REVIEW ARTICLE
Part III. Analysis of data gaps pertaining to enterotoxigenic *Escherichia coli* infections in low and medium human development index countries, 1984–2005

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SUMMARY

Enterotoxigenic *Escherichia coli* (ETEC) is a common cause of profuse watery diarrhoea in the developing world, often leading to severe dehydration or death. We found only 15 population-based studies in low and medium human development index (HDI) countries from 1984 to 2005 that evaluate disease incidence. Reported incidence ranged from 39 to 4460 infections/1000 persons per year. The peak incidence of ETEC appeared to occur between ages 6 and 18 months. A median of 14% (range 2–36%) of diarrhoeal specimens were positive for ETEC in 19 facility- and population-based studies conducted in all age groups and 13% (range 3–39%) in 51 studies conducted in children only. Heat-labile toxin (LT)-ETEC is thought to be less likely to cause disease than heat-stable toxin (ST)-ETEC or LT/ST-ETEC. Because population-based studies involve enhanced clinical management of patients and facility-based studies include only the most severe illnesses, reliable data on complications and mortality from ETEC infections was unavailable. To reduce gaps in the current understanding of ETEC incidence, complications and mortality, large population-based studies combined with facility-based studies covering a majority of the corresponding population are needed, especially in low-HDI countries. Moreover, a standard molecular definition of ETEC infection is needed to be able to compare results across study sites.

INTRODUCTION

Enterotoxigenic *Escherichia coli* (ETEC) causes an estimated 840 million gastrointestinal infections [1], and about 380 000 deaths worldwide each year [2]. Infection with ETEC leads to profuse watery

diarrhoea, and can be confused with cholera. ETEC is primarily spread through food or water contaminated with human waste, and is a frequent cause of outbreaks of diarrhoea. Prevention and control of ETEC infections is through the provision of safe food, water and sanitation, which has proved difficult to achieve on a large scale in the developing world. Currently no effective vaccine exists.

To identify contemporary gaps in the understanding of the global epidemiology of ETEC infection, we conducted a review of the recent scientific literature. We summarize below recent available data on

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This is the third of three papers, appearing in successive issues of the Journal, reviewing the analysis of data gaps pertaining to infections in low and medium human development index (HDI) countries.

morbidity and mortality burden, the age, geographic and temporal distribution of ETEC, the isolation of ETEC in relation to other diarrhoeagenic pathogens, and the frequency of the different ETEC toxin types. To provide context to its global epidemiology, we also describe ETEC diagnostics and preventive measures. Data gaps and existing research needs are then discussed.

BACKGROUND

Bacteriology

As members of the family Enterobacteriaceae, *Escherichia coli* are mostly Gram-negative, non-spore-forming, motile, lactose-fermenting rods. Although most *E. coli* are non-pathogenic commensal organisms that colonize animal and human intestines, some highly adapted strains can cause human illness.

Enterotoxigenic *E. coli* (ETEC), a cause of watery diarrhoea, belong to a large number of serotypes (defined by the lipopolysaccharide O antigen, flagellar H antigen and capsular K antigen types). They are defined by the ability to produce the heat-labile toxin (LT), heat-stable toxin (ST), or both (LT/ST). More than 20 colonization factors (CFs), found in adhesive fimbriae or bacterial cell surfaces, have also been identified. Immunity is poorly understood, but antibodies to both serotype-specific antigens and LT are thought to confer protective immunity [3]. Although a single individual can suffer repeated ETEC infections, persons living in endemic areas and exposed to multiple strains often develop immunity to a broad range of ETEC strains.

The infectious dose of ETEC is high, and estimated to range from 10^6 to 10^{10} organisms [4], although vulnerable populations such as children and the elderly may be susceptible to infection at lower doses. In order to cause diarrhoea, small intestine colonization, enterotoxin elaboration and action on enterocytes must all occur. The CFs promote small intestine colonization. Secretory watery diarrhoea can be caused by either LT or ST, both of which stimulate chloride secretion and inhibit chloride absorption in small intestine epithelial cells. LT is structurally and functionally similar to cholera toxin; ST is a very small protein that does not appear to elicit an immune response. The role of different CFs in determining severity of illness is poorly understood [5, 6].

The effect of antimicrobial use on the course of illness caused by ETEC is poorly understood, as diagnosis of ETEC is expensive and not readily available to most clinicians. Antimicrobial resistance to drugs such as ampicillin and trimethoprim–sulfamethoxazole is common. If antimicrobial agents are used, fluoroquinolones such as ciprofloxacin or norfloxacin are typically recommended [7, 8], although resistance to these antimicrobials has been documented as well [9], and their use in children is controversial.

Clinical features

The incubation period of ETEC is typically between 10 h and 3 days. Illnesses typically last for 3–5 days; shorter or longer duration illness can also occur [10]. ETEC infection is characterized by watery diarrhoea with either no fever or a low grade fever, but symptoms can also include abdominal cramping and vomiting. Severe, life-threatening dehydration can occur. Extraintestinal complications, such as bacteraemia, are not generally seen with ETEC infections.

Role of environmental and social factors

ETEC is found in both animal and human intestines. Although virulence factors, such as CFs and LT and ST subtypes, and infecting strains are generally species-specific [11, 12], animal strains have been reported to cause illness in humans [13]. Porcine ST toxin (STp) causes disease in humans, and diagnostic methods which differentiate STp from human ST toxin (STh) may help identify differences in the epidemiology of these two strains [14]. Unlike Shigellae or *E. coli* O157:H7, the high infectious dose of ETEC makes direct person-to-person spread rare. Instead, ETEC are primarily transmitted through contaminated food and water. In young infants who may become ill with a lower infectious dose than adults, the introduction of water or weaning foods has been shown to lead to ETEC infections as early as age 3 months [15]. Poor drinking water quality, lack of a sewage system and feeding of supplementary foods were found to be risk factors for ETEC infection in a study of Ecuadorian infants [16]. In Bangladesh, ETEC has also been found to be a major cause of epidemic watery diarrhoea during floods [17]. Countries with the highest incidence of ETEC infection tend to have rapid population growth, periurban areas with poor infrastructure combined with rapid

Table 1. *Terms used in literature search to identify gaps in data on enteric disease burden*

Burden of disease
   Epidemiology
   Morbidity
   Mortality
   Disease outbreaks
   Incidence
   Prevalence
   Seasons
   Population surveillance
   Age distribution
   Longitudinal survey
Pathogen-specific terms for Enterotoxigenic *E. coli*
   Escherichia coli
   E. coli infections
   Heat-stable toxin (*E. coli*)
   Enterotoxin heat-labile toxin

urbanization, sewage-contaminated drinking water and poor sanitary facilities. The epidemiology of ETEC is therefore related to poor socioeconomic conditions.

