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Laboratory Investigation of Hemorrhagic Colitis Outbreaks Associated with a Rare *Escherichia coli* Serotype

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Two outbreaks of hemorrhagic colitis, a newly recognized syndrome characterized by bloody diarrhea, severe abdominal pain, and little or no fever, occurred in 1982. No previously recognized pathogens were recovered from stool specimens from persons in either outbreak. However, a rare *E. coli* serotype, O157:H7, was isolated from 9 of 20 cases and from no controls. It was also recovered from a meat patty from the implicated lot eaten by persons in one outbreak. No recovery of this organism was made from stools collected 7 or more days after onset of illness; whereas 9 of 12 culture-positive stools had been collected within 4 days of onset of illness. The isolate was not invasive or toxigenic by standard tests, and all strains has a unique biotype. Plasmid profile analysis indicates that all outbreak-associated *E. coli* O157:H7 isolates are closely related. These results suggest that *E. coli* O157:H7 was the causative agent of illness in the two outbreaks.

Hemorrhagic colitis is a newly recognized syndrome characterized by severe crampy abdominal pains, watery diarrhea followed by grossly bloody diarrhea and little or no fever. Two outbreaks of hemorrhagic colitis that affected at least 47 people occurred in Oregon in February and March 1982 and in Michigan in May and June 1982 (26). The epidemiological investigation associated illness with eating hamburger sandwiches at outlets of the same fast food chain in both outbreaks. A rare *E. coli* serotype was identified as the cause of this unusual illness. This report describes the laboratory investigation of specimens from persons in both outbreaks and the identification of a previously unrecognized enteric pathogen that causes illness by yet undefined mechanisms.

MATERIALS AND METHODS

Stool specimens from cases were analyzed at local hospital laboratories and State health department laboratories of Oregon and Michigan for *Salmonella* ssp., *Shigella* ssp., *Campylobacter* ssp., ova, and parasites. In Oregon the local laboratories examined specimens for *Yersinia enterocolitica* as well. Additionally, specimens were collected from some of the cases and healthy control persons in both Oregon and Michigan for analysis at the Centers for Disease Control (CDC) laboratories. In Oregon, stool specimens were also collected from 45 patients who were seen at local hospital emergency rooms for diarrheal diseases.

Specimens analyzed at the CDC laboratories were examined for the following organisms: *Salmonella* ssp., *Shigella* ssp., *Escherichia coli*, *Klebsiella* ssp., *Y. enterocolitica*, *Campylobacter* ssp., *Vibrio* ssp., *Staphylococcus aureus*, *Bacillus* ssp., anaerobic bacteria including *Clostridium difficile*, and viruses. Methods used by the CDC laboratories for detection of these organisms were as follows. Stool specimens were maintained frozen at −70°C from the time of collection until examination. For selective enrichment of *Salmonella* ssp., stool specimens were inoculated into a tube of tetrathionate brilliant green broth. The tetrathionate brilliant green broth was incubated for 24 h at 37°C, and 1 ml was subcultured to a second tube of tetrathionate brilliant green broth. It was incubated for 24 h at 42°C and streaked to brilliant green agar. The brilliant green agar was incubated for 24 h at 37°C. If present, three colonies characteristic of *Salmonella* ssp. were selected from each plate and subcultured to triple sugar iron (TSI) and lysine-iron agar. Cultures typical of *Salmonella* ssp. were identified and serotyped by previously described methods (6). MacConkey agar and xylose-lysine-desoxycollate agar were directly inoculated with stool for the recovery of *Shigella* ssp. The plates were incubated for 24 h at 37°C, and three colonies suspected of being *Shigella* spp. were selected. Colonies were subcultured to TSI agar, and typical cultures were identified and serotyped (6). The MacConkey agar plate was also used to isolate *E. coli* and other organisms that grow on this medium. *E. coli* isolates were identified and serotyped by previously described methods (6, 12). In addition to typical *E. coli* and *Klebsiella*, 5 to 10 random isolates were identified from each MacConkey plate (6). To examine for *Y. enterocolitica*, specimens were inoculated to Salmonella-Shigella and MacConkey agar

