CRYPTOSPORIDIOSIS AMONG BANGLADESHI CHILDREN WITH DIARRHEA: A PROSPECTIVE, MATCHED, CASE-CONTROL STUDY OF CLINICAL FEATURES, EPIDEMIOLOGY AND SYSTEMIC ANTIBODY RESPONSES

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Abstract. We conducted a prospective case-control study to investigate the epidemiology, clinical features, and systemic antibody responses of cryptosporidiosis in Bangladeshi children. Forty-six children presenting to the International Center for Diarrheal Disease Research, Bangladesh in Dhaka, Bangladesh with diarrhea and Cryptosporidium spp. oocysts in the stool were enrolled as cases. Forty-six age-matched children with diarrhea, but without cryptosporidial infection, were enrolled as controls. Thirty cases and 23 controls returned for follow-up three weeks after discharge. Infection with Cryptosporidium spp. occurred most commonly in those less than two years of age, was accompanied by watery diarrhea and vomiting, and was more likely to be associated with persistent diarrhea. Other than duration of diarrhea, there were no significant differences in clinical or epidemiologic features between cases and controls. Cryptosporidium-specific serum IgM levels were significantly higher in cases compared with controls at presentation. In addition, there was a significant increase in serum Cryptosporidium-specific serum IgG levels over the three-week follow-up period in cases compared with controls. Within the case group, there was no difference between children with acute and persistent diarrhea in changes in IgG levels over the follow-up period. However, there was a significant difference between children with acute and persistent diarrhea in changes in both IgA and IgM levels, with persistent diarrhea being associated with a decrease in levels of both antibodies.

INTRODUCTION

The intestinal parasite Cryptosporidium spp. is an important cause of human diarrheal disease worldwide.\(^1\)\(^-\)\(^4\) Infection with this parasite is asymptomatic or mild and self-limiting in immunocompetent hosts, but can be severe and chronic in immunocompromised individuals, such as patients with acquired immunodeficiency syndrome or in severely malnourished children. The infection may be transmitted by direct person-to-person spread, contact with infected animals or by ingestion of contaminated food or water.

Cryptosporidium spp. are a leading cause of diarrhea, particularly persistent diarrhea, in children in developing countries.\(^1\) In these areas of the world, Cryptosporidium spp. infection has been reported to be more common in malnourished children than in well-nourished children and the consequences of the disease are more severe in the former than the latter. In countries such as Brazil, Peru and, Guinea-Bissau, cryptosporidial infection in early childhood has been reported to be associated with subsequent impairment in growth, physical fitness, and cognitive function.\(^5\)\(^-\)\(^7\)

In Bangladesh, Cryptosporidium spp. are a significant cause of diarrheal disease in young children.\(^8\)\(^-\)\(^11\) Researchers at the International Center for Diarrheal Disease Research, Bangladesh (ICDDR, B) in Dhaka, Bangladesh study the etiology, pathogenesis, treatment, and prevention of diarrheal diseases. Since 1979, there has been a surveillance system at the ICDDR, B in which every 25th patient (4% sample, 1979–1995) or 50th patient (2% sample, 1996–2003) is studied in detail. Retrospective case-control studies from this surveillance data as well as from other prospective studies from this institute have reported Cryptosporidium spp. infection in 1.4–3.5% of individuals, and have identified this parasite as one of the major enteropathogens significantly associated with diarrhea in young children.\(^8\)\(^-\)\(^11\) A recent study reported the presence of this parasite in 8.4% of diarrheal stool samples from Bangladeshi children.\(^12\) However, to date there have been no prospective case-control studies on the epidemiology of Cryptosporidium spp. infection in Bangladeshi children. In addition, there have been no previous studies of antibody responses to Cryptosporidium spp. in this population.

The purpose of this prospective age-matched case-control study was to describe the clinical and epidemiologic features of Cryptosporidium spp. infection in children ≤ 5 years of age presenting with diarrhea to the Clinical and Research Service Center of the ICDDR, B to investigate systemic antibody responses to Cryptosporidium spp., and to determine if there was any correlation between clinical, epidemiologic, or immunologic parameters and acute and persistent cryptosporidial diarrhea in this population.

