

Enterotoxigenic *Escherichia coli* in Central Canada

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During epidemiological studies carried out in urban and rural areas of the midwestern Canadian province of Manitoba, we cultured enterotoxigenic *Escherichia coli* (ETEC) from 16 (1.7%) of 945 diarrheal stools and 4 (0.3%) of 1,282 normal stools. ETEC was found in not more than 2.3% of diarrheal stools obtained from any population during any season. Diarrhea associated with ETEC persisted for a mean of 9 days. Two children were dehydrated and required intravenous fluid therapy, and one adult suffered a cholera-like syndrome. Half of the children required hospitalization for management of their diarrhea. Two adults and two children who harbored ETEC were completely asymptomatic. The pattern of toxin production correlated with serotype and the serotypes encountered were (with a few exceptions) similar to those found in other areas. We conclude that ETEC is an uncommon cause of diarrhea, both in rural and urban areas of central Canada. However, the possibility that ETEC might cause severe sporadic cases or epidemics of gastroenteritis remains.

Numerous recent studies have led to a better understanding of the epidemiological significance of enterotoxigenic *Escherichia coli* (ETEC) in human diarrheal disease. This organism has been found to be responsible for a considerable proportion of cases of endemic diarrhea (11, 20, 28, 35) both in adults and children in areas of poor environmental hygiene, particularly the tropics (20 to 50% in Bangladesh). Prospective studies have shown that ETEC is responsible for 30% or more of the cases of diarrhea which develop in travellers to tropical or subtropical countries (17, 26, 41). The role of ETEC in North America has been less clearly defined. ETEC has been implicated in waterborne outbreaks of diarrhea (4, 32) and a nursery epidemic (34), and it was also isolated from 16% of 64 children with endemic diarrhea on an Indian reservation in Arizona (38). A recent study of the etiology of pediatric diarrhea showed that ETEC was isolated from 4% of cases of diarrhea in Houston, Tex., and 7% of cases in Mexico City (31). By contrast, ETEC was uniformly absent in urban endemic diarrhea studied in Honolulu, Boston, and Washington, D.C. (7, 9, 25).

We report here the collated results of several surveys conducted to assess the role of ETEC in diarrhea of people resident in both urban and rural northern areas of the Canadian province of Manitoba. Despite the lack of chlorinated water supplies in some rural areas, ETEC was not

responsible for more than 2% of the cases of diarrhea in any population. However, people having diarrhea associated with ETEC suffered considerable morbidity. We also present an analysis of the serotypes, type of toxin produced, and antibiotic resistance in our isolates of ETEC.

MATERIALS AND METHODS

Patients surveyed. Table 1 summarizes the different populations studied for ETEC. The first group included 276 children hospitalized with diarrhea at the Childrens' Centre, Winnipeg, Manitoba, from May 1974, to November 1975. The etiology of diarrhea in this group has been reported previously (23). The second group consisted of families enrolled in a prospective study of diarrhea. Ninety-eight families, 166 children and 188 adults, were resident in Winnipeg; and 31 families, 80 children and 33 adults, lived in Berens River, Manitoba, an isolated northern community. Stool specimens were collected routinely from all family members at intervals of 3 months, and we attempted to obtain a specimen from all family members at the time of a diarrheal episode occurring in any family member. A total of 223 diarrheal and 1,282 normal stool specimens were obtained from this group between November 1976 and January 1979. The third group included patients, primarily North American Indians, resident in the rural North, who had stool cultures obtained during the course of a diarrheal illness. Fecal specimens from 374 such patients were obtained from May through August 1977, through the cooperation of the Provincial Laboratory of Manitoba and northern hospital laboratories. The fourth group was a miscellaneous group of 54 adults and 18 children with diarrhea whose stools were screened for ETEC on clinical grounds. Five ETEC isolates are not included in the survey data because they do not come