plates, which were incubated at 22°C for 48 h before examination. The stool specimen was also examined by cold enrichment in phosphate-buffered saline and held at 4°C for 3 weeks, at which time the suspension was inoculated to Salmonella-Shigella and MacConkey agar. Three colonies characteristic of *Y. enterocolitica* were selected from each plate and inoculated to TSI agar. Typical cultures were biochemically identified (23). Thiosulfate-citrate-bile salt-sucrose agar was inoculated for the recovery of vibrios. Agar plates were incubated for 24 h at 37°C, and three sucrose-negative and three sucrose-positive colonies, if present, were selected and subcultured to TSI agar. Colonies typical of *Vibrio parahaemolyticus* or *V. cholerae* were biochemically and serologically examined (34). For the recovery of *Staphylococcus aureus*, both mannitol-salt agar and 5% sheep-blood agar were inoculated. The mannitol-salt agar was incubated for 48 h at 37°C, and the blood agar was incubated for 24 h at 37°C. Three colonies typical of *S. aureus* were selected from each plate and biochemically identified (15). For *Campylobacter fetus* subspecies *fetus*, Skirrow's selective medium (31) was used. The plates were incubated at 37°C under microaerophilic conditions (5% oxygen) and were examined at 24, 48, and 72 h for colonies resembling *Campylobacter* ssp. For *C. jejuni*, both Skirrow's and Campy-BAP agar (3) were used and incubated microaerophilically at 42°C. Both plates were examined at 24, 48, and 72 h for *Campylobacter* ssp.-like colonies. Colonies that were positive for oxidase and had typical morphology and motility on wet mounts were considered presumptively positive and were biochemically confirmed (14). Specimens were examined for anaerobic bacteria by the Anaerobic Bacteria Branch, Hospital Infections Program, CDC, and included examination for *Clostridium difficile* and *C. difficile* toxins (5, 12a). *Bacillus* spp. were identified from rabbit or sheep blood agar plates and incubated at 37°C for 24 h (10). MacConkey-carbenicillin-adonitol (MCA) agar was also inoculated for selective enrichment of *Klebsiella* spp. MCA agar was incubated for 24 h at 37°C, and colonies resembling *Klebsiella* spp. were biochemically and serologically examined (6). The Gastroenteritis Laboratory Branch, Division of Hepatitis and Viral Enteritis, CDC, examined specimens for viruses by electron microscopy and immunoelectron microscopy by using patients' convalescent sera (13). Specimens were also examined for viruses by culture in Rhesus monkey and human fibroblast tissue cells by the Respiratory and Enterovirus Branch, Division of Viral Diseases, CDC (20).

Environmental studies. Food items including a cooked complete specialty hamburger, a frozen raw hamburger patty, and dehydrated onions were collected from the implicated restaurants during the outbreaks in Oregon and Michigan. A raw hamburger patty from the same lot of meat as that implicated in the Michigan outbreak had been held at the processing plant as part of a quality control program. It was also collected for examination. Foods were examined for *E. coli* O157:H7. A 10-g sample was suspended in 90 ml of physiological saline and blended. A 0.1-ml sample was subcultured to duplicate plates of MacConkey agar. Tenfold dilutions were made in physiological saline and similarly subcultured to MacConkey agar plates. All plates were incubated for 24 h at 37°C. The plates were counted, and all lactose-positive colonies were selected and identified.

Antimicrobial susceptibility testing. All *E. coli* O157:H7, *Klebsiella oxytoca*, and *B. pumilus* strains were tested for antimicrobial susceptibility by the modified Kirby and Bauer method (1) to the following antibiotics: nitrofurantoin, chloramphenicol, amikacin, trimethoprim-sulfamethoxazole, cephalothin, tetracycline, gentamicin, nalidixic acid, ampicillin, colistin, carbenicillin, kanamycin, cephaloridine, neomycin, streptomycin, sulfathiazole, tobramycin, and rifampin. The antimicrobial agents tested were selected for their usefulness as epidemiological markers.

