Enteroaggregative *Escherichia coli* as a Major Etiologic Agent in Traveler's Diarrhea in 3 Regions of the World

Javier A. Adachi,[1] Zhi-Dong Jiang,[1] John J. Mathewson,[1,a] Mangala P. Verenkar,[3] Sharon Thompson,[4] Francisco Martinez-Sandoval,[5] Robert Steffen,[6] Charles D. Ericsson,[1] and Herbert L. DuPont[1,2]

[1]Center for Infectious Diseases, University of Texas–Houston Medical School and School of Public Health, and [2]St. Luke's Episcopal Hospital and Baylor College of Medicine, Houston; [3]Goa Medical College, Bambolin, Goa, India; [4]Cornwell Regional Hospital, Montego Bay, Jamaica; [5]Universidad Autonoma de Guadalajara, Guadalajara, Mexico; and [6]University of Zurich, Zurich, Switzerland

(See the editorial commentary by Wanke on pages 1710–2)

Enteroaggregative *Escherichia coli* (EAEC) has been reported to cause traveler's diarrhea and persistent diarrhea in children in developing countries and in immunocompromised patients. To clarify the prevalence of EAEC in traveler's diarrhea, we studied 636 US, Canadian, or European travelers with diarrhea: 218 in Guadalajara, Mexico (June–August 1997 and 1998), 125 in Ocho Rios, Jamaica (September 1997–May 1998), and 293 in Goa, India (January 1997–April 1997 and October 1997–February 1998). Stool samples were tested for conventional enteropathogens. EAEC strains were identified by use of the HEp-2 assay. EAEC was isolated in 26% of cases of traveler's diarrhea (ranging from 19% in Goa to 33% in Guadalajara) and was second only to enterotoxigenic *E. coli* as the most common enteropathogen in all areas. Identification of EAEC reduced the number of cases for which the pathogen was unknown from 327 (51%) to 237 (37%) and explained 28% of cases with unknown etiology. EAEC was a major cause of traveler's diarrhea in 3 geographically distinct study areas.

Diarrhea is the major travel-related disease in terms of frequency and economic impact among people who travel from industrialized countries to high-risk tropical and subtropical developing regions of Latin America, Africa, and Asia, with incidence rates as high as 40%–

50% [1–3]. Various infectious agents have been identified as the primary cause of traveler's diarrhea; bacterial enteropathogens cause ~80% of cases of traveler's diarrhea with recognized etiology [1, 2]. Of these enteric bacteria, enterotoxigenic *Escherichia coli* (ETEC) has been identified as the most common organism, found in 20%–40% of travelers with diarrhea in different areas of the world [1, 2].

Enteroaggregative *E. coli* (EAEC) is a recently recognized pathogen within the group of *E. coli* that cause diarrhea [4]. Mathewson et al. [5] first recognized EAEC as a cause of traveler's diarrhea in 1985. To assess the worldwide prevalence, we used the HEp-2 cell assay [6, 7] to identify EAEC in stool samples from travelers with diarrhea in 3 different regions of the world.

POPULATION, MATERIALS, AND METHODS

Population. The study population included adults from the United States, Canada, of Europe with dia-

rrhea who visited one of our traveler's health clinics in Guadalajara, Mexico; Ocho Rios, Jamaica; or Goa, India. In Guadalajara, 94 students from the United States were enrolled in June–August 1997, and 124 more were recruited in June–August 1998. A total of 125 international tourists were enrolled in Jamaica in September 1997–May 1998. In Goa, 293 travelers with diarrhea were evaluated during 2 different periods of time: January–April 1997 and October 1997–February 1998. Subjects in Mexico and Jamaica participated in double-blind, randomized clinical trials that tested antidiarrheal compounds [8, 9]. Patients in Goa were recruited as part of a collaborative study of diarrhea conducted by the Center for Infectious Diseases at the University of Texas–Houston Medical School and School of Public Health and the Institute of Social and Preventive Medicine at the University of Zurich, Zurich, Switzerland [10].

In the studies, acute traveler's diarrhea was defined as the passage of ≥3 unformed stools in 24 h within 72 h of onset of symptoms, together with ≥1 additional clinical manifestation of enteric disease, such as nausea, vomiting, abdominal cramps or pain, tenesmus, stool urgency, or dysentery. Eligible subjects included men or women ≥18 years old. Subjects were not enrolled if they had taken an antimicrobial agent with expected activity against bacterial enteropathogens within the previous week.

