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Enteroadherent *Escherichia coli* as a Cause of Diarrhea among Children in Mexico

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Enteropathogenic *Escherichia coli* (EPEC) often exhibits localized adherence or diffuse adherence to HEp-2 cells. We recently provided evidence that HEp-2 cell-adherent or enteroadherent *E. coli* (EAEC) not belonging to EPEC serogroups was the cause of diarrhea among U.S. travelers to Mexico. In the present study, we looked for EAEC and EPEC in stool specimens from 154 children with acute diarrhea and 137 well children seen at several outpatient clinics in Guadalajara, Mexico. EAEC showing localized adherence (EAEC-L) was isolated from 13.0% of the patients and 0.7% of the controls ($P < 0.0001$). EAEC showing diffuse adherence (EAEC-D) was recovered from 20.8% of the patients and 7.3% of the controls ($P < 0.001$). EPEC was isolated from 4.5 and 6.7% of the patients and controls, respectively. Among all enteropathogens, only enterotoxigenic *E. coli* occurred as commonly (21.4%) as EAEC-D and EAEC-L did in children with diarrhea. Of the EAEC-L strains isolated from children with diarrhea, 20% belonged to recognized EPEC serogroups, and 3.1% of EAEC-D strains belonged to recognized EPEC serogroups. This study suggests that EAEC may be an important pediatric enteropathogen in Mexican children with diarrhea and further supports the observation that adherence to HEp-2 cells may be a marker of virulence independent of EPEC serogroup among *E. coli* strains.

Mannose-resistant adherence to HEp-2 tissue culture cells is a virulence characteristic originally found in strains of enteropathogenic *Escherichia coli* (EPEC) (1). Recently, we reported that HEp-2 cell-adherent *E. coli* causes diarrhea among U.S. travelers to Mexico (9, 10). The pathogenicity of these strains was further established with adult volunteers (11). Only 9% of the adherent strains isolated from our U.S. traveler population in Mexico belonged to traditional EPEC somatic (O) serogroups (10). For this reason, we have referred to any *E. coli* strain exhibiting mannose-resistant adherence to HEp-2 cells as enteroadherent *E. coli* (EAEC) to avoid reference to EPEC serotypes.

EPEC strains are well-known causes of diarrhea in children (5), but little is known about the importance of EAEC in pediatric populations. Since EAEC is a definition based on a virulence characteristic rather than an *E. coli* serotype, we sought to determine the importance of EAEC strains as causes of diarrhea among children in a developing nation.

MATERIALS AND METHODS

The subjects were children between the ages of 3 and 84 months with diarrhea (three or more unformed stools in 24 h) of no more than 48-h duration who were seen at public health clinics or the pediatric outpatient clinic of the Hospital General de Occidente, Guadalajara, Mexico, during the summer of 1985 (12a). Age-matched well subjects who served as controls were selected from children visiting the same clinics for routine childhood vaccinations. Control children were excluded if they or their family contacts had experienced diarrhea or had received an antimicrobial agent in the preceding 2 weeks. Dehydration was defined by clinical characteristics, e.g., tacky mucous membranes, sunken eyes or fontanelle, and poor skin turgor. Stool specimens from 154 children with acute diarrhea were studied for all enteric pathogens, whereas the 137 well children were studied only for the presence of diarrheagenic *E. coli*.

Specimens were processed at the microbiology laboratory of the Hospital General de Occidente within 4 h of collection. Recognized methods were used to identify *Salmonella* spp., *Shigella* spp., *Campylobacter jejuni*, *Aeromonas hydrophila*, *Giardia lamblia*, *Entamoeba histolytica*, cryptosporidia, and rotavirus. Each specimen obtained from the ill children was inoculated onto MacConkey, salmonella-shigella, Tergitol 7, and DNase plates (all from Difco Laboratories, Detroit, Mich.) and into 10 ml of Selenite broth (Difco). Specimens from the controls were inoculated only onto MacConkey agar. All these media were incubated overnight at 37°C. The Selenite broth was then restreaked to a MacConkey plate. Suspicious colonies growing on the plate media were identified by using standard biochemical tests (6). *Salmonella* spp. and *Shigella* spp. were confirmed by using commercial antisera (Difco). Campylobacter blood agar (Difco) was inoculated for the isolation of *C. jejuni*. These plates were incubated at 42°C for 24 to 72 h in a microaerophilic atmosphere produced by CampyPaks (BBL Microbiology Systems, Cockeysville, Md.). Suspicious colonies were identified by Gram stain, production of oxidase and catalase, resistance to cephalothin, and susceptibility to nalidixic acid. Enteropathogenic amoebae were identified by trichrome stain (15), and cryptosporidia were identified by a modified acid-fast stain (8). A portion of each illness specimen was frozen and shipped to Houston for examination for rotavirus by an enzyme-linked immunosorbent assay (16).

