JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2000, p. 3550–3554 Vol. 38, No. 10
0095-1137/00/$04.00+0

Diarrheagenic *Escherichia coli* and Acute and Persistent Diarrhea in Returned Travelers

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Received 16 March 2000/Returned for modification 6 June 2000/Accepted 8 August 2000

To determine the role of diarrheagenic *Escherichia coli* in acute and persistent diarrhea in returned travelers, a case control study was performed. Enterotoxigenic *E. coli* (ETEC) was detected in stool samples from 18 (10.7%) of 169 patients and 4 (3.7%) of 108 controls. Enteroaggregative *E. coli* (EAggEC) was detected in 16 (9.5%) patients and 7 (6.5%) controls. Diffuse adherent *E. coli* strains were commonly present in both patients (13%) and controls (13.9). *Campylobacter* and *Shigella* species were the other bacterial enteropathogens most commonly isolated (10% of patients, 2% of controls). Multivariate analysis showed that the presence of ETEC was associated with acute diarrhea (odds ratio [OR], 6.7; 95% confidence interval [CI], 1.5 to 29.1; $P = 0.005$), but not with persistent diarrhea (OR, 1.6; 95% CI, 0.4 to 7.4). EAggEC was significantly more often present in patients with acute diarrhea than in controls ($P = 0.009$), but no significant association remained after multivariate analysis. ETEC and EAggEC are frequently detected in returned travelers with diarrhea. The presence of ETEC strains is associated with acute but not with persistent diarrhea.

Millions of people travel from industrialized to developing countries each year. Traveler's diarrhea is the most common disorder encountered by these travelers during their journey and thereafter. While traveler's diarrhea generally lasts less than a week (7), approximately 3% of international travelers to high-risk areas develop persistent diarrhea. By definition, the duration of persistent diarrhea is at least 14 days (3), but it may last several months to a year. In approximately 50% of travelers with persistent diarrhea, diarrhea lasts more than 30 days (11). Besides travelers, persistent diarrhea is also a major cause of morbidity in expatriates and other long-term foreign residents in developing countries (1, 30).

The etiology of persistent diarrhea in travelers remains unknown in at least 50% of cases (11, 26). While the etiology of acute traveler's diarrhea has been studied extensively, there are only a few reports in which the etiology of persistent diarrhea in travelers and foreign residents has been studied. Although enterotoxigenic *Escherichia coli* (ETEC) is considered to be the most important cause of acute traveler's diarrhea (7), it has rarely been implicated as a cause of persistent diarrhea in travelers (30). In view of the possible association of enteroaggregative *E. coli* (EAggEC) with persistent diarrhea in children in developing countries (5, 10, 12), it has been suggested that EAggEC may also be a cause of persistent diarrhea in travelers.

We performed a case control study to determine the role of ETEC and EAggEC in acute and persistent diarrhea in returned travelers. In addition, the presence of diffuse adherent *E. coli* (DAEC) and enteropathogenic *E. coli* (EPEC) was determined.

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MATERIALS AND METHODS

Definitions. Diarrhea was defined as at least three loose stools in 24 h, any number of watery stools, or 1 or 2 loose stools in 24 h accompanied by at least one of the following symptoms: nausea, vomiting, abdominal cramps, or fever of >38°C.

Acute diarrhea was defined as diarrhea which lasted 14 days or less at the time of presentation. Persistent diarrhea was defined as diarrhea which lasted for more than 14 days at presentation. When diarrhea was present intermittently, it was considered persistent when diarrhea occurred during at least 6 days in a 2-week period.

Patients and controls. Patients were included who presented with diarrhea from December 1995 to August 1996 at the Outpatient Department for Tropical Diseases at the Academic Medical Centre, Amsterdam, The Netherlands, and the Leopold Institute of Tropical Medicine, Antwerp, Belgium. Cases were defined in terms of patients who developed diarrhea during a stay in tropical areas or within the first 2 weeks after their return.

Included as control subjects were the first consecutive individuals after each patient who, after a stay in tropical areas, presented at the outpatient departments for other reasons than diarrhea and who had not experienced diarrhea in the previous 2 months. Both patients and controls were requested to submit a stool sample at their first visit to the outpatient departments.

From both patients and controls, data were collected, including age, sex, geographic area visited, and duration of their journey, as well as date of return and use of antimicrobial therapy during or after the trip. From patients with diarrhea, a detailed clinical history was obtained.

