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Prospective Cohort Study of Enterotoxigenic *Escherichia coli* Infections in Argentinean Children

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In a follow-up study, enterotoxigenic *Escherichia coli* (ETEC) infections in 145 children from two communities located in northeastern Argentina were monitored for 2 years. The occurrence of diarrhea was monitored by weekly household visits. Of 730 fecal specimens collected, 137 (19%) corresponded to diarrheal episodes. ETEC was isolated from a significantly higher proportion of symptomatic (18.3%) than asymptomatic (13.3%) children ($P = 0.04541$). Individuals of up to 24 months of age were found to have a higher risk of developing ETEC diarrhea than older children (odds ratio [OR], 3.872; $P = 0.00021$). When the toxin profiles were considered, only heat stable enterotoxin (ST)-producing ETEC was directly associated with diarrhea ($P = 0.00035$). Fifty-five percent of the ETEC isolated from symptomatic children and 19% of the ETEC isolated from asymptomatic children expressed one of the colonization factors (CFs) investigated, i.e., CF antigen I (CFA/I), CFA/II, CFA/III, and CFA/IV; coli surface antigens CS7 and CS17; and putative CFs PCFO159, PCFO166, and PCFO20, indicating a clear association between diarrhea and ETEC strains that carry these factors ($P = 0.0000034$). The most frequently identified CFs were CFA/IV (16%), CFA/I (10%), and CS17 (9%). CFs were mostly associated with ETEC strains that produce ST and both heat-labile enterotoxin and ST. Logistic regression analysis, applied to remove confounding effects, revealed that the expression of CFs was associated with illness independently of the toxin type (OR, 4.81; $P = 0.0003$). When each CF was considered separately, CS17 was the only factor independently associated with illness (OR, 16.6; $P = 0.0151$). Most CFs (the exception was CFA/IV) fell within a limited array of serotypes, while the CF-negative isolates belonged to many different O:H types. These results demonstrate that some CFs are risk factors for the development of ETEC diarrhea.

Enteric infection caused by enterotoxigenic *Escherichia coli* (ETEC) is an important health problem among children in developing countries and among travelers from industrialized countries visiting tropical or subtropical areas of the world (1, 5, 6, 11, 15). The bacteria colonize and multiply in the small intestine and produce either a heat-labile enterotoxin (LT) or a heat-stable enterotoxin (ST), or both (3), inducing diarrheal disease. Colonization is usually associated with the presence of fimbrial surface structures called colonization factors (CFs). Three major CFs have been described and have been well characterized; they are referred to as CF antigen I (CFA/I), CFA/II, and CFA/IV (8). CFA/I is a single fimbrial antigen, while CFA/II and CFA/IV consist of three fimbrial antigens each: so-called coli surface antigens CS1, CS2, and CS3 and coli surface antigens CS4, CS5, and CS6, respectively (8). In addition, a number of putative CFs (PCFs) have been described and characterized to a certain extent, e.g., CFA/III, PCFO159, PCFO166, CS7, CS17, and PCFO20 (13, 23). Recently, a new designation of ETEC CFs was proposed by M. M. McConnell from consideration of the order in which the CFs were identified over time, i.e., CFA/I and CS1 to CS21 (8). Surveys of ETEC isolates have shown that most CFs are associated with a limited number of O:H serotypes and enterotoxin type (2, 24, 25).

Because the prevalence of these CFs varies according to the geographical region, a detailed description of the distribution of ETEC strains carrying the various CFs in different parts of the world is essential for the development of vaccines against diarrheal diseases in children in developing countries and in travelers to these areas.

The role of the CFs in the pathogenesis of ETEC still remains a matter of debate. Several reports have shown that some CFs are important virulence factors in ETEC strains; i.e., they induce a protective anticolonizing immunity in animal and human volunteers (9, 21). However, an epidemiological study has demonstrated that CFA/I, CFA/II, and CFA/IV do not represent a risk factor for the development of acute diarrhea (11).

