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Diarrhea Burden Due to Natural Infection with Enterotoxigenic *Escherichia coli* in a Birth Cohort in a Rural Egyptian Community

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Enterotoxigenic *Escherichia coli* (ETEC) is commonly associated with diarrhea in Egyptian children. Children less than 3 years old in Abu Homos, Egypt, had approximately five diarrheal episodes per child every year, and at least one of these episodes was due to ETEC. The epidemiology of ETEC diarrhea among children living in a rural Egyptian community was further evaluated in this study. Between January 2004 and April 2007, 348 neonates were enrolled and followed for 2 years. Children were visited twice weekly, and a stool sample was obtained every 2 weeks regardless of symptomatology. A stool sample was obtained whenever a child had diarrhea. From the routine stool culture, five *E. coli*-like colonies were selected and screened for heat-labile and heat-stable toxins by GM1 enzyme-linked immunosorbent assay (ELISA) and further typed for colonization factor antigens by dot blot assay. Incidence of ETEC infection was estimated among children with diarrhea (symptomatic) and without diarrhea (asymptomatic). Incidence of diarrhea and ETEC-associated diarrhea was 7.8 and 1.48 per child-year, respectively. High risk of repeated ETEC diarrhea was associated with being over 6 months of age, warm season, male gender, and crowded sleeping conditions. Exclusive breast-feeding was protective for repeated ETEC infection. ETEC-associated diarrhea remains common among children living in the Nile Delta. The protective role of breast-feeding demonstrates the importance of promoting exclusive breast-feeding during, at least, the first 6 months of life.

Diarrheal disease continues to be a significant cause of morbidity and mortality worldwide. As many as one million diarrhea deaths per year, with most recorded in children less than 5 years old, are estimated to occur in developing countries, ranking second among all causes of pediatric deaths worldwide (1). Evidence has recently emerged describing long-term consequences of early childhood diarrheal disease on growth, as well as on physical and cognitive development, that translates into extra burdens on economic and health care systems and society (2). Enterotoxigenic *Escherichia coli* (ETEC)-associated diarrhea is the most common bacterial diarrhea affecting children under 5 years old living in developing countries, as well as travelers to these countries (3). In these populations, ETEC causes an estimated 280 million diarrheal episodes and more than 400,000 deaths annually (4).

Because of regional variations in the epidemiology and bacteriologic characteristics of ETEC-associated diarrhea, local surveillance data are critical to assist vaccine development and protect against disease (5, 6). Unfortunately, such population-based studies in developing countries often are lacking (7); however, two large studies were conducted in Egypt: a population-based study for a cohort of children under 3 years of age (8) and a hospital-based study enrolling children <5 years of age presenting with severe diarrhea (9). The characterization of ETEC isolates obtained from both studies was reported (10, 11), while that of the current study was recently reported (12). The current study, a population-based study, was undertaken to narrow the knowledge gaps in the epidemiology of diarrhea, particularly ETEC-associated diarrhea, in Egyptian children almost 10 years later. Additionally, the current study aimed to identify the frequency of ETEC detection as a pathogen that causes disease versus detection as a pathogen that causes no disease (colonizer).

MATERIALS AND METHODS

Study population. Mapping and numbering of the houses and a house-to-house census were performed for six selected villages located in Abu Homos, a rural district located in the Nile Delta in northern Egypt. A census was performed for 1,916 households with a total population of 15,675 people. As part of the census, baseline sociodemographic and household hygiene information was collected. The study human use protocol NAMRU3.2003.0011 (IRB no.145) was approved by the U.S. Naval Medical Research Unit No. 3 Institutional Review Board in compliance with all applicable federal regulations governing the protection of human subjects.

Enrollment. At the commencement of the study in January 2004, children <3 months old living in the census area were eligible for enrollment. Thereafter, enrollment was restricted to infants in the first 28 days of life, and enrollment was continued until 348 children were enrolled. Written informed consent was obtained from the caregiver of the eligible child. At enrollment, children were examined by the study physician and anthropometric measurements were recorded. Participation in the study for both the enrolled child and the child's mother was completed when the

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child reached his/her second birthday or the completion of the study in April 2007, whichever occurred first.

Surveillance. Study children were visited twice weekly in their homes. On each visit, a study team member collected data on the health of the study child over the preceding 4 days using a standardized form. If a caregiver indicated that a child had diarrhea, information on the frequency of loose stools, associated symptoms, and feeding patterns was collected along with a stool specimen and two rectal swabs. In addition, every 2 weeks regardless of symptoms, a stool sample was collected from every child. Symptomatic children (S) were examined by a study physician, and if dehydration was noted, the child was referred to a village clinic for therapy and further evaluation. Study physicians classified the degree of dehydration according to the WHO criteria of "none," "some," or "severe" (13). Caregivers were instructed to bring the child to the field clinic at any time if the child had diarrhea or if it was perceived that medical assistance was needed.

