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Enterotoxigenic *Escherichia coli* diarrhoea: acquired immunity and transmission in an endemic area

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Enterotoxigenic Escherichia coli *(ETEC) are an important cause of diarrhoea in developing countries. Studies were made, in an endemic area of Bangladesh, of household contacts of patients with diarrhoea associated with* E. coli *producing heat-stable and heat-labile toxins (ST/LT) or heat-stable toxin (ST) only. It was found that 11% of contacts were infected in the 10-day study period, and that both the rate of infection and the proportion of infected persons with diarrhoea decreased with increasing age, suggesting the development of immunity. ETEC of the same serotype as that of the index patient were found in 9% of water sources used by index households, in a small number of food and drinking water specimens from the index homes, and in faeces from 3 healthy calves. The rate of infection of household members was highest in houses where there was contaminated food or water, which suggests that infection may take place in the home when contaminated water is brought in.*

Enterotoxigenic *Escherichia coli* (ETEC) organisms are important etiological agents of diarrhoea among residents of many developing countries (*1–5*). In a 2-year study conducted at a diarrhoea treatment centre in rural Bangladesh, ETEC were the most frequently found pathogens in patients of all ages and were second in importance only to rotavirus among patients less than 2 years old (*1*). Infection with ETEC may result in a severe cholera-like disease, or mild diarrhoea, or may be asymptomatic; however, the relative frequencies of these clinical states and the importance of acquired immunity are unknown (*4, 6*).

ETEC have been isolated from food and water, suggesting that these may be possible vehicles of infection (*4, 6, 7*). In the United States of America, a large outbreak of diarrhoea due to ETEC was related to consumption of contaminated water, the same serotype of ETEC being recovered from the affected persons and the water (*8*). In developing countries where ETEC disease is common, attempts to identify specific vehicles of infection have been unsuccessful (*4, 6*).

In an attempt to learn more about the clinical spectrum, epidemiological characteristics, and immunology of ETEC infection, we initiated a study in rural Bangladesh. In particular, the study aimed to find the proportion of infected persons who become ill and the variation in this proportion with age. On the basis of previous studies, the area is known to be endemic for ETEC infections (*1, 4, 5*).

METHODS

Study area

The study was conducted in the Matlab field research area of the International Centre for Diarrhoeal Diseases Research, Bangladesh (formerly the Cholera Research Laboratory). Matlab is 75 km south-east of Dacca, the capital of Bangladesh, and is a riverine, rural area that, at the time of the study, included 228 villages with 269 000 permanent residents. Since this population has been under continuous demographic surveillance for more than

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10 years, an accurate determination of each subject's age was possible (*9*).

Index patient selection

Between October 1977 and May 1978, all patients with diarrhoea who visited the central treatment facility and whose stool cultures were negative for *Salmonella*, *Shigella*, vibrios, and rotavirus were screened as potential ETEC index patients for a case–control study. Since the majority of ETEC in Bangladesh belong to a few well defined O:K:H serotypes (*10*, *11*), bacterial agglutination techniques followed by toxin tests were used to screen many of the *E. coli* isolates.

On admission, rectal swabs were taken from all patients and plated directly on MacConkey agar. For patients negative for other pathogens, 5 colonies with the appearance of *E. coli* were subjected to slide agglutination with a polyvalent antiserum containing serogroups O6, O8, O78, and O115. The polyvalent serum was made by pooling monovalent sera produced in rabbits after vaccination with the respective enterotoxigenic strains. Positive colonies were further subjected to slide and tube agglutination with monovalent O-group antisera (*12*). Patients positive for one of the four *E. coli* serogroups by tube agglutination were selected as index patients.

E. coli isolates that were positive by tube agglutination were assessed for their ability to produce heat-labile enterotoxin (LT) by the Chinese hamster ovary cell assay (*13*). Production of heat-stable enterotoxin (ST) was determined by the infant mouse assay (*14*).

The agglutination techniques proved to be an efficient method for selecting index patients since 85 of the 88 patients with positive agglutination tests had ETEC. The 3 patients with non-toxigenic *E. coli* were not included in the study, and the families of 2 patients were unwilling to participate, leaving 83 index patients in the study.

Epidemiological studies

The index patients were selected within 1–2 days of their visit to the treatment centre, and the patient's house was then visited. At the first visit and each day for the next 9 days, rectal swabs were obtained from all members of the index household, giving an average of 8.5 cultures per household member.

For each of the first 3 days, samples were taken from water sources used by any member of the household for drinking, bathing, or washing, from drinking water stored in the house, from left-over food, and by rectal swab from all animals owned or used by the household. Similar specimens were also collected from control households, which were systematically selected from the nearest adjacent residential compound and which had been free from diarrhoea in the preceding week. Specimens from water sources were obtained from the site at which water was taken by the family. Although some index and control families used the same water sources, they rarely used the same sites to take water.

