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Disease Burden Due to Enterotoxigenic *Escherichia coli* in the First 2 Years of Life in an Urban Community in Bangladesh▽

Firdausi Qadri,[1]* Amit Saha,[1] Tanvir Ahmed,[1] Abdullah Al Tarique,[1] Yasmin Ara Begum,[1] and Ann-Mari Svennerholm[2]

International Centre for Diarrheal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh,[1] and Göteborg University Vaccine Research Institute (GUVAX) and Department of Microbiology and Immunology, the Sahlgrenska Academy at Göteborg University, Box 435 S-40530, Göteborg, Sweden[2]

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A cohort of 321 children was followed from birth up to 2 years of age to determine the incidence of enterotoxigenic *Escherichia coli* (ETEC) in Bangladesh. The average number of diarrheal days and incidence rates were 6.6 and 2.3/child/year, respectively. ETEC was the most common pathogen and was isolated in 19.5% cases, with an incidence of 0.5 episode/child/year. The prevalence of rotavirus diarrhea was lower (10%). ETEC expressing the heat-stable enterotoxin (ST) was predominant. Strains isolated from diarrheal cases were positive for colonization factors (CFs) in higher frequency (66%) than from healthy children (33%) ($P < 0.001$). The heat-labile toxin (LT)-positive strains from healthy children were more often CF negative (92%) than those isolated from children with diarrhea (73%) ($P < 0.001$). In children with symptomatic or asymptomatic infections by CFA/I, CS1 plus CS3, CS2 plus CS3, or CS5 plus CS6 strains, a repeat episode of diarrhea or infection by the homologous CF type was uncommon. Repeat symptomatic infections were noted mostly for LT- and ST-expressing ETEC. ETEC diarrhea was more prevalent in children in the A and AB groups than in those in the O blood group ($P = 0.032$ to 0.023). Children with ETEC diarrhea were underweight and growth stunted at the 2-year follow-up period, showing the importance of strategies to prevent and decrease ETEC diarrheal morbidity in children.

Enterotoxigenic *Escherichia coli* (ETEC) is a common cause of acute watery diarrhea in children in developing countries (25). ETEC is a multivalent pathogen producing the heat-stable (ST) and/or heat-labile toxin (LT) as well as over 25 colonization factors (CFs). The ST phenotype of ETEC has been shown to be predominant (23, 25), while the most common of the CFs are CFA/I and CS1 to CS6 (14, 23, 25, 37). Studies in animals and in humans suggest that immunity against both LT and CFs may be important for protection against ETEC. However, results from different studies are conflicting regarding the relative importance of different toxin types and/or CFs on protection from further disease and infection (11, 18, 27, 32), and data from different settings are needed to elucidate whether ETEC strains expressing these different virulence factors may prevent against repeated episodes of diarrhea. The first longitudinal study to determine the relationship between ETEC and other enteric pathogens and the incidence of diarrhea of children in a birth cohort was carried out in Mexico (10). In the present study, we have evaluated the natural history of ETEC infections, with emphasis on the different phenotypes, during the first 2 years of life in a birth cohort of 321 children in an urban slum area in Dhaka, Bangladesh. This included evaluation of the incidence, seasonality, and occurrence of symptomatic and asymptomatic ETEC infections. We have also evaluated whether blood group and nutritional factors may predispose to ETEC diarrhea. For comparison, we have also studied the incidence of other common enteric infections.

MATERIALS AND METHODS

Study population. A prospective community-based study was conducted in an urban slum in Mirpur, Dhaka, Bangladesh, from April 2002 to October 2004. Of 695 pregnant mothers screened, 500 were registered and 321 newborn children were enrolled in the order in which they were born (Table 1). The inclusion criteria for the newborns were that they were of normal delivery with no congenital abnormality and with a weight of ≥2 kg at birth. Written informed consent was obtained from the parents. The research was approved by the Ethical Review Committee of the International Centre for Diarrheal Disease Research, Dhaka, Bangladesh (ICDDR, B).

Children were monitored by active surveillance. Field research assistants visited each child three times a week. The child's caretaker was interviewed for information on diarrhea and other illnesses as well as for feeding patterns. The children were brought to the field clinic at 3-month intervals for anthropometric and clinical evaluation and blood collection. They also attended the clinic when they had diarrhea or needed medical assistance. This was based on the perception of the caregiver or the study staff when on home visits that the child needed medical attention.