Plasmid isolation and analysis. All strains of *E. coli* O157:H7 were screened for the presence of plasmid DNA by a modification of the alkaline denaturation procedure of Birnboim and Doly (2). Further purification of plasmid DNA for enzyme digestion procedures was accomplished by the addition of RNase, extraction with phenol-chloroform, and a second precipitation with 2 volumes of cold ethanol. The restriction endonuclease *Hin*dIII was obtained from Bethesda Research Laboratories, Inc., Gaithersburg, Md., and enzymatic reactions were performed according to the manufacturer's instructions.

Plasmid DNA preparations were examined by vertical agarose gel electrophoresis (21). This method used 3-mm thick, 0.8% agarose gels and a buffer (89 mM Tris base, 2.5 mM disodium EDTA, 89 mM boric acid) run at 35 mA for 2 to 2.5 h. Gels were then stained with ethidium bromide, visualized by UV transillumination, and photographed.

Plasmid sizes were determined by reference to plasmids of known molecular weight included in each gel. These control plasmids consisted of R1 (62 megadaltons [Mdal]), RP4 (36 Mdal), S-a (23 Mdal), two plasmids from 2457 O S^r, an avirulent derivative of *Shigella flexneri* provided by Dennis Kopecko, Walter Reed Army Institute of Research, Washington, D.C. (105 and 140 Mdal), and eight plasmids from the *E. coli* strain V517 for small-molecular-weight plasmids that are described in detail elsewhere (18).

Pathogenicity tests. All colonies of *Enterobacteriaceae* from MacConkey, MCA, or blood agar were tested for enterotoxin production and invasiveness. For enterotoxin analyses, isolates were subcultured onto blood agar base slants for 18 h and then were inoculated into 5 ml of Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) with 0.6% yeast extract in screw-capped tubes. These were incubated overnight at 37°C on a roller drum (model TC-7; New Brunswick Scientific Co., New Brunswick, N.J.) rotating at 20 rpm. The broth cultures were centrifuged at 3,500 × *g* for 30 min, and the supernatants were filtered through 0.45-μm disposable filter units (Millex SLHA 0250S). The filtrates were then assayed for heat-labile enterotoxin by the Y1 adrenal cell test (27) and for heat-stable enterotoxin by the infant mouse assay (4). For the infant mouse assay, an intestinal/remaining carcass weight ratio of 0.083 or greater was considered positive (24). Isolates were also tested for heat-labile enterotoxin by a four-layer sandwich enzyme-linked immunosorbent assay (35) and the rabbit skin permeability test (20). In addition, genes for both heat-labile and heat-stable enterotoxins were assayed for by hybridization with genetic probes as described in detail elsewhere (25).

TABLE 1. Results of examination of frozen stools, Oregon, 1982

Sample	*E. coli* O157:H7	*K. oxytoca*	*Y. enterocolitica*	*B. pumilus*	*C. difficile*
Cases					
1	−	−	−	+	+
2	−	+	−	−	−
3	−	+	+	+	−
4	+	−	−	−	−
5	+	−	−	−	−
6	+	−	Not done	−	−
Controls					
1	−	−	−	−	−
2	−	−	−	−	−
3	−	−	−	−	−
4	−	−	−	−	−
5	−	−	−	−	−
6	−	−	−	−	−
7	−	−	−	+	−
8	−	−	−	−	−
9	−	−	−	−	−
10	−	−	−	−	−

[a] No growth on selective media; patient was taking antibiotics.

Isolates were tested for invasiveness by the Sereny test (30), by the HeLa cell assay (16), and by plasmid analysis for the presence of the 140-Mdal plasmid previously shown to be associated with invasiveness (11).

A strain of *E. coli* O157:H7 implicated as the pathogenic agent in this outbreak was tested for attachment to ileal brush border membranes of human origin by Michael Doyle of the Food Research Institute, University of Wisconsin (19). All *E. coli* O157:H7 strains were tested for colonization factor antigen (CFA/I) by mannose-resistant hemagglutination of human group A erythrocytes and for CFA/II by mannose-resistant hemagglutination of bovine erythocytes (7).