METHODS

Patients. This study was conducted from May 2001 to August 2002 at the Dhaka Hospital of the ICDDR, B, which treats approximately 100,000 patients each year. Patients recruited for the study were children ≤ 5 years of age who presented to the Dhaka Hospital of the ICDDR, B with diarrhea. Diarrhea was defined as three or more watery stools within a 24-hour period. A diarrheal episode was defined as diarrhea for at least 72 hours. The end of a diarrheal episode was defined as absence of diarrhea for 48 hours. Acute diarrhea was defined as a diarrheal episode lasting < 14 days. Persistent diarrhea was defined as a diarrheal episode lasting ≥ 14 days. Children in whom a stool microscopic examination confirmed the presence of Cryptosporidium spp. by modified acid-fast staining were enrolled as cases. An equal number of children with diarrhea but without evidence of Cryptosporid-
Cryptosporidiosis in Bangladeshi children with diarrhea

ium spp. in the stool by modified acid-fast staining were enrolled as controls. Control patients were identified as the next age appropriate child following a case presenting to the ICDDR, B and were age-matched by the following groups: ≤ 12 months, 13–24 months, and 25–60 months. Informed consent was obtained from the parents or guardians of all children recruited to the study according to the guidelines of the Ethical Review Committee of the ICDDR, B.

A complete history was obtained and a thorough physical examination, including assessment of dehydration and nutritional status, was performed on each patient.13,14 A questionnaire regarding demographic, clinical, and epidemiologic history was administered to a parent or guardian for each patient. Patients were treated with oral rehydration solutions (ORS) and discharged from the hospital at the earliest possible time with the advice to continue ORS at home until the diarrhea stopped. Both cases and controls were asked to return for follow-up three weeks after discharge. A modified acid-fast stain for Cryptosporidium spp. in stool was performed for all patients at follow-up.

Enzyme-linked immunosorbent assay. Serum IgG, IgM and IgA responses to Cryptosporidium antigens were assessed by an enzyme-linked immunosorbent assay (ELISA). Two milliliters of blood was drawn at the initial and follow-up visits from both cases and controls. Serum samples were stored at -80°C and shipped to Tufts-New England Medical Center, Boston on dry ice. Purified Cryptosporidium parvum oocysts (Iowa strain, genotype 2) were obtained from Pleasant Hill Farms (Troy, ID) and stored at 4°C in buffer containing 20 mM sodium phosphate, pH 7.2, 150 mM sodium chloride (PBS) until use. Before use, oocysts were treated with 1.75% sodium hypochlorite for 10 minutes on ice, then washed with PBS. Oocyst lysates were prepared by freezing and thawing 10⁸ oocysts/mL in PBS for 20 cycles in the presence of protease inhibitors (20 μM leupeptin, 1 μM pepstatin, 2 mM phenylmethylsulfonyl fluoride, and 10 μM (2S,3S)-3-N-[4-(guanidinobutyl)carbamoyl]-3-methylbutyl]carbamoyl)oxirane-2-carboxylic acid). Lysates were stored in aliquots at -80°C. The same batch of oocysts was used for all the assays in the study. Ninety-six-well microtiter plates (Nunc, Rochester, NY) were coated overnight in PBS. Plates were washed three times with PBS-0.05% Tween 20, and blocked with 1% bovine serum albumin (BSA) in PBS for two hours at 37°C. Serum diluted 1:100 in 1% BSA in PBS was added and the plates were incubated for one hour at 37°C. After three washes with PBS-0.05% Tween 20, alkaline phosphatase-conjugated goat-anti-human IgG (γ chain specific), IgA (α chain specific), or IgM (μ chain specific) (Southern Biotech, Birmingham, AL) diluted 1:2,000 (IgG), 1:5,000 (IgA), or 1:10,000 (IgM) in 1% BSA in PBS were added and the plates were incubated for one hour at 37°C. The plates were washed three times with PBS-0.05% Tween 20 and substrate solution containing p-nitrophenyl phosphate (Sigma, St. Louis, MO) (1 mg/mL in 100 mM Tris-HCl, pH 9.5, 100 mM NaCl, 5 mM MgCl₂) was added. After 30 minutes, absorbance at 405 nm (A₄₀₅ nm) was measured with a Bio-Rad Microplate Reader (Model 550; Bio-Rad Laboratories, Hercules, CA). To control for plate-to-plate variation, the same known Cryptosporidium-negative and positive serum samples were run on each plate. All samples were run in triplicate. The mean of three samples was determined and values were normalized between the plates by dividing the A₄₀₅ nm of each unknown sample by the A₂₄₀₅ nm of the positive control for that plate and multiplying by 100. Results were expressed as ELISA units.

