J HEALTH POPUL NUTR 2004 Dec;22(4):370-382
ISSN 1606-0997 $ 5.00+0.20

Prevalence of Enterotoxigenic *Escherichia coli*-associated Diarrhoea and Carrier State in the Developing World

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ABSTRACT

This study assesses the importance of enterotoxigenic *Escherichia coli* (ETEC) as a diarrhoeal agent in developing countries. Odds ratios were calculated for incurring ETEC-associated diarrhoea based on data reported between 1970 and 1999. Carriage of ETEC was associated with diarrhoea in children aged less than five years, except for hospitalized infants aged 0-11 month(s) and children aged 1-4 year(s) at outpatient clinics. Two hundred and eighty million episodes of diarrhoea due to ETEC were seen yearly among those aged less than five years, and close to 50 million children of this age group were asymptomatic carriers of ETEC. Every 7th diarrhoeal episode in children aged less than one year and close to 25% of diarrhoeal cases in children aged 1-4 year(s) were due to ETEC. A child born in a developing country is likely to experience 0.5 diarrhoeal episodes per year caused by ETEC until the age of five years, after which the yearly incidence drops to 0.1. To conclude, ETEC remains an important diarrhoeal pathogen among children in the developing world.

***Key words*:** *Escherichia coli*, Enterotoxigenic; Diarrhoea; Epidemiology; Odds ratio; Carrier rates; Child; Developing countries

INTRODUCTION

Enterotoxigenic *Escherichia coli* (ETEC), a subgroup of strains of *E. coli*, colonizes the small intestine and causes watery diarrhoea by the elaboration of a heat-stable enterotoxin (ST) and/or a heat-labile enterotoxin (LT) (1). Infection due to ETEC may result in severe cholera-like disease, moderate diarrhoea, or no symptoms at all. In fact, carriage of ETEC by healthy individuals is very common, making it difficult to estimate the influence this pathogen exerts on global health (2-4).

Although reports from the forties describe the existence of *E. coli* strains with the capacity to elicit watery diarrhoea in humans (5,6), it was only possible to differentiate with certainty between ETEC and other diarrhoeagenic *E. coli* after 1970 (7). Reliable ETEC diagnostic procedures must include detection of both ST and LT. Unfortunately, many epidemiological studies of diarrhoeal pathogens disregard ETEC because of the difficulty of diagnosis. The older methods for the detection of enterotoxins were bioassays requiring cell lines or live animals and could only be used for limited numbers of specimens. The advent of DNA hybridization techniques for the detection of enterotoxin genes has facilitated the screening of large numbers of isolates.

The major therapeutic problem of ETEC-associated diarrhoea is loss of fluid and subsequent dehydration. Hence, oral rehydration therapy, and in grave cases, replacement of intravenous fluid, are the cornerstones of treatment. Antibiotics may shorten the duration of diarrhoea due to ETEC (8), but the rapid emergence of resistant strains limits their usefulness (9).

ETEC is supposed to be a common cause of diarrhoea among children living in developing countries and visitors from regions where ETEC is not endemic

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(travellers' diarrhoea). Since the bacterium is also frequently isolated from stools of healthy individuals (2-4) or from specimens of diarrhoeal stool together with other enteric pathogens (10), it is complicated to assess its true pathogenic potential. This study had two main objectives: (a) to determine the carrier rates of ETEC in different population groups in developing countries and (b) to estimate odds ratios for developing diarrhoea in subjects colonized by ETEC.

Our analyses were based on data extracted from studies published between 1970 and 1999 that met predetermined inclusion criteria. The aim of this study was to determine the impact of ETEC on public health in the developing world.

MATERIALS AND METHODS

Literature search

A computer-based search was performed in Medline to retrieve scientific literature published between 1970 and July 1999 using the key words: ETEC, *Escherichia coli*, diarrhea, human, morbidity, and mortality, which yielded 3,302 articles, of which 664 were culled. Additional references were obtained from the citation lists of selected articles. A final selection of articles was made based on the criteria listed below.