METHODS

We systematically searched the English-language scientific literature published between 1984 and 2005, using the Medline database, restricting the search to low and medium development countries according to the Human Development Index, United Nations Development Programme (accessed at www.hdr. undp.org). A set of articles containing the key words epidemiol-, morbidity, mortality, disease outbreaks, incidence, prevalence, seasons, population surveillance, age distribution, and longitudinal survey was linked with a set of pathogen-specific terms (Table 1). The resulting cross-linked set was reviewed for publications that contained information addressing specific questions on morbidity, mortality, age distribution, geographic distribution, temporal distribution, pathogen-specific preventive measures, and diagnostics. Population-based studies with culture confirmation of cases were considered primary data sources. When these were lacking, hospital-based studies were considered. Publications were then evaluated for their contribution to an understanding of the global epidemiology of ETEC, and gaps in the data were identified.

Publications were sought with information on the incidence of the infection by region according to the 21 regions of the United Nations Department of Social and Economic Affairs, Population Division (http://esa.un.org/unpp/). We examined the correlation between the incidence of ETEC infection and the national *per capita* gross domestic product, adjusted for purchasing power parity in year 2000 dollars, at the time that the studies were conducted (http://unstats.unm.org/unsd/cdb/cdb_series_xrxx.asp?series_code = 29922). We searched for publications with information on the age-specific incidence, morbidity, seasonality, mortality, preventive measures and diagnostics. We examined the frequency of ETEC relative to other diarrhoea-causing pathogens including *Salmonella*, *Shigella*, *Vibrio cholerae* O1, *Campylobacter* and *Rotavirus*. In studies in which stools of healthy controls were tested in addition to those with diarrhoea, we report frequency of ETEC isolation only among those with diarrhoea, but discuss the impact of asymptomatic infection on estimates of disease incidence separately.

RESULTS

Morbidity

Incidence

In a 2004 review, the annual number of ETEC cases in the developing world was estimated at 840 million episodes, with another 50 million asymptomatic carriers in children aged <5 years [1]. Incidence of symptomatic infections was estimated at 500 cases/1000 person-years for children aged <5 years, and 100 cases/1000 person-years for older persons. These figures were derived by estimating the total number of diarrhoeal cases and multiplying that by the proportion attributable to ETEC, as determined by the yield of ETEC in stool among persons with diarrhoea found in a variety of studies and age groups.

In this review of the published literature from 1984 to 2005, we found 15 population-based studies of ETEC incidence in low and medium human development index (HDI) countries (Table 2) [18–32]. Fourteen of these studies were conducted in ten medium-HDI countries, representing Asia (8), Africa (2), and Latin America and the Caribbean (4). The fifteenth study was conducted in a low-HDI country, Guinea–Bissau.

Table 2. *Population-based studies of ETEC incidence published 1984–2005*

Region	Country	HDI	Study year(s)	Age groups	Size of cohort	Total person-years	Crude incidence*	Incidence calculation†	% of diarrhoea cases with ETEC	Pathogenicity Odds ratio (95% confidence interval) ST-ETEC	LT-ETEC	ST/LT-ETEC	Ref.
N Africa	Egypt	Med	1993–95	<3 yr	242	216	600	Author	20	2·3 (1·4–3·7)	1·5 (1·0–2·4)	n.s.	[18]
N Africa	Egypt	Med	1995–98	<3 yr	608	n.r.	1470	Author	n.r.				[19]
W Africa	Guinea-Bissau	Low	1997–98	<2 yr	200	201	4460	JK-calc	n.r.	1·8 (1·3–2·5)	0·8 (0·6–1·1)	1·2 (0·8–1·8)	[20]
SE Asia	Myanmar	Med	1982–83	<5 yr	1447	n.r.	300–1600	SKG-calc	18				[22]
SC Asia	India	Med	1985–86	<3 yr	794	768	39	SKG-calc	14				[21]
E Asia	China	Med	1986–87	All	19 410	n.r.	99	JK-calc‡	14	1·7 (1·1–2·6)	1·2 (0·9–1·7)	11·7 (3·6–46·3)	[25]
SE Asia	Thailand	Med	1988–89	<5 yr	482	n.r.	64	JK-calc	7				[23]
SE Asia	Thailand	Med	1988–89	<5 yr	452	n.r.	69	SKG-calc	7				[24]
SE Asia	Indonesia	Med	1993–94	<5 yr	297	n.r.	616	JK-calc	34				[26]
SE Asia	Indonesia	Med	1994	<5 yr	408	n.r.	300	Author	20				[27]
SE Asia	Vietnam	Med	1998–99	<5 yr	1655	n.r.	80	JK-calc	7				[28]
S America	Brazil	Med	1978–79	All	189	304	141	JK-calc	27				[29]
S America	Peru	Med	1982–84	<1 yr	153	n.r.	730	JK-calc‡	7	1·7 (1·3–2·2)	1·6 (1·1–2·2)	4·0 (2·9–5·6)	[32]
S America	Brazil	Med	1984–86	<5 yr	175	n.r.	1370	JK-calc	14				[30]
C America	Nicaragua	Med	1991–94	<2 yr	235	470	660	JK-calc‡	38	1·7 (1·0–3·0)	0·8 (0·6–1·3)	1·0 (0·4–2·2)	[31]

ETEC, Enterotoxigenic *E. coli*; HDI, human development index; ST, heat-stable toxin; LT, heat-labile toxin; n.r., not reported; n.s., data not sufficient.

* Crude incidence reported as number of cases/1000 persons per year.

† Author: incidence reported by author of paper; JK-calc or SKG-calc: incidence calculated by the investigators.

‡ Odds ratios for toxin types calculated using Epi-Info v.3.3.2, based on numbers of ill/well individuals with positive/negative toxin tests.