Five *E. coli* strains were picked from each stool specimen of both patients and controls. These strains were tested for heat-labile enterotoxin by Y-1 adrenal cell assay and for heat-stable enterotoxin by the suckling mouse assay in Houston (2, 3). All strains that were not enterotoxigenic were tested for mannose-resistant HEp-2 cell adherence and

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TABLE 1. Prevalence of enteropathogens among 154 children with acute diarrhea in Guadalajara, Mexico

Enteropathogen(s)	No. of patients (%)[a]
ETEC	33 (21.4)
EAEC-D	32 (20.8)
EAEC-L	20 (13.0)
Shigella spp.	10 (6.5)
Campylobacter jejuni	9 (5.8)
Giardia lamblia	7 (4.5)
EPEC	7 (4.5)
Entamoeba histolytica[b]	6 (3.9)
Cryptosporidia	4 (2.6)
Salmonella spp.	2 (1.3)
Rotavirus	2 (1.3)
Unknown agents	32 (20.8)

[a] Some patients had multiple pathogens.
[b] Trophozoites only.

for EPEC O serogroup. The HEp-2 cell adherence assay was a modification of the method of Cravioto et al. (1), as previously described (10). Briefly, a chamber slide (Miles Scientific, Div. Miles Laboratories, Inc., Naperville, Ill.) was seeded with HEp-2 cells, a continuous line of human epithelial cells. These cells were grown at 37°C in 5% CO_2 in Eagle minimal essential medium supplemented with 100 U of penicillin per ml, 100 μg of streptomycin per ml, 2 mM L-glutamine, and 10% fetal calf serum (KC Biologicals, Lexena, Kans.) until a nearly confluent monolayer had formed. Test strains were grown overnight in Trypticase soy broth (BBL) overnight at 37°C. Bacteria were incubated with a monolayer of HEp-2 cells for 1 h at 37°C, the monolayer was washed to remove any nonadherent bacteria, the tissue culture cells were incubated again for 2 h, and the culture was washed three times, fixed, and stained with carbolfuchsin. Microscopic examination allowed the determination of both localized and diffuse adherence of the *E. coli* to the HEp-2 cells (13). EPEC O serogrouping was done by the methods of Edward and Ewing (6, 10) with antisera prepared in our laboratory against the reference strains for each EPEC O serogroup. The EPEC serogroups sought were: O26, O44, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142, and O158. Statistical analysis was done by using the chi-square test (14).

RESULTS

Table 1 shows the prevalence of all enteropathogens isolated from the children with diarrhea. Enterotoxigenic *E. coli* (ETEC) was the most commonly identified pathogen. EAEC showing diffuse adherence (EAEC-D) was isolated with approximately equal frequency, and EAEC showing localized adherence (EAEC-L) was the third most frequently occurring agent. EPEC strains as identified by serogroup were isolated from seven of the children with diarrhea.

The prevalence of EAEC and EPEC strains among children with acute diarrhea and controls is shown in Table 2. EAEC-L was isolated from 20 of the patients but was isolated from only 1 of the well children ($P < 0.0001$). EAEC-D was recovered from 32 of the ill children and 10 of the controls ($P < 0.001$). EPEC strains as identified by serogroup were identified with approximately equal frequency among the well children (6.7%) and children with diarrhea (4.5%). ETEC strains were identified among 33 of the children with diarrhea. Of the controls, 24 also shed ETEC in their stools. When patients with only one enteropathogen were considered, the isolation rate of EAEC-L was significantly different ($P < 0.005$) from that for controls, and the isolation rate of EAEC-D was greater than that for the well children.

Only 1 of the 32 EAEC-D strains (3.1%) belonged to a recognized EPEC O serogroup (O44). Of the 20 EAEC-L strains, 4 (20.0%) belonged to EPEC serogroups. Two were O119, and two were O55. Four of seven EPEC strains (57.1%) isolated from the ill children exhibited localized adherence to HEp-2 cells, whereas none of the EPEC strains from the controls did. One of seven EPEC strains (14.3%) from patients was diffusely adherent, and two of nine EPEC strains (22.2%) from the controls were diffusely adherent. Seven of nine EPEC strains (77.8%) from the well children were nonadherent, but only two of seven EPEC strains (28.6%) isolated from children with diarrhea did not adhere to HEp-2 cells.