Sample collection and procedure. Fecal samples were collected every month and during episodes of diarrhea. During the first 6 months of life when breast-fed children passed stools with pasty characteristics, the mother's perception of diarrhea was used to identify cases. Cord blood was collected after birth, and venous blood was collected at intervals of 3 months. Weekly rectal swabs were tested for ETEC in a subgroup of 60 children to determine the rates of isolation in monthly versus weekly collections. For this purpose, every fifth child in the study was selected in the order in which the child enrolled in the study. Specimens were also collected from mothers at different time intervals, which included breast milk (quarterly, if available), stool (every 3 months), as well as blood (every 6 months). Stools from mothers were also screened when the mother or child had diarrhea.

Diarrhea was defined as three or more unformed stools in a 24-h period. Two episodes of diarrhea were considered different when they were separated by 3 symptom-free days. To determine, infection and reinfection rates from homologous CFs or LT and ST, only episodes of symptomatic or asymptomatic infec-

* Corresponding author. Mailing address: Laboratory Sciences Division, ICDDR, B, GPO Box 128, Dhaka 1000, Bangladesh. Phone: 880 2 8860523. Fax: 880 2 8823116. E-mail: fqadri@icddrb.org.

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TABLE 1. Characteristics of the study population

Parameter	Result for parameter
Children	
Total no.	321
Male/female ratio	157/164
Mean ± SD birth wt in g	2,791 ± 426
No. (%) with WAZ < −2SD at birth	47 (15)
No. (%) with LAZ < −2SD at birth	26 (8)
No. (%) for which follow-up completed	254 (79)
No. (%) with exclusive breast-feeding	76 (27)
No. (%) with partial breast-feeding	208 (73)
Parents	
Mean ± SD age of mother in yr	25 ± 4.94
No. of siblings	1–9
No. (%) with first child	88 (27.5)
% Maternal literacy	51
% Paternal literacy	60
Sociodemographic	
Avg no. of family members/household	5
% of families with monthly income ≤$60 U.S.	63
% of families using sanitary latrine	80
% of families using nontreated drinking water	79
% of families with poor housing facilities	84

tions separated by 2 weeks were calculated to separate one infection from another (32); in cases of children without diarrhea, isolation of ETEC strains of different phenotypes from both stools and weekly rectal swabs were calculated. Specimens were collected at home (53%) or at the clinic (47%).

Detection of pathogens. Stool specimens as well as rectal swabs collected in Cary Blair medium were transported to the laboratory within 4 to 5 h of collection. Stool samples obtained from the children were routinely screened for enteric pathogens, including *V. cholerae* O1/O139, *Salmonella* spp., *Shigella* spp. and *Campylobacter jejuni* by standard techniques (2, 23, 36). Rotavirus was tested in diarrheal samples (36) and in a subsample of stools from healthy children ($n = 180$). Microscopic examination of stools was carried out for the detection of enteric parasites (2).

Detection of toxins and CFs. The detection of LT and ST was carried out using GM1 enzyme-linked immunosorbent assay (ELISA) methods (23, 29, 31). Six lactose-fermenting *E. coli* colonies from MacConkey agar were inoculated on GM1-coated microtiter plates containing Luria-Bertani broth for 18 h. The supernatant was tested for ST using an inhibition ELISA procedure (30) and for LT by using an anti-LT monoclonal antibody (29).

ETEC strains were cultured on CF antigen agar (21) and tested for the expression of CFA/I, CS1 to CS8, CS12, CS14, CS17, CS19, and CS21 by monoclonal antibody-based dot blot assay (23). Control ETEC strains were used as reference strains (29). Other diarrheagenic *E. coli* strains were not analyzed in the study.

Anthropometric analyses. Nutritional status of children was assessed at 3-month intervals. Children were considered undernourished (< −2 standard deviations [−2SD] of the weight for age *Z* [WAZ] score) or with stunted growth (< −2SD of length for age *Z* [LAZ] score) by comparison with the NCHS reference for the same age and sex using Epi Info 6 (CDC, Atlanta, GA).

Blood grouping. Specimens were typed for ABO using a slide agglutination procedure. (Biotec Laboratories, Suffolk, United Kingdom).

Data analyses. A Fox pro-based (version 2.6) data management system was used. Analyses were carried out using appropriate statistical programs (Epi Info version 2000, SPSS for Windows version 10.00, or Sigma Stat).

RESULTS

Study population. On average there were five family members per household (Table 1). Maternal and paternal literacy rates were 51% and 60%, respectively. About 84% lived in substandard dwellings made of low-cost housing materials. About 93% of families used a piped water supply available at central points, and 21% treated their water. About 80% of families shared use of public latrines.

Of the 321 newborns enrolled in the study, 27.5% were firstborns. These children contributed 193,983 child-days of observation over the 2-year study period (16). Data from 254 children were available for analyses for the 2-year period. The dropout rate was 21% (67 of 321): 55 were lost due to outmigration or refusal by parents; 12 deaths occurred (median age, 6 months) of which 6 were from diarrhea.