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TABLE 1. *ETEC* isolated from different study groups

| Source of specimens | Stool type | No. of positive specimens/total | | | |
|---------------------|------------|---------------------------------|-----------------------|--------------|----------------------------|
| | | Miscellaneous | Hospitalized children | Family study | North-ern diarr-rhea study |
| Studies on children | Diarrheal | 0/18 | 3/276 | 4/183 | 7/336 |
| | Normal | 0 | 0 | 2/630 | 0 |
| Studies on adults | Diarrheal | 2/54 | | 0/40 | 0/38 |
| | Normal | 0 | | 2/652 | 0 |

from a definable population group. These are: (i) two O27:H- strains which were isolated from diarrheal stools in the enteric laboratory of the Health Sciences Center and tested for enterotoxin production when their serogroup became known; (ii) one O119:H- isolate found when 167 *E. coli* strains belonging to the "enteropathogenic serogroups" were tested for toxin production (24); (iii) two O6:H16 strains which were isolated from the postmortem bowel contents of two consecutive cases of sudden infant death syndrome. The above isolates have not been included in the calculation of the frequency of isolation of *ETEC* in diarrheal and normal stools. However, analysis of their biological properties and associated clinical syndrome is included when appropriate.

Microbiological methods. Fecal specimens were cultured for enteropathogens by standard techniques (14) as previously described (23). *Aeromonas hydrophila* was specifically sought in all fecal cultures obtained during the course of the family study and the Indian diarrhea study by screening all colonies on primary isolation plates with the oxidase test. All positive colonies were immediately subcultured, and *Aeromonas* species were identified by published procedures (5, 6, 13). All organisms belonging to the genus *Aeromonas* were tested for the production of cytotoxin in the HeLa cell system as previously described (6).

The antibiotic susceptibility of enterotoxigenic organisms was determined by the Kirby-Bauer disk diffusion method (2) with the following antibiotics: streptomycin, carbenicillin, cephalothin, kanamycin, gentamicin, tetracycline, chloramphenicol, and sulfadiazine. All fecal specimens obtained during the course of the family study were examined for the presence of rotavirus by direct electron microscopy (16). Only 25% of other specimens were so examined.

Enterotoxin assays. Five to ten colonies resembling *E. coli* were picked from primary isolation plates for enterotoxin testing. Production of heat-labile enterotoxin (LT) by *E. coli* in 2,227 fecal specimens was assayed in the mouse adrenal tumor cell system, either as previously described (8, 37) or by a rapid method with microculture plates (21). Only 294 of these fecal specimens were also tested for heat-stable enterotoxin (ST)-producing *E. coli*. One hundred and eight of these were tested in the suckling mouse system as described by Dean et al. (7), and 186 were tested by

pooling the supernatant of five colonies and testing the pool in a single group of mice (3).

Serotyping. The enterotoxigenic strains were serotyped in The Central Public Health Laboratory, Colindale, according to the internationally accepted scheme with antisera to *E. coli* O groups O1 to O164 and the flagellar antigens H1 to H56 (29). Nonmotile organisms are designated as H-.

RESULTS

Twenty-one *ETEC* strains (isolated from 20 patients) were found in the four study populations giving an overall isolation rate of 0.9% (Table 2). *ETEC* was isolated from 16/945 (1.7%) diarrheal stools and 4/1,282 (0.3%) normal stools ($\chi^2 = 10.15$, $P < 0.005$). The rate of isolation of *ETEC* from diarrheal stools of children (1.7%) was similar to the rate of adults (1.5%). *ETEC* was isolated significantly more frequently during the summer months (1.4% of specimens) than in the winter months (0.1%, $\chi^2 = 10.44$, $P < 0.005$). In Manitoba, November through April may well be considered winter. If only diarrheal specimens are considered, the rate during the summer was higher (2.2%) than in the winter (0.4%), but the difference is not significant ($\chi^2 = 3.44$, $P < 0.10$). In those subjects whose residence was known, *ETEC* was isolated from 7/404 (1.7%) specimens from urban Winnipeg and 16/534 (3.0%) from the rural North. This was not significantly different.

Two strains of *ETEC* were isolated from the postmortem bowel contents of two consecutive cases of sudden infant death syndrome. One of the children had vomited on the day before death, whereas the other had had several loose bowel movements. At autopsy, both children had slightly increased amounts of fluid in the small bowel, but had no evidence of dehydration or any pathological findings which explained the sudden death. Because of this unexpected finding, bowel contents of 54 subsequent cases of sudden infant death syndrome and 25 age-matched controls were screened for *ETEC*.

