ENTEROTOXIGENIC *ESCHERICHIA COLI* AND OTHER CAUSES OF INFECTIOUS PEDIATRIC DIARRHEAS IN JAKARTA, INDONESIA

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Abstract. A hospital stool survey of Indonesian children less than 5 years of age determined the prevalence of diarrhea caused by enterotoxigenic *Escherichia coli* (ETEC) and other bacterial enteropathogens, compared to non-diarrheic control patients. ETEC were the second most frequent cause of diarrhea, isolated from 16 of 194 (8.2%) of patient's stools compared to 2 of 97 (2.1%) of control stools. The highest prevalence was in infants 12 to 23 months of age (17.9%).

INTRODUCTION

Infectious diarrheal diseases are a leading cause of morbidity and mortality world-wide. Among the most severely affected are children living in the poorer developing countries where infectious diarrheas account for an estimated 4.6 million deaths annually in children under 5 years of age. In these populations, diarrhea mortality rates for the first year of life are as high as 50 per 1,000 live births (Guerrant *et al,* 1990; Snyder *et al,* 1982). Although substantial global progress has been made in reducing mortality due to diarrhea, the continued high morbidity of infectious diarrheas remains a major public health concern (Black *et al,* 1982; Cleason and Merson, 1990).

Enterotoxigenic strains of *Escherichia coli* (ETEC) are known to be a major cause of childhood diarrheas in Southeast Asia (Echeverria *et al,* 1989; Seriwatana *et al,* 1983), and diarrheas in travelers to this region (Taylor and Echeverria, 1986). Following ingestion of contaminated food or drink, the organisms may colonize the proximal small bowel, elaborating heat-stable (ST) or heat-labile enterotoxins (LT) or, infrequently, both enterotoxins. Both ST and LT cause a secretory type diarrheal response in the infected host, although by different mechanisms (Gross and Rowe, 1985; Lopez-Vidal *et al,* 1990). The extracellular production of these now well-characterized enterotoxins differentiate this group of organisms from other diarrheagenic forms of *E. coli,* including enteropathogenic *E. coli* (EPEC), enteroadherent *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and enterohemorrhagic *E. coli* (EHEC) (Burke and Gracey, 1991).

Early studies of infectious diarrheas in Indonesia demonstrated that EPEC were a frequent cause of endemic and epidemic pediatric diarrheas (Lie and Sahab, 1958; Lie *et al,* 1961). However, the importance of ETEC, and other pathogenic forms of *E. coli,* as a cause of diarrhea in Javanese children has yet to be investigated. Our principal objective in the current study was to estimate the prevalence of ETEC-associated diarrhea in a pediatric population of Jakarta, Indonesia.

MATERIALS AND METHODS

Study design

During the period of November 1988 through October 1989, we performed a pediatric stool survey of children hospitalized for diarrhea to determine the prevalence of ETEC-associated infections. A control group consisted of comparably aged children from the same hospital, who were visiting the immunization or outpatient clinics, or admitted for reasons other than diarrhea. Following parental informed consent, 194 hospitalized

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diarrheic patients and 97 non-diarrheic control patients (≤60 months of age) were enrolled for study. None of the cases or controls had received antibiotic therapy during the 7 days preceding enrolment. Cases were limited to non-persistent diarrheas which had onsets within 7 days of the study entry.

Bacteriological procedures

Stool or rectal swab specimens from diarrheic and control children were obtained on the day of admission, or at the time of presentation at the outpatient clinic. Specimens were transported to the laboratory in Cary-Blair medium (BBL, Cockeysville, MD) and processed within 4 hours using conventional enteric bacteriological techniques (Balows *et al,* 1991). Direct and enriched cultures were evaluated for the presence of common bacterial enteropathogens, including ETEC, *Salmonella* spp, *Shigella* spp, *Vibrio* spp, *Campylobacter* spp and *Aeromonas* spp. Isolates were identified using standard biochemical tests (Balows *et al,* 1991), or agglutination with specific antisera (Difco) when indicated. Specimens were not tested for other pathogenic types of *E. coli* (*eg* EPEC, EHEC, EIEC, EAEC), parasites, or rotavirus.