Statistical analysis. The Statistical Package for the Social Sciences (SPSS, Inc., Chicago, IL) was used for statistical analysis. Weight-for-height, height-for-age, and weight-for-age z scores were calculated using EPI Info (USD, Inc., Stone Mountain, GA). Dichotomous demographic and clinical characteristics were compared using the McNemar test and continuous variables were compared using the paired t-test (normally distributed variables) or the Wilcoxon signed rank test (non-normally distributed variables). Serum Cryptosporidium-specific IgG, IgA, and IgM ELISA units of cases only who presented with acute and persistent diarrhea (< or ≥ 14 days of diarrhea) were compared using an independent t-test. To preserve the matched analysis when comparing cases with controls at follow-up, we only analyzed data when both members of the pair presented for follow-up. Total duration of diarrhea and change in serum Cryptosporidium-specific IgG, IgA, and IgM ELISA units for cases and controls were compared using the paired t-test. Change in serum IgG, IgA, and IgM ELISA units for cases with acute and persistent diarrhea was compared using an independent t-test.

RESULTS

Clinical features. Forty-seven Cryptosporidium-infected patients were identified by screening of 1,672 stool samples. Forty-six of these patients were enrolled into the study as cases (parents of one child declined consent for participation in the study). An equal number of age-matched patients with diarrhea but without Cryptosporidium spp. in the stool (by modified acid-fast staining) were enrolled as controls. The salient clinical features of cases and controls are shown in Table 1.

Cryptosporidium spp. infection was most common in children less than two years of age (n = 44, 96%). There were only four (9%) Cryptosporidium-infected children less than six months of age. Watery diarrhea (96%) and vomiting (57%) were the most common presenting symptoms. However, on physical examination only six (13%) of the 46 cases were dehydrated. Although 17 (37%) Cryptosporidium-infected children presented with a history of fever, a temperature > 38°C was documented in only five (11%) of them. No other parasites were detected in the stools of cases; however, one (2%) of the controls was infected with Giardia lamblia, one (2%) with Entamoeba histolytica, and two (4%) with intestinal helminths.

The median duration of diarrhea at presentation was significantly greater in Cryptosporidium-infected cases than controls (nine versus three days; P < 0.001). Thirty (65%) cases and 23 (50%) controls returned for follow-up three weeks after discharge. The total duration of diarrhea at follow-up was also significantly greater in cases than controls (data available for 20 matched pairs; mean [SD] = 12 [4.8] days for cases versus 6 [2.9] days for controls; P < 0.001). Three patients in the case group and four in the control group reported new episodes of watery diarrhea that developed after discharge. Modified acid-fast staining was performed on stool samples obtained at follow-up from all cases and controls. Only one patient among the cases had Cryptosporidium spp.
in the stool at follow-up. This patient was asked to return for further follow-up after two weeks at which time stool microscopy was negative. Other than duration of diarrhea, there was no other significant difference in clinical parameters between cases and controls.

Thirteen Cryptosporidium-infected cases presented with persistent (≥14 days) diarrhea. Six Cryptosporidium-infected cases who presented with diarrhea for <14 days at enrollment continued to have diarrhea during the follow-up period for a total duration of diarrhea for ≥14 days, thus meeting the definition of persistent diarrhea. None of the controls had persistent diarrhea. There were no significant differences in any demographic or clinical characteristics including nutritional status between Cryptosporidium-infected cases with acute or persistent diarrhea. To determine if younger Cryptosporidium-infected children were more likely to develop persistent diarrhea, we compared children less than 12 months of age with those 13–60 months of age. However, there was no difference in the duration of diarrhea among the two groups.

**Epidemiology.** Epidemiologic features of cases and controls are summarized in Table 2. There were no significant differences between cases and controls with regard to contact with animals (cows, goats, chickens), type of water supply, food preparation within 10 yards of animal habitation, or family history of diarrheal illness. Similarly, there were no significant differences in epidemiologic parameters between Cryptosporidium-infected cases with acute and persistent diarrhea.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases (n = 46)</th>
<th>Controls (n = 46)</th>
<th>Matched analysis P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact with animals</td>
<td>7 (15%)</td>
<td>7 (15%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Food prepared within 10 yards of animal</td>
<td>9 (20%)</td>
<td>13 (28%)</td>
<td>0.48</td>
</tr>
<tr>
<td>Water supply</td>
<td></td>
<td></td>
<td>0.69</td>
</tr>
<tr>
<td>Tube well</td>
<td>28 (61%)</td>
<td>25 (54%)</td>
<td>0.30</td>
</tr>
<tr>
<td>Municipal water supply</td>
<td>18 (39%)</td>
<td>21 (46%)</td>
<td></td>
</tr>
<tr>
<td>No. of family members who eat from same utensils, median (interquartile range)</td>
<td>5 (4–5)</td>
<td>5 (3–6)</td>
<td>0.38</td>
</tr>
<tr>
<td>Other family members with diarrhea</td>
<td>5 (11%)</td>
<td>6 (13%)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