Table 3. *Age-specific incidence of ETEC infections in population-based studies, published 1984–2005*

Area	Asia	Africa	Africa
Region	SE Asia	N Africa	N Africa
Country	Vietnam	Egypt	Egypt
HDI	Medium	Medium	Medium
Study years	1998–99	1993–95	1995–98
Age range	<5 yr	<3 yr	<3 yr
ETEC incidence*	80	600	1470
0–5 months	250	1000	1670
6–11 months			2260
12–17 months	140	600	1990
18–23 months			1350
24–35 months	20	100	760
36–48 months	50		
49–60 months	20		
Reference	[28]	[18]	[19]

ETEC, Enterotoxigenic *E. coli*; HDI, human development index.
* Incidence reported as number of episodes/1000 person-years.
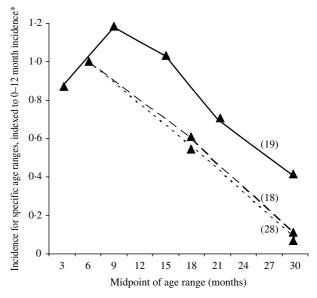

Fig. Age-specific incidence of ETEC infections in population-based studies, published 1984–2005. * In order to compare results in one figure, incidence is expressed as the incidence for the indicated age group, divided by the incidence for infants aged 0–12 months.

The incidence data presented in Table 2 were reported by the authors of three studies and calculated by the investigators based on data provided for the other 12 studies. Crude incidence estimates are given, without correction for age categories, sensitivity of diagnostic methods for ETEC, asymptomatic or LT-ETEC infection, or for the proportion of diarrhoeal episodes for which stool specimens were not collected. The study methodologies varied widely and incidence results are not comparable. In these 15 studies, longitudinal surveillance visits were conducted daily (3), three times a week (2), twice a week (5), weekly (4) or monthly (1). Eleven different definitions of diarrhoea were used and seven different algorithms were used for identification of ST and LT enterotoxins.

Among the two studies reporting on surveillance among all age groups, from China and Brazil, ETEC incidence was similar, 99 and 141 cases/1000 person-years. The other 13 studies included different subsets of children aged <5 years, and showed a wide range of incidence (39–4460 cases/1000 person-years), with no clear pattern by region, HDI level or population setting.

We did not find any published population-based studies of ETEC incidence from any country in sub-Saharan Africa or West Asia.

Age distribution

Neither of the two population-based studies of ETEC disease incidence which included all age groups reported age-specific incidence [25, 29]. However, three population-based studies of ETEC disease incidence among children only reported age-specific incidence (Table 3). All three studies showed the same pattern of peak incidence in the first year of life, with subsequent declines in incidence (Fig.). Rao *et al.* stratified infants by age and showed that the peak incidence is *after* 6 months and may last until age 18 months [19]. Children aged >6 months may be at increased risk due to reduced levels of maternal antibodies and increased consumption of contaminated water or foods.

Temporal distribution

Seventeen studies reported seasonality of ETEC infections. Of these, three reported no seasonal variation [32–34]. Another ten reported a higher incidence in the hot season [12, 22, 35–42]. The other four studies reported that ST-ETEC had a higher incidence in the hot season, but LT-ETEC showed no seasonal variation [18, 19, 43, 44]. No consistent patterns were seen when comparing wet and dry seasons.

Geographic distribution

The geographic distribution of ETEC is illustrated based on data from population-based studies presented in Table 2. Incidence ranges from 39 to 4460 cases/1000 person-years. Results from these specific study sites cannot be generalized to an entire country or geographic region. These rates are considerably higher than most other bacterial enteric infection rates, such as *Shigella* [45] and *Salmonella* Typhi [46]. No data were available for Eastern, Middle and Southern Africa and Western Asia. The highest ETEC incidence was reported from North and West Africa.

Frequency of ETEC relative to other diarrhoeagenic pathogens

We found 70 publications that described the frequency of ETEC in diarrhoea stools or the relative frequency of ETEC compared to other enteric pathogens. Table 4 summarizes these publications and includes the age of the population surveyed, the total number of stool samples or rectal swabs obtained, and where reported, the total proportion of samples yielding at least one pathogen. Studies varied widely with respect to the overall spectrum of pathogens tested. When available, the proportion of isolates yielding *Salmonella*, *Vibrio cholerae*, ETEC, *Campylobacter*, *Rotavirus*, and *Shigella* are reported. ETEC is assigned a rank among the pathogens based on data presented in Table 4 as well as data regarding other pathogens, such as enteropathogenic *E. coli*, which may have been presented in the studies under consideration.

The 70 studies represent Asia (42), Africa (16), Latin America and the Caribbean (10) and Oceania (2). One study was conducted in four different low- or medium-HDI countries [47] and is considered here as four distinct studies. Of 52 studies with relative frequency of pathogens other than ETEC, ETEC was the most frequently isolated pathogen in 21 (40%) studies and the second most frequently isolated in another 14 (27%) studies. ETEC was the first or second most frequently isolated pathogen in nine (75%) of the 12 studies conducted in Africa, 22 (65%) of 34 studies in Asia and three (50%) of six studies in Latin America and the Caribbean. Notably, only 37 (73%) of the 51 studies tested for and reported results of *Campylobacter* isolation, and only 32 (63%) tested for and reported results of *Rotavirus* infection. Most studies did not include identification of parasitic aetiologies of diarrhoea.

Among the 19 studies conducted with all age groups, a median of 14% of diarrhoeal specimens were positive for ETEC (range 2–36%). Among the 51 studies conducted among children only, a median of 13% of diarrhoeal episodes were positive for ETEC (interquartile range 3–39%).

Frequency of ETEC toxin phenotypic subgroups

Knowledge of the distribution of ETEC toxin phenotypic subgroups in a population may be useful to assess endemic disease incidence, as LT-ETEC is thought to be less likely to cause disease than ST-ETEC or LT/ST-ETEC [48]. Pathogenicity of LT-ETEC may be in part determined by the presence of specific CFs. The literature review yielded a total of 50 studies assessing the relative frequency of the three ETEC enterotoxin subgroups (Table 5). ST-ETEC was the most commonly detected subgroup in 27 of these, LT-ETEC in another 19, and ST/LT-ETEC in the remaining four. There was wide variability in the frequency of toxin phenotypic subgroups among ETEC isolates. The range (median) of frequency was 1–98% (30%) for LT-ETEC, 0–92% (45%) for ST-ETEC, and 0–52% (14%) for LT/ST-ETEC. This wide variation was seen in Africa, Asia and Latin America. No relationship between relative frequency of toxin phenotypic subgroups and study age group, year of study or study setting was noted.