Compilation of data

The proportions of diarrhoeic individuals and healthy subjects carrying ETEC were determined for each study. The lowest acceptable criterion for defining diarrhoeal disease was the presence of loose or diarrhoeal stool (11). We required that selected studies have well-defined age strata, containing at least 5 individuals in each age stratum to permit statistical analyses and that isolation frequencies of ETEC be presented for both ill and healthy individuals. The power of the selected studies was taken into consideration to allow for comparisons to be made between individuals from the various studies, which were very diverse in set-up and design. Thus, only studies containing more than 50 cases of diarrhoea were included, based on the assumption that if the true prevalence of ETEC-associated diarrhoea was 10% or higher, at least one case of ETEC-associated diarrhoea would be detected with probability of >99%.

Diagnostic criteria of infection due to ETEC

We only considered papers in which *E. coli* isolates were screened from stool for both ST and LT. The proper identification of *E. coli* was a necessary prerequisite since other Gram-negative bacteria, e.g. *Aeromonas, Morganella, Citrobacter, Yersinia, Klebsiella, Salmonella,* and *Campylobacter* species, are able to produce similar enterotoxins (12,13). We excluded articles in which the sole measure of the presence of enterotoxins was the detection of enterotoxin-specific serum antibodies. Such studies most likely grossly underestimate the isolation rate of ETEC since up to 50% of all strains produce ST only (14), a toxin that is too small to be immunogenic, and hence, does not give rise to antibody responses in humans. To rely on antibody responses to LT for diagnosis of LT-producing ETEC is also fraught with imprecision since detected antibodies might be directed against similar antigens, e.g. cholera toxin (15), or LT produced by other bacterial species (12,13).

Studies employing traditional, cell-culture-based assays of enterotoxin activity and studies using molecular tools for the detection of enterotoxin genes were accepted, despite the possible risk of varying concordance between DNA hybridization tests and biological assays (16,17).

Mixed infections, i.e. infections in which ETEC is isolated together with at least one additional enteropathogen, may be seen in up to 50% of all ETEC-infected persons (10). Since co-infections are the rule rather than the exception, we decided to include studies reporting such faecal specimens.

Calculation of odds ratios

To evaluate the pathogenicity of ETEC, we calculated the odds ratio for having diarrhoea upon isolation of ETEC in the stool. The proportions of diarrhoeic individuals and healthy subjects carrying ETEC were determined for each study. Odds ratios were automatically calculated by the software 'Epi Info' version 6.04b (CDC and WHO) using these figures. The same procedure was employed to calculate overall odds ratios for developing ETEC-associated diarrhoea among individuals belonging to specific age groups and settings. To this end, individuals from all studies were grouped together based on age and setting, and odds ratios were determined as described above.

RESULTS

In developing countries, enteric pathogens are frequently encountered in healthy individuals, making it difficult to determine their true aetiological role in

diarrhoeal diseases (2,18-20). To evaluate the pathogenic potential of ETEC, we chose to calculate the odds ratios for having diarrhoea among subjects carrying ETEC in their faeces. First, we compiled published data on faecal ETEC isolation rates (absolute numbers and percentages) from diarrhoeal cases and matched controls aged 0-11 month(s) (Table 1), 1-4 year(s) (Table 2), 5-15 years (Table 3), and >15 years (Table 3) (21). Next, we calculated diarrhoeal odds ratios for all these studies to estimate the risk of having diarrhoea when colonized with ETEC (Table 1-3). Finally, we pooled data from the different studies stratified by age [(0-11 month(s), 1-4 year(s), 5-15 years, and >15 years)] and setting (domicile, outpatient, or hospital) (Table 4).

Table 1. Isolation rates of ETEC from diarrhoeal cases and healthy controls aged 0-11 month(s)