* NA = not applicable. Cases and controls group matched by age.

**Table 2**

Demographic and clinical characteristics of cases and controls on presentation

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases (n = 46)</th>
<th>Controls (n = 46)</th>
<th>Matched analysis P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 6</td>
<td>4 (8%)</td>
<td>8 (17%)</td>
<td>NA</td>
</tr>
<tr>
<td>6–12</td>
<td>20 (44%)</td>
<td>16 (35%)</td>
<td></td>
</tr>
<tr>
<td>13–24</td>
<td>20 (44%)</td>
<td>20 (44%)</td>
<td></td>
</tr>
<tr>
<td>&gt; 25</td>
<td>2 (4%)</td>
<td>2 (4%)</td>
<td></td>
</tr>
<tr>
<td>Male sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of diarrhea prior to admission in days, median (interquartile range)</td>
<td>9 (5–15)</td>
<td>3 (1–4)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Frequency of diarrhea in 24 hours prior to admission, median (interquartile range)</td>
<td>10 (6–13)</td>
<td>10 (7–15)</td>
<td>0.32</td>
</tr>
<tr>
<td>Watery diarrhea</td>
<td>44 (96%)</td>
<td>46 (100%)</td>
<td>0.50</td>
</tr>
<tr>
<td>Vomiting</td>
<td>26 (57%)</td>
<td>27 (59%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Fever</td>
<td>17 (37%)</td>
<td>21 (46%)</td>
<td>0.56</td>
</tr>
<tr>
<td>History of breast-feeding</td>
<td>34 (74%)</td>
<td>40 (87%)</td>
<td>0.24</td>
</tr>
<tr>
<td>Nutritional status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight-for-age Z score, mean (SD)</td>
<td>−2.62 (0.96)</td>
<td>−2.36 (0.96)</td>
<td>0.13</td>
</tr>
<tr>
<td>Height-for-age Z score, mean (SD)</td>
<td>−1.54 (1.18)</td>
<td>−1.06 (2.21)</td>
<td>0.10</td>
</tr>
<tr>
<td>Weight-for-height Z score, mean (SD)</td>
<td>−2.07 (1.13)</td>
<td>−1.65 (2.15)</td>
<td>0.36</td>
</tr>
<tr>
<td>Dehydration</td>
<td>6 (13%)</td>
<td>8 (17%)</td>
<td>0.73</td>
</tr>
<tr>
<td>Temperature &gt; 38°C</td>
<td>5 (11%)</td>
<td>6 (13%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Radial pulse, rate/minute, mean (SD)</td>
<td>127 (12)</td>
<td>127 (14)</td>
<td>0.92</td>
</tr>
<tr>
<td>Respiration, rate/minute, mean (SD)</td>
<td>38 (8)</td>
<td>37 (6)</td>
<td>0.56</td>
</tr>
</tbody>
</table>

**Serum antibody response.** To assess systemic antibody responses to Cryptosporidium antigens, serum IgG, IgA, and IgM levels were determined by ELISA in both cases and controls at the initial as well as the follow-up visits. Figure 1 shows that serum IgM levels were significantly higher in cases compared with controls at the initial visit (mean [SD] ELISA units for IgM = 102 [35] for cases versus 87 [40] for controls; P = 0.024). There were no significant differences in IgG or IgA levels between cases and controls (IgG = 74 [64] for cases versus 68 [57] for controls; P = 0.72; IgA = 137 [81] for cases versus 122 [116] for controls; P = 0.5). To evaluate the serum antibody response over time, we determined the change in IgG, IgA, and IgM levels from the initial to the follow-up time points in the 19 matched case-control pairs who came for follow-up after three weeks. As shown in Figure 2, there was a significant increase in IgG levels over the three-week follow-up period in cases, compared with controls (mean [SD] change in ELISA units from initial to follow-up visits for IgG = 63 [77] for cases versus -10 [69] for controls; P = 0.01). There was no significant change in serum IgA and IgM levels for either cases or controls (IgA, mean [SD] change = 50 [103] for cases versus 16 [45] for controls; P = 0.19; IgM, mean [SD] change = 18 [40] for cases versus 8 [18] for controls; P = 0.31).