For ETEC detection, 5 lactose-positive colonies with typical appearance of *E. coli* were selected from primary MacConkey agar plates. Individual isolates were preserved in 20% glycerol in trypticase soy broth at -85°C until testing. LT-producing *E. coli* isolates were identified using the Y1 adrenal cell microtiter plate assay as described by Sack and Sack (1975) and Echeverria *et al* (1978). Forty-eight hour cultures in trypticase soy broth plus 0.6% yeast extract were used to inoculate individual wells containing the Y1 adrenal cells. A test was considered positive if >50% of cells were rounded after overnight incubation.

A DNA hybridization procedure (SNAP Hybridization Kit Manual, Molecular Biosystems, Inc, San Diego, CA) was used for ST detection using the Zeta-Probe membrane (Bio-Rad, Richmond, CA). The membranes were hybridized using a commercially prepared alkaline phosphatase labelled oligonucleotide probe (Du Pont ETEC-ST probe) (NEN Research Products, Boston, MA).

Statistical analysis

For statistical analyses, tests of proportions were done by Fisher Exact test; stratified analyses by the Mantel-Haenszel test; and calculation of 95% confidence limits by the method of Mehta, Patel, and Gray (1985).

RESULTS

Overall, bacterial enteropathogens were isolated from 55 of 194 (28.4%) diarrheic children and 20 of 97 (20.6%) control children. Of the 55 culture-positive diarrheic patients, 8 (14.5%) were mixed infections involving more than 1 enteropathogen. Frequencies of isolation for all detected enteropathogens are shown in Table 1. ETEC were isolated 4 times more frequently from diarrheic specimens than from control specimens (Table 1), although the overall association between ETEC and disease did not achieve statistical significance due to the relatively small sample size. After *Salmonella* spp, ETEC was the most frequent cause of diarrhea detected in this population, and LT was the most common ETEC toxin phenotype.

There were no ETEC isolates which produced both LT and ST. Age-specific analysis demonstrated a strong association with disease in children under 1 year of age (Table 2). Of the 9 ETEC infections occurring in diarrheic children <1 year of age, 7 (77.8%) occurred in infants 6 to 11 months old. When results were stratified by age and toxin phenotype (ST or LT), the risk of disease from ETEC-LT was found to be increased in 12 to 23 month old children compared to other age groups ($p=0.001$) (Table 3). For other diarrheic age groups, the prevalences of ETEC-ST and ETEC-LT were approximately equal.

ETEC-positive diarrheic patients demonstrated a wide range of clinical profiles. The most common features were watery stools (81.3%), sometimes with mucus (37.5%), often accompanied by fever (68.8%). Only 1 patient had bloody stools, and none reported vomiting. The frequencies of individual clinical signs among ETEC-positive patients did not differ significantly from other diarrheal patients, and there was no detectable difference between LT-positive and ST-positive patients.

Although ETEC-associated diarrheas occurred year-round, there appeared to be an increased prevalence during the dry season (May through August). Nine of the 18 (50.0%) ETEC isolates

Table 1

Enterotoxigenic *E. coli* (ETEC) and other enteric pathogens isolated from 194 hospitalized diarrheic children and 97 non-diarrheic control children.