RESULTS

All specimens examined at State and local laboratories in Oregon and Michigan were negative for *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., ova, and parasites, and all specimens examined in Oregon were negative for *Y. enterocolitica*. Specimens from 6 cases and 10 neighborhood age-matched controls from Oregon were examined at the CDC laboratories (Table 1). We listed only those organisms isolated that are either recognized pathogens such as *Y. enterocolitica* or those that were recovered from three or more cases from either the Oregon or the Michigan outbreak. In Oregon, *E. coli* O157:H7 was recovered from three of the six cases and from no controls (P = 0.036, Fisher's exact test, two tailed). No other organism was isolated from more than two patients. Case no. 1 was being treated with an antimicrobial agent to which this organism was susceptible. There was no growth on any selective medium inoculated with this patient's specimen, except for *Bacillus pumilus*, which was recovered only on blood agar. *Klebsiella oxytoca* was recovered from two of the six patients and from none of the controls by our laboratory (P > 0.10, Fisher's exact test, two tailed), but it was recovered from one of the controls by the Oregon State Laboratory before we received the specimens. *K. oxytoca* strains isolated from the two patients were

TABLE 2. Isolates from MacConkey, MCA, and blood agar, Oregon, 1982

Sample	*E. coli* O157:H7	*E. coli* not O157	*K. oxytoca*	*K. pneumoniae*
Cases				
1[a]	−	−	−	−
2	−	−	3/3[b]	−
3	−	−	5/5	−
4	3/5	2/5	−	−
5	4/5	1/5	−	−
6	5/5	−	−	−
Controls				
1	−	3/5	−	2/5
2	−	5/5	−	−
3	−	5/5	−	−
4	−	5/5	−	−
5	−	−	−	−
6	−	−	−	−
7	−	−	−	−
8	−	5/5	−	−
9	−	5/5	−	−
10	−	−	−	−

[a] No growth on selective media; patient was taking antibiotics.

[b] Number of colonies identified as this species/total number of colonies identified from MacConkey agar.

TABLE 3. Results of examination of frozen stools, Michigan, 1982

Sample	*E. coli* O157:H7	*K. oxytoca*	*S. aureus*	*B. pumilus*
Cases[a]				
1	+	+	−	−
2	+	−	−	−
3	+	+	−	−
4	+	−	+	−
5	+	−	−	−
6	−	−	Not done	−
7	−	−	Not done	−
8	−	−	Not done	−
9	−	−	Not done	−
10	+	−	Not done	−
11	−	−	Not done	+
12	−	+	Not done	+
13	−	−	Not done	+
14	−	−	Not done	−
Controls				
1	−	−	Not done	−
2	−	−	Not done	−
3	−	−	Not done	−
4	−	−	Not done	−

[a] Samples from cases 1 through 5 were received on 11 June 1982; those for cases 6 through 14 were received on 24 June 1982.

different serotypes and had different plasmid profiles. *B. pumilus* was recovered from two cases and from one control. The two *B. pumilus* strains recovered from the cases had different antimicrobial susceptibility patterns and plasmid profiles. *Y. enterocolitica* was recovered from one of the cases by the cold enrichment technique, but not from the plate inoculated directly with the specimen. *Clostridium difficile* was recovered from one patient, but tests for *C. difficile* toxin were negative. An additional *E. coli* O157:H7 isolate was identified from Oregon by screening isolates sent to CDC from the Oregon State Laboratory. Specimens from 45 patients who were seen at local emergency rooms for diarrheal diseases were also screened for *E. coli* O157:H7 and *K. oxytoca* to determine their prevalence in patients with diarrhea in that geographic area. *E. coli* O157:H7 was not isolated from any of the 45 patients. *K. oxytoca*, however, was recovered from two patients.

The identification of five colonies from MacConkey agar plates on the individual specimens from Oregon is shown in Table 2. In cases 4 through 6, *E. coli* O157:H7 was the predominant organism. In cases 2 and 3, *K. oxytoca* was the predominant organism, but strains from the two patients were different serotypes. Strains of *E. coli* other than serogroup O157 were the predominant organisms in controls.