Country	Ref no.	Study population	Study design	No. of diarrhoeal cases with ETEC/ total no. of diarrhoeal cases (%)	No. of healthy subjects with ETEC/total no. of controls (%)	Odds ratio	Confidence interval (95%)
Mexico	69	Domicile (R)	Prospective	58/170 (34.1)	48/170 (28.2)	1.32	0.81-2.14
Israel*	70	Domicile (R)	Prospective	20/159 (12.6)	104/857 (12.1)	1.04	0.60-1.78
Guinea-Bissau	71	Domicile (R)	Prospective	133/1219 (10.9)	40/511 (7.8)	1.44	0.98-2.12
Nicaragua	72	Domicile (U)	Prospective	154/643 (24.0)	203/1058 (19.2)	1.33	1.04-1.69
Peru	3	Domicile (U)	Prospective	70/952 (7.4)	138/1973 (7.0)	1.06	0.77-1.44
Chile	44	Domicile (U)	Prospective	22/162 (13.6)	10/159 (6.3)	2.34	1.01-5.51
Argentina	73	Domicile (R)	Prospective	21/95 (22.1)	75/572 (13.1)	1.88	1.05-3.33
Tanzania	74	Outpatient (R)	Case-control	30/108 (27.8)	4/100 (4.0)	9.23	3.04-37.3
Thailand	61	Outpatient (R)	Case-control	19/86 (22.1)	7/92 (7.6)	3.44	1.27-9.65
Brazil	62	Outpatient (U)	Case-control	33/500 (6.6)	9/500 (1.8)	3.86	1.75-8.76
Thailand	75	Outpatient (U)	Case-control	19/105 (18.1)	8/90 (8.9)	2.26	0.88-6.0
Brazil†	76	Outpatient (U)	Case-control	12/126 (9.5)	5/126 (4.0)	2.55	0.80-9.49
China	77	Outpatient (U)	Case-control	7/174 (4.0)	3/174 (1.7)	2.39	0.53-14.5
China‡	47	Hospital (U)	Case-control	41/180 (23.0)	18/90 (20.0)	1.14	0.60-2.19
Pakistan	78	Hospital (U)	Case-control	25/171 (14.6)	35/215 (16.2)	0.90	0.50-1.61
Brazil	79	Hospital (U)	Case-control	10/108 (9.2)	1/42 (2.3)	4.18	0.50-186
India	9	Hospital (U)	Case-control	17/218 (7.8)	1/102 (1.0)	7.95	1.10-162

*Bedouin children; †0-2 year(s) old; ‡Mean age: 1 year; ETEC=Enterotoxigenic *Escherichia coli*; R=Rural; U=Urban

Odds ratios above 1.0 with confidence intervals, excluding 1.0, indicate that ETEC has an aetiological role in diarrhoea. Thus, it may be concluded that, for the most studied age group of 0-4 year(s), colonization with ETEC was associated with a significantly increased risk of contracting diarrhoea, with a possible exception for children, aged 1-4 year(s), who attended outpatient clinics because of diarrhoea, and hospitalized children aged less than one year (Table 4). Unfortunately, few studies, comprising the age groups of 5-15 years and >15 years, included cases and controls, making it difficult to draw conclusions regarding the importance of ETEC as a diarrhoeal pathogen in these populations. One study does suggest that ETEC should be regarded as a possible diarrhoeal agent in adults with diarrhoea seeking treatment at outpatient clinics (Table 4). No suitable study on morbidity due to ETEC among adults in household settings was found.

Total numbers of diarrhoeal episodes of all aetiologies in different study populations

A review of 22 longitudinal community studies of stable populations in 12 developing countries in Asia, Latin America, and Africa revealed that the global median incidence of all types of diarrhoea in the developing world was 3.9 episodes/child/year for children aged 0-11 month(s) and 2.1 episodes/child/year for children aged 1-4 year(s) (Table 5) (22). Except for an epidemiological household-based study from China (23), hardly any studies have chosen to examine the yearly diarrhoeal incidence in the 5-15-year age groups and 16 years and above (Table 5). Based on recent estimates of the total world population (24), we could compute the number of diarrhoeal episodes of all aetiologies expected to strike infants, young children, older children, and adults in the developing world (Table 5).

A study conducted in a poor peri-urban community in Santiago, Chile, during the mid-1980s, revealed that 88% of diarrhoeal cases among 0-11-month old infants were treated at home, and 10% attended ambulatory treatment centres, whereas 1.5% were admitted to hospital (25). Among 1-4-year old children, 92% of diarrhoeic children remained at home, 7.8% sought treatment as outpatients, and 0.2% were admitted to hospital (25).