To explore the possibility that the serum Cryptosporidium-specific antibody response may be different among cases with acute and persistent diarrhea, we compared the change in IgG, IgM, and IgA levels from the initial to the follow-up visit in both groups. For this analysis, we included those cases for which initial and follow-up serum samples were available (30 of 46 cases) and who met the definition of acute (17 of 30
cases) or persistent (13 of 30 cases) diarrhea at the end of the diarrheal episode (as determined at follow-up). As shown in Figure 3, there was no difference between children with acute and persistent diarrhea in the change in IgG levels over the follow-up period (mean [SD] change in ELISA units from initial to follow-up visits for IgG = 74 [98] for cases with acute diarrhea versus 35 [41] for controls; \( P = 0.16 \)). However, there were significant differences in the changes in IgA and IgM levels in children with persistent versus acute diarrhea, with persistent diarrhea being associated with a decrease in levels of both antibodies (mean [SD] change in ELISA units from initial to follow-up visits for IgA = 96 [64] for cases with acute diarrhea versus -20 [124] for cases with persistent diarrhea; \( P = 0.007 \) and IgM, mean [SD] change in ELISA units from initial to follow-up visits = 37 [30] for cases with acute diarrhea versus -1 [37] for cases with persistent diarrhea; \( P = 0.006 \)).

**DISCUSSION**

This prospective, matched, case-control study of cryptosporidiosis in urban Bangladeshi children with diarrhea has

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**FIGURE 1.** Cryptosporidium-specific serum IgG, IgA, and IgM levels in infected cases and uninfected controls at presentation. Bars represent the mean IgG, IgA or IgM levels. Antibody levels are expressed in enzyme-linked immunosorbent assay (ELISA) units.

**FIGURE 2.** Change in Cryptosporidium-specific serum IgG levels in infected cases and uninfected controls over the three-week follow-up period. The first circle of each horizontal bar indicates IgG levels (in enzyme-linked immunosorbent assay [ELISA] units) for the individual patient on the day (after the start of diarrhea) when serum was obtained at the initial visit; the second circle indicates the antibody level on the day when serum was obtained at follow-up three weeks later. To preserve the matched analysis when comparing cases with controls at follow-up, data was analyzed and is shown only when both members of the pair presented for follow-up.
shown a number of similarities as well as differences in clinical and epidemiologic features when compared with previous studies from this country, as well as from other developing countries. In addition, the present study describes for the first time systemic antibody responses to Cryptosporidium spp. in Bangladesh.

In this study, Cryptosporidium spp. were more frequently detected in children less than two years of age (96%), a finding previously reported in studies from Bangladesh as well as from other countries.9,11,15–17 The most common clinical symptoms among the Cryptosporidium spp.–infected cases were watery diarrhea (96%) and vomiting (57%), which is consistent with previous studies from Bangladesh.9,11 However, dehydration, which was previously reported to occur in 73–81% of children with cryptosporidial diarrhea in Bangladesh,9,11 was only found in 13% of patients in the present study. This is likely to be due to the remarkably high rate of intake of ORS prior to presentation, which was reported in 100% of the cases.

The only significant difference in clinical parameters between cases and controls was the duration of diarrhea prior to admission, as well as the total duration of diarrhea determined at follow-up, which was greater in cases than controls. Thirteen (28%) of the 46 Cryptosporidium spp.–infected children presented with persistent diarrhea compared with none of the controls. Among the Cryptosporidium spp.–infected cases, 6 of the 33 children who initially presented with acute diarrhea developed persistent diarrhea on follow-up, compared with none in the control group. Thus, 19 of 46 Cryptosporidium spp.–infected patients (41%) presented with or subsequently developed persistent diarrhea compared with none of the controls. A similar association of Cryptosporidium spp. infection with persistent diarrhea has been previously reported from other developing countries.18,19 Rahman and others also found persistent diarrhea to be associated with Cryptosporidium spp. infection in Bangladeshi children from the ICDDR, B.10 However, a subsequent study from the same institution found no significant association of duration of diarrhea with Cryptosporidium spp infection.9