The results of examination of specimens from the Michigan outbreak are shown in Table 3. *E. coli* O157:H7 was recovered from all five cases in the first set of specimens collected soon after onset of illness and received at CDC on June 11 and from one of nine cases from specimens collected much later after onset of illness and received on June 24. *K. oxytoca* was recovered from two of five cases in the first set of specimens and one case in the second set. *S. aureus* was recovered from one case in the first set and *B. pumilus* from three cases in the second set. As with specimens from the Oregon outbreak, the *K. oxytoca* strains were different serotypes and had different plasmid profiles, and *B. pumilus* strains had two different antimicrobial susceptibility patterns and different plasmid profiles.

The results of identification of colonies from MacConkey agar and from MCA or blood agar (or both) are shown in Table 4. *E. coli* O157:H7 was the predominant organism in three of the six cases from which this organism was recovered. It was recovered only from the blood agar from patient no. 3. *E. coli* other than serogroup O157 was most frequently the predominant organism in the second set of specimens.

The intervals between onset of illness and date of stool collection were markedly different

TABLE 4. Isolates from MacConkey, MCA, and blood agar, Michigan, 1982

Sample	*E. coli* O157:H7	*E. coli* not O157	*K. oxytoca*	*K. pneumoniae*	*C. freundii*
Cases[a]					
1	1/5[b]	−	4/5	−	−
2	2/5	−	−	−	3/5
3	2/3[c]	1/5	1/5	3/5	−
4	4/5	1/5	−	−	−
5	5/5	−	−	−	−
6	−	5/5	−	−	−
7	−	5/5	−	−	−
8	−	5/5	−	−	−
9	−	5/5	−	−	−
10	4/4	−	−	−	−
11	−	−	−	−	−
12	−	5/5	2/3[d]	1/3[d]	−
13	−	−	−	−	−
14	−	5/5	−	−	−
Controls					
1	−	−	−	3/3[e]	−
2	−	−	−	5/5	−
3	−	−	−	−	−
4	−	5/5	−	−	−

[a] Receipt dates as in Table 3.
[b] Number of colonies identified as this species/total number of colonies identified from MacConkey's agar.
[c] Isolated from blood agar for this one specimen.
[d] Isolated from MCA agar.

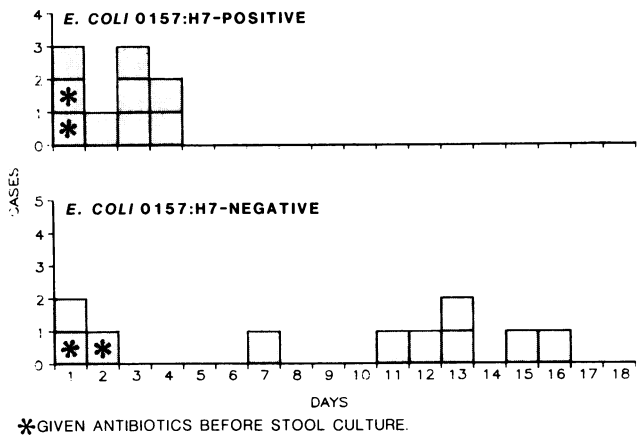

FIG. 1. Hemorrhagic colitis cases, by interval between onset of illness and date of stool collection in Oregon, February and March 1982, and Michigan, May and June 1982.

for cases from whom *E. coli* O157:H7 was and was not recovered (Fig. 1). *E. coli* O157:H7 was isolated from 9 of 12 stools collected within 4 days after onset of illness compared with none of 7 stools collected 7 or more days after onset of illness (P = 0.003, Fisher's exact test, two tailed). For patients not treated with antibiotics, the organism was recovered from seven of eight stools collected within 4 days after onset compared with none of seven stools collected 7 or more days after onset (P = 0.001, Fisher's exact test, two tailed).