In the prospective study described here, we have monitored for 2 years 145 children under 5 years of age living in an area in northeastern Argentina where ETEC is endemic. The ETEC isolates were analyzed for the presence of CFA/I, CS1 to CS7, CS8 (CFA/III), CS12 (PCFO159), CS14 (PCFO166), CS17, and CS18 (PCFO20), and the association of these ad-

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TABLE 1. Characteristics of the two cohorts monitored from September 1988 to August 1990

Community	No. (%) of children in the following age groups (mo):			Male/female ratio	No. of stool samples collected	Time of follow-up (mo)	% Diarrheal episodes[a]	% ETEC isolates[b]	% ETEC diarrheal episodes[c]
	0–12	12–24	24–60						
Zaimán (n = 91)	31 (34)	23 (25)	37 (41)	1.3	453	13.2	20	16.5	4.8
Las Dolores (n = 54)	16 (30)	14 (26)	24 (44)	1.1	277	13.8	16.7	13.3	4.0

[a] Number of diarrheal samples/total number of samples.
[b] Number of ETEC isolates/total number of samples.
[c] Number of ETEC strains isolated from diarrheal samples/total number of samples.

hesins with diarrhea was evaluated. In addition, the association between the expression of these fimbriae, production of enterotoxin, and the serotypes was also determined.

MATERIALS AND METHODS

Population studied. Between September 1988 and August 1990 a follow-up study was conducted in Zaimán and Las Dolores, two Argentinean communities located in the province of Misiones, which neighbors Brazil and Paraguay. Zaimán has a population of 1,600 and Las Dolores has a population of 1,560, with each community having 200 and 164 children under age 5 years, respectively. In Zaimán, water for drinking, cooking, hygienic practices, and recreation comes from groundwater wells fed by a stream of the same name, and only 16% of the population has adequate sanitation and waste treatment. On the other hand, in Las Dolores the households have no contact with the Zaimán stream; 62% of the population retrieve their water from wells, and the others retrieve their water from public taps. Of this population, 31% have bathroom facilities, while the remaining members of the population have only latrines (16).

In the present study, 91 children under 5 years of age from Zaimán and 54 children under 5 years of age from Las Dolores were recruited to be monitored for 2 years.

Sample collection. Children were prospectively monitored by active surveillance. Oral informed consent was obtained from the mothers of the children who participated in this study. Each participating child was visited weekly by a social worker who queried the caretaker about the occurrence of diarrheal illness. Fecal samples were collected from each child at the onset of the study and every 3rd month for 2 years. Additionally, a fecal specimen was collected whenever a child suffered from diarrhea. Diarrhea was defined as at least three watery or loose stools during a 24-h period. Two episodes of diarrhea were considered different when they were separated in time by 3 days without symptoms.

In order to investigate the persistence of an ETEC strain in the intestine, a fecal sample was collected as soon as a toxigenic *E. coli* was identified in the stools of any of the monitored children during the quarterly sampling.

An additional 241 stool samples (58 from Las Dolores and 183 from Zaimán) from children who were younger than age 5, who had an episode of diarrhea during the time of the study, and who did not belong to the population being monitored, were also collected.

Sample processing. Fecal specimens were inoculated onto MacConkey agar plates for selection of *E. coli* isolates. After overnight culture at 37°C, five *E. coli* colonies were incubated in individual deep-agar vials and were regularly sent to Buenos Aires for toxin and CF analyses. Immediately after arrival, the bacteria were subcultured in Casamino Acids medium for enterotoxin testing by means of ganglioside GM1–enzyme-linked immunosorbent assays (22) with direct culture. The ETEC strains were serotyped by Fritz and Ida Ørskov at the International Escherichia and Klebsiella Center, Statens Seruminstitut, Copenhagen, Denmark.

CF analysis. The ETEC strains to be assayed for CF expression were streaked onto Casamino Acids-yeast extract agar with bile salts (12). After overnight incubation at 37°C, they were tested for CFA/I, CFA/II, CFA/IV, and their subcomponents by slide agglutination (2) or dot blot tests (24) as described previously and were maintained at −70°C in Trypticase soy broth (Difco, Detroit, Mich.) supplemented with 15% glycerol. All the ETEC strains were also sent on agar slants to the University of Göteborg, Göteborg, Sweden, where they were tested for the presence of CS7, CS8, CS12, CS14, CS17, and CS18 by dot blot tests.

The following monoclonal antibodies, which were produced as described previously (24), were used for CF analysis: CFA/I 1:6, CS1 6:1, CS2 10:3, CS3 10:2, CS4 4:6, CS5 1:1, CS7 5:2, CFA/III 3:3, PCFO159 1:1, PCFO166 1:6, and CS17 8:1. In addition, absorbed polyclonal antiserum R1868 (23) was used to screen for CS18.