Number of diarrhoeal episodes due to ETEC in various settings

The total number of diarrhoeal cases attributable to ETEC (Tables 6 and 7) was arrived at by multiplying the percentage of episodes from which ETEC was identified in different age groups and study populations (Table 4), by the total number of diarrhoeal cases in each study population and age group (Table 5).

Table 2. Isolation rates of ETEC from diarrhoeal cases and healthy controls aged 1-4 year(s)

Country	Ref no.	Study population	Study design	No. of diarrhoeal cases with ETEC/ total no. of diarrhoeal cases (%)	No. of healthy subjects with ETEC/total no. of controls (%)	Odds ratio	Confidence interval (95%)
El Salvador	20	Domicile (R)	Prospective	13/85 (15.3)	14/78 (17.9)	0.83	0.33-2.03
India	68	Domicile (R)	Prospective	115/302 (38.0)	6/72 (8.3)	6.76	2.81-19.6
Bangladesh	49	Domicile (R)	Prospective	248/920 (27.0)	88/2,060 (4.3)	8.27	6.34-10.8
Australia*	80	Domicile (R)	Prospective	28/173 (16.2)	33/349 (9.5)	1.85	1.04-3.28
Argentina	73	Domicile (R)	Prospective	21/95 (22.1)	75/572 (13.1)	1.88	1.05-3.33
Chile	44	Domicile (U)	Prospective	109/919 (11.9)	64/904 (7.1)	1.77	1.26-2.47
Brazil†	19	Domicile (U)	Prospective	7/50 (14.0)	6/40 (15.0)	0.92	0.25-3.47
Burma	81	Domicile (R)	Case-control	112/356 (31.5)	24/308 (7.8)	5.43	3.31-8.97
Iran	43	Outpatient (U)	Case control	76/443 (17.1)	1/37 (2.7)	7.46	1.21-306
Nepal	82	Outpatient (U)	Case-control	38/124 (31.0)	22/103 (21.0)	1.63	0.85-3.12
China‡	47	Outpatient (U)	Case-control	41/221 (18.6)	18/100 (18.0)	1.04	0.54-2.01
Iran	43	Hospital (U)	Case-control	122/715 (17.1)	3/42 (7.1)	2.67	0.83-13.7
Kuwait	83	Hospital (U)	Case-control	50/621 (8.0)	1/151 (0.7)	13.1	2.21-532

*Aborigines; †Acute and persistent diarrhoea, 0-5 year(s) of age; ‡Mean age: 1.2 years
ETEC=Enterotoxigenic *Escherichia coli*; R=Rural; U=Urban

Table 3. Isolation rates of ETEC from diarrhoeal cases and healthy controls aged 5-15 years and >15 years

Country	Ref no.	Study population	Study design	No. of diarrhoeal cases with ETEC/ total no. of diarrhoeal cases (%)	No. of healthy subjects with ETEC/total no. of controls (%)	Odds ratio	Confidence interval (95%)
5-15 years							
Chile	44	Domicile (U)	Prospective	14/157 (8.9)	10/157 (6.4)	1.44	0.58-3.62
Thailand	61	Outpatient (R)	Case-control	7/40 (17.5)	16/399 (4.0)	5.08	1.75-14.4
Djibouti*	84	Outpatient (U)	Case-control	23/209 (11.0)	10/100 (10.0)	1.11	0.48-2.63
Korea†	85	Hospital (U)	Case-control	52/231 (22.5)	14/104 (13.5)	1.87	0.94-3.74
>15 years							
Thailand	61	Outpatient (R)	Case-control	15/107 (14.0)	24/831 (2.9)	4.85	2.34-9.99
Brazil	86	Hospital (U)	Case-control	20/43 (46.5)	12/31 (39.7)	1.38	0.49-3.92

*Mean age: 13 years; †Children aged less than 15 years

We extrapolated these data to calculate overall numbers of diarrhoeal cases in the developing world expected to occur in home settings, treatment centres, or hospitals (Table 5), an approach employed in a recent report on the global burden of shigellosis (26).