E. coli O157:H7 was not recovered from food items collected from the implicated restaurants in Oregon and Michigan. It was recovered, however, from a raw hamburger patty from the same lot as that used at the Michigan outlets during the outbreak period. It was present at a level of 50 organisms per g.

All isolates tested failed to produce LT or ST, were negative with gene probes for these toxins, and were not invasive. All of the *E. coli* O157:H7 strains tested were CFA/I negative and CFA/II negative by mannose-resistant hemagglutination. The single strain of *E. coli* O157:H7 tested did not attach to human ileal brush border membranes. All of the *E. coli* O157:H7 and *K. oxytoca* strains were susceptible to the antimicrobial agents tested.

Biochemical reactions of the *E. coli* O157:H7 strains are shown in Table 5. The reactions are typical of *E. coli*, with the exception of sorbitol fermentation. The organism does not ferment sorbitol; whereas 93% of *E. coli* of human origin are sorbitol positive (6). This organism is also nonhemolytic on sheep and rabbit blood agar.

To determine the frequency of isolation of *E. coli* O157:H7, *E. coli* serotyping records of the U.S. Department of Agriculture Animal Laboratories at Ames, Iowa, the Pennsylvania State University Veterinary Research Laboratory, and the CDC Enteric Reference Laboratories were reviewed to search for previous isolates of *E. coli* O157:H7. The Ames, Iowa, laboratory and the Pennsylvania State Laboratory reported no previous identification of *E. coli* O157:H7 from animal sources in the United States. The CDC Enteric Reference Laboratories had detected only 1 strain of *E. coli* O157:H7 from among over 3,000 *E. coli* isolates serotyped since 1973 when this service was initiated. It was recovered from a patient in California in 1975 with a bloody diarrhea syndrome similar to that described in the Oregon and Michigan outbreaks.

The plasmid profiles of *E. coli* O157:H7 strains recovered from patients in the Oregon and Michigan outbreaks are identical (Fig. 2). They both contain a larger plasmid which is approximately 72 Mdal and a smaller one which is less than 1 Mdal. All of the outbreak-associated isolates, including the isolate from the hamburger, contained those same plasmids. The profile of the isolate recovered from the sporadic case in 1975 is similar to the outbreak-associated strains, but contains one additional plasmid that is 6 Mdal. Plasmid profiles of the six *E. coli* O157 isolates belonging to H groups other than H7 were different from the *E. coli* O157:H7 profiles.

Agarose gel electrophoresis of purified plasmid DNA and lambda phage DNA (standard) that have been digested by the restriction endonuclease *Hin*dIII is shown in Fig. 3. All Oregon outbreak strains have an *Hin*dIII digest pattern composed of 13 fragments. The Michigan strains have a 12-fragment digest. This difference has been reproducible and stable in all of the strains obtained from each state.

TABLE 5. Biochemical reactions of *E. coli* O157:H7 strains, Michigan and Oregon, 1982

Test	Reaction[a]
Indole	+
Methyl red	+
Voges-Proskauer	−
Simmon citrate	−
H_2S (TSI)	−
Urease	−
Phenylalanine deaminase	−
Lysine decarboxylase	+
Arginine dihydrolase	+2 −4
Ornithine decarboxylase	+
Motility	+
Gelatin 22°C	−
KCN (growth)	−
Malonate	−
D-Glucose	+
Gas from glucose	+
Lactose	+
Sucrose	+
D-Mannitol	+
Dulcitol	+
Salicin	−
Adonitol	−
L-Inositol	−
D-Sorbitol	−
L-Arabinose	+
Raffinose	+
L-Rhamnose	+
Maltose	+
D-Xylose	+
Trehalose	+
Cellobiose	−
α-CH_3 glucoside	−
Erythritol	−
Esculin	−
Melibiose	+
D-Arabitol	−
Glycerol	−
Mucate	+
Tartrate (Jordan)	+
Sodium acetate	+
Lipase (corn oil)	−
Nitrate nitrite	+
Oxidase	−
Christensen citrate	+3 −4

[a] +, Positive in 1 or 2 days; −, negative after 7 days; +2 −4, = positive after 2 to 4 days.

