I
1	O116:H21	II
5	O121:H7	I
1	O145:NM[b]	II
1	O153:H25[b]	I
9	O157:H7[b]	II
7	O157:H7[b]	I, II
1	O157:H7[b]	I
1	O157:NM[b]	I, II
1	O163:H19[b]	II
6	O171:H2	II
2	OX3:H21[b]	II
3	OX3:NM	II
2	ORough:H2	II
1	ORough:H8	II
4	ORough:NM[b]	II
1	Ound[c]:NM	I
1	Ound:Hund[c]	I

[a] Two serotypes of non-O157 SLTEC were recovered from two animals; three serotypes were recovered from one animal.
[b] Serotype associated with human disease.
[c] und, Undetermined.

DISCUSSION

Our results support previous evidence that cattle are a major reservoir of *E. coli* serotype O157:H7 and other SLT-producing *E. coli*. During a milkborne outbreak of gastroenteritis and HUS caused by *E. coli* O157:H7 in Ontario, Canada, the organism was recovered from the feces of patients and young animals of dairy herds associated with the outbreak (3). We identified *E. coli* O157:H7 on 45% (10 of 22) of the dairy farms examined, but found it more frequently on case than on control farms. We recovered the organism from 2.8% of healthy heifers and calves and 0.15% of healthy adult cows. Other studies have shown similar rates of isolation. In a study of randomly selected animals at slaughter in Ontario, Canada, *E. coli* O157:H7 was recovered from 3 of 200 beef cattle (1.5%), 1 of 200 dairy cows (0.5%), and none of 200 veal calves (5a). In the United Kingdom, the organism was isolated from 2 of 207 cows at slaughter (1%) (5); in Spain, it was isolated from 1 of 78 calves with diarrhea (1.3%) (10); and in Germany, it was isolated from none of 47 healthy dairy cows and 2 of 212 healthy bulls (1%) (22).

We also presented evidence that dairy cattle may serve as a reservoir of non-O157 SLTEC. We found SLTEC on 80% of the farms examined. They were present in 8.4% of the adult cows and 19.0% of the heifers and calves examined. Similar results were reported in a Canadian study of randomly selected cattle at slaughter. SLTEC was isolated from 10.5% of beef cattle, 19.5% of dairy cows, and 3.5% of veal calves (5a). In a study of healthy cattle in Germany, SLTEC was recovered from 17% of dairy cows and 9.4% of bulls (22). We recovered 28 different serotypes of SLTEC. Thirteen (O22:H8, O26:H11, O45:H2, O84:H2, O103:H2, O111:NM, O145:NM, O153:H25, O157:H7, O157:NM, O163:H19, OX3:H21, and ORough:NM) have been previously associated with human disease. Other investigators have also found that many of the SLTEC isolates recovered from cattle were of serogroups previously associated with human disease (10, 16, 20, 22, 26).

Karmali et al. (14) have shown that the colony sweep method is more sensitive than testing individual colonies for SLT is. We also found specimens that were positive for SLTEC by the colony sweep method that were not confirmed by culture.

It appears that, although individual animal infection with *E. coli* serotype O157:H7 is transient, herd infection may be maintained. When selected animals that were positive for *E. coli* O157:H7 were retested at later times, the length of

excretion varied from 8 to at least 46 days. On a return visit 1 year later, an animal that had been initially positive was negative, but an additional heifer was positive. Plasmid profile and toxin testing data from the Wisconsin investigation support the transient nature of excretion by cattle. During the initial investigation, soon after the onset of one patient's illness, *E. coli* O157:H7 isolates with the same plasmid profile and toxin type were recovered from the patient and from a heifer on that farm. Three weeks later, isolates from a calf and a milk specimen from that same farm were of a different toxin type and plasmid profile. The intermittent excretion of *E. coli* O157:H7 by cattle probably accounts for the difficulty in recovering the same strain of *E. coli* O157:H7 from human cases and the incriminated food or animal sources. In outbreak investigations, specimens from incriminated sources are usually collected a substantial length of time after the cases have occurred. By that time, the source animal may no longer be excreting the strain or another animal may have begun excreting a different strain.

Both *E. coli* serotype O157:H7 and other SLTEC isolates were recovered significantly more often from young animals than they were from adults. Howe et al. (13) recovered a variety of *E. coli* serotypes of unknown SLT status from 100% of calves, but from only 24% of adult cows. The reasons for the differences in recovery of SLTEC and *E. coli*, in general, from young animals and adults are unknown, but may reflect differences in ruminal development, diet, resistance to infection, or other factors. We found no significant difference in the rate of recovery of *E. coli* O157:H7 between calves and heifers; this suggests that those factors that influence colonization by *E. coli* O157:H7 occur later in the life cycle of dairy cows. It is interesting that products of apparently healthy adult animals, beef and raw milk, are the most frequent source of human disease (11), although the animals themselves have a relatively low level of fecal carriage.

Our findings suggest that dairy cattle are an important reservoir of *E. coli* serotype O157:H7 and other SLTEC isolates that cause human disease. It is clear from our data that apparently healthy cattle carry SLTEC. Other investigators found SLTEC, including *E. coli* O157:H7, in both ill (10, 16, 24, 26) and well (3, 5, 5a, 22) animals. In a study in Sri Lanka, however, SLTEC was recovered significantly more often from cattle and water buffalo calves with diarrhea than it was from well animals (20, 21), suggesting that SLTEC may cause illness in cattle as well as in humans.

It is not clear why dairy herds would be more likely than beef herds to harbor the organism, although epidemiologic evidence to date suggests that dairy animals may be the primary reservoir of *E. coli* serotype O157:H7 in the United States. Further investigations of infected herds are necessary to understand the ecology of this organism in dairy and beef herds, the mechanisms by which meat and milk become contaminated, and the potential for herd-based control measures to prevent this growing public health problem.

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