TABLE 2. Source of *ETEC* by season

| Stool type | Source | No. of specimens positive/total (%) | | |
|------------|----------|-------------------------------------|---------------------|---------------|
| | | Winter ^a | Summer ^b | Total |
| Diarrheal | Children | 0/197 | 14/616 (2.2) | 14/813 (1.7) |
| | Adults | 1/54 (1.8) ^c | 1/78 (1.3) | 2/132 (1.5) |
| | Total | 1/251 (0.4) | 15/694 (2.2) | 16/945 (1.7) |
| Normal | Children | 0/315 | 2/315 (0.6) | 2/630 (0.3) |
| | Adults | 0/331 | 2/321 (0.6) | 2/652 (0.3) |
| | Total | 0/646 | 4/636 (0.6) | 4/1,282 (0.3) |

^a Winter is defined as November to April.

^b Summer is defined as May to October.

^c The *ETEC*-associated winter diarrhea in this adult was acquired in Mexico

ETEC was not isolated from any of these subsequent postmortem specimens.

The clinical features of patients harboring ETEC ranged from a mild illness to life-threatening severe diarrhea seen in one adult (22) (Table 3).

Ten children were hospitalized because of their diarrhea, and six were seen as outpatients; only one was not seen by a physician. Two of the hospitalized children required intravenous fluids and were transferred from northern hospitals to the Childrens' Centre, Winnipeg, because of the severity of their illness. The diarrhea was often prolonged. The total duration of diarrhea ranged from 2 to 10 days (mean, 9.9 days) in the 19 symptomatic adults and children. Two adults and two children had no diarrhea.

Other pathogens were isolated relatively frequently from the stools of patients with ETEC. In some cases these additional pathogens may have increased the severity of diarrhea. Thus, two patients had *Shigella* species, four had cytotoxic enterotoxin-producing *A. hydrophila* (6), and one had both rotavirus and *Salmonella typhimurium* together with ETEC. The adult with the cholera-like diarrhea was one of the patients who also had *A. hydrophila* in her stool (22). The infant with rotavirus and *Salmonella* had moderate dehydration and acidosis. Diarrhea was associated with each pattern of toxin production: ST only, LT only, or both. There did not appear to be a correlation between the type of toxin produced and the severity of diarrhea.

ST production was detected in only 10 of the 26 ETEC strains: 6 produced both LT and ST, and 4 produced ST only. Two of the four ST-only strains were detected in the "pooled" assay (3). The other two had been negative when originally tested by this method. They were recognized as ST-producing when tested individ-

ually after it was known that they were in the O27 serogroup. All six LT-ST strains were originally recognized as LT positive; ST production was detected because of the policy of ST testing all LT-positive strains.

The 26 ETEC strains were confined to a limited number of serotypes (Table 4). There was some correlation between serotype and the type of toxin produced. All four O6:H16 strains produced both LT and ST. All four O27 strains produced ST only, whereas both O8:H9 strains

TABLE 4. Laboratory characteristics of ETEC

| Serotype | No. of isolates ^a | No. associated with diarrhea | ST only | LT only | ST and LT | Antibiotic resistance ^b |
|------------------------------------|------------------------------|------------------------------|---------|---------|-----------|------------------------------------|
| O6:H16 | 4 | 2 ^c | 0 | 0 | 4 | 1 (Tc) |
| O27:H- | 3 | 3 | 3 | 0 | 0 | None |
| O27:H7 | 1 | 1 | 1 | 0 | 0 | 1 (Tc) |
| E2981:H40 | 3 | 3 | 0 | 3 | 0 | None |
| O8:H9 | 2 | 1 | 0 | 2 | 0 | 2 (Cb,Cf, Sm,Su) |
| O159: (H34,H21, H4) | 3 | 3 | 0 | 2 | 1 | 1 (Cb,Cf) 1 (Cb,Su) |
| O109:H- ^d | 3 | 2 | 0 | 3 | 0 | 3 (Tc, Sm) |
| O75: (H-,H10) | 2 | 2 | 0 | 2 | 0 | None |
| O untypable: (H-,H8,H 20) | 3 | 1 | 0 | 3 | 0 | 1 (Tc, Sm) |
| O119:H- | 1 | 1 | 0 | 1 | 0 | 1 (Cb) |
| O63:H- | 1 | 1 | 0 | 0 | 1 | 1 (Tc) |

^a Includes two ETEC, O6:H16 and O63:H-, isolated from the same patient.

^b Tc, Tetracycline; Cb, carbenicillin; Cf, cephalothin; Sm, streptomycin; Su, sulfadiazine.

^c Two of the four O6:H16 strains were isolated from cases of sudden infant death syndrome and are not counted as being associated with diarrhea.