Definitions. An asymptomatic control (AS) was defined as a child without a loose or liquid stool for a period, including the day of the biweekly, routine, nondiarrhea sample and 3 days before or after. Cases were defined as patients with episodes of diarrhea detected during the twice-weekly visits. A diarrheal day was defined as the occurrence of at least three nonformed stools (or at least one, if bloody) in a 24-h period. For breast-fed children, diarrhea was defined as at least one bloody stool or an increase in frequency or a reduction in consistency of the stools, compared with a caregiver's assessment of normal for the child. The duration of a diarrheal episode was defined as beginning on the first diarrheal day after at least three consecutive nondiarrheal days and ending when followed by at least three consecutive nondiarrheal days. An acute diarrheal episode is defined as a diarrheal episode of less than 14 days' duration; a persistent diarrheal episode was defined as a diarrheal episode lasting more than 14 days. Bloody diarrhea (dysentery) was noted if the caregiver reported visible blood in the stool.

Pathogens, including ETEC, were considered to be associated with a diarrheal episode if they were detected on any day of a diarrheal episode. An ETEC-associated diarrheal episode denoted an episode in which at least one lactose-fermenting colony obtained from a diarrheal specimen expressed the heat-labile (LT) enterotoxin, heat-stable (ST) enterotoxin, or both. An ETEC-associated diarrheal episode was considered to have a copathogen if *Shigella* spp., *Campylobacter* spp., *Salmonella* spp., or rotavirus was identified in a fecal specimen collected during the episode.

Seasons of the year were divided into cool (November to April) and warm (May to October) months. A child was defined as being breast-fed if she/he received any breast milk and as exclusively breast-fed if that was the only source of nutrition. Socioeconomic status of the family was expressed as a score from 0 to 29 based on the type of household (apartment versus detached home) and the number of expensive items found in the household (washing machine, car/truck, radio, television, etc.) (14). A score of <8 points was defined as low socioeconomic status (15), while a score of ≥ 8 points represented the moderate-and-above socioeconomic status (nonpoor) (16). Assessment of crowding was based on the number of residents per sleeping room and was categorized by tertiles into low (0.5 to <3.5 persons per room), medium (≥ 3.5 and <4.75), and high (≥ 4.75).

Specimen collection and transportation. A stool sample and two rectal swabs were collected during twice-weekly visits when a child had diarrhea and collected every 2 weeks if no diarrhea was reported. Stool if collected or rectal swabs were placed in both Cary-Blair (CB) and buffered-glycerol-saline (BGS) transport media. All specimens were transported immediately on ice packs to the Abu Homos field laboratory. Rectal swabs were refrigerated at 2 to 8°C; stool samples were aliquoted in 3 cryotubes (2 ml each) and stored at −20°C. Within 3 days of collection, specimens were sent to the U.S. Naval Medical Research Unit No. 3, Cairo, Egypt (NAMRU-3), where the rectal swabs were evaluated and stool samples were transferred to a −70°C freezer for long-term storage.

Laboratory evaluation and procedures. Fecal material from the rectal swabs was plated onto MacConkey-lactose agar and *Salmonella-Shigella* (SS) agar; CB swab material was also plated onto *Campylobacter* blood agar with Skirrow's supplement (Oxoid, Hampshire, United Kingdom) and thiosulfate citrate bile salts sucrose (TCBS) agar (Becton Dickenson, Cockeysville, MD, USA). Plates were incubated at 35 to 37°C overnight and checked for growth after 16 to 24 h. *Campylobacter* plates were incubated at 42°C in a candle jar for 48 h before checking for growth. Conventional microbiological procedures (17) were used to identify enteric bacterial pathogens, including *Shigella*, *Salmonella*, *Campylobacter*, and *Vibrio* spp.

From the MacConkey-lactose agar plate, five lactose-fermenting (red) colonies with *E. coli*-like morphology were preserved in Trypticase soy broth containing 15% glycerol and were stored at −70°C until they were tested. *E. coli*-like colonies were evaluated for expression of LT and ST using GM1 enzyme-linked immunosorbent assays (ELISAs) (18, 19). Colonies positive for either enterotoxin were further tested for the expression of colonization factor antigens (CFA), including CFA/I, CS1 to CS8, CS12, CS14, and CS17, using an immunodot blot method (20, 21). Rotavirus was tested in frozen stool samples using a commercially available ELISA (Premier Rotaclone; Meridian Bioscience, Cincinnati, OH, USA) according to the manufacturer's instructions.

Analysis. Crude and adjusted diarrhea incidence rates were calculated by dividing the number of episodes by the number of person-years at risk (total person-years of follow-up minus the duration of diarrheal episodes).

Logistic regression analyses were performed to determine the odds of incidence of ETEC diarrhea in children, given covariates such as gender, crowding, absence of a household latrine, absence of a household garbage container, breast-feeding status (exclusive and not exclusive versus no breast-feeding), and socioeconomic status of the families. All exploratory variables with a significance level of less than 0.05 were included in the multivariate models and were adjusted for age and season.

Calculation of time to first episode was performed using Kaplan-Meier (KM) analysis for the cohort. To measure the hazard of acquiring the infection as repeated ETEC infection, logistic regression models were used adjusting for confounding factors such as child's age, child's gender, season, maternal education, breast-feeding, household crowding, and socioeconomic status. The analysis was restricted to episodes associated with ETEC, *Campylobacter*, *Shigella*, *Salmonella*, and rotavirus.

The ETEC-associated diarrhea-attributable fraction of all diarrheal episodes was calculated using the following formula (22): proportion of ETEC-associated diarrhea = number of children with ETEC-associated diarrhea/number of children with all types of diarrhea $\times$ 100.

Variables with significant crude relative risk, as well as multiple observations of individual subjects, were used in adjusting the relative risk. SAS software (version 9.1; SAS Institute Inc., Cary, NC, USA) was used for all analyses.