The highest incidence of ETEC-associated diarrhoea was observed in children aged 0-4 year(s). Every year, 280 million cases of diarrhoea due to ETEC arose in this age group (Table 6). The incidence of this disease entity dropped to ≃110 million per year for individuals

Table 4. Isolation rates of ETEC and odds ratios for diarrhoeal illness in different age groups and study populations

Age group	Study population	No. of studies	No. of diarrhoeal cases with ETEC/ total no. of diarrhoeal cases (%)	No. of healthy subjects with ETEC/total no. of controls (%)	Odds ratio	Confidence interval (95%)
0-11 month(s)	Domicile	7	478/3,300 (14.5)	618/5,300 (11.7)	1.24*	1.12-1.46
	Outpatient	6	120/1,099 (10.9)	36/1,082 (3.3)	3.56*	2.39-5.32
	Hospital	4	93/677 (13.7)	55/449 (12.2)	1.14	0.76-1.66
1-4 year(s)	Domicile	8	653/2,900 (22.5)	310/4,383 (7.1)	3.82*	3.30-4.42
	Outpatient	3	155/788 (19.7)	41/240 (17.1)	1.19	0.90-1.77
	Hospital	2	172/1,336 (12.9)	4/193 (2.1)	6.21*	2.34-23.3
5-15 years	Domicile	1	14/157 (8.9)	10/157 (6.4)	1.44	0.58-3.62
	Outpatient	2	42/241 (17.4)	58/239 (24.3)	0.72	0.45-1.13
	Hospital	1	52/231 (22.5)	14/104 (13.5)	1.87	0.94-3.74
>15 years	Outpatient	1	15/107 (14.0)	24/831 (2.9)	4.85*	2.34-9.99
	Hospital	1	20/43 (46.5)	12/31 (39.7)	1.38	0.49-3.92

*The confidence interval of calculated odds ratio does not include the digit 1; ETEC=Enterotoxigenic *Escherichia coli*

Table 5. Burden of diarrhoea of all aetiologies in different settings and age groups in the developing world (22-24)*

Estimate	Age groups			
	0-11 month(s)	1-4 year(s)	5-15 years	>15 years
Total population (1994)	125,000,000	450,000,000	1,010,985,000	2,976,058,000
No. of diarrhoeal episodes/person/year	3.9	2.1	0.65	0.50
Total no. of diarrhoeal episodes/year	487,500,000	945,000,000	657,140,250	1,488,029,000
No. of domicile cases	429,975,000	868,455,000	ND	ND
No. of outpatient cases	50,212,500	74,655,000	ND	ND
No. of hospital cases	7,312,500	1,890,000	ND	ND

*Figures derived from these publications; ND=Not done

aged 5-15 years (Table 7). It is not clear how prominent ETEC-associated diarrhoea is among the adult population, but our figures, albeit based on a single study, suggest that it may be of importance (Table 7).

We also predicted the yearly risk the individual children within the various age intervals ran of contracting ETEC-associated diarrhoea. This figure was arrived at by multiplying the expected number of diarrhoeal episodes/person/year of all aetiologies (Table 5) by the percentage of ETEC-associated diarrhoeal episodes predicted to occur in different age groups (Tables 6 and 7). It was seen that, for the youngest children, the predicted number of ETEC-mediated diarrhoeal episodes was 0.5 episodes/child/year (for 0-11-month old and 1-4-year old children) (Table 6), whereas for the older children the number dropped to ~0.1 episodes/child/year (Table 7).

ETEC carrier rates

High isolation rates of ETEC are found in stools of asymptomatic individuals of all ages. We tried to estimate the numbers of healthy children colonized with ETEC by multiplying the proportion of healthy children carrying ETEC reported in the prospective domicilary studies with the total numbers of children in different age groups (Table 8). Since there was more bias in the selection of controls used in outpatient and hospital studies, we did not base our assessment of ETEC carriage on those studies. Our estimates indicate that close to 50 million children aged 0-4 year(s) are at any time colonized with ETEC. When comparing the isolation rates of ETEC from sick and healthy individuals, it was observed that the confidence intervals of odds ratios computed for children aged five years and above always included 1.0, indicating that there was no significant difference between cases and controls in this age group (Table 4).