Five of the 15 population-based studies of ETEC incidence obtained sufficient data to determine the pathogenicity of different toxin types (Table 2) [18, 20, 25, 31, 32]. In all five of these studies, ill patients were more likely to have ST-ETEC isolated from their stools than well patients. However, in only two of five studies LT-ETEC was more likely to be isolated from ill patients, and in two or four studies ST/LT-ETEC was more likely to be isolated from ill patients. These results are consistent with facility-based case-control studies which suggest that LT-ETEC, or certain strains of LT-ETEC may be infrequently pathogenic [48].

Three studies described the relative frequency of toxin phenotypic subgroups during three different ETEC outbreaks in India [9, 49, 50]. All ETEC isolates from each outbreak were positive for LT and negative for ST, suggesting that LT-ETEC does cause clinical illness. Outbreaks of watery diarrhoea due to ETEC may often go unrecognized because of the unavailability of tests for LT and ST outside research settings.

Table 4a. *Relative frequency of endemic ETEC isolation, community- and facility-based studies conducted among all age groups, published 1984–2005*

Area	Region	Country	HDI	Study type	Study years	No. of samples tested	% pos. for any pathogen	% ETEC	% Sa	% VC	% RV	% Sh	% Ca	ETEC rank	Ref.
Africa	E Africa	Djibouti	Low	Clinic	1989	209	n.r.	11	3	n.t.	n.t.	8	3	1	[80]
Africa	W Africa	Nigeria	Low	Hospital	1995–96	852	21	2	17	n.t.	n.t.	21	n.t.	4	[85]
Asia	SE Asia	Thailand	Med	Cohort	1982–83	177	n.r.	10	n.t.	n.t.	n.t.	4	n.r.	1	[38]
Asia	SC Asia	India	Med	Hospital	1982–83	240	n.r.	12	0	33	8·8	5	7	2	[86]
Asia	SE Asia	Thailand	Med	Clinic	1982–83	299	30	17	n.t.	2	n.t.	9	n.t.	1	[87]
Asia	SE Asia	Philippines	Med	Hospital	1983	1021	n.r.	7·8	12	4·1	27	10	4	3	[33]
Asia	SC Asia	Bangladesh	Med	Hospital	1983–84	2635	69	14	1	39	n.t.	11	11	2	[39]
Asia	SC Asia	Iran	Med	Clinic	1984	273	n.r.	22	n.t.	n.t.	n.t.	n.t.	n.t.	n.c.	[88]
Asia	E Asia	China	Med	Cohort	1986–87	2265	39	14	0	0·8	n.t.	11	3	1	[25]
Asia	SE Asia	Thailand	Med	Hospital	1991	363	n.r.	7	8	n.t.	n.t.	16	5	3	[89]
Asia	SC Asia	Bangladesh	Med	Hospital	1995	113	87	36	1	24	10	8	5	1	[90]
Asia	SE Asia	Laos	Med	Hospital	1996–97	880	43	20	1	0	n.t.	17	4	1	[43]
Asia	SC Asia	Bangladesh	Med	Hospital	1996–98	4662	n.r.	14	n.t.	n.t.	n.t.	n.t.	n.t.	n.c.	[37]
Asia	SC Asia	Indonesia	Med	Hospital	2000–01	489	n.r.	15	3	0·2	n.t.	3	0	1	[44]
Asia	SE Asia	Indonesia	Med	Hospital	n.r.	1169	n.r.	19	n.t.	n.t.	n.t.	n.t.	n.t.	n.c.	[82]
Latin America	S America	Brazil	Med	Cohort	1978–79	110	62	27	5	n.t.	n.t.	15	n.t.	1	[29]
Latin America	S America	Peru	Med	Hospital/ clinic	1992–93	143	52	22	4	31	n.t.	3	3	2	[91]

ETEC, Enterotoxigenic *E. coli*; HDI, human development index; Sa, *Salmonella* (includes typhoidal and non-typhoidal serotypes); VC, *Vibrio cholerae*; RV, Rotavirus numbers; Sh, *Shigella*; Ca, Campylobacter; n.r., not reported; n.t., not tested; n.c., not calculable.

Table 4b. *Relative endemic ETEC isolation frequency, community- and facility-based studies conducted among restricted age groups, published 1984–2005*