Stool examination. After a qualified patient signed a written consent form, a stool specimen was collected. These samples were submitted to the field-site laboratory, where they were examined by published methods [5] for conventional bacterial enteric pathogens, including *Shigella* species, *Salmonella* species, *Vibrio* species, *Campylobacter jejuni*, *Yersinia enterocolitica*, *Aeromonas* species, and *Plesiomonas shigelloides*. *Entamoeba histolytica*, *Cryptosporidium* species, and *Giardia lamblia* were identified by means of EIA. The presence of rotavirus and other viral enteric pathogens were not sought in this study. Five *E. coli*–like colonies were retrieved from MacConkey agar plates from each stool sample and were inoculated into individual peptone stabs. They were transported to the Center for Infectious Diseases, University of Texas–Houston, for further identification. Oligonucleotide probes for heat-labile and heat-stable enterotoxins of ETEC were hybridized with the 5 *E. coli*–like colonies for the detection of ETEC [11].

HEp-2 adherence assay. At least 3 of the 5 *E. coli*–like colonies per stool sample were tested for the presence of EAEC by looking for a characteristic pattern of adherence to cultured HEp-2 cells [4, 6, 7, 12]. We used the method of Cravioto et al. [6], which was demonstrated to be the optimal procedure, according to a comparative study by Vial et al. [12]. In brief, a chamber slide (Dynatek) was seeded with HEp-2 cells (ATCC) that had been grown at 37°C in 5% CO_2 on minimum essential medium (Gibco BRL) supplemented with 10% fetal calf serum. *E. coli* to be tested were grown overnight at 37°C in tryptic soy broth (BBL Microbiology) with 1% D-mannose. The cell culture medium in the chamber slide then was replaced with minimum essential medium containing 1% D-mannose without antibiotics; *E. coli* was added and was incubated at 37°C for 3 h. The slide was washed vigorously 3 times with PBS, was fixed with 100% methanol, and was stained with Wright-Giemsa. Positive and negative controls were included in each assay. Finally, each *E. coli* strain was twice examined in a blinded fashion. A sample was interpreted as positive for EAEC if it showed the characteristic "stacked-brick" aggregative appearance, as described by Nataro et al. [7].

Pulsed-field gel electrophoresis (PFGE). Thirty EAEC strains from Guadalajara, Mexico (obtained in the summer of 1998), were tested by means of PFGE [13], to look for interstrain variability.

RESULTS

Table 1 shows the prevalence of the various enteric pathogens in the 3 study areas. EAEC was identified in 162 (26%) of 636 cases of traveler's diarrhea—19% in Goa, 26% in Jamaica, and 33% in Guadalajara. Seventy-three of the 162 EAEC cases (45%) were isolated in Guadalajara: 19 patients in 1997 and 54 during the summer of 1998. EAEC accounted for 26% of cases of traveler's diarrhea when the 3 areas were combined, and only ETEC was more prevalent, found in 30% of the cases. ETEC was responsible for 48% (193 of 399) of the pathogen-identifiable illness, whereas EAEC was found in 41% of the cases with an established etiology. Overall, the number of cases caused by all the other bacterial enteric pathogens combined

Table 1. Prevalence of enteropathogens in cases of traveler's diarrhea at 3 locations.

| Infecting pathogen(s) | No. of patients (prevalence, %), by location | | | |
	Guadalajara, Mexico	Ocho Rios, Jamaica	Goa, India	Total
ETEC	83 (38)	37 (30)	73 (25)	193 (30)
EAEC	73 (33)	33 (26)	56 (19)	162 (26)
Others[a]	10 (5)	4 (3)	30 (10)	44 (7)
Mixed[b]	40 (18)	7 (6)	79 (27)	126 (20)
None identified[c]	76 (35)	53 (42)	108 (37)	237 (37)
Total[d]	218 (100)	125 (100)	293 (100)	636 (100)

NOTE. EAEC, enteroaggregative *Escherichia coli;* ETEC, enterotoxigenic *E. coli.*

[a] Includes *Salmonella* species, *Shigella* species, *Campylobacter jejuni, Vibrio* species, *Aeromonas hydrophila, Plesiomonas shigelloides, Entamoeba histolytica, Cryptosporidium parvum, Giardia lamblia,* and rotavirus.

[b] Mixed infections comprised patients with >1 enteric pathogen.

[c] No pathogen indicates cases of traveler's diarrhea with a nonidentified etiologic agent.

[d] Some of the patients had mixed infections.

was less than the number of cases caused by ETEC or EAEC, which were isolated from 148 (23%) of all the subjects with traveler's diarrhea. In general, most of the defined non-ETEC, non-EAEC pathogens identified in our study were obtained from stool samples from tourists with traveler's diarrhea visiting Goa.

In the 3 regions studied, EAEC was the only pathogen isolated from 90 (14%) of 636 patients and was found to be mixed with other enteric pathogens in 72 (11%) patients. Coinfection of EAEC and ETEC accounted for 41 of these 72 mixed infections (table 2). Infection by EAEC alone occurred more often in Jamaica (79%) and Guadalajara (59%) than in Goa (38%). Coinfection by EAEC and ETEC occurred most commonly in Guadalajara, whereas mixed infection with other enteropathogens was more common in Goa and Jamaica.