Swabs from household contacts and animals were kept in chilled Cary-Blair transport medium and plated on MacConkey agar within 4 hours of collection. Water specimens were kept chilled and spread on MacConkey agar after serial 10-fold dilution. Ten colonies with the typical morphology of *E. coli* from each plate were subjected to slide agglutination with the monovalent O antiserum specific for the index patient. The testing of 10 colonies from each culture should have been sufficient to include more than 80% of the serotypes present in the intestine of a healthy person or in an environmental specimen (*15*). The O-groups of the slide agglutination-positive isolates were confirmed by tube agglutination, and were then tested for enterotoxin production in the same manner as the isolates from the index patients.

Blood specimens were collected from members of index patient households on the first and last household visits, and *E. coli* LT antitoxin titres were determined by the adrenal cell assay (*16*).

Analysis

ETEC isolated from index patients, family members, and environmental specimens were later confirmed as *E. coli* and their O:H serotype determined (*12*). ETEC from family or environmental cultures were included in the analysis only if they were the same O:H and toxin type as the index patient isolates.

RESULTS

Of the 83 index patients selected, 35% were less than 2 years old, 10% were 2–9 years old, and 55% were 10 or more years old. A total of 54 had ETEC that produced both ST and LT and 29 had ETEC that produced only ST. The serotypes of ETEC from index patients are listed in Table 1.

Of the contacts of index patients with a ST/LT infection, 11% were infected with ETEC of the same serotype and toxin type (Table 2). The proportion of persons infected was highest for children less than 2 years old and decreased with increase in age. Furthermore infected children under 5 years of age were significantly more often ill than older children and adults (Fisher's exact test, $P = 0.02$).

In the 29 families with an ST index infection, 10% of contacts were infected (Table 2), and here again, children less than 5 years old were more likely to be

Table 1. Serotype and toxin type of ETEC isolated from index patients

Serotype	Toxin type	
	ST/LT	ST
O6 : H16	20	4
O6[a]	–	1
O8 : H9	7	1
O78 : H10	1	1
O78 : H11	3	1
O78 : H12	16	15
O78 : H18	–	1
O78	–	3
O115 : H21	–	1
O115 : H40	1	1
O115 : H51	6	–
Total	54	29

[a] Non-motile.

Table 2. Proportion of household contacts of index patients with ETEC and diarrhoea

Age of contact (years)	No. of contacts	Infected contacts		Infected contacts with diarrhoea	
		No.	%	No.	%
ST/LT (54 families)					
< 2	24	7	29	5	71
2–4	31	7	23	3	43
5–19	138	15	11	3	20
20 +	112	6	5	1	17
Total	305	35	11	12	34
ST (29 families)					
< 2	9	2	22	2	100
2–4	20	3	15	2	67
5–19	56	5	9	1	20
20 +	56	4	7	0	0
Total	141	14	10	5	36

infected and were significantly more often ill than older children and adults (Fisher's exact test, $P = 0.02$).

Of the 49 infections with ETEC, 43% were detected on day 1 and 88% by day 4; 17 persons had diarrhoea, and one child had mild dehydration, while the others did not become dehydrated.

Table 3. Geometric mean titre of *E. coli* LT antitoxin in serum samples from household contacts with ST/LT *E. coli* and diarrhoea

Household contact	No.	Geometric mean titre (units per ml)	
		Acute	Convalescent
Not infected	183	15.7	16.1
Infected – no diarrhoea	23	16.0	17.0
Infected – diarrhoea	11	40.2	77.8[a]

[a] Paired t-test, $P < 0.05$.

Serum samples taken from 217 household contacts during the acute and convalescent phases of illness were tested for *E. coli* LT antitoxin. Fourfold rises in titre were found in 2 of 11 persons with *E. coli* diarrhoea and in 2 of 21 persons who had diarrhoea, but who had not been culture positive for ETEC. Table 3 illustrates the acute- and convalescent-phase geometric mean titres for household contacts of ST/LT patients. No change in mean titre was noted for the uninfected group or the group who were infected but not ill. The group with *E. coli* diarrhoea, however, did demonstrate a significant rise in titre in the convalescent phase. The relatively high mean titre of the acute-phase serum in this group probably reflects the titres found in young Bangladeshi children, who made up a large proportion of the group (Fig. 1).

