INTRODUCTION

Global mortality due to diarrhea among children less than five years of age during the period 1978–1987 has been estimated at 3.3 million deaths per year, with a case fatality rate of 0.3%.1 In Indonesia, diarrheal disease mortality rates have been similar, ranging from 1–18 per 1,000 children less than five years old, with the highest death rates among those younger than five years old, with the highest death rates among those younger than 12 months of age.2,3 While a number of enteropathogens have been associated with travelers’ diarrhea, enterotoxigenic Escherichia coli (ETEC) is the most frequent cause and is isolated from over half the cases. ETEC isolates are also a primary cause of diarrhea in infants and young children in developing tropical countries.4,5

Enterotoxigenic Escherichia coli isolates are identified by their ability to produce heat-labile (LT) and/or heat-stable (ST) enterotoxins.6 These bacteria colonize the small bowel epithelium by means of thread-like appendages (fimbriae) and by mechanisms yet to be completely established, resulting in diarrhea. These fimbriae are antigenically diverse and have been called colonization factor antigens (CFA).7 Recognized CFAs associated with human ETEC isolates are CFA/I, CFA/I, and CFA/IV. CFA/I is a single fimbrial antigen,8 while CFA/II and CFA/IV have been shown to consist of three distinct fimbrial antigens called coli surface (CS) antigens. CFA/II is composed of antigens CS1, CS2, and CS3.9 CFA/IV comprises antigens CS4, CS5, and CS6.10 A number of other putative colonization factors (PCF) have been described and characterized. These include those referred to as CFA/III, CS7, PCFO159, PCFO166, PCFO148, PCFO9, and PCF 8786.11,12 Currently there is no licensed vaccine for use against ETEC diarrhea. However, there is an oral cholera vaccine composed of killed whole-cell Vibrio cholera, plus the B subunit of cholera toxin, which provides some cross-protection against LT- and LT/ST-producing strains of ETEC.13,14 The basis for the cross-protective efficacy of the cholera vaccine lies in the fact that the LT of ETEC is antigenically similar to cholera toxin in that it can elicit sufficient cross-reacting antibodies to LT.15 Although anti-LT immunity is important in disease prevention, many ETEC isolates produce ST without LT.16 ST is poorly antigenic and unrelated to LT, so other common antigens must be evaluated as potential vaccine components.

The role of CFAs in the pathogenesis of ETEC-associated diarrhea has been well documented in both experimental and human volunteer studies.8,17,18 It has been shown that active or passive immunization with ETEC strains harboring CFAs induced protective immunity against diarrhea in animal models.17,18 In addition, immunization of human volunteers with purified CFA/I or CFA/II was shown to induce specific serum and intestinal antibody responses leading to protection against diarrhea.19 This observation argues for the inclusion of CFAs into a multivalent vaccine formula.20 Since the composition of CFAs expressed by ETECs varies from country to country,21 we reasoned that identification of the toxin-associated CFAs of ETEC isolated from a diarrheal disease case-control study in Jakarta, Indonesia, would provide useful information for this geographical region. Knowledge from such a study may be useful for determining study sites for the evaluation of potential ETEC vaccines.

MATERIALS AND METHODS

Study population. Three hospitals (Harapan Kita, Friendship, and Sumber Waras) and two Pusat Kesehatan Masyarakat (PUSKESMAS) (Community Health Centers) in Ja-
overnight incubation at 37°C in nitol selenite broth (MSB) for enrichment. Isolates from a subset of patients were inoculated into alkaline peptone water (APW) and mannitol-salt-Campylobacter, Escherichia coli,

72-hr in a microaerophilic atmosphere. Cultures were examined for the following agents: Vibrio cholerae, Salmonella, Shigella, Campylobacter, and E. coli.

Toxin and CFA determination. Fecal specimens collected from patients were cultured on MacConkey agar plates for selection of E. coli isolates. After overnight culture, 5 E. coli lactose-fermenting colonies from each patient were picked and stored on nutrient agar stab cultures until toxin and CFA determination.

Within 2-hr of passage, the stool samples from ill and control cases were delivered to the NAMRU-2 microbiology laboratory where numbers were assigned.