RESULTS

Characteristics of villages and study population. The two groups of villages were populated with 15,675 people in 1,916 households. The majority of the heads of household were farmers. The mean age for mothers enrolled in the study was 25; 44% were illiterate, and only 6% worked outside the home. The mean age for fathers of children enrolled in the study was 31; approximately two-thirds (65%) were illiterate and 89% worked outside the home. Sixty percent of the 348 families involved in the study had moderate socioeconomic status, with a mean $\pm$ standard deviation (SD) socioeconomic score of 9.8 $\pm$ 1.5, and 39% had low socioeconomic status, with a mean socioeconomic score of 5.6 $\pm$ 1.7. The majority of the families (85%) lived in village houses which consisted of a one-floor building with a barn in the backyard. Fifty-four percent of houses had cement tile floors, compared with a

floor of earth, sand, or other material. Houses typically utilized electricity (99%), and almost all houses had a municipal water source for drinking and cooking. A municipal water source was the main source for washing and bathing in 91% of houses. Eighty-eight percent of houses had nonflush latrines. Fifty-five percent of all latrines drained to a sealed pit, and the rest drained to a sewage system. Only 19% of houses had garbage containers either inside or outside the house. Of the 414 potentially eligible children, seven were excluded, as they were nonpermanent residents of the area, and 59 refused to participate. Of the 348 children enrolled, 285 (82%) completed the study. The remaining children either moved from the study area ($n = 18$), refused to continue follow-up ($n = 38$), or died ($n = 7$; two accidents, two postoperative, two congenital anomalies, and one due to encephalitis). Mean duration of the follow-up period for all children was 20.3 months. Females represented 51% of enrolled children.

Over the study period, questionnaires were successfully obtained during 54,950 twice-weekly visits (95.3% of planned visits); of these, diarrhea was noted to have occurred in 7,596 visits (13.8%). From the diarrheal visits, 6,440 (84.8%) stool specimens and 7,582 (99.8%) rectal swabs were collected. In total, 77% of scheduled biweekly (routine) visits were completed in full (questionnaire completed and sample collected), during which 9,539 stool samples were collected when the child was not sick; the median number of stool samples collected from each child was 31 (interquartile range [IQR], 23 to 37).

ETEC infection. A total of 316 children (91%) were infected with ETEC at least once during the period of follow up, while the vast majority of children ($n = 269$) had at least one symptomatic ETEC infection. On the other hand, 47 children never experienced an ETEC diarrheal episode but were colonized with ETEC expressing a limited number of CFA types. A total of 208 ETEC isolates were recovered from this group throughout the study period; 50% of the isolates express LT enterotoxins, and the other 50% were ST alone (42%) or associated with LT (8%). The most common CFAs were CFA4 (12%), including CS6 (6%), CFA/I (5%), and CS12 (5%). Of interest, the children who did not have any ETEC-associated diarrhea also did not have any *Campylobacter*-, *Shigella*-, or *Salmonella*-associated diarrhea during the first 6 months of life. However, they had four episodes of rotavirus-associated diarrhea during the same period. In the 47 children who never had symptomatic ETEC infection during the study, the incidence rates of *Campylobacter*- and rotavirus-associated diarrhea were much lower than the incidence rates of both pathogens in the children who had ETEC-associated diarrhea (0.23 versus 0.63 episodes/child-year and 0.26 compare to 0.41 episodes/child-year, respectively).

Incidence and characteristics of ETEC diarrhea. ETEC was the most common pathogen isolated from stool samples (756/4,001 or 18.9%), resulting in an incidence rate of 1.5 episodes per child-year. Over the course of the study, 77% of study children had at least one episode of ETEC-associated diarrhea, with 50% of children having an episode by 7 months of age. A total of 632 episodes were due to ETEC as a sole pathogen, with an incidence rate of 1.24 episodes per child-year. Copathogens were isolated with ETEC in 124 diarrheal episodes (16.4%) and included *Campylobacter* ($n = 57$, 46%), rotavirus ($n = 40$, 32%), *Shigella* ($n = 16$, 13%), and *Salmonella* spp. ($n = 1$, <1%). In 10 episodes (8%), ETEC was isolated together with more than one pathogen. Of the total diarrheal episodes associated with ETEC, 13.6% were preceded by a positive ETEC isolation from a healthy stool sample collected from the same child during the 2 weeks prior to the episode. The median duration between a positive ETEC detection from a healthy stool sample to the occurrence of an ETEC episode was 10 (IQR, 6 to 16) days.

Nine percent of all initial episodes of diarrhea were due to ETEC, which represented 56% of all initial episodes with identified pathogens. The mean age of children who had initial infection with ETEC was 7.5 months.

The incidence of ETEC-associated diarrhea, calculated as episodes/child-years of follow-up, was 1.17 episodes per child-year in the first 6 months of life, peaked at the second 6 months at 1.83 episodes/child-year, and then declined to 1.59 and 1.31 episodes/child-year in the age groups 12 to 17 and 18 to 23 months, respectively (Table 1). Overall incidence of ETEC diarrhea was the same in the first and second years of life at 1.5 episodes/child-year each; however, during the first 6 months of life, 12% of diarrheal episodes were due to ETEC (ETEC-attributable fraction of diarrheal episodes), and this percentage increased gradually to 24% by the end of the second year of life. Incidence of ETEC diarrhea occurred more frequently during warmer months, with an incidence rate of 2.1 episodes/child-year, compared to that of cooler months, with an incidence rate of 0.75 episodes/child-year ($P < 0.001$) (Table 1).