Incidence of diarrhea. Of 7,617 stools, 16% were from diarrheal children. In addition rectal swabs ($n = 4,313$) from a subgroup of children were screened for ETEC. About 89% of the children in the cohort suffered from one or more episodes of diarrhea (range, 1 to 14 episodes), and the average number of diarrheal days was 6.6 days/child/year. The incidence rate of diarrhea was 2.43/child/year. Vomiting and fever were observed in 19% and 14% of children, respectively. The duration of diarrhea was about 3 days in 80% of cases. Most children suffered from mild or moderate dehydration (severe dehydration seen in less than 1%). Children were hospitalized with acute watery diarrhea of various clinical severities (three children with severe dehydration, five with some, and four with no signs of dehydration).

ETEC diarrhea. ETEC was a first or primary cause of diarrheal agent isolated in the cohort in 37% (107 of 283) of children. The youngest child with ETEC diarrhea was 6 days old (median age, 12 months), with an incidence of 0.5 episode/child/year. The incidence increased from 0.2 episode in the first 6 months to 0.7 episode in the second 6 months and then plateaued to 0.5 episode. Of these, 0.5% ($n = 1$) suffered from severe dehydration and 10% ($n = 24$) suffered from some dehydration (mild to moderate), while the rest ($n = 217$) showed no signs of dehydration. Children up to 12 months of age had higher incidence of a primary case of ETEC diarrhea than children in the second year. There were 95 children in the first year, compared to 48 children in the second year of the 143 children with a primary episode of ETEC diarrhea (odds ratio [OR], 2.18; 95% confidence interval [CI], 1.42 to 3.35; $P < 0.0001$). The prevalence of ETEC diarrhea was lowest in the first 6 months of life (11%) and increased thereafter.

However, the proportions of diarrheas due to ETEC were comparable during the first (19.2%) and second (20%) years of life. ETEC was a cause of at least one diarrheal episode in 51% of the children (143 of 283 studied). Among these 143 children, 42% of the children suffered from more than one episode in the first 2 years. ETEC was also isolated from diarrheal stools (11 of 97; 11%) and 3-month routine stools (130 of 2,336; 5.5%) from mothers. In nine cases, when a child had ETEC diarrhea, the mother was also infected with ETEC of the same toxin and CF type (CFA/I, $n = 2$; CS5 plus CS6, $n = 2$; CF negative, $n = 5$). From children of mothers with ETEC diarrhea (11 cases), ETEC was isolated in 3 cases, although the strains were not of similar phenotypes.

Other enteric pathogens isolated. Rotavirus was a first or primary cause of diarrhea and was detected in 28% of children (80 of 283 children), and 38.5% of the children suffered from at least one episode (109 of 283 children). Four percent suffered from severe dehydration, 19% showed some dehydration, and 77% showed no dehydration. Primary rotavirus isolation was higher in the first year (73 of 109 [67%]) than in the second year (36 of 109 [33%]), and the overall prevalence was 10% (121 of 1,181). The highest rate of isolation was at 8