**Bacterial strains and cultivation.** Although ETEC was isolated from patients reporting diarrhea, and from other unrelated cases for use as controls. For the purpose of this study, diarrhea was defined as the passage of 3 or more stools in a 24-hr period. The number of stools passed during the previous 24-hr period was determined historically as further evidence of diarrhea illness. When diarrhea was reported, a stool specimen was obtained by the nurse. If diarrhea was serious, intravenous rehydration therapy was immediately instituted. Dehydration was diagnosed clinically based on the finding of dry mucous membranes, lack of tears when crying, or decrease in skin turgor.

Within 2-hr of passage, the stool samples from ill and control cases were delivered to the NAMRU-2 microbiology laboratory where numbers were assigned.

**Table 1**

Presence of CFA/I, CFA/II, CFA/IV, PCFO159/CS5 and PCFO166 in relation to toxin production in enterotoxigenic Escherichia coli (ETEC) strains isolated in Jakarta, Indonesia

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Case (n = 160)</th>
<th>Control (n = 53)</th>
<th>Case (n = 226)</th>
<th>Control (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST</td>
<td>18 (11.3)</td>
<td>3 (5.7)</td>
<td>21 (9.2)</td>
<td></td>
</tr>
<tr>
<td>LT</td>
<td>6 (3.8)</td>
<td>2 (9.5)</td>
<td>8 (3.5)</td>
<td></td>
</tr>
<tr>
<td>ST/LT</td>
<td>1 (0.6)</td>
<td>1 (1.9)</td>
<td>1 (0.4)</td>
<td></td>
</tr>
<tr>
<td>PCFO159</td>
<td>–</td>
<td>–</td>
<td>1 (0.4)</td>
<td></td>
</tr>
<tr>
<td>PCFO159 &amp; CS5</td>
<td>–</td>
<td>–</td>
<td>2 (15.4)</td>
<td>2 (9.0)</td>
</tr>
<tr>
<td>PCFO166</td>
<td>3 (1.9)</td>
<td>1 (7.7)</td>
<td>4 (1.8)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>129 (81)</td>
<td>15 (88)</td>
<td>47 (89)</td>
<td>183 (81)</td>
</tr>
</tbody>
</table>

*P values between ETEC cases and controls < 0.05.

**RESULTS**

**Pathogens.** Of the 6,615 E. coli isolates processed from 1,323 patients, 246 patients (19%) were positive for ETEC. Of the 246 ETEC isolates, only 9 (4%) were positive for the other pathogens (1 Shigella spp., 6 Vibrio cholera, 2 Salmonella spp.) (data not shown). No parasites or rotavirus were found.

**Toxins.** The distributions of toxins produced by the 246 ETEC isolates are shown in Table 1. Both ST and LT toxins were produced by 5% of the isolates, ST alone was produced by 72% of the isolates, and LT alone was produced by 23% of the isolates. Of the 1,169 symptomatic diarrhea cases in which ETEC was isolated, 160 (13.7%) of the patient isolates were associated with ST, 53 (4.5%) of the patient isolates with LT, while 13 (1.1%) of the patient isolates were associated with ST and LT.
attributed to both ST/LT. Of the 154 control isolates, 17 (11%) were associated with ST, 3 (2.0%) were associated with LT, while none of the ETEC from the controls were associated with both ST and LT (Table 1).

**CFA expression.** Of the 246 ETEC isolates, 21 (8.5%) expressed CFA/I, 14 (5.7%) elaborated CFA/IV, 3 (1.2%) showed CFA/II, 3 (1.2%) expressed PCFO159 and CS5, while 4 (1.6%) elaborated PCFO166 only. Two hundred and one (82%) of the ETEC isolates showed no association with either CFAs or PCFs (Table 1).

**Association of toxins and CFAs.** The correlation between ST and LT expression with synthesis of CFA subtypes is illustrated in Table 1. As seen, a greater percentage of the ST and LT ETEC detected was associated with CFA/I. There were not enough of the other CFA subtypes to derive any meaningful correlation.