ETEC was found in 13 water sources used by index households, but in none used by control households (Table 4). For both ST and ST/LT infections, the sources used by the case households were significantly more often contaminated than those used by the matched controls. Since several sources were positive for 2 or 3 days, there were 17 positive cultures from

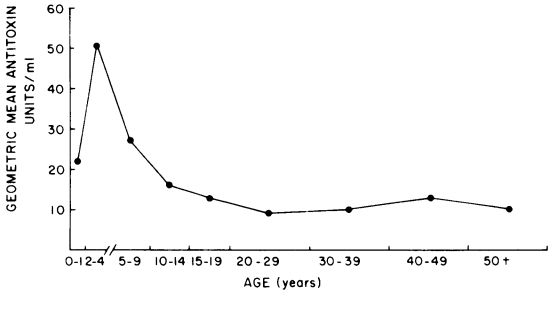

Fig. 1. *E. coli* LT antitoxic antibody levels in acute-phase sera of index patients, according to age.

Table 4. Isolation of ETEC from water sources of case and control households

Toxin type	No. of cultures		ETEC-positive sources		Significance
	Case	Control	Case	Control	
ST/LT	97	94	8 (8%)	0	$P = 0.004$
ST	55	50	5 (9%)	0	$P = 0.04$

water sources, of which 12 (71%) had fewer than 100 *E. coli* colony-forming units (cfu) per ml, while 5 had 100–1000 cfu per ml. The frequency of ETEC isolation from the 455 water cultures varied according to the type of water source; the positive rate for ditch water was 6%; tank water, 5%; canal water, 4%; tubewell water, 2%; and river water, 0%. These percentages were not significantly different. Of the 13 contaminated water sources, 7 were used for drinking water and other purposes, while the remaining 6 provided water that was not used for drinking but only for bathing, washing utensils, and cooking.

Other specimens, such as those from stored drinking water and food, were less often found to contain ETEC, although again the trend was for more positive specimens in case homes than in control homes (Table 5). In 2 case households and 1 control household, drinking water stored in the house was positive, and positive food samples were found in 4 case households. All drinking water cultures had fewer than 100 *E. coli* cfu per ml, while 3 of the 4 food specimens had 100–1000 cfu per ml. Cultures from 3 of 459 apparently healthy calves or cows contained ETEC of the same serotype and toxin variety as those of the index patient (2 were O6:H16 ST/LT *E. coli* and 1 was an O78:H12 ST *E. coli*).

The possible importance of exposure to positive environmental specimens is examined in Table 6. The risk of infection for members of households using a positive water source or potentially exposed to ETEC-contaminated cow dung was only slightly higher than that for persons in homes where all environmental specimens were negative. In contrast, persons exposed to contaminated food or drinking water at home had a risk of infection 3 times that of the other 2 groups.

Table 6. Rate of infection of household members exposed to ETEC in food, water, or animals of that household

ETEC-positive specimens	No. of household members	Household members infected with ETEC	
		No.	%
None	361	30	8.3[a]
Water source or animal only	30	3	10.0[b]
Drinking water or cooked food in house	55	16	29.0[a, b]
Total	446	49	11.0

[a] $\chi^2 = 18.9$, $P < 0.001$; relative risk, 3.5.
[b] $\chi^2 = 3.1$, $P < 0.08$; relative risk, 2.9.

DISCUSSION

Studies of household contacts of patients with ETEC diarrhoea have shown that 10–11% of contacts were infected with ETEC during a 10-day study period. The highest infection rates were in the youngest age groups, suggesting extensive exposure to ETEC early in life in this endemic area. The decreasing rate of infection with age could be due to more limited exposure to ETEC in older children and adults or to the development of immunity which prevents intestinal colonization with ETEC. The presence of contaminated food and water in the home suggests that all contacts were exposed to similar vehicles of ETEC. In the youngest age groups, infected household contacts nearly always had diarrhoea, while the proportion of infected persons who became ill decreased significantly with increasing age, for both ST

Table 5. Isolation of ETEC from stored drinking water, food, and animals

Toxin type	Stored drinking water (no. positive/no. tested)		Leftover food (no. positive/no. tested)		Cows and calves (no. positive/no. tested)		Goats and kids (no. positive/no. tested)	
	Case	Control	Case	Control	Case	Control	Case	Control
ST/LT	2/158	1/158	2/194	0/148	2/163	0/150	0/108	0/79
ST	0/86	0/84	2/93	0/72	1/74	0/72	0/19	0/21
Total	3/486		4/507		3/459		0/227	

and ST/LT ETEC. This finding suggests that immunity to these organisms does develop.

The mechanism of immunity to ETEC is unknown, but the development of serum antitoxic antibodies at the time of clinical illness, and the apparent protection given by antitoxin in some animal models, has led to speculation that these antibodies may have a protective role (*17, 18*). Our studies found a rise in antitoxic antibodies in patients who developed illness associated with ST/LT ETEC; however, the presence of substantial serum antitoxic antibody prior to infection did not appear to protect from illness. This observation on naturally acquired illness in an area with endemic ETEC supports similar findings in US volunteers, which indicated that serum antitoxic antibodies did not protect against subsequent challenge with LT-producing ETEC (*19*). Although systemic antibody may not reflect local intestinal immunity, the apparent development of immunity to ETEC producing only ST, which is only slightly immunogenic, indicates that immune mechanisms other than antitoxic antibody must be operative (*20*). The decreasing rate of infection with age observed in this study suggests that antibacterial or anticolonization mechanisms of immunity may be important.