There were no significant differences in clinical or epidemiologic parameters among Cryptosporidium spp.–infected patients with acute or persistent diarrhea. This finding is similar to that reported by Newman and others, who were unable to detect any difference in demographic or nutritional characteristics among Cryptosporidium spp.–infected children with acute and persistent diarrhea in northeastern Brazil.20

Lack of breast-feeding has been reported to be associated with cryptosporidiosis in many studies in developing countries, including Bangladesh.9,17,21 However, some prospective studies have not shown a protective effect of breast-feeding.15,20,22 and one study found that Cryptosporidium spp. infection was actually more common in breast-fed children.23 In the present study, although breast-feeding was less common among the cases (74%) compared with controls (87%), there was no statistically significant difference between the two groups. However, in our study, there were only four (8%) cases less than six months old (when children are typically exclusively breast-fed), an observation similar to that in a previous study in which only two (3%) of 68 Cryptosporidium spp. infections occurred in infants less than six months old.9 In this earlier study from the ICDDR, B, non-breast-fed children were reported to have a 2.3 times higher risk of acquiring Cryptosporidium spp. infection.9 Specifically, 21 (31%) patients who were infected with Cryptosporidium spp. compared with 26 (13%) controls were ≥ 24 months old (P = 0.001), an age group in which it is likely that breast-feeding is less common. When these investigators compared breast-feeding between cases and controls, they did not stratify by age group; thus it is possible that the higher age (24–60 months old) could be the confounding factor that suggests less breast-feeding among the cases.

A number of previous studies have reported an association between cryptosporidiosis and malnutrition (reviewed by Griffiths4). The study by Rahman and others from the ICDDR, B found that 36% of Cryptosporidium spp.–infected cases were malnourished compared with 16% of uninfected unmatched controls.10 Similarly, a study from the same institution reported by Bhattacharya and others found that malnutrition was significantly more evident in Cryptosporidium spp.–infected children than controls.9 In that study, stunting but not wasting carried a 2–4-fold increased risk for the presence of Cryptosporidium spp. in children with diarrhea. In contrast, malnutrition was not common in either cases or controls in the present study and there was no difference in nutritional status between the two groups. A study in urban Zambian children also found no significant association of cryptosporidial infection with nutritional status.23

None of the Cryptosporidium spp.–infected patients were co-infected with other parasites in the present study. How-
ever, previous reports from developing countries, including Bangladesh, have found high rates of co-infection with other enteropathogens such as *Shigella* spp., *Campylobacter* spp., *Vibrio cholerae*, enterotoxigenic *Escherichia coli*, and *Aeromonas* spp.8,11,24 In a retrospective case-control study of enteropathogens associated with childhood diarrhea from the same institution as the present study, Albert and others reported that 73% of *Cryptosporidium* spp.–infected patients were co-infected with other pathogens.8

Cryptosporidiosis is one of the most commonly identified infections in human immunodeficiency virus (HIV)–infected individuals with diarrheal disease.2,4 The HIV status of both cases and controls in this study was not assessed. However, there were no obvious risk factors for HIV infection in the patients studied and the incidence of HIV infection in Bangladesh has been reported to be low.25

Since waterborne transmission is a major route of infection with *Cryptosporidium* spp., it was of interest to determine whether there was any difference in the type of water supply between cases and controls. The cases and controls in this study came from an urban area in which the two main sources of water are tube wells and the municipal water supply. However, there was no significant difference in water source when comparing cases and controls. Two other studies from urban or semi-urban areas in Brazil16 and Guinea Bissau24 have also shown no association of cryptosporidiosis with source or type of water supply.

Cryptosporidiosis is a zoonotic infection and previous studies have reported an association of this disease with the presence of domestic animals in the household.21 A study by Rahman and others described a high prevalence of cryptosporidiosis in calves and their handlers in Bangladesh.26 In our study, however, there was no significant difference between the cases and controls in contact with domestic animals such as cows, goats, and chickens, or the presence of animals within 10 yards of food preparation. However, in this urban area of Bangladesh, the prevalence of domestic animals in the household is not as high as in rural areas. Previous studies of cryptosporidiosis in Mexico16 and Brazil27 also found no association of exposure to domestic animals.