Investigation of stool samples. Stool samples from patients and controls were collected on the day of presentation at the outpatient department, immediately transported to the laboratory, stored at 4°C, and processed within 24 h.

Detection of diarrheagenic *E. coli*. Stool samples were inoculated onto cysteine lactose electrolyte-deficient (CLED) agar plates, and after incubation for 18 h at 37°C, a "sweep" of the complete bacterial growth on the agar was collected with a sterile cotton swab and stored in glycerol-peptone at −70°C, as described previously (28).

Detection of diarrheagenic *E. coli* was performed in the Medical Microbiology laboratory in Amsterdam. The frozen sweeps collected in Antwerp were transported on dry ice to Amsterdam within 1 day. To test the sweep, after thawing, the material was inoculated on a CLED agar plate and a second sweep was made. This sweep was diluted in phosphate-buffered saline and subjected to PCR for detection of ETEC as described previously (28). In addition, 3 μl of the diluted sweep was spotted on nylon filters (Hybond-N; Amersham Nederland BV) overlying nutrient agar plates and grown for 6 h at 37°C. Growth on the filters was lysed and denatured as described previously (28). Membranes, containing positive and negative controls, were hybridized with digoxigenin (Boehringer Mannheim BV, Almere, The Netherlands)-labeled polynucleotide DNA probes for detection of EAggEC (4), DAEC (*daaC*) (6), and attaching-effacing *E. coli* (*eae*) (17).

All stored sweeps which yielded weak to strong signals after hybridization were

TABLE 1. Characteristics of patients and controls

Characteristic	Result		
	Patients (n = 169)	Controls (n = 108)	P value[a]
Mean ± SD age (range in yr)	34 ± 11.6 (2–75)	39 ± 14.1 (7–70)	0.005
No. male/female	88/81	61/47	NS
No. (%) with destination			NS
Maghreb, Middle East	9 (5.3)	2 (1.9)	
Africa	39 (23.4)	42 (38.9)	
Central and South America	26 (15.6)	14 (13.0)	
Indian Subcontinent	43 (25.8)	22 (20.4)	
Southeast Asia	42 (25.2)	21 (19.4)	
Multiple geographic areas	8 (4.8)	7 (6.5)	
No. (%) with duration of stay			
<14 days	138 (81.7)	54 (50.0)	
15–91 days	16 (9.5)	23 (21.3)	<0.001
>91 days	15 (8.9)	31 (28.7)	
No. (%) with interval between return and presentation			
<14 days	89 (54.6)	43 (41.4)	
15–91 days	57 (35.0)	34 (32.7)	0.003
>91 days	17 (10.4)	27 (26.0)	
No. (%) with prior use of antibiotics/no. tested	46/164[b] (28.1)	9/52[b] (17.3)	NS
No. (%) with prior use of imidazoles/no. tested	35/167[b] (21)	3/52[b] (6.5)	0.012
No. at Amsterdam/Antwerp	63/106	53/55	NS[c]

[a] Pearson χ^2 test. NS, not significant.
[b] Data available for 164 or 167 of 169 patients and for 52 of 108 controls.
[c] Yates χ^2 test.

again inoculated on CLED agar plates, and three to five *E. coli*-like colonies were isolated and identified biochemically as *E. coli*. These colonies were tested in a 6-h HEp-2 adherence assay for phenotypical confirmation of their adherence pattern (31). Only colonies which yielded an aggregative or diffuse adherence pattern in the HEp-2 test, in addition to positive hybridization with the EAggEC probe or the *daaC* probe, were considered EAggEC or DAEC, respectively. Isolates which showed an adherence pattern compatible with both EAggEC and DAEC were categorized separately and designated DAAA (diffuse adherent-aggregative adherent). Strains were considered EPEC if they were positive after hybridization with the *eae* probe and showed localized adherence in the HEp-2 test.