The LT-ETEC incidence rate was 0.65 episodes/child-year. The incidence of ETEC continued to rise after the first 6 months of life, peaking at 12 to 17 months of age (Table 1). Risk of LT-ETEC was significantly higher in the warm season (adjusted relative risk [RRa] = 1.89; 95% confidence interval [CI], 1.50 to 2.38; $P < 0.001$) (Table 2).

The ST-ETEC incidence rate was 0.57 episodes/child-year (Table 1). ST-ETEC diarrhea risk increased in the age group of 6 to 11 months compared to that in the first 6 months of life (RRa = 1.37; 95% CI, 0.98 to 1.90) and was four times more likely to occur in warm months (RRa = 3.91; 95% CI, 2.91 to 5.25; $P < 0.001$) than in cold months (Table 2).

The LT/ST-ETEC incidence rate was 0.26 episodes/child-year (Table 1). LT/ST-ETEC diarrhea risk was 6 times higher in the warm season (RRa = 6.04; 95% CI, 3.63 to 10.06; $P < 0.001$) and increased significantly among males compared to females (RRa = 1.81; 95% CI, 1.26 to 2.60; $P < 0.01$). However, the risk did not differ among different age groups (Table 2).

Exclusive breast-feeding was associated with a significant decrease in the risk of ST- and LT/ST-ETEC-associated diarrhea (47% and 19%, respectively; Table 2).

Approximately half of the children with ETEC-associated diarrhea reported fever, and about 25% reported vomiting. Mild to moderate dehydration was detected in 2.3% of episodes of ETEC-associated diarrhea. Five children had rectal prolapse that was thought to be related to their episode of ETEC-associated diarrhea. ETEC diarrhea presented as acute in 92% of episodes, as dysentery in 3.4%, and as persistent in 4.5%. Comparison of ETEC clinical characteristics and other pathogens detected was presented in Table 3.

ETEC-associated asymptomatic infection. Of the 348 children, 303 children (87%) had at least one asymptomatic infection of ETEC. LT-ETEC was the most common ETEC-associated enterotoxin isolated from asymptomatic children (56% [721/1,299 isolates]) followed by ST-ETEC (35% [449/1,299 isolates]) and LT/ST-ETEC (10% [129/1,299 isolates]) in asymptomatic stool samples.

TABLE 1 Incidence rate and incidence relative risk of ETEC diarrhea by demographic characteristics, Abu Homos, Egypt, 2004 to 2007

Feature	ETEC		
	IR (no. of episodes)[c]	Crude IRR (95% CI)	Adjusted IRR (95% CI)[d]
Child's age (mo)			
0–5	1.17 (148)	1.00	1.00
6–11	1.83 (238)	1.54 (1.25, 1.89)[g]	1.35 (1.07, 1.68)[f]
12–17	1.59 (203)	1.31 (1.06, 1.62)[e]	1.15 (0.91, 1.45)
18–23	1.31 (167)	1.08 (0.86, 1.34)	0.87 (0.66, 1.15)
Child's gender			
Male	1.63 (411)	1.24 (1.07, 1.43)[f]	1.23 (1.07, 1.43)[f]
Female	1.33 (345)	1.0	1.0
Breast-feeding	0.79 (36)	0.53 (0.37, 0.76)[g]	0.50 (0.33, 0.77)[f]
Exclusively	1.56 (523)	1.05 (0.89, 1.23)	0.83 (0.68, 1.02)
Not exclusively	1.52 (197)	1.0	1.0
Not breast-fed			
Season at follow-up[a]			
Warm	2.13 (576)	2.90 (2.46, 3.43)[g]	2.89 (2.45, 3.42)[g]
Cold	0.75 (180)	1.0	1.0
Maternal education			
Some schooling	1.32 (281)	0.82 (0.71, 0.95)[e]	0.83 (0.71, 0.97)[e]
No schooling	1.59 (475)	1.0	1.0
Garbage container in house			
Yes	1.27 (119)	0.84 (0.69, 1.02)	0.92 (0.75, 1.13)
No	1.52 (637)	1.0	1.0
Sanitary latrine			
Yes	1.53 (629)	1.22 (1.0, 1.48)[e]	1.21 (1.002, 1.47)[e]
No	1.26 (127)	1.0	1.0
Crowding[b]			
≥ 4.75	1.47 (125)	1.11 (0.90, 1.36)	1.08 (0.87, 1.33)
≥ 3.5 to < 4.75	1.65 (312)	1.23 (1.05, 1.44)[e]	1.18 (1.01, 1.39)[e]
< 3.5	1.35 (319)	1.0	1.0
Socioeconomic status			
Poor	1.47 (178)	1.0 (0.85, 1.18)	0.93 (0.78, 1.10)
Nonpoor	1.48 (578)	1.0	1.0

[a] Warm season from May to October; cold season from November to April.
[b] No. of persons per bedroom.
[c] IR, incidence rate. Episodes per child-years of follow-up.
[d] IRR, incidence relative risk. Adjusted for statistically significant univariate predictors, including child's age (6-month intervals), child's gender, season of follow-up, breast-feeding status, maternal education, sanitation, and crowding, using multivariate Poisson regression.
[e] $P < 0.05$.
[f] $P < 0.01$.
[g] $P < 0.001$.