DISCUSSION

This study almost exclusively focuses on individuals living in developing countries. ETEC is only exceptionally a cause of childhood diarrhoea in industrialized countries

Table 6. Annual burden of diarrhoea due to ETEC among children aged up to 4 years in different settings in the developing world

Setting	Age group		
	0-11 month(s)	1-4 year(s)	Total 0-4 year(s)
Domicile			
Total no. of diarrhoeal cases	429,975,000	868,455,000	1,298,430,000
% of ETEC episodes	14.5	22.5	19.8
No. of diarrhoeal cases due to ETEC	62,346,375	195,402,380	257,748,755
Outpatient			
Total no. of diarrhoeal cases	50,212,500	74,655,000	124,867,500
% of ETEC episodes	10.9	19.7*	16.2
No. of diarrhoeal cases due to ETEC	5,473,162	14,707,035	20,180,197
Hospital			
Total no. of diarrhoeal cases	7,312,500	1,890,000	9,202,500
% of ETEC episodes	13.7*	12.9	13.5
No. of diarrhoeal cases due to ETEC	1,001,812	243,810	1,245,622
All settings			
Total no. of diarrhoeal cases	487,500,000	945,000,000	1,432,500,000
% of ETEC episodes	14.1	22.2	19.4
No. of diarrhoeal cases due to ETEC	68,821,349	210,353,225	279,174,574
No. of diarrhoeal episodes due to ETEC/person/year	0.54	0.47	ND

*ETEC was not significantly more often isolated from individuals with diarrhoea than from controls;
ETEC=Enterotoxigenic *Escherichia coli*; ND=Not done

Table 7. Annual burden of diarrhoea due to ETEC for persons aged 5 years and above in the developing world

Estimate	Age group		
	5-15 years	>15 years	Total >5 years
No. of diarrhoeal cases due to ETEC	114,342,400	446,408,700	560,751,100
Total no. of diarrhoeal cases	657,140,250	1,488,029,000	2,045,169,250
% of ETEC episodes	17.4	30.0	27.0
No. of diarrhoeal cases due to ETEC/person/year	0.11	0.15	ND

ETEC=Enterotoxigenic *Escherichia coli;* ND=Not done

Table 8. Percentage and total number of ETEC carriers among healthy individuals in different age groups

Estimate	Age group		
	0-11 month(s)	1-4 year(s)	Total 0-4 year(s)
Total no. of children	125,000,000	450,000,000	575,000,000
% of healthy children colonized with ETEC	11.7	7.1	8.1
Total no. of healthy children colonized with ETEC	14,625,000	31,950,000	46,575,000

ETEC=Enterotoxigenic *Escherichia coli*

(27-33). Further, food-borne outbreaks of ETEC are very rare in this economically-privileged part of the world (34-36). The predominant way for residents of the developed world to contract infections due to ETEC is through travels to developing countries (37,38). A large number of studies have also established that ETEC-associated diarrhoea is a frequent problem among military personnel deployed to regions of ETEC endemicity (39,40). However, we disregarded the contribution of ETEC-associated diarrhoea among western travellers, military personnel, and expatriates to total global morbidity due to ETEC.

Our survey of studies indicates that the incidence of diarrhoea due to ETEC is high in children aged less than one year (69 million episodes per year), remains high among children aged 1-4 year(s) (210 million per year for the whole age bracket, average of 52 million

per year), and declines thereafter, giving an incidence of 114 million per year for the entire age group of 5-15 years (an average of about 10 million per year). Our findings are supported by a multicentre study of morbidity due to ETEC, initiated by WHO in five developing countries, which revealed that ETEC was detected at a relatively constant rate from diarrhoeal cases during the first three years of life (41). Nevertheless, other studies have repor-ted that the incidence of ETEC-associated disease varies within this age interval (10,11,42,43).

The commonality of ETEC-associated diarrhoea is also reflected by our estimates, indicating that a child residing in a developing country will, on average, experience 2.5 episodes of diarrhoea due to ETEC during the first four years of life (0.5 episodes per child per year until the age of five years), after which the frequency of ETEC-mediated diarrhoea declines sharply, amounting to 0.1 episodes per year from the age of five years and onward. Similarly, a longitudinal study following two cohorts of children of low socioeconomic level established that one in two children had experienced an episode of ETEC-associated diarrhoea by the age of two years, and 90% had done so by the age of five years (44). It is difficult to make a reliable estimate of morbidity due to ETEC among adults due to a paucity of adequate studies, although ETEC-associated diarrhoea does seem to be a disease of adulthood too (45). More studies are warranted to elucidate this point.