The following reference strains were included in the different assays: H10407 (CFA/I positive [CFA/I$^+$], LT positive [LT+], and ST positive [ST+]), kindly provided by D. G. Evans, Houston, Tex.; E1392-75 (CS1+, CS3+, LT+, and ST+), 58R597 (CS2+, LT+, and ST+), E11881A (CS4+, CS6+, LT+, and ST+), and E17018 (CS5+, CS6+, ST+, and LT+), kindly provided by B. Rowe, London, United Kingdom; 31-10A (CS8+, CS6+, and LT+), 31-10B [CS8 negative [CS8−], CS6−, and LT negative [LT−], 350C1A (CS12+, ST+, and LT+), 350C1B (CS12−, ST negative [ST−], and LT−), E7476A (CS14+ and ST+), E7476B (CS14− and ST−), 334A (CS7+, ST+, and LT+), 334C (CS7−, ST−, and LT−), E20738A (CS17+, LT+), and E20738B (CS17− and LT−), kindly provided by M. M. McConnell, Public Health Laboratory Service, London, United Kingdom; ARG-2 (CS18+, LT+, and ST+); and ARG-2/1 (CS18−, LT+, and ST+) (23).

Statistical analysis. The univariate association of categorical variables with symptomatic ETEC infection was examined by Fisher's exact probability test or the chi-square test with Yates' correction by using Epi Info, version 5. Multivariate analysis was applied by using the Cox logistic regression model (4) with BMDP statistical software (University of California at Berkeley). The sex and age of the infected children and the toxins and CFs of the ETEC strains were included as variables. Variables were included in the model if they met a 5% significance level of inclusion and maintained a minimum of 5% significance at later steps of the selection process. Odds ratio (OR) estimates with associated 95% confidence intervals (CIs) were also calculated with this model.

Some children contributed with samples from more than one diarrheal episode. However, only one sample was taken from each episode and thus the samples were considered to be independent.

RESULTS

Surveillance of the two communities. A total of 145 children from two communities with populations with low socioeconomic levels were enrolled in the 2-year follow-up study described here. There was a dropout rate of 16.5% after the first sampling (23% from Zaimán and 5% from Las Dolores). The two populations had similar characteristics. Accordingly, no statistically significant differences ($P > 0.5$) in either age or sex distribution were found (Table 1). Similarly, no significant differences in the proportion of diarrheal episodes ($P = 0.28396$), the frequency of ETEC strains isolated ($P = 0.29010$), and the ETEC-associated diarrhea episodes ($P = 0.85907$) were found. Thus, the two communities were considered as a single cohort. The total number of child-months of follow-up was 1,946. The incidence of diarrhea was estimated to be 0.84 per child-year; that of ETEC diarrhea was 0.2 and that of all ETEC infections was 0.69.

Persistence of ETEC in the intestine. In order to investigate the persistence of an ETEC strain in the intestine, a stool sample was collected as soon as a toxigenic *E. coli* strain was identified in any of the monitored children during the routine stool sampling. The period of time between retrieval of the two samples varied from 30 to 40 days due to the procedure used for toxin analysis. A total of 43 stool specimens were analyzed. In only six patients was an ETEC strain isolated after the previous ETEC infection. However, these strains differed either in the toxin profile or in the serotype from the corresponding original ETEC strain, indicating that the children had new rather than persistent ETEC infections.

Frequency of ETEC reinfection. Forty children had one ETEC infection, 18 had two ETEC infections, and 9 had three or more ETEC infections. ETEC-associated diarrheal episodes were recorded in 28 of these 67 children. Only three children had more than one symptomatic ETEC infection. In a single

TABLE 2. Identification of CFs on ETEC strains isolated from children with and without diarrhea

CF	No. (%) of children:			*P* value
	With diarrhea (n = 68)	Without diarrhea (n = 79)	Total (n = 147)	
CFA/I	7 (10)	2	9	0.1068880[a]
CS1-CS3	0	2	2	0.5437739[a]
CS2-CS3	2	0	2	0.4117483[a]
CS5-CS6	5 (7)	0	5	0.0453430[a]
CS6	6 (9)	2	8	0.1894894[a]
CS7	3	1	4	0.6014660[a]
CS8	1	2	3	0.8955217[a]
CS12	5 (7)	5 (6)	10	0.9341057[a]
CS14	3	0	3	0.1931669[a]
CS17	6 (9)	1	10	0.0789201[a]
Total (%)	38 (55)	15 (19)	53 (36)	0.0000034[b]

[a] Determined by Fisher's exact probability test.
[b] Determined by the chi-square test.