Detection of other enteropathogenic bacteria. All stool samples were inoculated onto *Salmonella-Shigella* (SS) agar, CLED agar, Hektoen enteric agar (HEA), MacConkey agar containing potassium-tellurite, and thiosulfate-citrate-bile salts-sucrose agar and incubated for 24 to 48 h at 37°C. For enrichment, Hajna GramNegative broth was inoculated, and after incubation for 24 h at 42°C, cells were subcultured onto SS agar and HEA. For selective enrichment of *Salmonella* species, feces was inoculated onto a semisolid selective-motility enrichment medium (14), incubated at 37°C, and subcultured onto SS agar. *Yersinia* species were isolated by inoculation of cefsulodin-irgasan-novobiocin (CIN) agar, which was incubated for 48 h at room temperature. In addition, glucose broth was inoculated and incubated for 1 week at 4°C and subcultured onto CIN agar. For detection of *Campylobacter* species, Butzler *Campylobacter* selective medium was inoculated, and plates were incubated for 48 h at 42°C under microaerophilic conditions. For isolation of *Plesiomonas shigelloides*, mannitol-MacConkey agar plates were inoculated and incubated for 18 to 36 h at 37 and 42°C. All isolates were further characterized by standard microbiological identification methods and with commercially available antisera. For detection of *Aeromonas* species, blood agar plates containing 10 μg of ampicillin per ml were inoculated and incubated for 24 to 48 h at 37°C and biochemically characterized as described by Kuijper et al. (20). The methods used for bacterial culture in Amsterdam and Antwerp were identical.

Parasitological examination. Parasitological investigation of stool samples was performed by direct examination of stool in eosin solution. In addition, samples were concentrated as described by Ridley (25) and examined after staining with potassium-iodine. Stool samples were examined for the presence of ova, cysts, and cyclospora. Identical protocols for identification of parasites were used in Amsterdam and Antwerp.

Data collection and analysis. Clinical data from each patient and control were collected by using a questionnaire in the Epi-info format (Amsterdam) or by direct registration in dBase (Antwerp). Data were merged into Epi-info, version 6.0 (Centers for Disease Control and Prevention, Atlanta, Ga.). Statistical analysis was performed with Stata, version 4 (Stata Corporation, College Station, Tex.).

For comparisons of categorical and numerical variables, χ^2 tests with Yates' continuity correction for 2 × 2 tables and unpaired Student t tests were used, respectively. Associations of diarrheagenic *E. coli* with acute and persistent diarrhea were assessed by unmatched case control analysis. For this approach, cases were defined as patients with diarrhea with a negative stool examination for other enteropathogenic bacteria than diarrheagenic *E. coli* (including nontyphoid *Salmonella*, *Shigella*, *Campylobacter*, *Aeromonas*, *Plesiomonas*, *Yersinia enterocolitica*, and *Vibrio cholerae*). Patients for whom these examinations were not performed completely, were excluded. Patients were allowed to have a positive stool examination with *Giardia lamblia* and *Blastocystis hominis*, because the role of these parasites in the etiology of diarrhea in travelers is debatable (7). In addition, the sensitivity of microscopic examination of a single stool specimen for these parasites is relatively low (19). The association of cyclospora with diarrhea was considered sufficiently strong to exclude patients who were cyclospora positive (15).

Crude and adjusted odds ratios (ORs) were calculated separately for associations of case control status with the presence of ETEC, EAggEC and DAEC, and EPEC and DAAA and for each of these associations after stratification for potential confounding variables. These included sex, age, travel destination, duration of stay, interval between return to The Netherlands or Belgium, and presentation at the outpatient department, season of return, recent use of antibiotics, presence of *G. lamblia* or *B. hominis*, presence of ETEC (for EAggEC-associated diarrhea), and institute of study inclusion (Amsterdam or Antwerp). The stratification for travel destination was based on destination-specific diarrhea risk categories for Dutch travelers (8). If crude and adjusted ORs differed by more than 15% in the simple stratified analysis, the confounding effect was further assessed in a multivariate model by using logistic regression. For each association under study, a final model was constructed including all variables displaying a meaningful confounding effect; the contribution of the presence of the pathogen to the model was expressed as an OR with a 95% confidence interval (CI) and assessed by the likelihood ratio test for exclusion from that model (16). For all statistical tests, $P < 0.05$ was considered significant.

RESULTS

A total of 171 patients and 109 controls were included. Of these, 63 patients and 53 controls were included in Amsterdam and 108 patients and 56 controls were included in Antwerp. Two patients and one control were excluded because of inconsistent data collection. Characteristics of patients and controls are presented in Table 1. Patients were younger than controls. The duration of stay abroad of patients was significantly shorter than that of controls, and patients presented at the outpatient department within a significantly shorter period after return than controls. In addition, patients used more imidazoles prior to presentation than controls.