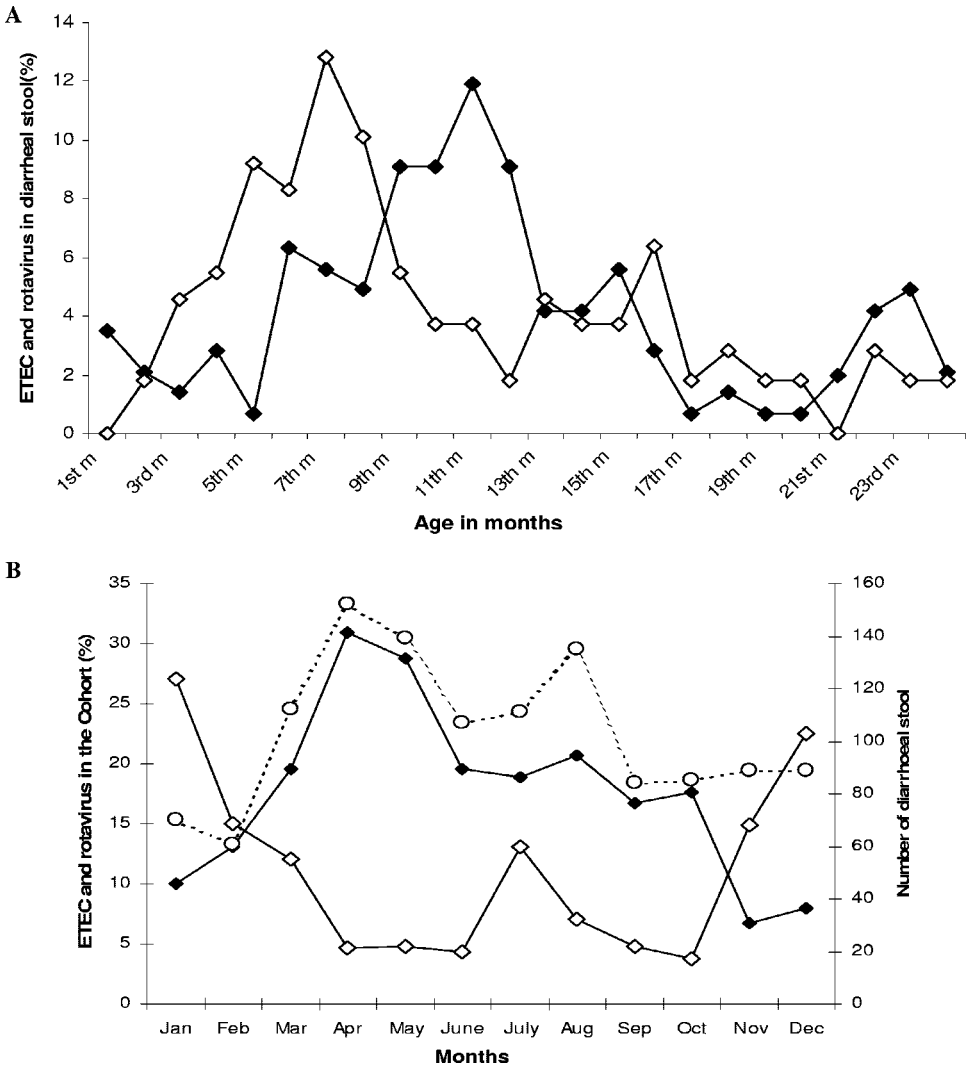

FIG. 1. (A) ETEC (◆) and rotavirus (◇) causing a primary diarrheal episode during the first 2 years of life. The prevalence (%) of children positive for the respective pathogens during each month is shown. (B) Seasonality of isolation of ETEC (◆) and rotavirus (◇) in total diarrheal stools (○) from the children in the cohort over the 2-year surveillance period.

months (Fig. 1A), and the prevalence decreased thereafter (OR, 1.90; 95% CI, 1.20 to 2.99; $P < 0.005$). Only 12 children had repeat episodes of rotavirus diarrhea (11%).

V. cholerae O1 and *C. jejuni* were isolated from 3 (0.24%) and 31 (2.5%) diarrheal stools, respectively (Table 2). No *Shigella* spp. and *Salmonella* spp. were isolated. Enteric parasites were detected in 14% of stools (Table 2), which included *Giardia lamblia* (0.75%), *Ascaris lumbricoides* (11%), *Trichuris trichuris* (1.7%), and *Endolimax nana* (0.75%). *Entamoeba histolytica* was not detected.

In children with ETEC diarrhea, rotavirus was also concomitantly detected in 15 cases.

Isolation of enteric pathogens from stools of healthy children. Enteric pathogens were isolated from 76% of the children. However, ETEC was more frequently isolated from diarrheal children (19.5%) than healthy children (8%) (OR, 2.76; 95% CI, 2.32 to 3.27; $P < 0.001$] (Table 2). Rotavirus was

TABLE 2. Enteric pathogens identified in diarrheal stools and in stool specimens from healthy children

Pathogenic agent	No. (%) of stools with agent/no. tested	
	Diarrheal	Nondiarrheal
ETEC[a]	242/1,234 (19.5)[c]	528/6,383 (8)
Shigella spp.	0	0
C. jejuni	31/1,234 (2.5)	93/6,383 (7.5)[c]
Salmonella spp.	0	0
V. cholerae	3/1,234 (0.24)	2/6,383 (0.03)
Rotavirus	121/1,181 (10.2)[c]	3/180 (1.7)
Enteric parasites[b]	57/403 (14.1)	283/792 (35.6)[c]

[a] Of the pathogens tested, ETEC was isolated from 51% of diarrheal children and was a primary or first cause of diarrhea in 37% of cases.
[b] Tested by routine microscopic examination of stools.
[c] Statistically significant difference in isolation between the two groups (P = 0.008 to 0.001).