TABLE 3. Influence of age, toxin, and CFs on the prevalence of ETEC diarrhea on the basis of a multiple logistic regression model

Variable	OR	95% CI	*P* value
Age (mo)			0.0303[a]
0–12 vs 13–24	0.645[a]	0.224–1.850[a]	
≥25 vs 13–24	0.278[a]	0.104–0.740[a]	
LT production	0.337[a]	0.134–0.845[a]	0.0263[a]
CF production	4.81[a]	2.14–10.8[a]	0.0003[a]
CS17 production	16.6[b]	1.82–151[b]	0.0151[b]

[a] The variables analyzed were sex, age, and LT, ST, and CF production.
[b] The variables analyzed were sex, age, and LT, ST, CFA/I, CS1-CS2, CS1-CS3, CS7, CS8, CS12, CS14, and CS17 production.

child, an ETEC infection was followed 3 months later by another infection caused by a strain with the same CF (CS6).

Association between diarrhea and presence of toxins and CFs in ETEC strains. The analyses of the effects of the different variables associated with ETEC diarrheal episodes were performed with 730 stool samples from the monitored population plus an additional 241 samples. This extra group involved specimens from 183 children younger than age 5 living in Zaimán and 58 children younger than age 5 living in Las Dolores, who had an episode of diarrhea during the time of the study and who did not belong to the monitored population. The asymptomatic ETEC infections were those in which the organism was isolated from a child who did not have any diarrhea on the day of collection or during the previous week. Data for eight cases of diarrhea in which an additional pathogen was isolated together with ETEC (four cases, *Shigella* spp.; four cases, other ETEC) were excluded from this analysis. Thus, a total of 147 ETEC strains isolated from 964 stool specimens (371 diarrheal and 593 nondiarrheal samples) were analyzed.

A significantly higher proportion of ETEC strains was isolated from children with diarrhea (18.3%) than from asymptomatic children (13.3%) (P = 0.04541). The distribution of toxins produced by ETEC strains that caused diarrhea (53% LT, 40% ST, and 7% LT and ST) varied from the distribution of toxins produced by ETEC strains isolated from children with asymptomatic infections (74% LT, 13% ST, and 13% LT and ST), this difference being significant only for ST-producing ETEC strains (OR, 4.544; P = 0.00035).

Comparison of the CF profiles of the ETEC strains isolated from symptomatic and asymptomatic children showed a clear association between ETEC strains carrying any of the adherence factors and diarrhea (P = 0.0000034) (Table 2). When each adhesin was studied separately, CS5-CS6 was detected at a significantly higher frequency in children with diarrhea (P = 0.045343), and the association of CS17 with diarrhea approached statistical significance (P = 0.078920).

Among the ETEC strains isolated from symptomatic children, CFA/IV (CS5 and CS6) was the most frequently identified fimbria (16%), followed by CFA/I (10%) and CS17 (9%) while the remaining CFs were found on only a few strains (Table 2). CS4 and CS18 were not identified on any of the ETEC strains isolated.

A direct correlation was found between the presence of CFs and ST-producing ETEC strains (OR, 4.394; P = 0.00012) and LT- and ST-producing ETEC strains (OR, 3.3; P = 0.03577), but an inverse association was found with LT-producing ETEC strains (OR, 0.165, P = 0.00001).

Since the pathogenicity of ETEC has been shown to differ by age among young children, we analyzed the odds of getting diarrhea from an ETEC infection with respect to age. Children up to 24 months of age turned out to have a higher risk of developing diarrhea than older children (OR, 3.872; P = 0.00021). In addition, when comparing the frequency of occurrence of CFs on the ETEC strains isolated from children with and without diarrhea in different age groups, i.e., ≤12, 13 to 24, and over 25 months, we found that although CFs were found on a higher proportion of ETEC strains isolated from children with diarrhea than on those isolated from asymptomatic children in all age groups, this difference (58 versus 12%) was significant only for children younger than age 24 months (OR, 10.012; P = 0.00003).