Fifty-three (31%) patients presented with acute diarrhea, 73 (43%) patients had diarrhea for 14 days to 3 months, and 43 (25%) patients had diarrhea for more than 3 months.

Bacterial enteropathogens, except for diarrheagenic *E. coli*, were isolated from 18 patients and 3 controls. The rates of detection of these pathogens in patients with acute and persistent diarrhea are shown in Table 2. *Campylobacter* and *Shigella* species were isolated in patients with both acute and persistent diarrhea. No *Plesiomonas shigelloides*, *Aeromonas*

TABLE 2. Detection rates of non-*E. coli* bacterial enteropathogens and parasites in fecal samples from 169 patients and 108 controls returning from (sub)tropical areas

Organism	No. positive/no. studied (%)		
	Diarrhea ≤14 days	Diarrhea >14 days	Controls
Campylobacter jejuni	3/52 (5.8)	7/114 (6.1)	1/98 (1.0)
Shigella sp.	3/52 (5.8)	4/114 (3.5)	1/99 (1.0)
Salmonella sp.	0/52	1/114 (0.9)	1/99 (1.0)
Giardia lamblia	1/49 (2.0)	19/116 (16.4)	5/102 (4.9)
Cyclospora	1/48 (2.1)	4/115 (3.5)	0/100
Entamoeba histolytica trophozoites	0/48	1/115 (0.9)	0/100
Blastocystis hominis	2/48 (4.2)	11/115 (9.6)	3/102 (2.9)

TABLE 3. Detection rates of diarrheagenic *E. coli* in stool samples obtained from patients and controls returning from (sub)tropical areas

Organism type	No. (%) with detection	
	Patients (n = 169)[a]	Controls (n = 108)
ETEC	18 (10.7)	4 (3.7)
EAggEC	16 (9.5)	7 (6.5)
DAEC	22 (13.0)	15 (13.9)
EPEC	2 (1.2)	6 (5.6)
DAAA	2 (1.2)	3 (2.8)

[a] For associations of diarrheagenic *E. coli* with diarrhea, see Table 5.

species, or *Yersinia* species were isolated. Bacteriological stool investigation was incomplete in 4 patients and 11 controls.

Giardia lamblia was identified in 20 patients and 5 controls, *Blastocystis hominis* was identified in 13 patients and 3 controls, and cyclospora was identified in 5 patients and 0 controls (Table 2). The parasitological stool investigation was incomplete in six patients and eight controls. If stool investigations were incomplete, this was generally due to the submission of amounts of stools too small to perform all investigations.

ETEC was isolated in 18 patients and 4 controls (Table 3). EAggEC was isolated in 16 patients and 7 controls. In five patients, both ETEC and EAggEC were present. DAEC was found in 22 patients and 15 controls, while EPEC was found in 2 patients and 6 controls. In two patients and three controls, DAAA was identified (Table 3). Mixed infections with other bacteria were present in three patients with ETEC infection (one each with *Salmonella* sp., *Shigella* sp., and *Campylobacter jejuni*) and one control with ETEC (*Campylobacter jejuni*). Mixed infection with cyclospora occurred in one patient with ETEC. Mixed infections with other bacteria were present in one patient and one control with EAggEC, both with *Shigella* sp.

The association of infection with diarrheagenic *E. coli* and diarrhea was analyzed by multivariate analysis with logistic regression. Crude ORs and results of stratified analysis to assess potential confounding variables are shown in Table 4. ORs, adjusted for confounding in logistic regression models, are shown in Table 5. Infection with ETEC was significantly associated with diarrhea of less than 14 days of duration (P = 0.005; Table 5), but not with persistent diarrhea. EAggEC was found significantly more frequently in patients with acute diarrhea than in controls (P = 0.009; Table 4). However, no significant association of EAggEC infection with diarrhea remained after multivariate analysis (Table 5), which was mainly due to the fact that coinfection of EAggEC with ETEC occurred in five patients with acute diarrhea. In 9 of 15 patients with EAggEC infection and no other bacterial causes of diarrhea (except ETEC), diarrhea was acute, and in 6 patients diarrhea was persistent, lasting 14 days to 3 months in 4 of them.

Isolation of DAEC was not associated with acute or persistent diarrhea (Table 5). EPEC and DAAA were detected in too few patients and controls to permit statistical analysis.