TABLE 3. Toxins and colonization factors of ETEC isolated from stools of symptomatically or asymptomatically infected children

Toxin and CF type(s) produced	Symptomatic		Asymptomatic		*P*
	Total no. (%) of isolates	No. (%) isolates	Total no. (%) of isolates	No. (%) isolates	
ST	117 (49)		242 (46)		
CFA/I		26 (22)[a]		33 (13.6)	0.05
CS1 + CS3		3 (2.5)		7 (2.9)	
CS2 + CS3		4 (3.4)		8 (3.3)	
CS4 + CS6		3 (2.5)		2 (0.8)	
CS5 + CS6		8 (6.8)[a]		4 (1.6)	0.025
CS3		1 (1)		1 (0.5)	
CS6		22 (19)		36 (14.9)	
CS12		1 (1)		1 (0.5)	
CS14		7 (6.0)		12 (5.0)	
CS17		0 (0)		2 (0.8)	
CS19		2 (1.7)		0 (0)	
CS21		10 (8.5)		4 (1.6)	
CF negative		30 (25.6)		132 (54.5)[a]	0.001
LT/ST	73 (30)		101 (19)		
CFA/I		0 (0)		5 (5.0)	
CS1 + CS3		11 (15.1)		18 (17.8)	
CS2 + CS3		6 (8.3)		6 (5.9)	
CS4 + CS6		4 (5.5)		1 (1.0)	
CS5 + CS6		17 (23.4)[a]		9 (9.0)	0.016
CS6		2 (2.7)		3 (3.0)	
CS12		3 (4.1)		6 (5.9)	
CS14		5 (6.8)		1 (1.0)	
CS19		9 (12.5)[a]		0 (0)	0.001
CS21		1 (0.8)		0 (0)	
CF negative		15 (20.6)		52 (51.4)[a]	0.001
LT	52 (21)		185 (35)		
CS6		1 (2.0)		1 (0.5)	
CS7		9 (17.2)[a]		3 (1.6)	0.001
CS8		0 (0)		1 (0.5)	
CS12		0 (0)		2 (1.1)	
CS14		0 (0)		1 (0.5)	
CS17		3 (5.8)		7 (3.8)	
CS19		1 (2.0)		0 (0)	
CF negative		38 (73.0)		170 (92.0)[a]	0.001

[a] Statistically significant difference when ETEC strains isolated from symptomatic and asymptomatic cases were compared ($P = 0.05$ to <0.001).

isolated in only 3/180 monthly stools tested from healthy children (Table 2). *C. jejuni* and enteric parasites were more prevalent in control than in diarrheal stools. About 10% of rectal swabs from 60 healthy children were positive for ETEC, suggesting that the monthly surveillance that showed a prevalence of 8% did not markedly underestimate the true rate of asymptomatic infections.

Toxins and CFs on ETEC. Of the ETEC strains from diarrheal stools, 49% expressed ST, 30% expressed LT/ST, and 21% expressed LT only, and of the ETEC strains isolated from asymptomatic infections, 46% produced ST, 35% expressed LT, and 19% expressed both LT and ST (Table 3). LT/ST-expressing strains were more prevalent in diarrheal than healthy children (OR, 1.83; 95% CI, 1.27 to 2.63; $P < 0.001$), while LT-expressing ETEC strains were more common in healthy children (OR, 0.51; 95% CI, 0.35 to 0.73; $P < 0.001$) (Table 3). About 73% of LT ETEC strains isolated from stools of symptomatic children and 92% of LT ETEC strains isolated from asymptomatic children were negative for the 14 CFs tested.

The CFs studied were detected in a higher frequency in children with symptomatic infection: 66% in diarrheal children and 33% in control children (OR, 3.90; 95% CI, 2.79 to 5.45; $P < 0.001$) (Table 3). About 10% of ETEC strains from diarrheal stools ($n = 242$) expressed CFA/I, CS6, or CS5 plus CS6, and of those strains isolated from healthy children, CS6 and CFA/I were prevalent (≅7.0% each on 528 strains positive for ETEC) (Table 3). CS19-positive ETEC strains were only isolated from diarrheal cases, whereas the other CFs were found in more or less similar frequencies in the specimens. CF-positive ETEC strains in all three ETEC toxin types were more prevalent in diarrheal stools ($P < 0.001$ for all three comparisons).