Colonization with nonsymptomatic ETEC infections was directly related to the age of the child, with 13% of ETEC colonization being in children under 6 months of age, increasing to 32% at 23 months of age. Consequently, the prevalence of colonization with ETEC-associated enterotoxins detected increased gradually from 11%, 17%, and 13% for LT, ST, and LT/ST, respectively, in the age group of less than 6 months, to be 36%, 26%, and 36%, respectively, in the age group of 18 to 23 months (Table 4).

Prevalence of ETEC colonization did not differ according to gender. Prevalence of enterotoxins detected also did not differ according to gender, except for the LT/ST enterotoxin, which was more frequent among females (58%; $P = 0.02$; Table 4).

Asymptomatic ETEC infection was more frequent during warmer months (61%). Enterotoxin expression was also significantly higher during warmer months ($P < 0.0001$); 54% of LT enterotoxin was expressed during summer months, while the percentages were 69% and 67% for ST and LT/ST, respectively (Table 4).

Risk factors for ETEC infection. For diarrheal episodes due to ETEC, crude relative rates for the association between selected sociodemographic or environmental factors and the incidence of ETEC diarrhea were statistically significant for age, gender, season, maternal education, breast-feeding, and crowding (Table 1). The variables included in the final multivariate model were age,

TABLE 2 Incidence rate and incidence relative risk of ETEC phenotype by selected features, Abu Homos, Egypt, 2004 to 2007

Feature	ETEC toxin phenotype								
	LT only			ST only			LT/ST		
	IR (no. of episodes)[c]	Crude IRR	Adjusted IRR[d]	IR (no. of episodes)	Crude IRR	Adjusted IRR	IR (no. of episodes)	Crude IRR	Adjusted IRR
Child age (mo)									
0–5	0.46 (58)	1.00	1.00	0.51 (65)	1.00	1.00	0.20 (25)	1.0	1.0
6–11	0.72 (93)	1.53 (1.11, 2.13)[e]	1.48 (1.02, 2.15)[e]	0.84 (109)	1.61 (1.18, 2.18)[f]	1.37 (0.98, 1.90)	0.28 (36)	1.38 (0.83, 2.29)	1.09 (0.64, 1.85)
12–17	0.77 (99)	1.64 (1.18, 2.26)[f]	1.57 (1.08, 2.29)[e]	0.50 (64)	0.94 (0.67, 1.33)	0.82 (0.56, 1.18)	0.31 (40)	1.53 (0.93, 2.53)	1.17 (0.69, 1.98)
18–23	0.66 (84)	1.38 (0.99, 1.93)	1.24 (0.81, 1.91)	0.42 (53)	0.78 (0.54, 1.12)	0.65 (0.41, 1.01)	0.24 (30)	1.15 (0.67, 1.95)	0.74 (0.40, 1.39)
Child's gender									
Male	0.70 (175)	1.14 (0.92, 1.42)	1.16 (0.93, 1.44)	0.60 (152)	1.14 (0.90, 1.43)	1.12 (0.89, 1.41)	0.33 (84)	1.87 (1.30, 2.65)[g]	1.81 (1.26, 2.60)[f]
Female	0.61 (159)	1.0	1.0	0.54 (139)	1.0	1.0	0.18 (47)	1.0	1.0
Breast-feeding									
Exclusively	0.42 (19)	0.59 (0.36, 0.96)[e]	0.76 (0.41, 1.40)	0.31 (14)	0.62 (0.35, 1.11)	0.47 (0.24, 0.91)[e]	0.07 (3)	0.23 (0.07, 0.74)[e]	0.19 (0.05, 0.67)[e]
Not exclusively	0.66 (221)	0.93 (0.73, 1.18)	0.86 (0.64, 1.15)	0.63 (212)	128 (0.97, 1.70)	0.89 (0.64, 1.26)	0.27 (90)	0.93 (0.64, 1.36)	0.66 (0.42, 1.04)
Not breast-fed	0.72 (94)	1.0	1.0	0.50 (65)	1.0	1.0	0.29 (38)	1.0	1.0
Season at follow-up[a]									
Warm	0.83 (225)	1.87 (1.49, 2.36)[g]	1.89 (1.50, 2.38)[g]	0.88 (237)	3.98 (2.96, 5.35)[g]	3.91 (2.91, 5.25)[g]	0.42 (114)	6.09 (3.66, 10.13)[g]	6.04 (3.63, 10.06)[g]
Cold	0.45 (109)	1.0	1.0	0.22 (54)	1.0	1.0	0.07 (17)	1.0	1.0
Maternal education									
Some schooling	0.59 (126)	0.84 (0.68, 1.05)	0.83 (0.66, 1.04)	0.52 (111)	0.85 (0.68, 1.09)	0.87 (0.68, 1.11)	0.21 (44)	0.70 (0.49, 1.01)	0.75 (0.52, 1.10)
No schooling	0.70 (208)	1.0	1.0	0.60 (180)	1.0	1.0	0.29 (87)	1.0	1.0
Garbage container in house									
Yes	0.61 (57)	0.92 (0.70, 1.23)	0.99 (0.74, 1.34)	0.45 (42)	0.76 (0.55, 1.05)	0.80 (0.57, 1.12)	0.21 (20)	0.81 (0.50, 1.30)	1.03 (0.63, 1.68)
No	0.66 (277)	1.0	1.0	0.60 (249)	1.0	1.0	0.27 (111)	1.0	1.0
Sanitary latrine									
Yes	0.67 (275)	1.14 (0.87, 1.52)	1.15 (0.87, 1.53)	0.59 (243)	1.25 (0.92, 1.70)	1.23 (0.90, 1.68)	0.27 (111)	1.37 (0.85, 2.20)	1.36 (0.84, 2.20)
No	0.58 (59)	1.0	1.0	0.48 (48)	1.0	1.0	0.20 (20)	1.0	1.0
Crowding[b]									
≥4.75	0.65 (55)	1.10 (0.80, 1.50)	1.10 (0.80, 1.51)	0.60 (51)	1.12 (0.81, 1.55)	1.07 (0.77, 1.49)	0.22 (19)	1.12 (0.66, 1.91)	1.06 (0.61, 1.82)
≥3.5 to <4.75	0.72 (137)	1.21 (0.96, 1.54)	1.21 (0.95, 1.54)	0.59 (111)	1.08 (0.84, 1.40)	1.04 (0.80, 1.35)	0.34 (64)	1.68 (1.15, 2.44)[f]	1.50 (1.02, 2.22)[e]
<3.5	0.60 (142)	1.0	1.0	0.54 (129)	1.0	1.0	0.20 (48)	1.0	1.0
Socioeconomic status									
Poor	0.57 (69)	0.85 (0.65, 1.10)	0.79 (0.60, 1.03)	0.57 (69)	1.01 (0.77, 1.32)	0.95 (0.72, 1.26)	0.33 (40)	1.43 (0.98, 2.07)	1.24 (0.84, 1.83)
Nonpoor	0.68 (265)	1.0	1.0	0.57 (222)	1.0	1.0	0.23 (91)	1.0	1.0