^d All three isolates were from the same family.

TABLE 3. Clinical features associated with ETEC

| Source | Diarrhea syndrome | No. of cases | No. dehydrated | Intravenous fluids required | Duration of diarrhea (days) | Other pathogens isolated from stool |
|-----------------------|----------------------------|--------------|----------------|-----------------------------|-----------------------------|---|
| Adults | Travellers | 1 | 0 | 0 | 6 | <i>S. sonnei</i> |
| | Cholera-like | 1 | 1 | 1 | 10 | <i>A. hydrophila</i> ^a |
| | No diarrhea | 2 | | | | |
| Children ^b | Mild (3-5 stools/day) | 6 | 0 | 0 | 2-19 | 2 <i>A. hydrophila</i> |
| | Moderate (6-10 stools/day) | 9 | 0 | 0 | 6-20 | 1 <i>S. flexneri</i> (type 2) |
| | Severe (11-20 stools/day) | 2 | 2 | 2 | 7-16 | 1 case had both rotavirus and <i>S. typhimurium</i> |
| | No diarrhea | 2 | | | | |

^a All strains of *A. hydrophila* in this table produced cytotoxin.

^b Excludes two infants with sudden infant death syndrome.

produced LT only. Three isolates, all epidemiologically unrelated and isolated over a 3-year period, had the flagellar antigen H40 and had a serologically identical, but as yet unrecorded O antigen, temporarily designated E2981. An antiserum against one of these organisms agglutinated the other two strains to a high titer. Thus, all three strains were serologically identical and produced LT only. Although the somatic antigen E2981 was distinct from the 164 recognized O antigens, it did show some cross reactivity with the antigen O15. Three strains of the O group 159 had different H antigens.

Five organisms were epidemiologically related. Three of these were the O109:H- isolates which had been isolated from children in the same family in Berens River, Manitoba. These organisms were also identical with respect to antibiotic resistance pattern and type of toxin produced. Two ETEC strains were isolated from one patient (O6:H16 and O63:H-) who developed diarrhea in Mexico. These two strains were the only ones associated with foreign travel, with the exception of an O?:H- strain isolated from an asymptomatic adult 5 days after he returned from a Caribbean cruise. Twelve (46%) of the ETEC isolates were resistant to one or more antibiotics, although this includes the three epidemiologically related O109:H- isolates.

DISCUSSION

Several outbreaks of diarrhea associated with ETEC have been reported from North America and Europe (4, 9, 32, 34). However, ETEC has been convincingly associated with endemic diarrhea in only one study. ETEC was shown to account for 16% of cases of diarrhea on a rural Indian reservation in Arizona (38). Because many rural northern communities in Manitoba do not have chlorinated water or adequate sewage disposal, we thought that ETEC might be a significant cause of diarrhea in these areas. A previous study (23) showed that other bacterial diarrheas (those due to *Shigella* and enteropathogenic *Escherichia coli*) were more common in rural northern communities, particularly in the summer. In the present study, diarrhea due to ETEC showed a similar pattern. However, ETEC did not account for more than 2.3% of cases of diarrhea under any circumstances. This low frequency would account for the failure to isolate ETEC at all in three other studies of diarrhea in American children (7, 9, 25) and is very similar to the 4% found by Pickering et al. in Houston, Tex. (31).

The frequency of isolation of ETEC from asymptomatic individuals (0.3%) is lower than that reported from Bangladesh (1.5% of relatives of patients with LT-producing ETEC and con-

trol patients) (35) and from travellers to Mexico who were asymptomatic (6%) (26). Simultaneous cultures from members of the family of individuals colonized with ETEC were taken prospectively in six epidemiologically distinct episodes. In only one of these was simultaneous colonization of multiple family members demonstrated. This suggests that cross-infection in a household does not occur frequently and is borne out by similar results in two studies done in tropical countries (26, 35).

Although we failed to isolate ETEC from several samples of river water obtained from Berens River, testing was not extensive enough to rule this out as a source of the organism. It is worth noting, however, that from the same water samples cytotoxic enterotoxin-producing *A. hydrophila* were isolated without difficulty (6). ETEC has been isolated from food in the United States (39). However, the significance of this finding is uncertain because the quantity of organisms was not determined and the majority of the strains were not serologically typical of ETEC isolated from cases of human diarrhea. It is worth noting, however, that an outbreak of enteroinvasive *E. coli* diarrhea was traced to contaminated cheese (42). Thus, the significance of ETEC isolated from food may only be determined by searching for their presence in food-associated diarrheal outbreaks.