**Age and sex distribution of LT- and ST-synthesizing ETEC isolates.** Age and sex of 240 of the 246 patients from whom ETEC strains were isolated were available for analysis and are illustrated in Table 2a and 2b. As seen, a high proportion of the ST (43%, 74/172), LT (69%, 38/55), and ST/LT (61%, 8/13) synthesizing ETEC were from patients < 5 yrs of age, although 50% of the ETEC was identified from children 0–5 years of age. There did not appear to be any sex difference in the frequency of ST- and ST/LT-synthesizing ETEC isolates across the various ages of the patients. However, there did appear to be appropriately twice as many males with LT-only ETEC isolates (15% in males and 8% in females). The reason for this male predominance for LT-synthesizing ETEC is not clear at present, and it is possible that the sample size may not be large enough to derive conclusions from such a finding. It should be noted that there were 131 as compared to 109 cases of ETEC from children < 1 yr of age, although 50% of the ETEC was identified from children 0–2 years of age. In Indonesia, diarrhea disease mortality rates have been similar, ranging from 1–18/1,000 in children < 5 yrs, with the highest death rates among those less than 12 months of age. The current study shows that ETEC-associated diarrhea occurred most frequently among children less than 1 yr of age, supporting previously reported findings in Indonesia.

Twenty-three percent of the ETEC isolates from this study expressed LT toxin, with or without concomitant ST, a rate that was not similar to that observed in a 1982 study of diarrhea among residents of Kebon Bawang, an urban, densely populated section of North Jakarta. In that study, ETEC was found in 16% of diarrheal cases identified by active community surveillance, with 50% of ETEC cases in children less than two years of age. The current study shows a high proportion of children with ETEC diarrhea; more than 50% in children 0–5 years and 15.4% of ETEC cases were observed among children who were 0–2 years of age. In Indonesia, diarrhea disease mortality rates have been similar, ranging from 1–18/1,000 in children < 5 yrs, with the highest death rates among those less than 12 months of age. The current study shows that ETEC-associated diarrhea occurred most frequently among children less than 1 yr of age, supporting previously reported findings in Indonesia.

**DISCUSSION**

The prevalence of ETEC isolated from the stool samples of diarrheic patients was 19%. This result is similar to that observed in a 1982 study of diarrhea among residents of Kebon Bawang, an urban, densely populated section of North Jakarta. In that study, ETEC was found in 16% of diarrheal cases identified by active community surveillance, with 50% of ETEC cases in children less than two years of age. The current study shows a high proportion of children with ETEC diarrhea; more than 50% in children 0–5 years and 15.4% of ETEC cases were observed among children who were 0–2 years of age. In Indonesia, diarrhea disease mortality rates have been similar, ranging from 1–18/1,000 in children < 5 yrs, with the highest death rates among those less than 12 months of age. The current study shows that ETEC-associated diarrhea occurred most frequently among children less than 1 yr of age, supporting previously reported findings in Indonesia.

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### Table 2A

<table>
<thead>
<tr>
<th>ETEC</th>
<th>0-1 yr (n = 36)</th>
<th>&gt;1-5 yr (n = 35)</th>
<th>&gt;5-12 yr (n = 11)</th>
<th>&gt;12-25 yr (n = 15)</th>
<th>&gt;25-60 yr (n = 6)</th>
<th>Total (n = 220)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST</td>
<td>16 (29)</td>
<td>13 (24)</td>
<td>3 (27)</td>
<td>3 (27)</td>
<td>5 (33)</td>
<td>31 (37)</td>
</tr>
<tr>
<td>LT</td>
<td>5 (9)</td>
<td>7 (13)</td>
<td>1 (9)</td>
<td>2 (18)</td>
<td>3 (20)</td>
<td>15 (8)</td>
</tr>
<tr>
<td>ST/LT</td>
<td>1 (2)</td>
<td>4 (7)</td>
<td>2 (18)</td>
<td>2 (18)</td>
<td>2 (18)</td>
<td>7 (3)</td>
</tr>
</tbody>
</table>