Seasonality of isolation. In the urban community studied, ETEC diarrhea peaked in the spring and autumn months from March to October, while rotavirus predominated from November to February (Fig. 1B). Isolation of ETEC from diarrheal children was higher during March to June than between July and October. The isolation of ST and LT/ST ETEC strains (60%) was higher than that of LT (40%) in the earlier part of the year (March to June) ($P = 0.014$), whereas LT ETEC (53%) was more prominent than the ST- and LT/ST-express-

TABLE 4. Association between malnutrition and ETEC diarrhea in children in the cohort[a]

Age (mo)	% of children with ETEC diarrhea[b]			
	WAZ		LAZ	
	< −2SD	≥ −2SD	< −2SD	≥ −2SD
6	10.8	3.4	6.9	3.6
12	17.0†	6.9	13.8*	8.4
18	14.5†	7.5	13.0*	8.8
24	13.3†	8	12.6*	9.1

[a] Assessed in comparison with the NCHS data in children with ETEC only (excluding mixed infection with rotavirus). The underweight (WAZ) and stunted growth (LAZ) levels were determined every 3 months in the cohort.

[b] * and † indicate statistically significant differences in the percentages of children with ETEC diarrhea who were undernourished (< −2SD) or growth stunted (< −2SD) compared to those who were not. *, $P = 0.05$ to 0.001; †, $P < 0.001$.

ing ETEC strains between July and October (25%) ($P < 0.001$). A similar distribution of toxin types was seen on ETEC strains isolated from routine sampling of monthly stools. Between March and June, ST/LT and ST ETEC strains (51%) were more prominent than LT ETEC strains (40%) ($P = 0.019$); between July and October, the LT phenotypes (47%) were more prominent than the ST and LT/ST phenotypes (29%) ($P < 0.001$).

In children with diarrhea, higher isolation of the predominant CF-positive ETEC strains was seen in the spring (CFA/I [$n = 21$], 15.6%, and CS5 plus CS6 [$n = 20$], 14.8% of 135 ETEC strains isolated) than in the latter half of the year (CFA/I [$n = 3$], 3.7%, and CS5 plus CS6 [$n = 4$], 4.9% of 81 ETEC strains isolated) ($P = 044$ to 0.014). Seasonality in the isolation of CS6- or CS1- to CS3-expressing ETEC strains was not seen for CS6- or CS1- to CS3-positive ETEC strains from diarrheal children (P not significant). Differences in seasonal distribution of CF-positive ETEC strains were not seen for any of the major CF types for strains isolated from healthy children.

Anthropometric measurements and nutritional status. Only newborn children weighing ≥2 kg were enrolled: 15% were underweight, and 8% were growth stunted at birth. At 2 years of age, 55% were underweight and 38% were growth stunted. Cumulative analyses of nutritional status and relationship with ETEC diarrhea at 6-month intervals showed that by 12 months of age a higher percentage of children with ETEC diarrhea were underweight (< −2SD) and growth stunted (< −2SD) compared to children with non-ETEC diarrhea ($P < 0.001$) (Table 4), which persisted at 18 and 24 months of age. At 2 years, more children who had experienced ETEC diarrhea were growth stunted (45% [60 of 134]) compared to those who had not had ETEC diarrhea (31% [37 of 120]) ($P = 0.035$). No association was found between the incidence of rotavirus and nutritional status.

ABO blood group and ETEC diarrhea. In the cohort, 34% were blood type O, 24% were type A, 30% were type B, and 12% were type AB (Table 5), which was representative of the ABO group in the general population (22). Children with blood type A or AB were more susceptible to ETEC diarrhea than those with blood group O ($P = 0.032$ to 0.023). Children in the A or AB blood groups were more often infected with ST-expressing ETEC (33/63 and 18/30, respectively) than those in the O blood group (31 ST ETEC of 89 children) ($P = 0.046$ to 0.027). However, the overall frequencies of diarrheal episodes were quite similar in all four groups (A = 4.2, B = 4.4, AB = 4.5, and O = 4.5 episodes/child).

Repeat infection with ETEC. We also wanted to determine if a primary ETEC infection prevented reinfection with ETEC of a similar CF or toxin type. This was analyzed for ETEC strains expressing the most frequent CFs, LT, ST, or ST/LT (Table 6). A diarrheal infection with CFA/I ($n = 24$), CS1 plus CS3 ($n = 11$), CS2 plus CS3 ($n = 10$), or CS5 plus CS6 ($n = 24$) ETEC was not followed by a repeat episode of diarrhea caused by a homologous CF type, whereas repeated diarrheal episodes were seen with CS6 expressing ETEC (3 of 19 [16%]). Also asymptomatic infections with any of the CF ETEC, including CS6, seemed to prevent further diarrhea due to ETEC of the same CF type. Infections with LT-producing ETEC ($n = 32$), on the other hand, were frequently followed by LT ETEC diarrhea, both in children with an initial symptomatic infection (8 of 32 [25%]) and in those with asymptomatic infection (12 of 141 [9%]). Infections with ST or ST/LT strains were not protective from symptomatic or asymptomatic infections with these toxin phenotypes. Protection was only seen when strains expressed CFA/I, CS1 plus CS3, CS2 plus CS3, and CS5 plus CS6. These children, however, were infected in the course of the follow-up period with other CF-expressing ETEC strains, and protection from these different CFs was not seen.