Because there is a strong association between an ST-only toxin profile and diarrhea, as well as an ST-only toxin profile and the expression of CFs, in addition to age and disease, all these factors were simultaneously included in a statistical model. Thus, multiple regression analysis in which even sex, in addition to toxin profile, CF, and age, was included as an explanatory variable was used. The results of this analysis (Table 3) revealed that age and expression of CFs were associated with diarrhea. When each CF was considered individually in a separate analysis, only CS17 was found to be independently related to symptomatic infection (Table 3). On the other hand, LT production alone was not found to be a risk factor for the development of diarrhea.

Association between CFs, toxins, and serotypes of ETEC strains. The association between the expression of different fimbriae, the production of enterotoxins, and the serotypes of the ETEC strains was also studied (Table 4). Data for an additional 12 ETEC strains, which were excluded from the previous analyses because they were isolated together with other enteropathogens from children with diarrheal episodes, were added to the analysis, whose results are presented in Table 4. CFA/I, CFA/IV, and CS14 were mainly expressed by ST-producing ETEC strains and CFA/II was mainly expressed by LT- and ST-producing ETEC strains, whereas CS7 and CS17 were identified only on LT-producing ETEC strains. Except for CS5 and CS6, which were expressed on strains of various serotypes and with various toxin profiles, the other CFs were associated with strains of a limited number of O:H and toxin types. On the other hand, the CF-negative strains belonged to many different serogroups, with O8, O9, and O20 being among the most prevalent.

TABLE 4. Expression of CFs in relation to enterotoxin profile and serotypes in 159 ETEC strains isolated from children with and without diarrhea living in northeastern Argentina

CF	No. (%) of strains with the following enterotoxin profile:			Serotype(s) (no. of strains)
	LT (n = 100)	ST (n = 42)	LT and ST (n = 17)	
CFA/I	0	11	0	O153:H45 (5), 078:H12 (4), rough:H45 (1), O8:H25 (1)
CS1-CS3	0	0	2	O6:H16 (2)
CS2-CS3	0	0	3	O6:H16 (1), O6:H− (1), rough:H− (1)
CS5-CS6	0	6	0	O29:H21 (4), O114:H21 (1), O127:H6 (1)
CS6	2	6	0	O9:H− (2), O27:H7 (2), O27:H− (1), O20:H− (1), O148:H28 (1), O172:H6 (1)
CS7	5	0	0	O114:H49 (2), O114:H− (1), O127:H40 (1), rough:H33 (1)
CS8	1	0	1	O102:H10 (1), rough:H33 (1);
CS8-CS6	2	0	0	O25:H− (2)
CS12	5	0	5	O159:H4 (8), O102:H10 (1), O?:H49 (1)
CS14	0	4	0	O78:H18 (3), O78:H− (1)
CS17	8	0	0	O144:H21 (3), O114:H− (2), O8:H9 (1), O8:H− (1), O12:H33 (1)
Any CF	23 (23)	27 (64)	11 (64)	
No CF	77 (77)	15 (36)	6 (36)	O9:H− (10), O20:H− (4), O15:H40 (4), O64:H5 (4), O?:H32 (4), O8:H− (4), O8:H10 (3), O159:H4 (3), O″K48″:H− (3), O?:H2 (3), rough:H− (3), ND[a] (14), others (39)

[a] ND, not determined.

DISCUSSION

The results of the present investigation support the observations from other epidemiological studies that in Argentina, as in other Latin American countries, ETEC represents an important cause of childhood morbidity, particularly in populations with low socioeconomic levels (5, 6, 10, 11, 20). Thus, ETEC was isolated from about 18% of the diarrheal episodes suffered by children under 5 years of age in these two communities in northeastern Argentina; this figure was comparable to those for other areas where ETEC strains are endemic (1, 10).