[a] Warm season from May to October; cold season from November to April.
[b] Number of person per bedroom.
[c] Episodes per child-years of follow-up.
[d] Adjusted for statistically significant univariate predictors, including child's age, child's gender, season of follow-up, and crowding, using multivariate Poisson regression.
[e] $P < 0.05$.
[f] $P < 0.01$.
[g] $P < 0.001$.

gender, breast-feeding, maternal education, crowding, season, sanitary latrine, garbage container in the house, and socioeconomic status (SES). Relative rates for the association between each variable and the incidence of ETEC diarrhea, adjusted for the mentioned variables, and their 95% CIs are shown in Table 1. The variables found to be significantly associated with the increase in the incidence of ETEC diarrhea were age (the second 6 months of life compared to the first 6 months of life), warm season, male gender, and crowding in sleeping rooms. On the other hand, exclusive breast-feeding and having a mother with some education (able to read and write) were associated with lower incidence of diarrhea (Table 1).

Identification of ETEC and other enteric organisms. Potential enteric pathogens were identified in 21% (2,017/9,539) of the samples collected from children who were asymptomatic at the time of collection, compared to 30% (1,202/4,001) of stools collected from children with diarrhea. ETEC was the most common pathogen cultured from either asymptomatic or symptomatic children (13.6% for AS versus 18.9% for symptomatic), followed by *Campylobacter* spp. (7.2% for AS versus 8.0% for symptomatic), *Shigella* spp. (0.2% for AS versus 1.2% for symptomatic), and *Salmonella* spp. (0.1% for AS versus 0.2% for symptomatic). Rotavirus was detected in the stool of 5.4% of children with diarrhea, but asymptomatic children were not tested for rotavirus.

Diarrhea incidence rates for ETEC, *Campylobacter* spp., *Shigella*, and rotavirus in the first 6 months of life were 1.17, 0.56, 0.01, and 0.38 episodes/child-year, respectively, compared to 1.31, 0.63, 0.09, and 0.41 episodes/child-year in the age group of 18 to 23 months. Pathogen-specific incidence rates in episodes per child-year were 1.5 for ETEC, 0.63 for *Campylobacter* spp., 0.09 for *Shigella*, and 0.41 for rotavirus.

Overall characteristics, pathogen distribution, and incidence of diarrhea. Of the 511 child-years at risk of diarrhea, 4,001 episodes of diarrhea were detected in the study cohort. For the 285

TABLE 3 Clinical characteristics of diarrheal episodes by diarrhea type, Abu Homos cohort, 2004 to 2007 (copathogen excluded)