Effect of breast-feeding on diarrhea. All children were breast-fed (27% exclusively and the rest with water, honey, or sugar syrup) until 6 months of age. About 61% were breast-fed at various frequencies up to 2 years of age. There was no

TABLE 5. Association between number of total diarrheal and ETEC diarrheal episodes and blood group in children in the cohort

ABO blood group	No. (%) of:		P (OR; 95% CI)	Total no. of episodes		P (OR; 95% CI)
	Children[a]	Subjects with ETEC diarrhea		Diarrheal	ETEC	
A	63 (24)	33 (24)	A vs O = 0.621 (1.18; 0.59–2.36)	267	61	A vs O = 0.032[b] (1.56; 1.04–2.35)
B	79 (30)	37 (28)	B vs O = 0.84 (0.94; 0.49–1.81)	347	66	B vs O = 0.315 (1.24; 0.83–1.84)
AB	30 (12)	21 (15)	AB vs O = 0.065 (2.50; 0.95–6.66)	135	34	AB vs O = 0.023[b] (1.77; 1.08–2.91)
O	89 (34)	43 (32)	O vs A, AB, and B = 0.48 (0.83; 0.48–1.43)	401	64	O vs A, AB, and B = 0.029[c] (0.69; 0.50–0.96)

[a] Blood groups were determined for only 261 children.

[b] Statistically significant differences in frequency of ETEC diarrheal episodes between children of blood group A compared to O and between children of group AB compared to O.

[c] Statistically significant differences seen in frequency of ETEC diarrheal episodes between children of group O compared to groups A, AB, and B.

TABLE 6. Repeated infection with ETEC expressing homologous colonization factors or the LT and ST after an initial symptomatic or asymptomatic episode

Initial infection[a]	Repeated infection[b]	No. of initial episodes	
		Symptomatic	Asymptomatic
CFA/I		24	47
	Symptomatic	0	1
	Asymptomatic	1	2
CS1 + CS3		11	27
	Symptomatic	0	0
	Asymptomatic	0	2
CS2 + CS3		10	12
	Symptomatic	0	0
	Asymptomatic	1	0
CS5 + CS6		24	20
	Symptomatic	0	0
	Asymptomatic	0	0
CS6		19	50
	Symptomatic	3	0
	Asymptomatic	2	11
LT		32	141
	Symptomatic	8	12
	Asymptomatic	17	119
ST		64	153
	Symptomatic	19	34
	Asymptomatic	54	135
ST/LT		53	84
	Symptomatic	6	7
	Asymptomatic	17	44

[a] The LT-positive strains from symptomatic and asymptomatic infections were mostly CF negative (82%). Of the ST strains (ST and ST/LT), 38% of strains were CF negative in symptomatic and asymptomatic infections. Only the most frequently isolated CF phenotypes were analyzed. To determine infection and reinfection rates from homologous CFs (LT or ST), episodes of symptomatic or asymptomatic infections separated by 2 weeks were calculated only for isolations in stools and both stools and rectal swabs from healthy children.

[b] Repeat episode of a symptomatic diarrheal or asymptomatic infection with the same ETEC phenotype as that in the initial infection.

difference in the prevalence of diarrhea or ETEC diarrhea among children who were exclusively breast-fed compared to those who got mixed feedings when analyzed for the first 6, 12, and 24 months of the study (*P* not significant) (data not shown).

DISCUSSION

The study shows that in a densely populated urban housing area in Bangladesh, the majority of children suffer from diarrhea ranging from a minimum of 1 to 14 episodes and ETEC was the major cause. Almost half of the children suffered from ETEC diarrhea, and repeated infections were common. ETEC was also isolated from diarrheal and routine stools of mothers, showing that the fecal/oral route of transmission and carriage are factors that contribute to high rates of infection.

The incidence and prevalence of ETEC diarrhea were similar to the rates predicted in other settings (4, 11, 24, 35); furthermore, ETEC was detected in significantly higher frequencies in diarrheal children than from healthy children. In community-based studies in developing countries, spanning different continents, ETEC has also been found to be the one of the common pathogens isolated from young children in different studies (2, 3, 11, 26, 34). However, in a cohort of children in Guinea Bissau (32), where stools from both healthy and control children were analyzed, rotavirus was the most common.