Area	Region	Country	HDI	Study type	Study years	Age range	No. samples tested	% pos. for any pathogen	% ETEC	% Sa	% VC	% RV	% Sh	% Ca	ETEC rank	Ref.
Asia	SC Asia	India	Med	Hospital	1986–88	<6 mo.	218	n.r.	12	n.t.	n.t.	n.t.	n.t.	n.t.	n.c.	[92]
Asia	E Asia	China	Med	Clinic	1989	<1 yr	174	34	4			13	1	10	2	[74]
Latin America	S America	Peru	Med	Cohort	1982–84	<1 yr	952	41	7	1	0	3	2	10	2	[32]
Latin America	S America	Brazil	Med	Hospital	1985–86	<1 yr	500	55	7	8		14	5	3	4	[36]
Africa	W Africa	Gambia	Low	Cohort	1981–84	<2 yr	516	28	10	1	0	6	2	4	1	[76]
Africa	S Africa	South Africa	Med	Hospital	1982–83	<2 yr	478	46	4	7	2	26	4	11	4	[71]
Latin America	S America	Brazil	Med	Clinic	1989	<2 yr	126	83	10	n.t.	n.t.	n.t.	n.t.	n.t.	n.c.	[79]
Latin America	C America	Nicaragua	Med	Cohort	1991–94	<2 yr	808	n.r.	38	n.t.	n.t.	n.t.	n.t.	n.t.	n.c.	[31]
Africa	N Africa	Egypt	Med	Cohort	1993–95	<3 yr	628	n.r.	20	n.t.	n.t.	n.t.	n.t.	n.t.	n.c.	[18]
Africa	N Africa	Egypt	Med	Cohort	1995–98	<3 yr	2201	n.r.	28	n.t.	n.t.	n.t.	n.t.	n.t.	n.c.	[60]
Africa	S Africa	South Africa	Med	Hospital	n.r.	<3 yr	78	77	39	4	0	13	1	15	1	[93]
Latin America	S America	Brazil	Med	Hospital	1994–95	<3 yr	199	n.r.	4	n.t.	n.t.	n.t.	n.t.	n.t.	n.c.	[75]
Asia	E Asia	China	Med	Clinic	1982–85	<3 yr	594	n.r.	6	5	0	13	18	17	4	[47]
Asia	SE Asia	Myanmar	Med	Clinic	1982–85	<3 yr	813	n.r.	26	1	1	22	3	2	1	[47]
Asia	S Asia	India	Med	Clinic	1982–85	<3 yr	915	n.r.	14	4	2	18	20	15	4	[47]
Asia	S Asia	Pakistan	Low	Clinic	1982–85	<3 yr	758	n.r.	17	3	2	14	6	10	1	[47]
Asia	S Asia	Pakistan	Low	Hospital	1983–84	<3 yr	250	76	14	3·2	n.t.	10	4	n.t.	2	[94]
Asia	SC Asia	India	Med	Cohort	1985–86	<3 yr	222	54	14	3	0	2	2	6	1	[21]
Asia	SC Asia	India	Med	Cohort	1985–86	<3 yr	179	44	15	3	0	2	2	6	1	[95]
Asia	W Asia	Iraq	NR	Hospital	1988–89	<3 yr	304	n.r.	13	n.t.	n.t.	n.t.	n.t.	n.t.	n.c.	[96]
Asia	SC Asia	Bangladesh	Med	Hospital	1988–89	<3 yr	969	64	16	<1	5	29	3	27	3	[97]
Africa	N Africa	Egypt	Med	Hospital/clinic	1986	<5 yr	151	n.r.	17	7		18	5	7	2	[98]
Africa	W Africa	Nigeria	Low	Clinic	1989–90	<5 yr	215	75	14	3		22	5	n.t.	2	[78]
Africa	E Africa	Tanzania	Low	Clinic	1990	<5 yr	394	n.r.	20	n.t.	n.t.	n.t.	n.t.	18	n.c.	[34]
Africa	E Africa	Tanzania	Low	Hospital	1997	<5 yr	103	63	16	0	n.t.	4	13	n.t.	1	[81]
Africa	W Africa	Nigeria	Low	Clinic	n.r.	<5 yr	187	n.r.	3	1	n.t.	n.t.	1	n.t.	1	[73]
Africa	E Africa	Kenya	Low	Hospital	n.r.	<5 yr	300	n.r.	6	n.t.	n.t.	n.t.	n.t.	n.t.	n.c.	[77]
Asia	SE Asia	Thailand	Med	Clinic	1982	<5 yr	221	n.r.	14	1	2	n.t.	9	n.t.	1	[99]
Asia	SE Asia	Myanmar	Med	Cohort	1982–83	<5 yr	501	n.r.	18	1	n.t.	5	2	2	1	[22]
Asia	SE Asia	Thailand	Med	Clinic	1985–86	<5 yr	1230	63	9	12	1	20	13	13	5	[70]
Asia	SE Asia	Thailand	Med	Clinic	1985–86	<5 yr	1230	n.r.	13	n.t.	n.t.	n.t.	n.t.	n.t.	n.c.	[100]
Asia	SE Asia	Thailand	Med	Cohort	1988–89	<5 yr	345	54	7	13	0	12	6	14	4	[23]
Asia	SE Asia	Thailand	Med	Cohort	1988–89	<5 yr	345	56	7	9	0	9	5	9	4	[24]
Asia	SE Asia	Indonesia	Med	Hospital	1988–89	<5 yr	194	28	8	19	3		2	6	2	[101]
Asia	W Asia	Jordan	Med	Hospital	1993–94	<5 yr	256	66	6	5	0	33	5	2	4	[54]
Asia	SE Asia	Vietnam	Med	Cohort	1998–99	<5 yr	2160	22	7	1	n.t.	n.t.	6	7	2	[28]
Asia	SC Asia	Bangladesh	Med	Hospital	1991–92	<5 yr	451	n.r.	12	1	10	17	10	n.t.	3	[41]

Asia	SC Asia	Bangladesh	Med	Hospital	1993–94	<5 yr	75	814	17	2	5	20	9	17	3	[40]
Asia	SE Asia	Indonesia	Med	Cohort	1993–94	<5 yr	n.r.	179	34	n.t.	n.t.	n.t.	n.t.	n.t.	n.c.	[26]
Asia	SE Asia	Indonesia	Med	Cohort	1994	<5 yr	n.r.	241	14	2	7	n.t.	1	2	1	[27]
Latin America	S America	Brazil	Med	Cohort	1984–86	<5 yr	53	90	14	n.t.	n.t.	21	2		3	[30]
Latin America	S America	Bolivia	Med	Hospital	1991–92	<7 yr	n.r.	195	3	1	8	n.t.	3		6	[102]
Latin America	S America	Brazil	Med	Clinic	1993	<5 yr	n.r.	76	16	n.t.	n.t.	n.t.	n.t.	n.t.	n.c.	[103]
Asia	SC Asia	Bangladesh	Med	Hospital	1982–83	<8 yr	59	104	9	11	11	17	5	20	5	[72]
Oceania	Melanesia	N. Caledonia	n.r.	Hospital	1990	<10 yr	n.r.	448	5	n.c.	n.c.	n.c.	n.c.	n.t.	n.c.	[104]
Oceania	Melanesia	Vanuatu	Med	Hospital/clinic	1984	<10 yr	n.t.	109	27	n.t.	n.t.	n.t.	n.t.	n.t.	n.c.	[84]
Asia	SE Asia	Thailand	Med	Clinic	1996–00	<12 yr	n.r.	2100	3	n.t.	n.t.	n.t.	n.t.	n.t.	n.c.	[105]
Africa	Middle Africa	CAR	Low	Hospital/clinic	1981–82	<15 yr	64	1197	3	5	18	18	3	11	5	[106]
Africa	E Africa	Somalia	n.r.	Hospital	1983–84	<15 yr	61	1667	11	4	6	25	9	8	2	[35]
Asia	SC Asia	Iran	Med	Hospital	1986–87	Children*	n.r.	715	17	9	n.t.	n.t.	6	n.t.	2	[69]
Asia	SC Asia	Iran	Med	Clinic	1986–87	Children*	n.r.	443	17	4	n.t.	n.t.	4	n.t.	2	[69]
Asia	E Asia	China	Med	Hospital/clinic	1989	Children*	56·5	221	20	12	7	7	3	2	1	[83]

ETEC, Enterotoxigenic E. coli; HDI, human development index; Sa, Salmonella (includes typhoidal and non-typhoidal serotypes); VC, Vibrio cholerae; RV, Rotavirus; Sh, Shigella; Ca, Campylobacter; CAR, Central African Republic; n.r., not reported; n.t., not tested; n.c., not calculable.
* Children: the corresponding study did not describe the age range of children included in the study.