Clinical characteristic	No. (%) of diarrheal episodes				
	All diarrhea (n = 4,001)	ETEC (n = 622)	*Campylobacter* (n = 238)	*Shigella* (n = 40)	Rotavirus (n = 151)
Vomiting	1,112 (27.8)	157 (25.2)	63 (26.5)	8 (20.0)	94 (62.3)[c]
Fever	1,822 (45.5)	297 (47.8)	113 (47.5)	23 (57.5)	93 (61.6)[b]
Chills	50 (1.3)	11 (1.8)	3 (1.3)	0 (0.0)	6 (4.0)[a]
Blood in stool	168 (4.2)	23 (3.7)	20 (8.4)[b]	4 (10.0)	5 (3.3)
Increased thirst	1,755 (43.9)	311 (50.0)	97 (40.8)	28 (70.0)[a]	98 (64.9)
Rectal prolapse	15 (0.4)	5 (0.8)	1 (0.4)	0 (0.0)	1 (0.7)
Treated with ORS	1,486 (37.1)	263 (42.3)	89 (37.4)	16 (40.0)	94 (62.3)[c]
Convulsions	1 (0.02)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Dehydration	34 (0.9)	14 (2.3)	0 (0.0)	1 (2.5)	7 (4.6)[a]
Episode type					
Acute	3,757 (93.9)	573 (92.1)	209 (87.8)	36 (90.0)	145 (96.0)[a]
Dysentery	156 (3.9)	21 (3.4)	15 (6.3)	3 (7.5)	5 (3.3)
Persistent	88 (2.2)	28 (4.5)	14 (5.9)	1 (2.5)	1 (0.7)

[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.

children who completed the follow-up period, 94.4% suffered from at least one episode of diarrhea. Children experienced a mean (±SD) of 13.2 (±7) episodes of diarrhea during the study, with the first episode occurring at 2 months (±2 months). The overall incidence of diarrhea was 7.8 episodes per child-year, decreasing from 9.4 episodes per child-year in the first year of life to 7.1 and 5.4 episodes per child-year in the following first and second 6 months of the second year, respectively. Diarrheal incidence was slightly higher in boys than in girls (8.4 episodes/child-year compared to 7.3 episodes/child-year. In the warm season, the incidence rate of diarrhea was 9.5 episodes/child-year compared to 5.9 episodes/child-year in the cold season. Exclusively breast-fed children had a statistically significant lower incidence of diarrhea (7.3 episodes/child-year) than nonexclusive breastfed children (8.5 episodes/child-year). Most diarrheal episodes were acute (94%), while 2.2% were persistent. Only 4.2% of episodes were described as bloody diarrhea. Fever was reported by the caregiver in 46% of the episodes, and 28% of episodes were associated with vomiting. Thirty-four episodes of diarrhea (0.9%) were associated with dehydration (Table 3).

Symptomatic ETEC infection and nutritional status. Nine anthropometric measures were performed every 2 months during the 2-year period of follow-up. At least one measure was performed for 311 children, and 173 children had all the anthropometric surveys.

Cumulative analysis showed that by the end of 2 years of follow-up, a significantly higher proportion of children who had ETEC-associated diarrhea (12.4%, 32/258) were stunted (height, less than 2 SD) compared to children who did not have any ETEC-associated diarrhea (7.6%, 4/53) ($P < 0.001$). For children who completed all the surveys, the proportion of stunted children increased when the number of ETEC-associated diarrheal episodes increased; 5.7% when ETEC episodes were three or less, 17.2% when between 4 and 6 episodes, and 18.4% when more than 7 episodes. The correlation between the number of ETEC episodes and the proportion of stunting was suggested to be significant ($P = 0.055$).

TABLE 4 Epidemiological characteristics of colonized ETEC infection among children less than 2 years old, Abu Homos, Egypt, 2004 to 2007

Characteristic	No. (%) of samples			
	LT	ST	LT/ST	Total
Age (mo)				
0–5	77 (10.7)	76 (16.9)	17 (13.2)	170 (13.1)
6–11	168 (23.3)	138 (30.8)	35 (27.1)	341 (26.3)
12–17	215 (29.8)	120 (26.7)	31 (24)	366 (28.2)
18–23	261 (36.2)	115 (25.6)	46 (35.7)	422 (32.4)
Total	721 (100)	449 (100)	129 (100)	1,299 (100)
Season				
Warm	390 (54.1)[b]	312 (69.5)[b]	86 (66.7)	788 (60.7)
Cold	331 (45.4)	137 (30.5)	43 (33.3)	511 (39.3)
Gender				
Female	354 (49.1)	215 (47.9)	76 (58.9)[a]	645 (49.7)
Male	367 (50.9)	234 (52.1)	53 (41.1)	654 (50.3)

[a] $P < 0.05$.
[b] $P < 0.001$.

DISCUSSION

Diarrhea epidemiology and enteric pathogen distribution have been studied in many different epidemiological settings in Egypt; two previous cohort studies have been published reporting a very high incidence of diarrhea among children residing in rural communities in Egypt (8, 16). A cohort study was conducted from 1995 to 1998, in which diarrhea incidence was 5.5 episodes/child-year and ETEC was the most common pathogen isolated, with an incidence of 1.5 episodes/child-year; ETEC incidence rates (in episodes/child-year) in a previous cohort and the current cohort for 0 to 6 months were 1.67 and 1.17, respectively, for 6 to 11 months were 2.26 and 1.83, respectively, for 12 to 17 months were 1.99 and 1.59, respectively, and for 18 to 23 months were 1.35 and 1.31, respectively. The ETEC incidence rates in the previous and current cohort are comparable (8).

In the current study, ETEC remained the most common organism isolated. ETEC was isolated from 18.9% (756/4,001) of diarrheal episodes suffered by children less than 2 years old; this

figure was comparable to studies from other developing countries (23–25).

ETEC is one pathotype of diarrheagenic *Escherichia coli* (DEC) bacteria, which consists also of enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), and enteroinvasive *E. coli* (EIEC). The focus of the current study was on ETEC, and testing to evaluate for other pathogenic *E. coli* was not performed. However, one study from Egypt indicated that the prevalence of EPEC is 5.2% in children under 5 years old who were hospitalized with diarrhea (26).