The clinical characteristics of the children with diarrhea caused by the various types of *E. coli* are shown in Table 3. Patients with multiple pathogens were excluded from this analysis. Diarrheal disease caused by *E. coli* occurred primarily in children less than 2 years of age. Patients with diarrhea due to ETEC were slightly older than those with diarrhea due to EAEC-L and EAEC-D. Children with EAEC-D had the greatest number of unformed stools in the 24 h prior to being seen. All the patients had had diarrhea for approximately 40 h before presentation. Other characteristics of these patients are shown in Table 3; overall, diarrhea due to EAEC-D appeared to be the most severe.

DISCUSSION

These data suggest that in this pediatric population in Mexico EAEC-L and EAEC-D are important causes of acute summertime diarrhea. The two pathogens occurred significantly more often in stool specimens from children with diarrhea than in the stools of children who did not have the illness. In addition, a minority of EAEC-D (3.1%) and EAEC-L (20.0%) strains belonged to classical EPEC serogroups. EPEC as recognized by serogrouping was recovered as frequently from stool specimens obtained from well children as from the children who had diarrhea. We were surprised to find ETEC almost as often in well children as in those with diarrhea, but this has also been reported from studies with U.S. students who had been in Mexico for at least 1 year (4). Four of seven EPEC strains isolated in cases of illness showed localized adherence to HEp-2 cells, compared with none of nine EPEC strains in the controls. A relationship could not be demonstrated between EPEC serogroup and diffuse adherence pattern among *E. coli* strains isolated from patients with diarrhea.

TABLE 2. Occurrence of EAEC, EPEC, and ETEC among children with and without diarrhea

Pathogen	No. of isolates (%) in children:		
	With diarrhea (n = 154)	With diarrhea and single pathogen	Without diarrhea (n = 137)
EAEC-L	20 (13.0)[a]	14 (9.1)[b]	1 (0.7)
EAEC-D	32 (20.8)[c]	19 (12.3)	10 (7.3)
EPEC	7 (4.5)	5 (3.2)	9 (6.7)
ETEC	33 (21.4)	24 (15.6)	24 (17.5)

[a] $P < 0.0001$.
[b] $P < 0.005$.
[c] $P < 0.001$.

TABLE 3. Clinical disease of children with diarrhea due to various types of *E. coli*

Pathogen (*n*)[a]	Age of children (mo)		No. of unformed stools in last 24 h		No. (%) of children with:			
	Mean (range)	Median	Mean (range)	Median	Fever <100.5°F (<38.1°C)	Dehydration	Gross blood in stool	Fecal leukocytes
EAEC-L (14)	16.3 (4–57)	11.0	5.4 (3–11)	5.0	1 (7.1)	6 (42.9)	0 (0.0)	6 (42.9)
EAEC-D (19)	10.1 (3–18)	10.0	7.4 (3–15)	7.0	6 (31.6)	12 (63.2)	2 (10.5)	7 (36.8)
ETEC (24)	21.3 (3–86)	13.0	6.7 (3–20)	6.0	6 (25.0)	11 (45.8)	3 (12.0)	6 (25.0)

[a] Stated pathogen was the only enteropathogen identified in stool specimen. Patients with multiple pathogens were excluded from the analysis. *n*, Number of children with the pathogen.

Although the number of cases studied was small, this study indicates that HEp-2 cell adherence may be a better marker than EPEC serogroup of pathogenicity among *E. coli* strains that cause pediatric diarrhea in Mexico. Nataro et al. (12) found that EPEC strains exhibiting localized adherence occurred significantly more often in Peruvian infants with diarrhea than in those without the illness. In the same study, nonadherent EPEC as identified by serogroup alone was isolated as often from the well controls as from ill infants. In a volunteer challenge study, the pathogenicity for adults of a locally adherent EPEC strain was established (7). Diarrhea was produced after the adherent strains were ingested, whereas the same strain cured of the plasmid encoding HEp-2 cell adherence showed reduced virulence (7). A diffusely adherent EPEC strain also caused diarrhea but at a decreased frequency.

These findings further support results of our previous studies among U.S. travelers to Mexico (9, 10) indicating that EAEC independent of EPEC serogroup is the cause of diarrhea in Mexico. The pathogenicity of two of the isolates obtained from U.S. students who acquired diarrhea in Mexico has been confirmed in adults by volunteer challenge experiments (11). EAEC is a definition based on a virulence characteristic rather than on *E. coli* serotype. In the present study, we have provided evidence that EAEC is an important pathogen in a pediatric population in Mexico. Further studies are needed in other populations to determine the importance of EAEC-L and EAEC-D as enteropathogens and to determine the relationship between HEp-2 cell adherence and serotype among *E. coli* strains.

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