Complications

Extraintestinal complications do not generally occur with ETEC infections. The most concerning acute complication is severe dehydration due to profuse watery diarrhoea and vomiting. In a hospital-based study in Bangladesh from 1996 to 2002, 36% of 478 adults and 3% of 1107 children admitted with ETEC infection had severe dehydration [48]. This is consistent with reports that adults, at least in the Indian subcontinent, frequently present with a severe dehydrating cholera-like illness, although this also could be due to longer delays in reaching a treatment facility [37]. In India, one outbreak of severe watery diarrhoea thought to be due to cholera was found to be due to ETEC [49] and another was found to be due to both cholera and ETEC [9].

ETEC infections may also cause malnutrition and poor weight gain. In one study in rural Bangladesh, ETEC infections were shown to decrease 60-day weight gain in children [51]. In Brazil, childhood diarrhoea was shown to be associated with long-term impaired physical fitness and cognitive function [52], although this has not been studied specifically with ETEC infections.

Mortality

Annual diarrhoeal deaths among children aged <5 years are estimated at 2·5 million based on studies conducted between 1992 and 2000 [53]. If ETEC is responsible for 13% of these deaths, the median proportion of ETEC among diarrhoea cases in this review, 325 000 children (interquartile range 175 000–425 000) aged <5 years would be estimated to die each year from ETEC.

Of the 15 population-based studies of ETEC, only two discuss mortality. In Vietnam, 2160 cases of diarrhoea were identified over one year in 1655 children aged <5 years; of 25 ETEC cases, there were no deaths [28]. In Nicaragua, 885 cases of diarrhoea were reported in the first two years of life among 235 children; of 310 ETEC cases detected among 808 specimens, there were no deaths [31]. Of the hospital-based studies of endemic disease referenced in this report, one discusses mortality; among 265 children admitted with acute diarrhoea, none died [54]. In three epidemics attributed to ETEC in India [9, 49, 50], no patient deaths were reported. If patients with severe ETEC infections reach a treatment facility, the mortality rate should be extremely low. However, most infections may go untreated, and the

Table 5. *Relative frequency of ETEC enterotoxin subgroups in endemic disease, published 1984–2005*

Area	Region	Country	HDI	Study type	Study years	Age range	No. of diarrhoea samples tested	% of diarrhoea specimens with ETEC	% LT only	% ST only	% LT/ST	Ref.
Africa	Middle Africa	CAR	Low	Hospital/clinic	1981–82	<15 yr	1197	3	44	29	26	[106]
Africa	N Africa	Egypt	Med	Cohort	1981–83	All	3513	24	61	36	4	[107]
Africa	W Africa	Gambia	Low	Cohort	1981–84	<2 yr	516	10	53	39	6	[76]
Africa	N Africa	Egypt	Med	Hospital/clinic	1986	<5 yr	151	17	30	61	9	[98]
Africa	E Africa	Djibouti	Low	Clinic	1989	All	209	11	44	44	13	[80]
Africa	W Africa	Nigeria	Low	Clinic	1989–90	<5 yr	215	14	19	74	7	[78]
Africa	N Africa	Egypt	Med	Cohort	1993–95	<3 yr	628	20	34	53	13	[18]
Africa	W Africa	Nigeria	Low	Hospital	1995–96	All	852	2	24	35	41	[85]
Africa	N Africa	Egypt	Med	Cohort	1995–98	<3 yr	2201	28	24	61	7	[60]
Africa	E Africa	Tanzania	Low	Hospital	1997	<5 yr	103	16	31	56	13	[81]
Africa	W Africa	Nigeria	Low	Clinic	n.r	<5 yr	187	3	17	83	0	[73]
Africa	S Africa	South Africa	Med	Hospital	n.r.	<3 yr	78	39	77	13	10	[93]
Asia	SC Asia	India	Med	Hospital	1982–83	All	240	12	48	21	31	[86]
Asia	SC Asia	Bangladesh	Med	Hospital	1982–83	<8 yr	104	9	22	56	22	[72]
Asia	SC Asia	Bangladesh	Med	Hospital	1983–84	All	2635	14	29	50	21	[39]
Asia	S Asia	Pakistan	Low	Hospital	1983–84	<3 yr	250	14	40	49	11	[42]
Asia	SC Asia	Iran	Med	Clinic	1984	All	273	22	17	60	23	[88]
Asia	SE Asia	Thailand	Med	Clinic	1985–86	<5 yr	1230	9	44	33	22	[70]
Asia	SE Asia	Thailand	Med	Clinic	1985–86	<5 yr	1230	13	47	30	23	[100]
Asia	SC Asia	India	Med	Cohort	1985–86	<3 yr	222	15	48	30	19	[21]
Asia	SC Asia	India	Med	Cohort	1985–86	<3 yr	222	14	57	27	17	[21]
Asia	SC Asia	Iran	Med	Clinic	1986–87	All	443	17	1	92	7	[69]
Asia	SC Asia	Iran	Med	Hospital	1986–87	Children	715	17	12	77	11	[69]
Asia	E Asia	China	Med	Cohort	1986–87	All	2265	14	43	32	25	[25]
Asia	SC Asia	India	Med	Hospital	1986–88	<6 mo.	218	12	58	31	12	[92]
Asia	W Asia	Iraq	Med	Hospital	1988–89	<3 yr	304	13	69	21	10	[96]
Asia	SE Asia	Indonesia	Med	Hospital	1988–89	<5 yr	194	8·2	69	31	0	[101]
Asia	E Asia	China	Med	Clinic	1989	<1 yr	174	4	71	0	29	[74]
Asia	E Asia	China	Med	Hospital/clinic	1989	Children	221	20	98	3	0	[83]
Asia	SE Asia	Thailand	Med	Hospital	1990	All	363	7	18	56	26	[99]
Asia	SC Asia	Bangladesh	Med	Hospital	1991–92	<5 yr	451	12	15	57	24	[41]
Asia	SC Asia	Bangladesh	Med	Hospital	1993–94	<5 yr	814	17	27	50	23	[40]
Asia	W Asia	Jordan	Med	Hospital	1993–94	<5 yr	256	6	60	27	13	[54]
Asia	SE Asia	Indonesia	Med	Cohort	1994	<5 yr	241	20	34	52	24	[27]
Asia	SE Asia	Laos	Med	Hospital	1996–97	All	880	20	13	84	3	[43]
Asia	SC Asia	Bangladesh	Med	Hospital	1996–98	All	4662	14	25	49	25	[37]