The distributions of patients with ETEC and EAggEC among the various geographic regions visited were similar. The highest rates of detection occurred among visitors to Africa (ETEC, 21%; EAggEC, 15%) and South and Central America (ETEC, 15%; EAggEC, 27%), and the lowest detection rate occurred among visitors to Southeast Asia (ETEC, 7%; EAggEC, 5%).

TABLE 4. Association of diarrheagenic *E. coli* with diarrhea according to crude and adjusted ORs[a]

	Result for group (no. with diarrhea/no. tested) with diarrhea duration of:							
	ETEC		EAggEC		DAEC		Other *E. coli*[b]	
OR	≤14 days (10/43)	>14 days (4/99)	≤14 days (9/43)	>14 days (6/99)	≤14 days (4/43)	>14 days (12/99)	≤14 days (2/43)	>14 days (1/99)
Crude (95% CI) [P value]	7.88 (2.12–28.6) [<0.001]	1.1 (0.27–4.52) [0.90]	3.82 (1.29–11.36) [0.009]	0.93 (0.3–2.88) [0.90]	0.64 (0.2–2.1) [0.45]	0.86 (0.38–1.93) [0.71]	0.54 (0.11–2.61) [0.43]	0.11 (0.01–0.94) [0.01]
Stratified[c]								
Age	8.19	1.31[d]	3.68	0.96	0.63	0.67	0.54	0.09
Sex	9.32[d]	1.03	3.68	1.0	0.64	0.84	0.58	0.12
Destination	7.73	1.27	3.8	0.70[d]	0.63	0.9	0.51	0.08[d]
Duration of stay	7.43	1.10	4.37	0.93	0.58	0.75	0.48	0.09
Period between return and presentation	6.43[d]	1.39[d]	3.58	0.95	0.54	0.81	0.31	0.1
Prior antibiotics	7.89	1.24	2.49[d]	0.59[d]	0.97[d]	0.98[d]	1.21	0.24[d]
Parasitic infection	7.37	1.31	3.6	1.0	0.65	0.98	0.62	0.12
Season of return	8.26	1.04	3.78	0.87	0.52	0.79	0.68	0.13
Season of presentation	8.23	1.06	3.87	1.04	0.62	0.79	0.53	0.11
Institute	8.6	1.09	4.68	0.93	0.48[d]	0.86	0.37	0.12
ETEC infection	NA[e]	NA	2.88[d]	0.93	0.68	0.86	0.56	0.11
EAggEC infection	6.73	1.09	NA	NA	ND[f]	ND	ND	ND

[a] Crude and adjusted ORs after simple stratified analysis for potential confounding variables.
[b] Other categories include EPEC and DAAA.
[c] The categories of the stratifying variables are defined as follows: age, 0 to 29, 30 to 49, and ≥50 years; destination, Indian Subcontinent and North Africa, Central and South America and Southeast Asia, and Subsaharan Africa and multiple regions; duration of stay, <3 months, 3 months to 1 year, and >1 year; season, January to March, April to June, July to September, and October to December; parasitic infection, *Giardia lamblia*, other, and missing data. For the remaining variables, see Table 1.
[d] Change in OR of >15% (increase or decrease) in simple stratified analysis and tested in the multivariate model.
[e] NA, not applicable.
[f] ND, not determined.

TABLE 5. Results of multivariate analysis of association of diarrheagenic *E. coli* with diarrhea

Parameter	Result for group[a]			Variables in model[b]
	Diarrhea ≤14 days (n = 43)	Diarrhea >14 days (n = 99)	Controls (n = 108)	
No. (%) infected with ETEC	10 (23.3)	4 (4)	4 (3.7)	
Adjusted OR	6.7AB[c]	1.62BC		Sex, period between return and presentation, age
95% CI	1.54–29.09	0.35–7.39		
No. (%) infected with EAggEC	9 (20.9)	6 (6.1)	7 (6.5)	
Adjusted OR	1.74DE	0.42DF		Prior antibiotics, ETEC infection, destination
95% CI	0.48–6.26	0.12–1.64		
No. (%) infected with DAEC	4 (9.3)	12 (12.1)	15 (13.9)	
Adjusted OR	0.9DG	0.76D		Prior antibiotics, institute
95% CI	0.24–3.86	0.27–2.2		

[a] Letters show the relevant variables as follows: A, sex; B, period between return and presentation; C, age; D, prior antibiotics; E, ETEC infection; F, destination; G, institute.
[b] See Table 4 for results of simple stratified analysis for each variable.
[c] $P = 0.005$.