Enteropathogen isolated	Number of patients positive (%)	
	Diarrheic (n = 194)	Non-diarrheic (n = 97)
ETEC total	16 (8.2)	2 (2.1)
LT	11 (5.7)	2 (2.1)
ST	5 (2.6)	0 (0.0)
Salmonella spp total	37 (19.1)	5 (5.2)
Salmonella group B	25 (12.9)	1 (1.0)
Salmonella group C	3 (1.5)	2 (2.1)
Salmonella group E	9 (4.6)	2 (2.1)
Campylobacter jejuni	12 (6.2)	9 (9.3)
Shigella spp total	3 (1.5)	0 (0.0)
Sh. sonnei	2 (1.0)	0 (0.0)
Sh. flexneri	1 (0.5)	0 (0.0)
Vibrio spp total	6 (3.1)	0 (0.0)
V. cholerae-01	5 (2.6)	0 (0.0)
V. cholerae-non 01	1 (0.5)	0 (0.0)
Aeromonas spp	2 (1.0)	8 (8.2)
A. hydrophila	1 (0.5)	2 (2.1)
A. sobria	1 (0.5)	4 (4.1)
A. caviae	0 (0.0)	2 (2.1)
Concomitant multiple infections:		
ETEC + *Salmonella*	2 (1.0)	0 (0.0)
ETEC + *C. jejuni*	2 (1.0)	0 (0.0)
ETEC + *V. cholerae* 01	1 (0.5)	0 (0.0)
ETEC + *Shigella*	1 (0.5)	0 (0.0)
ETEC + *Aeromonas*	1 (0.5)	0 (0.0)
Salmonella + *C. jejuni*	3 (1.5)	0 (0.0)
Salmonella + *Aeromonas*	1 (0.5)	1 (1.0)
C. jejuni + *Aeromonas*	0 (0.0)	1 (1.0)
Overall	55 (28.4)	20 (20.6)

were made during July or August. There was no detectable seasonal trend for specific toxin types.

DISCUSSION

The current study has shown that ETEC are a significant cause of severe diarrheas in Indonesian children, especially during the first year of life. The prevalence of ETEC in these hospitalized diarrhea patients (8.2%) was lower than that reported for some other tropical developing countries where sanitation infrastructures may be lacking or safe drinking water may not be sufficient (Guerrant *et al,* 1983; Merson *et al,* 1976; Taylor and Echeverria, 1986). Despite the overall moderate prevalences of infection, the high frequency of fecal isolation among 12 to 23 month old diarrheic children (17.9%) leaves little doubt that these organisms contribute substantially to diarrhea-associated infant morbidity and mortality in Indonesia.

Table 2

Age-specific prevalence of isolation of enterotoxigenic *E. coli* (ETEC) among diarrheic and non-diarrheic children ≤ 5 years of age.

Age (mo)	N positive/n tested (%) Diarrheic	Non-diarrheic	p of diff	Odds ratio	95% CL[a]
0-11	9/133 (6.8)	0/74 (0.0)	0.017	>5.30[b]	undef[a]
12-23	7/39 (17.9)	2/20 (10.0)	0.347	1.97	0.32-21.17
24-60	0/22 (0.0)	0/3 (0.0)	1.000	undef	undef
Overall	16/194 (8.2)	2/97 (2.8)	0.051	4.93	1.06-43.68

[a] *Abbreviations:* CL, confidence limits; undef, undefined because of division by zero.

[b] Estimated. Because there were no ETEC isolates among the 0-11 month old controls, an odds ratio could not be calculated for this stratum; this estimate is based on the hypothetical situation that 1 ETEC isolation occurred in this group.

Table 3

Age-specific prevalence of enterotoxigenic *E. coli* (ETEC-ST or ETEC-LT) for diarrheic and non-diarrheic Indonesian children.

	No. (%) of children ETEC[a]-positive					
	Diarrheic			Non-diarrheic		
Age (mo)	N tested	ST[a]	LT[a]	N tested	ST	LT
0-11	133	4 (3.0)	5 (3.8)	74	0 (0.0)	0 (0.0)
12-23	39	1 (2.6)	6 (15.4)	20	0 (0.0)	2 (10.0)
24-60	22	0 (0.0)	0 (0.0)	3	0 (0.0)	0 (0.0)
Overall	194	5 (2.6)	11 (5.7)	97	0 (0.0)	2 (2.1)

[a] *Abbreviations:* ETEC, enterotoxigenic *E. coli;* ST, heat stable toxin producing; LT, heat labile toxin producing.

Other hospital- and community-based studies of ETEC-associated diarrhea are underway in Jakarta. It is hoped that these and future investigations will provide Southeast Asian public health officials and healthcare providers additional information which can be used to reduce the childhood morbidity attributable to ETEC infection.

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