In the present study, rates of isolation of *V. cholerae* as well as *Shigella* or *Salmonella* were low to negligible. The low isolation rates of these pathogens are probably not due to methodological problems, since the specimens were cultured within a few hours after collection and the microbiological techniques used in this study are similar to those used in the routine surveillance system of laboratories at ICDDR, B (3, 23). The low prevalences of *V. cholerae*, *Shigella*, and *Salmonella* are possibly age related and have been observed in other studies (23, 26). Enteric parasites were isolated from diarrheal stools and control specimens. We used only routine microscopic examination for detection of parasites in this study and could not use more sensitive methods (15) that can give a more accurate estimation of prevalence.

The seasonality of ETEC diarrhea in the community was similar to results of hospital-based studies in Bangladesh (3, 23). In addition, we observed seasonality for toxin types and the CFs CFA/I and CS5 plus CS6. In the spring months, the prevalence of the ST and prominent CFs was highest. A high preponderance of ST ETEC strains was seen in the spring in Egypt (26). ETEC strains producing ST were most common in children with symptomatic infections (almost 80% were ST- or LT/ST-expressing strains), with CFs predominantly being present on these strains. ETEC strains expressing LT alone were, however, isolated frequently from routine monthly stools which were usually CF negative compared to the LT/ST or ST toxin phenotypes. The low prevalence of CFs on LT ETEC strains has been observed in Argentina, Bangladesh, and Egypt (23, 26, 34).

A considerably higher proportion of ETEC strains isolated from diarrheal stools were CF positive in comparison to controls, supporting the concept that possession of CFs is needed for induction of virulence and studies are needed to determine if there are clones that are pathogenic (1, 11–13, 19). We also found a high frequency of ETEC reinfections. However, initial infections with ETEC strains expressing CFA/I, CS5 plus CS6, and combinations of CS1 to CS3 were not followed by symptomatic or asymptomatic infections with ETEC strains expressing the homologous CF. Our data on the capacity of major CFs to prevent reinfection fit in well with those available from animal and human studies and support the present strategy of including them in ETEC vaccines (28). However, this is discordant with results observed from epidemiological studies in Guinea Bissau, which focused on both weekly isolations from children and infections rather than disease (27).

Symptomatic or asymptomatic infection with LT ETEC was often followed by reinfection of the same toxin type. Although studies (9, 27) show that vaccination or natural infection by the LT phenotype is protective, our study was unable to demonstrate a role of LT ETEC in preventing reinfection. The reasons for these discrepant results are obscure, but they may be explained by the different study designs and community set-

tings and also because strains in different locations may produce different levels of toxins and disease severity. We also observed that ETEC of the ST or the ST/LT phenotype did not protect from further ETEC infections of the same toxin phenotype.

Studies including those in Bangladesh (8) and Mexico (11, 20) have suggested that breast-feeding is associated with a reduced risk of ETEC diarrhea in infancy. However, it has also been shown that this protection does not last over the first 2 to 3 years of life (8, 26). In the present cohort, all children were breast-fed in the first 6 months after birth and we also observed that the rate of ETEC diarrhea was lower during this period compared to rest of the study period, which suggests an overall protective effect of breast-feeding only in infancy.

We could not detect rates of ETEC diarrhea in the children in our cohort who were exclusively breast-fed lower than those in children given other types of liquid and weaning food together with breast milk in the initial 6 months. No difference was seen in the rates of diarrhea among these two groups of children in the rest of the 2-year study period.

ETEC diarrhea appeared to be associated with the nutritional status of the children. Thus, those children who had experienced one or more episodes of diarrhea due to ETEC as a single pathogen were significantly more malnourished and growth stunted by 2 years of age than those without any episode of ETEC disease. This is in agreement with earlier studies which have also shown that ETEC diarrheal episodes in early childhood may have a negative effect on the growth of the children (6). However, additional factors that may dispose these children to malnutrition include other enteric infections as well their overall lower socioeconomic living conditions and contaminated environment (11).

In our study, we found ETEC diarrheal episodes were more common in children in the AB or A group than those in the O blood group. A higher incidence of *Entamoeba histolytica*-associated diarrhea has been seen in those in the O and AB groups (16), while a relationship has been found with cholera and the O blood group but not ETEC diarrhea (7, 33). It has recently been shown that CFA/I binds to glycospingolipids that are associated with blood group antigens that may be expressed on epithelial cells in the small intestine in humans (5, 17). Thus, the presence of certain blood group antigens on intestinal epithelial cells may predispose to ETEC expressing certain CFs.

An improved understanding of the natural history of ETEC disease during early childhood in areas of high endemicity and factors that may predispose to these diarrheas may assist in the development of interventions such as effective vaccines for developing countries. The results from this study may contribute to such knowledge.

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