Another relevant conclusion that can be drawn from the results of this study is the fact that some CFs are important virulence factors in ETEC strains. Accordingly, ETEC strains that possess CFs were isolated at a significantly higher frequency from symptomatic children than from asymptomatic children. This conclusion has major implications, especially considering the fact that other studies have found no apparent association. Thus, López-Vidal et al. (11), in an epidemiological study carried out in urban Mexico, found that the presence of CFA/I, CFA/II, and CFA/IV does not represent a risk factor for the development of acute diarrhea. On the other hand, Cravioto et al. (6) found that in rural Mexico ETEC strains that produce CFs were more likely to cause diarrhea, while Paniagua et al. (15) found that in Nicaragua only CFA/I was significantly associated with disease. In the present study, we have found that disentangling the relative effects of the different variables with a multivariate model was crucial prior to arriving at any conclusion. In this regard, the presence of a CF in an ETEC strain was found to be closely correlated with diarrhea, independently of the toxin profile.

The capacity of the CFs that were formerly called PCFs and that were analyzed in this study, i.e., CS7, CS8, CS12, CS14, and CS17, to colonize and to induce an immune response has been shown in an animal model (21). However, this is the first study in which they are evaluated as a risk factor for diarrhea in children. Our data from the multivariate analysis show that ETEC strains that produce CS17 are independently associated with diarrhea. Although LT was not found to be a risk factor for diarrhea, LT-producing ETEC strains that express CFs, such as CS17, appeared to be strongly associated with illness.

The distribution of CFs in the ETEC strains is in itself important for ongoing efforts toward the development of vaccines against childhood diarrhea. Thus, results from such studies from different parts of the world will define the most important CFs to be covered by a vaccine. Previous surveys of ETEC strains from different areas have shown variations in the prevalence of the CFs produced by *E. coli* strains of 29 to 76%. In this study we found that 55% of the ETEC strains isolated from children with diarrhea produce some of the CFs sought. This frequency is lower than those found in some countries (5, 11, 20) and in our own previous studies performed in Argentina (2, 24). This is most likely due to geographical and temporal variations rather than to a low sensitivity of our detection system, as suggested by other investigators (25), since we have found a large number of ETEC strains that belong to serotypes which were never reported to be associated with any CF. In analogy with previous studies in Mexico (11) and India (20), the CFs most frequently identified were CFA/IV (16%), CFA/I (10%), and CS17 (9%).

CFs were mainly identified on strains producing ST or LT and ST; this finding is consistent with the fact that the plasmids coding for the CFs investigated in this study also usually code for ST (7). Furthermore, as previously shown for Argentinean ETEC isolates (2), CFA/I was produced by strains of serotypes O153:H45 and O78:H12.

As reported previously, CS1-, CS2-, and CS3-positive strains were found in serotype O6:H16 or O6:H− strains and were almost exclusively associated with the LT and ST production profile. Consistent with this observation, a recent molecular epidemiological study demonstrates that LT- and ST-producing ETEC O6:H16 strains are widespread (14). In analogy with previous studies in Latin America (5, 11), relatively few ETEC isolates expressed CFA/II. This is related to the low proportion of O6 strains, which accounted for only 2.5% of the total ETEC isolates.

It has been described previously that the ETEC isolates that produce both LT and ST are more often associated with diarrhea than those that produce only one enterotoxin. Nevertheless, the results from our univariate analysis show that LT- and

ST-producing ETEC strains were not significantly associated with symptomatic infection.

The association of ETEC with diarrhea varied according to the age of the children. Thus, the younger the child, the higher the risk of getting a symptomatic ETEC infection. Children older than 2 years are likely to be colonized with ETEC several times and therefore have acquired partial or complete immunity against ETEC diarrhea. Consistent with this, increased titers of antibodies against LT, CFA/I, and CFA/II were detected in another investigation (16) in which immunity against these virulence factors was investigated in the same population evaluated in the present study.

A specific vaccine that contains the most prevalent adhesins which were identified in this study, i.e., CFA/IV and CFA/I for use in humans, may cover as many as 65% of the strains that produce ST only and both ST and LT isolated from children with diarrhea, but only 3% of those that produce only LT (data not shown). These findings suggest that immunity against these LT only-producing strains, which belong to a much broader spectrum of serotypes and adhesin types than the ST-producing ETEC isolates, must be provided by the LT toxoid rather than by a number of different adhesins. Thus, recombinantly produced cholera toxin B subunit (CTB) is included in the candidate ETEC vaccine presently undergoing clinical trials. This orally administered vaccine has already been proven to be safe in Egyptian adults (17) and American students traveling to Mexico (19) and to induce a good immune response against CTB, CFA/I, CS1, CS2, and CFA/IV in Swedish and Egyptian volunteers (9, 18).

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