The incidence of ETEC-associated diarrhea was consistently high during the first and second year of life (1.5 episodes/child-year, each) regardless of the study; however, the incidence of other diarrhea-associated pathogens decreased in the second year of life, except for *Shigella* spp., which increased from 0.06 to 1.2 episodes/child-year. This explains the finding that the ETEC-associated diarrhea-attributable fraction was 12% of all diarrheal episodes in the first 6 months of life and increased to 24% of all diarrheal episodes in children 18 to 24 months of age. The attributable fraction of ETEC to overall diarrhea is comparable to that previously reported from Egypt (16, 27) and other developing countries in Africa, Asia, and Latin America (7, 28). These figures imply the importance of controlling ETEC-associated diarrhea on the burden of overall diarrheal disease.

This study revealed that the mean age for the first ETEC-associated diarrheal episode was 7.5 months and that the risk of first infection is primarily age under 9 months; this finding was similar to that in other studies (8, 29). However, in Bangladesh, the children were older (12 months of age) when they had their first reported infection (27). The finding that ETEC-associated diarrhea accounted for 56% of all initial pathogen-specific episodes is comparable to another study from Egypt (8) and higher than that reported from Bangladesh (37%) (25). The highest incidence of ETEC-associated diarrhea was in the age group of 6 to 12 months, and most of this burden was due to ST-ETEC- and LT/ST-ETEC-associated diarrhea. Vaccines targeting ETEC infection are presently in development (30); these results indicate that candidate vaccines need to confer immunity at an age early enough to decrease the burden of ETEC-associated diarrhea during infancy.

Similar to previous studies from Egypt and other developing countries, the overall incidence of ETEC-associated diarrhea was consistently higher during warmer months and among males (8, 31). However, two previous birth cohorts from Egypt reported that only ST-ETEC diarrhea had a higher incidence during summer months and not LT-ETEC-associated diarrhea, which had no seasonal variations (8, 16). In contrast, a third cohort found that ST-ETEC diarrhea was common during cooler months and that LT-ETEC-associated diarrhea was more frequent during warmer months (32). The current study revealed that not only did ST-ETEC- and LT/ST-ETEC-associated diarrhea have a higher incidence during warmer months, but so did LT-ETEC-associated diarrhea. Differences in the incidence of toxin-specific ETEC infection according to seasonality observed in studies from Egypt suggest the importance of continuous monitoring of disease incidence in different seasons and monitoring the effect of climatic changes on disease incidence. It is possible that differences in seasonality noted in the study were due in part to circulation of different ETEC clones, with particular toxin types, at different times of the year. Additionally, other unrecognized factors may have affected the seasonal distribution noted in the study.

Risk factors that were identified as significant ETEC-associated infections were male sex, age, and warm season. Crowding in sleeping rooms posed a significant risk only for repeated ETEC-associated infections. Additionally, exclusive breast-feeding was protective from repeated infections, mainly for ST-ETEC- and LT/ST-ETEC-associated diarrhea. Exclusive breast-feeding appeared to delay the age of the initial ETEC-associated infection. This finding was in agreement with other studies (8, 31) yet disagrees with similar studies from Egypt and other developing countries (8, 16, 32). A recent study identified that human colostrum-derived phagocytes eliminate ETEC opsonized by colostrum supernatant (33). A fully breast-fed infant has secretory immunoglobulin A (sIgA) antibodies, the predominant antibody in human milk. These sIgA antibodies have been shown to protect against ETEC infection. Furthermore, human breast milk is rich in receptor analogues for certain epithelial structures which microbes need for attachment to host tissues as an initial step in infections (34). Correa et al. demonstrated that human colostrum contains sIgA antibodies which are reactive to colonization factors I and II of ETEC (35). The lack of protection detected in other studies may be explained by the high burden of ETEC-associated diarrhea in infancy; these authors suggest that breastfeeding cannot be depended on for reduction in the incidence of ETEC-associated diarrhea.

The association between ETEC diarrhea and stunting has been addressed previously (25). In the current study, children who experienced one or more episodes of diarrhea due to ETEC were significantly more stunted by 2 years of age than children without any episodes of ETEC-associated diarrhea. This result is in agreement with a previous study from Bangladesh (25). Negative effects of ETEC diarrhea on the growth of the children may be confounded by additional factors that may dispose these children to malnutrition, such as socioeconomic status and home environment. Previous studies have shown that children with stunted growth at the age of 2 years have poor cognitive functioning at age 9 years (2, 36). This finding needs confirmation from countries where ETEC is endemic, such as Egypt.

Conclusion. Diarrhea is still of high morbidity among children less than 2 years old living in the Nile Delta, with ETEC-associated diarrhea still the most common cause of infectious diarrhea. This study highlighted the importance of exclusive breast-feeding and improving mothers' education in decreasing the incidence of diarrhea. Intentions to implement programs to reduce diarrhea incidence may target those two factors. With increasing evidence of long-term consequences of early childhood diarrhea, which include permanent shortfalls in physical and cognitive development (2), more studies are required to identify the impact of diarrhea on those long-term consequences. It is of vital importance for proper childhood development to continue monitoring diarrhea incidence and to identify its impact on child development with the application of adequate intervention strategies to reduce diarrhea morbidity and mortality.

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