DISCUSSION

Two outbreaks of a clinically distinct diarrheal illness, referred to as hemorrhagic colitis, led to epidemiological and laboratory investigations, which identified a rare serotype of *E. coli*, O157:H7, as the probable cause of the illness (26). This *E. coli* serotype has not been previously recognized as an enteric pathogen. Sporadic cases of bloody diarrheal illness with clinical manifestations similar to those seen in hemorrhagic colitis have been reported from Japan (28) and the United States (32). Although various possible etiologies have been suggested, no recognized pathogens have been identified; in the above studies the specimens examined were not compared with appropriate controls. The two outbreaks provided an opportunity to examine a relatively large number of freshly collected stool specimens from cases and controls with no illness and with other forms of diarrhea. These fresh specimens were extensively evaluated for common and uncommon microorganisms on routine and special media. Other possible vehicles of transmission identified by epidemiological investigations were examined for the presence of these organisms.

The distinguishing features of hemorrhagic colitis led to a more rigorous search for unusual or hypothesized causes of this syndrome. Other organisms, such as *K. oxytoca* and *B. pumilus*, were isolated and were each initially viewed as possible causative agents. *K. oxytoca* has previously been suggested as an agent in an antibiotic-associated bloody diarrheal disease in Japan (33). It did not appear, however, that either of these organisms was the etiological agent in the two outbreaks. *K. oxytoca* and *B. pumilus* were both recovered from ill patients in Michigan and Oregon, but both were recovered from a healthy control. *K. oxytoca* was also recovered from two patients with nonbloody diarrhea in Oregon. Also, *K. oxytoca* and *B. pumilus* organisms recovered from patients in both outbreaks were different strains.

We identified a single recognized pathogen in no more than one patient from each outbreak. *C. difficile* was recovered from one patient in Oregon, but the stool specimen did not contain *C. difficile* toxin. *Y. enterocolitica* was also recov-

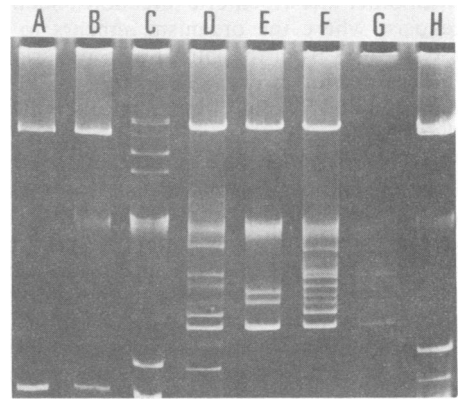

FIG. 2. Agarose gel electrophoresis of plasmid DNA from *E. coli* strains of serogroup O157: A, O157:H7 isolated in Oregon; B, O157:H7 isolated in Michigan; C, molecular weight standards (not including V517); D, O157:H45; E, O157:H45; F, O157:H45; G, O157:H45; H, O157:H38.

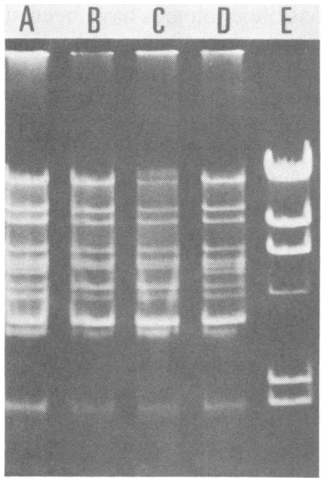

FIG. 3. Agarose gel electrophoresis of plasmid DNA after digestion with the restriction endonuclease *Hin*dIII. Lanes: A, O157:H7 isolated in Oregon; B, O157:H7 isolated in Michigan; C, O157:H7 isolated from hamburger in Michigan; D, O157:H7 isolated in Calfornia in 1975; E, lambda phage DNA.

ered from one patient in Oregon. It was not recovered on direct plating, however, possibly indicating that the organism was present in small numbers.