In these studies, ETEC of the same serotype as that of the index patient were found frequently in water sources used by the index family, but not in those used by neighbouring control families. These sources provided water for various purposes, such as drinking, cooking, washing utensils, and bathing. Household contacts had a substantially higher risk of infection when there was contaminated water or food in the house than if they simply used a contaminated water source. This suggests that important exposures leading to infection may take place in the home, when contaminated water is used for drinking or preparing food. Since ETEC-positive household water was always associated with positive source water, it is likely that contamination took place at the source rather than in the house. This observation is similar to the finding in a study of El Tor cholera transmission in the same area, that the presence of *Vibrio cholerae* in household water is primarily due to contamination at the source rather than in-house exposure to an infected individual (*21*). However, in contrast to the observation that food is not a major vehicle for cholera (*21*), our studies suggest that it may be important in ETEC infection.

The level of *E. coli* found in ETEC-contaminated specimens (< 1000 cfu per ml) was lower than that used to induce disease in American volunteers; however, it is possible that, in the natural setting, a lower level of ETEC will result in illness. Reduced gastric acid or buffering of the gastric acid, e.g., by food, could result in enhanced susceptibility to ETEC infection (*19*).

ETEC are also frequent causes of diarrhoeal illness in young pigs and calves (*22, 23*). Although the ETEC associated with disease in various animals and man belong to different serogroups characteristic of the host species, an ETEC of a serotype that commonly causes diarrhoea in man has been isolated from a healthy pig (*6, 10*). The use of cow dung as cooking fuel by most rural Bangladeshi families necessitates frequent handling of dung by family members, especially women and older children. The isolation, from three animals, of ETEC of serotypes associated with human disease suggests that hands or water or food contaminated by animal faeces are possible vehicles of infection. Since 2 of the 3 families with an ETEC-positive animal also had contaminated food or water in their home, it is difficult to distinguish the possible routes of infection. As animals in this area drink from and often bathe and defecate in water used by the family members, they may also be occasional reservoirs of human serotypes of ETEC and may facilitate transmission of the organisms by faecal pollution of drinking water.

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RÉSUMÉ

DIARRHÉES À *ESCHERICHIA COLI* ENTÉROTOXIGÈNES: IMMUNITÉ ACQUISE ET TRANSMISSION DANS UNE ZONE D'ENDÉMIE

Les *Escherichia coli* producteurs d'entérotoxines (ETEC) représentent une importante cause de diarrhée dans de nombreux pays en développement. Afin de mieux connaître le spectre des manifestations cliniques, les caractéristiques épidémiologiques et l'immunologie de l'infection à ETEC, nous avons étudié les contacts domestiques de cas initiaux qui avaient présenté une diarrhée associée à la présence de *E. coli* produisant des toxines thermostable et thermolabile

(ST/LT) ou seulement la toxine thermostable (ST). Dans 83 familles étudiées (où il y avait eu 54 cas ST/LT et 29 ST), 11% des contacts ont été trouvés infectés par des ETEC au cours de la période d'étude de 10 jours. Les taux d'infection et de maladie les plus élevés se rencontraient dans les groupes d'âge les plus jeunes. La proportion de personnes infectées qui contractaient la maladie s'abaissait notablement en fonction de l'accroissement de l'âge, ce qui donne à penser que, dans cette zone d'endémie, il s'établit une immunité contre les ETEC tant ST/LT que ST. On ignore le mécanisme de cette immunité, mais l'existence d'anticorps sériques antitoxiques avant l'infection ne semble pas protéger de la maladie.

Des ETEC du même sérotype que ceux du cas initial ont été trouvés dans 9% des sources d'eau utilisées par les familles de ces malades, mais non dans les sources utilisées par les familles voisines témoins. Des ETEC ont été également découverts dans un petit nombre d'échantillons d'aliments et d'eau de boisson dans les maisons, et les personnes vivant dans ces ménages étaient exposées au plus haut risque d'infection. Il semble donc que les eaux de surface sont souvent contaminées par des ETEC, probablement en raison d'une pollution fécale, mais que des expositions importantes entraînant une infection peuvent se produire à domicile lorsque de l'eau contaminée y est introduite pour la boisson ou que des aliments sont préparés avec une telle eau. L'isolement, à partir de trois veaux en bonne santé, de ETEC d'un sérotype qui provoquait la maladie chez l'homme donne à penser que les animaux peuvent être occasionnellement des réservoirs de ETEC de sérotypes humains et que les mains, l'eau ou les aliments contaminés par des excréments d'animaux constituent des véhicules possibles de l'infection.

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