Asia	SE Asia	Thailand	Med	Clinic	1996–2000	2100	3	39	54	8	[105]
Asia	SC Asia	Indonesia	Med	Hospital	2000–01	489	15	29	70	1	[44]
Asia	SE Asia	Indonesia	Med	Hospital	n.r.	1169	19	23	71	6	[82]
Latin America	S America	Brazil	Med	Cohort	1978–79	110	27	20	60	20	[29]
Latin America	S America	Peru	Med	Cohort	1982–84	952	7	50	36	14	[32]
Latin America	S America	Brazil	Med	Hospital	1985–86	500	7	42	45	12	[36]
Latin America	S America	Brazil	Med	Cohort	1986	90	14	43	14	43	[30]
Latin America	S America	Brazil	Med	Clinic	1989	126	10	50	8	42	[79]
Latin America	C America	Nicaragua	Med	Cohort	1991–94	808	38	24	34	42	[31]
Latin America	S America	Peru	Med	Hospital/clinic	1992–93	143	22	16	68	16	[91]
Latin America	S America	Brazil	Med	Clinic	1993	76	16	19	31	50	[103]
Oceania	Melanesia	Vanuatu	Med	Hospital/clinic	1984	109	27	24	24	52	[84]
Oceania	Melanesia	New Caledonia	n.r.	Hospital	1990	448	5	21	71	8	[104]

ETEC, Enterotoxigenic *E. coli*; HDI, human development index; LT, heat-labile toxin; ST, heat-stable toxin; CAR, Central African Republic; n.r., not reported.

case-fatality rate (CFR) for untreated infections is unknown.

Pathogen-specific preventive measures

Vaccine strategies

Efforts to develop an effective and useful ETEC vaccine are ongoing. Many different antigens have been considered in vaccine development including O antigens, H antigens, CFs and toxins. At least 78 O antigens and 34 H antigens have been identified in ETEC isolates, with no predominant serotype or group of serotypes [55, 56]. O and H antigens are known to confer protective immunity [57], and infection with multiple serotypes is believed to confer immunity to the predominant ETEC strains present in a community. However, because of the large number of O and H antigens, these have not been considered practical targets for vaccine development unless common epitopes can be identified [3, 55]. The ST toxin is not immunogenic because of its small size (only 19 amino acids), and efforts to design a ST-conjugate antigen that confers protective immunity against ST-ETEC have not yet been successful [56].

A 1988 study showed 67% short-term, 3-month protection against illness from LT-ETEC by a B subunit whole-cell oral cholera vaccine [58], providing hope for the development of an ETEC vaccine. Current experimental ETEC vaccines currently target the LT toxin as well as some of the CFs [3]. However, this strategy is limited because of the small proportion of ETEC illnesses attributable to LT-ETEC, the large number of CFs, the high proportion of ETEC isolates without identifiable CFs, the finding that CFs may not effectively confer protective immunity [59], and the lack of effectiveness of a candidate vaccine in a recent trial [60]. The success of future vaccine development efforts will depend on the ability to find or design the best vaccine antigens for protective immunity, and to find effective methods of vaccine delivery for optimal antigen presentation [61].

Non-vaccine strategies

Both young age and pre-existing malnutrition have been found to be associated with more severe ETEC infections [62, 63]. The effect of micronutrient deficiencies such as vitamin A and zinc on risk for severe ETEC infection is unknown. Exclusive breastfeeding

of infants has been shown to protect against severe ETEC infections, but this effect was not seen with partial breastfeeding or with children aged >1 year [64].

Non-vaccine measures for the prevention of ETEC include exclusive breastfeeding of infants [64, 65], sanitary preparation of weaning foods for infants [65], promotion of handwashing and hygiene, control of houseflies [66], sanitary disposal of human faeces, provision of safe drinking water at the point of consumption through improved water supplies, household water disinfection and safe water storage [67], sanitary food preparation and storage [10], and the provision of safe drinking water during flood disasters [68].

Diagnostics

Testing for both LT and ST in *E. coli* isolates is the established method for the identification of ETEC. Traditionally, enterotoxins have been detected with immunoassays or bioassays. Because of the large number of serotypes associated with ETEC, serotyping is not as useful for diagnosis. Because it is faster, detection of the LT or ST gene through the use of DNA probes or PCR amplification has become common. In incidence studies, the differences in sensitivity of different diagnostic methods may partially explain the wide variety in incidence estimates, relative frequency of ETEC in diarrhoeal stools and relative frequency of ETEC toxin types. However, as all ETEC diagnostic methods are widely unavailable in most clinical laboratories in both the developing and developed world, ETEC is likely under-recognized outside of research settings.

DATA GAPS AND DISCUSSION

This review describes the available data and highlights the major gaps in data (below) regarding the global epidemiology of ETEC infections. A successful research agenda based on these data gaps could be expected to help appropriately direct resources for prevention and control activities in order to more efficiently reduce the burden of illness due to ETEC. This research can be performed for multiple diarrhoeagenic pathogens simultaneously in order to both reduce costs and better understand the relative contribution of different pathogens to age-specific morbidity and mortality.