DISCUSSION

The aim of this study was to evaluate the potential associations between diarrheagenic *E. coli* and diarrhea of short and prolonged duration in returned travelers.

In 31% of patients, diarrhea lasted 14 days or less, and in these patients, a significant association of ETEC with diarrhea was found. This observation is compatible with the well established association of ETEC with classical traveler's diarrhea (7) and with findings from other studies of returned travelers (13, 18, 22). While ETEC was the pathogen most commonly detected in our patients, it should be taken into account that we used a highly sensitive method for detection of ETEC in stool samples (28). This method probably has a higher sensitivity than routine stool culture for detection of other common enteropathogens, such as *Campylobacter* or *Shigella*.

In patients with acute diarrhea, the frequency of EAggEC was similar to the frequency of ETEC. The role of EAggEC as a cause of diarrhea in travelers is not well established. Enteroadherent *E. coli* strains, which include both EAggEC and DAEC, have been associated with traveler's diarrhea in adults traveling to Mexico (21). However, at the time of that study, no distinction was made between localized, aggregative, and diffuse adhesion of adherent strains, and the contribution of EAggEC in this group is unknown. Increased colonization with EAggEC was observed in adult travelers to Mexico, when stool samples before and after the journey were compared, but no association with diarrhea was found (9). Finally, in a recent study of 165 returned Spanish travelers with diarrhea and their healthy travel companions, EAggEC was associated with diarrhea, which clinically resembled diarrhea caused by ETEC (13).

The detection rate of EAggEC in our study was similar to that found in the Spanish travelers (13). However, we found polymicrobial infection, particularly coinfection with ETEC, in 6 of 17 (35%) patients with EAggEC. Coinfection with ETEC and EAggEC may be a common phenomenon, considering the comparable distributions of the two categories of *E. coli* among the geographic regions. In addition, the uses of antibiotics by patients with EAggEC and controls were different (Table 4). Both factors account for the lack of association of EAggEC with acute diarrhea in our multivariate model (Table 5).

The majority of the patients (68%) presented with persistent diarrhea; illness lasting more than 3 months was present in 25%. Neither ETEC nor EAggEC was associated with persistent diarrhea. In a study in Nepal, ETEC was found in 19% of 37 travelers and foreign residents, who had diarrhea for more than 14 days. However, this study was performed in an area of endemicity, and no control group of individuals without diarrhea was included (30). EAggEC has been associated with persistent diarrhea in children in areas of endemicity in a number of studies (5, 10, 12), but to our knowledge, no data are available demonstrating an association of EAggEC in travelers with persistent diarrhea.

DAEC was detected at a relatively high frequency among patients and controls. The potential role of DAEC as an enteric pathogen is not well established (23). DAEC was detected in stool samples in 20% of both adult patients with ulcerative colitis and healthy controls in The Netherlands, who had not traveled during the year before the investigation (27). The latter findings suggest that DAEC strains are commonly present in stool samples from adults in The Netherlands and explain the lack of an association with diarrhea in travelers from tropical areas.

Since EPEC, similar to ETEC, is a common cause of childhood diarrhea in developing countries, several studies have investigated the role of EPEC in acute traveler's diarrhea (9, 13, 22, 24), using either serotyping or DNA probes for detection of EPEC. In none of these studies, was EPEC significantly associated with diarrhea, which is confirmed by our study. In addition, our results indicate that EPEC is also not associated with persistent diarrhea in travelers. The category of *E. coli* strains which hybridize with both the *daaC* probe and the EAggEC fragment probe and which show a mixed adherence pattern to HEp-2 cells, has been recognized before (29). We chose to evaluate the individuals with such strains separately from those infected with DAEC or EAggEC, since it is not clear to which category of diarrheagenic *E. coli* the strains belong and their pathogenic potential has not been evaluated.

The presence of other diarrheagenic *E. coli*, in particular verotoxigenic *E. coli* and enteroinvasive *E. coli*, was not investigated in the current study. However, we did not detect any of these pathogens in a pilot study among 50 returned travelers (C. Schultsz, R. van Ketel, P. Speelman, G. J. Pool, and J. Dankert, Abstr. 34th Intersci. Conf. Antimicrob. Agents Chemother., abstr. J220, p. 269, 1994), and therefore we did not expect to find any association with diarrhea in the present study.