**Total no. (%)** 56 (25) 55 (25) 11 (5) 15 (7) 83 (38) 220 (100)

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### Table 2B

<table>
<thead>
<tr>
<th>ETEC</th>
<th>0-1 yr (n = 36)</th>
<th>&gt;1-5 yr (n = 35)</th>
<th>&gt;5-12 yr (n = 0)</th>
<th>&gt;12-25 yr (n = 15)</th>
<th>&gt;25-60 yr (n = 6)</th>
<th>Total (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST</td>
<td>1 (20)</td>
<td>1 (33)</td>
<td>(-)</td>
<td>1 (20)</td>
<td>3 (60)</td>
<td>6 (30)</td>
</tr>
<tr>
<td>LT</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>ST/LT</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
</tbody>
</table>

**Total no. (%)** 5 (25) 3 (15) 0 (0) 5 (25) 7 (35) 20 (100)

**ST** = heat-stable toxin.

**LT** = heat-labile toxin.

**F** = female.

**M** = male.
didates. However, it has been shown that immunization with LT toxoid has resulted in protection against both LT/ST- and LT-only ETEC infections.31

Reported surveys of ETEC strains from various parts of the world have shown wide variation in the number of E. coli strains with adherence factors. In a clinical study conducted in Bangladesh,29 75% of the ETEC strains expressed CFA/I or CFA/II, compared to 32.5% of the ETEC strains isolated from various categories of patients in Southeast Asia possessing CFA/I, CFA/II, or CFA/IV.30 On the other hand, ETEC isolates from Thai patients showed an overall 29% rate for CFAs.31 In Mexico City, 46% of the ETEC strains expressed CFAs, with 18% CFA/I, 5% CFA/II, and 23% CFA/IV, as compared to ETEC strains from Argentina expressing 23% CFA/I, 12% CFA/II, and 17% CFA/IV.32 In the present study, only 19% of the strains expressed CFAs: 9% expressed CFA/I, 1% expressed CFA/II, 6% CFA/IV, and 3% expressed PCFO159/O166. The difference between our results and those mentioned previously may be due to differences in the expression of CFAs on ETEC in various geographical regions. Another reason could be due to the laboratory techniques employed in the identification of CFA/toxin. It has been reported that a low percentage of strains carrying CFAs and enterotoxins in certain areas may also be due to repeated subculturing of strains and subsequent loss of plasmids,13,15 or the occurrence of a strain that has not been previously characterized phenotypically.14 In a recent review,14 it was reported that a high proportion of ETEC isolates from Peru and Thailand were found to be lacking CFAs, even with an enzyme-linked immunosorbent assay, indicating that ETEC isolated from these sites may have CFAs that have not yet been defined. The relatively low percentage of PCF-expressing strains (3%) found in our study is in agreement with the reports of Viboud and others12 and McConnell and others35 who found 9.5% and 17% of the ETEC strains tested, expressed PCFs. The low prevalence of PCFs could be attributed to many factors, such as loss of plasmids resulting from repeated subculturing or long-term storage.35 However, the strains used in our study were subcultured only once, so it is unlikely that the low percentage of PCF found could be attributed to plasmid loss alone.

Enterotoxigenic E. coli is a leading cause of diarrhea in young children in developing countries. The disease is characterized by watery diarrhea, cholera-like diarrhea, abdominal cramps, nausea, and vomiting, with or without fever. In many cases, extreme fluid loss (up to 10 times per day) accompanies the disease, consequently, dehydration occurs if fluids are not replenished.36 Earlier studies have implicated ETEC with watery, cholera-like diarrhea in India and Brazil.37,38 In this study, 81% of the patients who had ETEC diarrhea reported watery stools. This could mean that 81% of the ETEC-associated diarrhea patients were dehydrated, though treatment data were not available at the time of analysis. In Indonesia, the most common treatment for diarrhea is oral rehydration. In a study in Bangladesh, dehydration occurred in 43% of the adults and 20% of the children with ETEC.29

Two hundred and forty-six (19%) of the 1,323 patients with diarrhea had ETEC isolated from their stool samples. These data are in agreement with those of Richie and others26 who reported 19.6% ETEC isolation among children in Jakarta, Indonesia. The high proportion of patients without CFAs or CS antigens indicates the necessity to continue to survey for CFAs for inclusion in the ETEC vaccine that could be used to immunize travelers or residents of Southeast Asia. This study demonstrates a high incidence of ETEC diarrhea among residents of Jakarta and suggests that Jakarta could be a suitable site for the ETEC vaccine efficacy trial.

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