No patient was found to excrete ETEC chronically. However, ETEC was cultured from the feces of one patient 20 days after the cessation of diarrhea (22). This apparently short duration of colonization has been demonstrated in other studies (17, 26) and may relate either to efficient elimination by the host or to the fact that the enterotoxigenic strain must represent at least 10% of the fecal aerobic gram-negative flora to be detected by present methods. Thus, the investigation of the importance of a carrier state in the epidemiology of ETEC awaits the development of techniques for detection of small numbers of these organisms in the fecal or small bowel flora.

The ETEC strains isolated in this study included serotypes similar to those previously reported: O6:H16, O8:H9, and O159 (10, 12, 18, 19, 27, 30, 33). Serogroups O15, O25, and O78, found frequently in ETEC isolated in Asia and Mexico (12, 27, 30), were not isolated. The finding of four epidemiologically unrelated isolates with the somatic antigen O27, as well as three serologically identical strains with an as yet unrecorded O antigen (E2981), is unusual. Several groups (12, 26, 40) have pointed out a striking correlation between the toxin produced and the O:H serotype of the organism. Similar results were noted in this study for the O6:H16 isolates,

all of which produced LT and ST. We also noted that the three E2981:H40 isolates all produced LT only, and all four O27 isolates produced ST only. The basis for this correlation between enterotoxigenicity and serotype is unknown.

Two O27:H- isolates had been negative for ST production when tested by the pooled supernatant method (3), but positive when tested individually. This suggests that the pooled supernatant method of testing for ST may occasionally yield false-negative results.

Twelve of the twenty-six isolates were resistant to at least two antibiotics. As a comparison, *E. coli* strains shown to be nontoxigenic and not of enteropathogenic serotypes from 31 children with diarrhea (23) were tested for susceptibility to the same antibiotics. Five were resistant to one antibiotic, and 15 were resistant to at least two antibiotics. Thus, enterotoxigenicity was not found to be specifically associated with antibiotic resistance.

The isolation of ETEC from the small bowel contents of two children who died of the sudden infant death syndrome deserves comment. Recently, *Clostridium botulinum* has been found in the bowel contents of approximately 4% of cases of sudden infant death syndrome (1). When ETEC was found in two consecutive cases of sudden infant death syndrome, we wondered whether the toxins produced by ETEC might also be associated with a proportion of cases. This, however, has not been borne out by subsequent studies, although this could be due to failure to examine a sufficient number of cases.

Our summary of the clinical features associated with ETEC in 23 patients confirms the finding that ETEC is only very rarely associated with a life-threatening diarrhea in North America (15, 16, 38). However, considerable morbidity was associated with ETEC diarrhea, since 10 children and one adult were hospitalized. The mean duration of diarrhea in the hospitalized children was 9.3 days (range, 2 to 19 days). This is longer than the duration of diarrhea associated with *Shigella flexneri* which was the organism associated with the most severe diarrhea in our previous study (23). It seems fair to say that the diarrhea associated with ETEC is more severe than that seen with other gastrointestinal pathogens such as rotavirus, *Shigella*, and enteropathogenic *E. coli*, although the frequency of coexistent pathogens may in part account for this.

Since *E. coli* from only 294 fecal specimens were tested for ST, and 186 of these by the pooled method, it is possible that some ST-only ETEC strains were not detected and that our rate is an underestimate of the total ETEC. Four ST-only ETEC were detected in 294 specimens

(1.4%), a rate which might represent approximately 30 ST-only ETEC in the entire 2,227 specimens tested for LT-producing *E. coli*, and an approximate doubling of the overall isolation rate of ETEC. Better or more rapid methods for detecting ST-only ETEC may be required before a more complete understanding of the epidemiology of ST-only ETEC is achieved.

In spite of these considerations, it does not appear that routine screening for ETEC in a hospital microbiology laboratory in North America is warranted at this time. However, the fact that this organism accounts for slightly less than 2% of diarrhea indicates that there is always the possibility that it may cause diarrheal outbreaks. These have been amply documented in the past in North America (4, 32, 34). *E. coli* strains which are implicated in outbreaks of diarrhea should, therefore, be tested for production of both LT and ST.

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