Based on the epidemic investigations and the following laboratory data, *E. coli* O157:H7 was the likely etiological agent of hemorrhagic colitis. (i) The organism was isolated from 9 of 20 cases and from no controls. (ii) No recovery of this organism was made from stools collected 7 or more days after onset of illness, whereas 9 of 12 culture-positive stools had been collected within 4 days of onset of illness. This is consistent with other known enteric infections, such as shigellosis, where the organism appears to be rapidly cleared from the gut. It also underlines the necessity for prompt collection of stool specimens. (iii) Plasmid profile analysis indicated that all outbreak-associated and sporadic *E. coli* O157:H7 isolates were closely related. The Oregon and Michigan isolates could be distinguished by plasmid DNA restriction patterns. However, the one fragment difference probably represents a clonal variant of an otherwise homogenous serotype. The profile of the sporadic isolate recovered in 1975 suggested that it is the epidemic strain that has acquired a plasmid or that the epidemic isolates have lost a plasmid.

From our results, it is not clear how *E. coli* O157:H7 causes diarrhea. Preliminary animal studies have indicated that it is pathogenic for infant rabbits, but its pathogenic mechanism is unknown (9). *E. coli* strains that cause diarrhea are usually divided into three groups, enterotoxigenic, enteroinvasive, and enteropathogenic. The primary mechanism by which enterotoxigenic and enteroinvasive strains cause diarrhea is known. These pathogenic groups are generally limited to a small number of *E. coli* serotypes. Enteroinvasive *E. coli* causes disease by direct invasion of intestinal mucosa. Enteroinvasive *E. coli* causes a bloody diarrhea which is indistinguishable from shigellosis clinically, and often resembles *Shigella* spp. in many phenotypic characteristics. These organisms are frequently nonmotile, non-gas producers like *Shigella* spp. and often share common somatic antigens with *Shigella* spp. *E. coli* O157:H7 is not one of the enteroinvasive serotypes and in our studies was negative in tests used to detect invasiveness. The enterotoxigenic *E. coli* causes disease by the production of heat-labile or heat-stable enterotoxin. These *E. coli* organisms cause cholera-like or watery diarrhea. *E. coli* O157:H7 is not among the serotypes previously reported to have been associated with enterotoxigenic *E. coli* causing disease in humans or animals. Additionally, it did not produce heat-labile or heat-stable toxins by several well-established tests or contain genes coding for these toxins.

Enteropathogenic *E. coli* strains have been epidemiologically associated with a nonbloody diarrhea occurring in children under the age of 2 years. However, enteropathogenic *E. coli* strains can cause natural adult disease (29) and has caused watery diarrhea in adult volunteers (17). *E. coli* O157:H7 is not one of the traditional enteropathogenic *E. coli* serotypes and appears to cause a distinctly different clinical picture. Our investigation did elucidate two similarities between *E. coli* O157:H7 and enteropathogenic *E. coli* strains. Serotyping *E. coli* isolates was essential to the eventual identification of an organism epidemiologically associated with outbreaks of hemorrhagic colitis. Second, as early as 1952, Rappaport and Henig described a sorbitol-base MacConkey agar to differentiate sorbitol-negative enteropathogenic *E. coli* strains (8). We also found sorbitol to be a useful strain marker for *E. coli* O157:H7. All *E. coli* O157:H7 isolates recovered from persons associated with the two outbreaks failed to ferment sorbitol within 7 days. However, some isolates subsequently examined from sporadic cases have fermented sorbitol as early as 4 days. For laboratories that cannot serotype *E. coli*, screening for sorbitol fermentation is a valuable and simple tool to be employed before they send isolates to a reference laboratory for serotyping.

In conclusion, this laboratory investigation identified a rare *E. coli* serotype, O157:H7, as the most likely cause of the unusual illness in two outbreaks. The pathogenic mechanism of *E. coli* O157:H7 is presently unknown. Based on

our past experience with other *E. coli* strains, we may expect to identify other serotypes associated with this illness. This organism may be the prototype of a group of *E. coli* strains which produce disease by a previously undescribed mechanism.

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