The chief aim of this study was to calculate the odds ratios for incurring diarrhoea in individuals colonized with ETEC. Although we could have estimated the relative risks for morbidity due to ETEC instead of odds ratios for the few prospective studies that were available, we chose to calculate odds ratios throughout, so that case-control and prospective studies could be analyzed together. Longitudinal analyses were seldom performed in the prospective studies and, hence, did not take into account the effect of autocorrelation within individuals. However, we judged this to be an acceptable approximation for the purposes of this report.

It is important to realize that an important bias may have been introduced into our survey since it is based on the compilation of a large number of studies performed in various regions of the developing world that differ with respect to socioeconomic development. Thus, our calculations of morbidity due to ETEC are, by necessity, crude estimates. Another caveat is that the actual number of *E. coli* colonies the various studies chose to screen for enterotoxins might have influenced the sensitivity of detection, since non-ETEC are, as a rule, also present in faecal specimens. Most studies picked 3-5 colonies per specimen, a procedure which may miss 8-50% of ETEC cases (18).

We estimated that the odds ratio for ETEC-associated diarrhoea varied between 1.2 and 6.2 for the numerically most important group of individuals who are prone to infections due to ETEC, i.e. children aged less than five years in developing countries. We could not establish that ETEC colonization was associated with an increased risk of developing diarrhoea for two subpopulations of small children, namely hospitalized children aged less than one year and children aged 1-4 year(s) attending outpatient clinics. These findings can be interpreted to indicate that ETEC-associated diarrhoea is generally not a severe disease requiring care at treatment centres. Hence, a positive association between carriage of ETEC and diarrhoea is difficult to demonstrate at treatment centres, but more easily revealed for (the majority of) sick children treated at home.

Although ETEC-associated diarrhoea particularly strikes small children, it must be emphasized that, at any time, close to 50 million children aged 0-4 year(s) are colonized with ETEC without displaying any symptoms of diarrhoea at all. Several studies have documented that asymptomatic carriers of ETEC are, on average, a few years older than those individuals who succumb to symptomatic infections due to ETEC (46-48). Our analyses support this claim, since the odds ratios for ETEC as a cause of diarrhoea in children older than four years were non-significant, i.e. confidence intervals included the digit 1.0. Consequently, estimates of diarrhoeal incidence attributed to ETEC must be questioned for this age group; it is likely that a significant fraction of these individuals happens to be colonized by ETEC and have diarrhoea of other aetiologies.

It is well-known that the incidence of ETEC-associated diarrhoea diminishes with age in endemic areas (49,50). Children aged less than five years were more likely to develop diarrhoea due to ETEC compared to the older children or adults residing in the same household (51). However, these data can only indirectly support the notion that the drop in incidence is due to development of protective immunity in the host. Nevertheless, an experimental study involving American volunteers demonstrated that primary infection with an ETEC strain gave protection against reinfection with the same

strain (52). These facts have lent optimism to efforts aimed at elaborating vaccines against ETEC-associated disease.

It was more common for children staying at home to be unaffected by ETEC carriage when they were aged less than one year compared to when they were aged 1-4 year(s). Hence, 7/7 of domicile studies among children aged 0-11 month(s) had odds ratios with confidence intervals including the digit 1, whereas this was only the case for 2/7 of studies involving older children. The reason for this is unknown, but breastfeeding may be a plausible explanation. The beneficial effect of breastmilk in diminishing the incidence of diarrhoea in infants colonized with ETEC may be attributed to avoidance of contaminated food and drink and to protective factors present in milk (53).

A protective effect of breastfeeding against ETEC-associated diarrhoea was seen in about half of studies dedicated to this topic. Thus, exclusive breastfeeding halved the risk of developing severe infection due to ETEC in Bangladeshi children during the first year of life, but no protective effect was seen for mixed feeding during the second and third years of life (54). Two studies from Egypt failed to document a protective effect of breastfeeding against ETEC-associated diarrhoea (11,55), as did a study from Java (56). In contrast, Long and co-workers found that formula-fed children had a threefold higher incidence of LT-ETEC-associated diarrhoea than strictly breastfed infants and twice as high as mixed-fed infants (57). Similarly, Cruz *et al*. could demonstrate that infants infected with LT-producing ETEC were significantly less likely to suffer from LT-induced diarrhoea if they were fed breastmilk containing high IgA titres against LT compared to infected children who received breastmilk containing low IgA titres to LT (58).