We found a potential cause of diarrhea in less than 50% of patients. ETEC and EAggEC were the (putative) enteropathogens isolated most frequently. Twenty-eight percent of patients used antibiotics during the course of their disease, which may

have influenced the detection rates of pathogens. This detection rate is, however, comparable with rates reported by others (18). Several reports indicate that persistent diarrhea, which has developed after a bout of acute diarrhea and for which no cause is found, is self-limited, although symptoms may persist for periods as long as a year (2, 11, 26). Patients with persistent diarrhea after travel, without identifiable cause, should therefore not receive recurrent antimicrobial therapy.

ACKNOWLEDGMENTS

This work was supported by financial gifts from the Cornelis Visser Stichting and Hoechst Marion Roussel.

We are indebted to E. J. Kuijper, T. van Gool, and E. van Dijck for their crucial contributions to the analyses of stool samples. We also thank all of the physicians of the outpatient departments for their contribution to this study, X. Boon for technical assistance, and M. D. de Jong for critically reviewing the manuscript.

REFERENCES

1. **Addiss, D. G., R. V. Tauxe, and K. W. Bernard.** 1990. Chronic diarrhoeal illness in US Peace Corps volunteers. Int. J. Epidemiol. **19:**217–218.
2. **Afzalpurkar, R. G., L. R. Schiller, K. H. Little, W. C. Santangelo, and J. S. Fordtran.** 1992. The self-limited nature of chronic idiopathic diarrhea. N. Engl. J. Med. **327:**1849–1852.
3. **Anonymous.** 1988. Persistent diarrhoea in children in developing countries: memorandum from a WHO meeting. Bull. W.H.O. **66:**709–717.
4. **Baudry, B., S. J. Savarino, P. Vial, J. B. Kaper, and M. M. Levine.** 1990. A sensitive and specific DNA probe to identify enteroaggregative *Escherichia coli*, a recently discovered diarrheal pathogen. J. Infect. Dis. **161:**1249–1251.
5. **Bhan, M. K., P. Raj, M. M. Levine, J. B. Kaper, N. Bhandari, R. Srivastava, R. Kumar, and S. Sazawal.** 1989. Enteroaggregative *Escherichia coli* associated with persistent diarrhea in a cohort of rural children in India. J. Infect. Dis. **159:**1061–1064.
6. **Bilge, S. S., C. R. Clausen, W. Lau, and S. L. Moseley.** 1989. Molecular characterization of a fimbrial adhesin, F1845, mediating diffuse adherence of diarrhea-associated *Escherichia coli* to HEp-2 cells. J. Bacteriol. **171:**4281–4289.
7. **Black, R. E.** 1990. Epidemiology of travelers' diarrhea and relative importance of various pathogens. Rev. Infect. Dis. **12**(Suppl. 1)**:**S73–S79.
8. **Cobelens, F. G., A. Leentvaar-Kuijpers, J. Kleijnen, and R. A. Coutinho.** 1998. Incidence and risk factors of diarrhoea in Dutch travellers: consequences for priorities in pre-travel health advice. Trop. Med. Int. Health **3:** 896–903.
9. **Cohen, M. B., J. A. Hawkins, L. S. Weckbach, J. L. Staneck, M. M. Levine, and J. E. Heck.** 1993. Colonization by enteroaggregative *Escherichia coli* in travelers with and without diarrhea. J. Clin. Microbiol. **31:**351–353.
10. **Cravioto, A., A. Tello, A. Navarro, J. Ruiz, H. Villafan, F. Uribe, and C. Eslava.** 1991. Association of *Escherichia coli* HEp-2 adherence patterns with type and duration of diarrhoea. Lancet **337:**262–264.
11. **DuPont, H. L., and E. G. Capsuto.** 1996. Persistent diarrhea in travelers. Clin. Infect. Dis. **22:**124–128.
12. **Fang, G. D., A. A. Lima, C. V. Martins, J. P. Nataro, and R. L. Guerrant.** 1995. Etiology and epidemiology of persistent diarrhea in northeastern Brazil: a hospital-based, prospective, case-control study. J. Pediatr. Gastroenterol. Nutr. **21:**137–144.