The low incidence of diarrhea in immediate family members residing under the same roof as the *Cryptosporidium* spp.–infected children in this population (11%) is in keeping with previous findings of a low frequency of intra-familial transmission of this infection in Bangladesh.19 This is in contrast to other studies in developing and developed countries, which have documented a high rate of intra-familial transmission.20

A number of previous studies have investigated serum antibody responses to *Cryptosporidium* spp. in individuals in developed countries26–32 as well as developing areas such as the Philippines,33 China,34 Brazil,34 Ecuador,35 Peru,36 Venezuela,36 Thailand,37 and a Bedouin population in Israel.38 Many of these studies have used seropositivity to estimate the prevalence of cryptosporidiosis in the population under study. However, to date there have been no reports from Bangladesh on serum antibody responses to *Cryptosporidium* spp.

In the present study, we sought to evaluate the *Cryptosporidium*–specific serum antibody response in infected cases and uninfected controls. The results indicated that serum IgM levels were significantly higher in cases than controls at the time of presentation. This result is consistent with the presence of a recent infection in which the antibody response is usually of the IgM isotype. Studies from Thailand,37 the Philippines,33 and Israel34 found no significant difference in antibody levels between *Cryptosporidium*-infected children and uninfected children. However, the study population and the study design were different in these studies compared with those of our study. In the present study, there was a significant increase in IgG levels after three weeks in cases compared with controls, a finding that is consistent with development of an IgG response over time. In the study from the Philippines, there was no increase in antibody levels in patients after one and six weeks of follow-up. In the study from Israel, which measured *Cryptosporidium*-specific serum antibody levels in a cohort of 52 children (25 of whom had documented *Cryptosporidium* spp. infection) from birth to 23 months of age, mean antibody levels increased after 12 and 23 months; however, differences, if any, between *Cryptosporidium*-infected children and uninfected children were not reported.

In this initial study, we sought to obtain an overall idea of the systemic antibody response to as many *Cryptosporidium* antigens as possible using an ELISA with oocyst lysate as antigen. Previous studies have used either oocyst lysate or specific native or recombinant proteins as antigens for ELISA.28,30,31,34,36,38 Some studies suggest that use of specific cryptosporidial antigens such as the immunodominant 15/17-kD or 23/27-kD group of proteins is more sensitive and specific in evaluating the antibody response.28,31 Others suggest that the Western blot is more sensitive than the ELISA.32 However, a number of studies have detected an adequate response by ELISA using oocyst lysate.30,34,36,38 We are currently performing direct comparisons of serum isotype–specific antibody responses using oocyst lysate and specific recombinant antigens by ELISA and Western blot in this population.

None of the previous studies reported differences in serum antibody response between acute and persistent diarrhea associated with cryptosporidiosis. In the present study, there was no significant difference in the change in IgG levels over the follow-up period in cases with persistent diarrhea compared with those with acute diarrhea, whereas there was a significant difference in the change in IgA and IgM levels in cases with persistent diarrhea (decrease in levels) compared with those with acute diarrhea (increase in levels). The reason for the decrease in IgA in patients with persistent diarrhea is not clear. Since serum IgA is mostly derived from mucosal sources, it is possible that this may represent a diminished mucosal IgA response, which may contribute to persistent infection in these patients. However, one of the limitations of this study is the fact that *Cryptosporidium*-infected cases (particularly some with persistent diarrhea) presented at varying times after the onset of diarrhea. This resulted in differences in the time at which the initial serum samples were obtained, making it difficult to compare acute and convalescent antibody levels among them.

In conclusion, in these Bangladeshi children, *Cryptosporidium* spp. infection occurred most commonly in those less than two years of age, was accompanied by watery diarrhea and vomiting, and was associated with a significantly longer duration of diarrhea. Unlike previous studies from the same institution, in the present study, there was no significant association of cryptosporidial infection with lack of breastfeeding or with malnutrition. Serum *Cryptosporidium*-specific
IgM levels were higher in cases than in controls, and there was a significant increase in serum IgG levels over the three-week follow-up period in cases compared with controls. Cases with acute and persistent diarrhea were significantly different in the change in IgA and IgM levels over the follow-up period, with persistent diarrhea being associated with a decrease in serum IgA and IgM levels. Future community-based studies in which larger numbers of patients can be studied earlier at a more consistent point in the course of their disease and with a higher follow-up rate may help to further elucidate the clinical features, epidemiology and immune response in cryptosporidiosis in this population.

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