A possibility, which has not been explored so far, is maturational differences in expression of intestinal receptors for colonization factors, which protect older pigs from contracting diarrhoea mediated by porcine ETEC strains (59). Age-dependent differences in receptor patterns have also been described in rabbits (60). It is unknown whether similar age-related differences in receptor expression exist in human beings, and if so, whether this could explain the increased resistance of older individuals to ETEC-associated diarrhoea. The fact that adult travellers from industrialized countries often contract ETEC-associated diarrhoea may speak against this possibility.

The incidence of both symptomatic and asymptomatic infections due to ETEC has a seasonal variation: most studies report higher isolation rates of ETEC during the hot and wet seasons (2,61,62). Studies of epidemiology of ETEC may, thus, overestimate or underestimate the incidence of diarrhoea due to ETEC depending on when the studies were conducted. One factor, which has not received due attention, is the time of the year when cohorts of children monitored for diarrhoeal disease were born. It is beginning to be appreciated that the health status of growing children is significantly influenced by season of their birth and that this effect is particularly important for children raised in poor surroundings (63,64). Hence, longitudinal studies that describe the impact of season on the isolation rate of ETEC, while taking into account the effect of age and birth date, are needed. Such studies may help clarify the dynamic distribution of ETEC in developing countries and increase the understanding of the relationship between healthy and sick carriers of ETEC.

Socioeconomic factors are of central importance for ETEC-associated morbidity since transmission of infections due to ETEC occurs mainly through ingestion of contaminated drinking-water and food (3). In rural Egypt, ownership of a household latrine halved the risk of incurring a symptomatic infection due to ETEC (11). Conversely, patients suffering from diarrhoea due to ETEC lacked an indoor tap significantly more often than did patients with diarrhoea of other aetiologies or controls (65). Nonetheless, considerable transmission of ETEC was observed in a cohort of Chilean children who had access to potable water (44). Gender appears to impact on the risk of contracting ETEC-associated diarrhoea as documented by two studies which found that girls were 1.5-2 times more likely to have diarrhoea due to ETEC than boys (47,66). A plausible explanation may be that girls are more underprivileged than boys in developing countries. Since children living in poverty are the main victims of disease due to ETEC, measures directed towards reducing faecal contamination of their environment, e.g. construction of latrines, access to potable water, and promotion of breastfeeding, are essential.

It is rare to find studies of ETEC-associated morbidity in which multivariate analyses are used (11,44, 55,57). The study of Abu-Elyazeed and co-workers in Egypt showed that faecal isolation of ETEC was associated with diarrhoeal symptoms with an odds ratio of 2.0 (confidence interval: 1.4-3.0) (11). This odds ratio

was adjusted for season, age, socioeconomic status, presence of sanitary latrine, and occurrence of breastfeeding usng multivariate logistic regression. Hopefully, future studies of the epidemiology of ETEC will use this approach to help determine which measures will be most effective in endeavours to eliminate ETEC-associated disease.

Is it worthwhile to attempt to eradicate ETEC to promote public health in developing countries? This may not seem to be the case since the majority of diarrhoeal episodes caused by this pathogen can be treated at home and mortality figures are difficult to obtain. However, ETEC-associated diarrhoea has been shown to exert a negative impact on short-term weight gain in Bangladeshi children (67). Furthermore, ETEC is frequently isolated from infants with chronic diarrhoea, a condition known to be associated with higher mortality than short-term diarrhoea (3). Finally, an Indian study revealed that ETEC was more frequently isolated from malnourished children than from children of normal nutritional grade (68). Thus, it appears as if ETEC intervention measures could increase the health status of least-privileged children living in developing countries.

ACKNOWLEDGEMENTS

The World Health Organization provided financial support for this study. We are grateful to Agnes Wold and Ia Adlerberth for critical reading of the manuscript.

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