13. **Gascon, J., M. Vargas, L. Quinto, M. Corachan, M. T. Jimenez de Anta, and J. Vila.** 1998. Enteroaggregative *Escherichia coli* strains as a cause of traveler's diarrhea: a case-control study. J. Infect. Dis. **177:**1409–1412.
14. **Goossens, H., G. Wauters, M. de Boeck, M. Janssens, and J. P. Butzler.** 1984. Semisolid selective-motility enrichment medium for isolation of salmonellae from fecal specimens. J. Clin. Microbiol. **19:**940–941.
15. **Hoge, C. W., D. Shlim, R. Rajah, J. Triplett, M. Shear, J. G. Rabold, and P. Echeverria.** 1993. Epidemiology of diarrhoeal illness associated with coccidian-like organism among travellers and foreign residents in Nepal. Lancet **341:**1175–1179.
16. **Hosmer, D. W., and S. Lemeshow.** 1989. Applied logistic regression. John Wiley & Sons, Inc., New York, N.Y.
17. **Jerse, E. A., J. Yu, B. D. Tall, and J. B. Kaper.** 1990. A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. Proc. Natl. Acad. Sci. USA **87:** 7839–7843.
18. **Jertborn, M., and A.-M. Svennerholm.** 1991. Enterotoxin-producing bacteria isolated from Swedish travellers with diarrhoea. Scand. J. Infect. Dis. **23:** 473–479.
19. **Koneman, E. W., S. D. Allen, W. M. Janda, P. C. Schreckenberger, and W. C. Winn.** 1992. Diagnostic microbiology, p. 879–961. J.B. Lippincott Company, Philadelphia, Pa.
20. **Kuijper, E. J., A. G. Steigerwalt, B. S. C. I. M. Schoenmakers, M. F. Peeters, H. C. Zanen, and D. J. Brenner.** 1989. Phenotypic characterization and DNA relatedness in human fecal isolates of *Aeromonas* spp. J. Clin. Microbiol. **27:** 132–138.
21. **Mathewson, J. J., H. L. DuPont, D. R. Morgan, S. A. Thornton, and C. D. Ericsson.** 1983. Enteroadherent *Escherichia coli* associated with travellers' diarrhoea. Lancet **i:**1048.
22. **Mattila, L.** 1994. Clinical features and duration of traveler's diarrhea in relation to its etiology. Clin. Infect. Dis. **19:**728–734.
23. **Nataro, J. P., and J. B. Kaper.** 1998. Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. **11:**142–201.
24. **Rademaker, C. M. A., M. R. L. Krul, W. H. Jansen, N. M. Vos, I. M. Hoepelman, M. Rozenberg-Arska, and J. Verhoef.** 1991. Analysis of Escherichia coli isolates from subjects with travellers' diarrhoea using DNA-probes and serotyping. Eur. J. Clin. Microbiol. Infect. Dis. **10:**625–629.
25. **Ridley, D. S., and B. C. Hawgood.** 1956. Concentration of faecal samples for direct smear investigation. J. Clin. Pathol. **9:**74–76.
26. **Schultsz, C., and A. De Geus.** 1992. Characteristics and aetiology of diarrhoea in travellers returned from the (sub)tropics. Eur. J. Int. Med. **2:** 217–222.
27. **Schultsz, C., M. Moussa, R. J. Van Ketel, G. N. J. Tytgat, and J. Dankert.** 1997. Frequency of pathogenic and enteroadherent *Escherichia coli* in patients with inflammatory bowel disease and controls. J. Clin. Pathol. **50:** 573–579.
28. **Schultsz, C., G. J. Pool, R. van Ketel, B. de Wever, P. Speelman, and J. Dankert.** 1994. Detection of enterotoxigenic *Escherichia coli* in stool samples by using nonradioactively labeled oligonucleotide DNA probes and PCR. J. Clin. Microbiol. **32:**2393–2397.
29. **Smith, H. R., S. M. Scotland, G. A. Willshaw, B. Rowe, A. Cravioto, and C. Eslava.** 1994. Isolates of Escherichia coli O44:H18 of diverse origin are enteroaggregative. J. Infect. Dis. **170:**1610–1613.
30. **Taylor, D. N., R. Houston, D. R. Shlim, M. Bhaibulaya, B. L. P. Ungar, and P. Echeverria.** 1988. Etiology of diarrhea among travelers and foreign residents in Nepal. JAMA **260:**1245–1248.
31. **Vial, P. A., J. J. Mathewson, H. L. DuPont, L. Guers, and M. M. Levine.** 1990. Comparison of two assay methods for patterns of adherence to HEp-2 cells of *Escherichia coli* from patients with diarrhea. J. Clin. Microbiol. **28:** 882–885.