The identification of EAEC as an enteric pathogen explained the etiology of ≤28% of cases of diarrhea in patients who otherwise had an unidentified pathogen. By looking for EAEC in all diarrhea samples using the HEp-2 call adherence assay, we were able to reduce the number of patients with an unknown etiologic agent from 51% of 636 patients to 37%. This reduction was similar in Guadalajara (55%–35%) and in Jamaica (63%–42%). Because of the lower number of patients with EAEC as the only pathogen identified, this reduction was less evident in Goa (44%–37%).

Finally, we tested 30 of 32 strains of EAEC that were isolated as the only pathogen from subjects with traveler's diarrhea in Guadalajara during the summer of 1998 for strain differences. By use of PFGE, our results showed a highly heterogeneous DNA pattern among the EAEC strains, with no more than 2 similar bands between the different isolates.

DISCUSSION

EAEC was identified in the present study as an important cause of traveler's diarrhea in Mexico, Jamaica, and India. EAEC was the second most common enteropathogen isolated at all 3 locations.

Because coinfection with EAEC and other recognized enteric pathogens was common (72 of 162 patients), it is not possible to define EAEC as the true pathogen in these patients. However, in 90 of 162 patients, EAEC was the sole pathogen, and identifying this pathogen explained the etiology of ∼30% of the cases for which the pathogen was unknown. Identification of EAEC as a pathogen that causes diarrhea helps to explain a previous observation by our group, that diarrhea in patients for which no pathogen is identified improves with antimicrobial therapy [14, 15].

In 1985, Mathewson et al. [5] first described EAEC to be responsible for 14.9% of diarrhea in a US student population visiting Guadalajara. The results of the present study, taken

Table 2. Prevalence of infection with enteroaggregative *Escherichia coli* and coinfection with other enteric pathogens in patients with diarrhea at 3 locations.

Infecting pathogen(s)	No. of patients (prevalence, %), by location			
	Guadalajara, Mexico	Ocho Rios, Jamaica	Goa, India	Total
EAEC alone	43 (59)	26 (79)	21 (38)	90 (56)
EAEC and ETEC	26 (36)	1 (3)	14 (25)	41 (25)
EAEC and others[a]	4 (5)	6 (18)	21 (38)	31 (19)
Total EAEC	73 (100)	33 (100)	56 (100)	162 (100)

NOTE. EAEC, enteroaggregative *E. coli*; ETEC, enterotoxigenic *E. coli*.
[a] Mixed infection with EAEC and non-ETEC enteric pathogens.

with this finding, confirm that EAEC is a consistently important pathogen causing traveler's diarrhea in Guadalajara, Mexico.

Although EAEC was the second most common pathogen identified in Goa, it was least commonly the sole enteric pathogen and was most commonly isolated with other enteropathogens, compared with the pattern of infection in Guadalajara and Jamaica. It appears that exposure to multiple enteropathogens is more common in Goa than in the other locations.

Although different candidate virulence factors have been reported in EAEC strains, such as the production of EAEC heat-stable toxin, plasmid-encoded enterotoxin or a novel cryo-hemagglutinin, or the presence of a novel flagellin or an aggregative adherence fimbria I or II, most EAEC strains have none of these factors [16–20]. These data and the finding of no homogeneous DNA pattern in the EAEC strains from Guadalajara support the probability that EAEC strains are a group of heterogeneous *E. coli* that share few characteristics, with the exception of the distinctive aggregative adherence to HEp-2 cells. Further studies of the molecular biology of these bacteria should provide additional knowledge about its epidemiology, diagnosis, prevention, and treatment.

Although some studies have questioned the pathogenic role of these strains because of the similar frequency of isolating EAEC from patients with diarrhea and from asymptomatic control subjects [21, 22], the findings of more recent studies support the hypothesis that it has a true pathogenic role. This role has been confirmed in outbreaks and in volunteer studies [5, 23], but the strains have heterogeneous virulence [23].

EAEC has been implicated as an etiologic agent in persistent diarrhea in children in developing countries [24, 25] and in patients with AIDS-associated chronic diarrhea in the United States [26] and in Africa [27]. On the basis of the present study and others [28, 29], the organism appears to be an important cause of traveler's diarrhea. It is uncertain how important EAEC is as a cause of sporadic and epidemic diarrhea in industrialized areas [30]. Further evidence of the organism's pathogenicity is the finding that therapy with fluoroquinolones shortened the length of bouts of diarrhea in patients with AIDS [26] and in

international travelers with EAEC diarrhea [28]. Our study indicates the importance of EAEC as a cause of traveler's diarrhea in these diverse regions of the world.

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