The major data needs for incidence, including age, temporal and geographic distribution are:

- population-based surveillance for ETEC incidence in low-HDI countries, especially in sub-Saharan Africa, including testing in both ill and well participants;
- population-based surveillance, with incidence reported for total population as well as by toxin type and age group, including young (<6 months) and older (6–12 months) infants, including testing in both ill and well participants;
- population based-surveillance among multiple sites in a country or region to determine the generalizability of data from a specific study site;
- temporal distribution of ETEC over multi-year periods, with reporting using consistent descriptors and contextual environmental information such as rainfall and flooding;
- comprehensive stool analyses, using state-of-the-art diagnostic methods, including isolation of *Shigella*, *Campylobacter*, enteropathogenic *E. coli* (EPEC), *Rotavirus*, and parasitic infections, in studies of relative frequency of diarrhoeagenic pathogens, including testing in both ill and well participants;
- further characterization of the pathogenicity of different ETEC toxin types and strains in both primary and repeat infections, as well as the impact of infection with multiple pathogens on disease incidence and severity.

Diarrhoea incidence is extremely variable and estimates of pathogen-specific incidence may not be generalizable to populations outside of a specific study site, or to the same study population at a different season, or even the same season in a different year. Estimates of ETEC disease incidence are further complicated by a number of other pathogen-specific factors. The frequency of asymptomatic infection ranges from 0% to 22% [22, 25, 30–36, 40–42, 44, 47, 69–84], and as many as 40% of ill persons with ETEC have other pathogens in their stools, making it difficult to know which pathogen caused the illness [40]. Furthermore, multiple case-control studies have shown that cases are no more likely to have LT-ETEC than controls; therefore, LT-ETEC is thought to less commonly cause ETEC disease or severe ETEC disease than ST-ETEC or LT/ST-ETEC [48, 60]. ST-ETEC may be more pathogenic because the heat-stable toxin does not cause an immune response, and the pathogenicity of LT-ETEC

may be largely limited to first infections of a given strain.

Because of these pathogen-specific factors, ETEC disease incidence estimates from population-based studies may be artificially elevated as they generally do not control for asymptomatic infection or infection with multiple pathogens. At the same time, ETEC is clinically under-recognized as a pathogen, as most clinical laboratories in both resource-poor and resource-rich countries lack the capacity to test for it.

Perhaps the most striking data gap is the absence of population-based data from low-HDI countries, especially in sub-Saharan Africa. These countries may have a high incidence of ETEC infection, and understanding this epidemiology will assist in projecting the impact of pathogen-specific and non-specific preventive measures.

A better understanding of age-specific incidence is also needed; only one study describes the incidence in both young and older infants. Further data may help define age groups at highest risk of infection and complications, as well as the role of breastfeeding in preventing ETEC infection.

In the developing world, the incidence of diarrhoea varies greatly by place, season, and even by year independent of season. Simultaneous studies in multiple sites in one country and studies over multiple years would determine the extent of this variability for ETEC infections, and would improve interpretation of existing data and planning of prevention and control programmes.

Finally, studies of relative frequency of diarrhoeagenic pathogens often do not include identification of *Campylobacter*, *Rotavirus*, EPEC or parasites, do not use highly sensitive PCR techniques for the identification of *Shigella*, and do not present results controlling for asymptomatic infections. Addressing these limitations in future studies will improve our understanding of the relative contribution of different pathogens to disease incidence.

The major data needs regarding mortality and complications from ETEC infections are:

- determination of the economic and social burden due to ETEC infections and mortality;
- calculation of CFRs and mortality rates in population-based studies;
- rates of complications in population-based studies of ETEC infection;

- further studies of frequency of moderate or severe dehydration in adults compared to children and the reasons for any differences;
- studies of long-term complications of ETEC infections, including physical fitness, language ability and cognitive function.

Data on complications and mortality due to ETEC infections is extremely limited, and is generally limited to facility-based studies. Calculation of age-specific case-fatality and complication rates may be achieved by studying a discrete number of health facilities providing care for the majority of a given population, in conjunction with a simultaneous population-based incidence study. The clinical severity of ETEC infections is variable, depending both on the host as well as the bacterium and the specific CFs and toxins present, and such studies may improve the understanding of this variability. Such studies could also be done examining multiple pathogens simultaneously in order to minimize costs and provide data on the relative contribution of different pathogens to serious morbidity and mortality.

Data on long-term complications of ETEC infections is also lacking, and longitudinal cohort studies can be conducted examining the relationship between the frequency and severity of multiple ETEC infections and long-term effects on physical, language and cognitive abilities.

The major data needs regarding preventive measures for ETEC infections are:

- further research into the immunology of natural protection from ETEC, as well as methods of vaccine delivery and optimal vaccine antigens;
- effect of vitamin A and zinc deficiency or supplementation on risk of severe ETEC infections;
- determination of proportion of ETEC infections attributable to different routes of transmission in different settings;
- evaluation of the cost-effectiveness and sustainability of large scale introduction of non-vaccine preventive measures in endemic settings, and the feasibility of rapid introduction of non-vaccine preventive measures in epidemic settings.

Such research is critical to define the most effective prevention measures. Research also needs to continue on cost-effective, sustainable, large-scale models for implementation of more general

prevention strategies, such as provision of safe food and water.

The major needs regarding diagnostics for ETEC infections are:

- standardization of the molecular definition of ETEC, based on either PCR or DNA probes for enterotoxins, for surveillance purposes;
- evaluation of the performance of PCR compared to ELISA for toxin identification;
- development of correction factors for other diagnostic methods to enable comparison of incidence results.

Existing data regarding the epidemiology of ETEC infections rests on the quality and consistency of diagnostic methods. In order to be able to compare results from different studies, a standardized molecular definition of ETEC for surveillance purposes is urgently needed. Moreover, because different diagnostic methods are available in different sites, correction factors for different diagnostic methods need to be developed to allow comparison of results to the gold standard. An international microbiological standard, along with a centralized system of laboratory training and quality assurance, would form the foundation for addressing the other data gaps.

Although much is known about the burden of ETEC infections, there are still substantial gaps in knowledge, including large geographic areas and important age groups. Filling in these gaps will require extensive planning and investment, but studies can simultaneously fill in similar gaps for other important infectious causes of diarrhoea. This information is essential in order to direct research into effective interventions, and to target the interventions for maximum impact.

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DECLARATION OF INTEREST

None.

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