PREVALENCE AND IMMUNE RESPONSE TO ENTAMOEBA HISTOLYTICA INFECTION IN PRESCHOOL CHILDREN IN BANGLADESH

RASHIDUL HAQUE, IBNEKARIM M. ALI, AND WILLIAM A. PETRI, JR.

Abstract. Entamoeba histolytica infection was present in 5% and E. dispar in 13% of asymptomatic 2–5-year-old children from an urban slum of Dhaka, Bangladesh. Entamoeba dispar–infected children were no more likely than uninfected children to have serum antibodies to lectin. In contrast, all children infected with E. histolytica had serum antibodies to lectin. This anti-lectin response included antibodies against the carbohydrate recognition domain, which have been demonstrated in animal models to confer passive protection from amebiasis. Antibodies to lectin persisted in the sera of 17 children with E. histolytica infection over one year of follow-up, during which time E. histolytica infection cleared without treatment in 15, and with anti-amebic medication in two. We conclude that half of the children in this population have serologic evidence of amebiasis by five years of age, and that an anti-lectin serum antibody response is associated with limitation of E. histolytica infection to the colon.

Amebiasis is defined as infection with the protozoan parasite Entamoeba histolytica. It is now generally accepted that what was earlier known as E. histolytica actually comprises two genetically distinct but morphologically indistinguishable species, E. histolytica and E. dispar, previously known as pathogenic and non-pathogenic E. histolytica, respectively. The World Health Organization has reaffirmed the definition of amebiasis as infection with E. histolytica sensu stricto with or without clinical manifestations. Only E. histolytica can cause intestinal and extraintestinal disease. Amebiasis is a common problem in the developing world. For example, at least 8.4% of the population of Mexico had evidence of prior invasive amebiasis (based on the Mexican National Serosurvey) with an estimated one million cases of amebiasis and 1,000 deaths annually.

The two species can be differentiated by biochemical, immunologic, and molecular biological methods. Recently, antigen detection tests have been developed that rapidly detect intestinal amebic infection (E. histolytica/E. dispar) and differentiate pathogenic E. histolytica from nonpathogenic E. dispar. Stool antigen detection tests have proven to be sensitive and specific means for diagnosis of E. histolytica and E. dispar infection. While detection of E. histolytica infection in stool samples is useful to determine the point prevalence of infection, serologic tests for anti-amebic antibodies can be used to assess a population’s cumulative experience with E. histolytica infection. Serologic tests for anti-amebic antibodies are a marker of current or past infection with E. histolytica. A purified E. histolytica surface antigen, the galactose/N-acetyl-D-galactosamine (Gal/GalNAc)–inhibitable adherence lectin, has been studied for recognition by human immune serum in an ELISA. It was shown that antibodies to Gal/GalNAc lectin are associated with the occurrence of invasive amebiasis or presumably asymptomatic past infection by E. histolytica. The aim of our study was to determine the prevalence of E. histolytica infection based on the presence of antibodies against the Gal/GalNAc lectin of E. histolytica, as well as by the detection of E. histolytica in stool specimens in preschool children 2–5 years of age in a slum of Dhaka, Bangladesh.

MATERIALS AND METHODS

Study area. Preschool children 2–5 years of age enrolled in this study were from Mirpur, an urban slum of Dhaka. The majority of the inhabitants are of Bihari ethnic origin, who settled in Mirpur after the liberation of Bangladesh from Pakistan in 1971. The area is densely populated with approximately 50,000 residents. The use of human subjects was approved by the Ethical Review Committee of the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B) and the Human Investigation Committee of the University of Virginia. Informed consent was obtained from the parents of the children.

Sample collection. A list of all children 2–5 years of age was made and stool samples were collected using wide mouth stool containers. Serum samples were collected by trained personnel using sterile butterfly needles and 5-ml syringes and were placed into 5-ml serum separator tubes. Blood samples were centrifuged for 5 min at 1,200 × g and the resulting sera were frozen at −20°C until assayed for anti-lectin IgG antibodies.

Microscopy and culture. Fresh stool samples were examined for the presence of blood visible to the naked eye, and a smear of feces in 0.9% saline was examined microscopically for blood and for the presence of E. histolytica–E. dispar complex cysts and trophozoites. In addition, stool samples were concentrated using a formal-ether concentration technique for identification of cysts. Fresh stool samples were cultured for Entamoeba species in Robinson’s medium within 6 hr of collection. After 48 hr, a drop of culture sediment was examined microscopically for the presence of E. histolytica–E. disperscomplex trophozoites.

Stool antigen detection. The Entamoeba test (designed to detect but not differentiate the antigens of E. histolytica and E. dispar in stool specimens) and the E. histolytica test (designed to detect specifically E. histolytica in stool) were performed according to the manufacturer’s instructions (TechLab, Inc., Blacksburg, VA). The basis of these tests is antigenic differences in the GalNAc lectin between E. histolytica and E. dispar, which are detected by monoclonal antibodies. All stool samples that were positive by the Entamoeba test were tested with the E. histolytica test for spe-
specific identification of E. histolytica infection. A stool sample positive by the Entamoeba test and negative by the E. histolytica test was considered a E. dispar infection.

Polymerase chain reaction (PCR) test. The PCR for detection of E. histolytica and E. dispar infection in stool samples were carried out according to the protocol previously described by Haque and others.3 The nested PCR test is based on the amplification of the small subunit rRNA gene of E. histolytica and E. dispar.

Enzyme-linked immunosorbent assay for detection of serum anti-lectin antibodies. The anti-lectin IgG ELISA procedure was modified from that of Ravdin and others.9 Wells of 96-well microtiter plates were coated with purified lectin or with the lectin carbohydrate recognition domain (CRD; amino acids 895–998 of the lectin heavy subunit; TechLab, Inc.). Test sera were added at a 1:1,000 dilution in phosphate-buffered saline (PBS)–Tween 20, 1% bovine serum albumin (final volume = 100 µl) for 2 hr at room temperature. Wells were washed four times with PBS-Tween 20, and the plates were incubated with 100 µl of a 1:5000 dilution of horseradish peroxidase–conjugated goat anti-human IgG for 1 hr at room temperature. The wells were washed four times in PBS-Tween, followed by the addition of substrate. The optical densities (ODs) of the microtiter wells were measured at 450 nm with an ELISA plate reader (TiterTek Multiskan; Flow Laboratories, McLean, VA). To obtain a high level of specificity, the results were corrected for nonspecific background by subtracting the OD from wells in which serum samples were not added but otherwise exposed to the identical procedure described. A sample was considered positive if the OD reading was greater than the mean + three standard deviations of the reading of 34 serum samples of children 2 years of age whose stool samples were negative for Entamoeba infection by microscopy, culture, and antigen detection, and for which antibody levels were comparable with negative sera from a non-endemic country (United States).

RESULTS

During the study period from November 1996 to March 1997, single stool samples from 680 children who agreed to participate in the study from the community were collected and examined for the presence of E. histolytica and E. dispar infection. Serum samples were collected from 232 of those 680 children and tested for the presence of antibodies to lectin. Serum was collected from a subset of the 680 children who were heavily infected with Ascaris lumbricoides. The median age of the 680 children was 39 months. Of 680 children, 366 were male (53.8%) and 314 were female (46.2%). The overall prevalence of asymptomatic colonization with E. histolytica-E. dispar complex infection was 17.3% as determined by antigen detection and 4.8% and 10.4% as determined by microscopy and culture, respectively (Table 1).

Prevalence of E. histolytica infection was 4.7% and there was no significant difference in the prevalence of infection in the different age groups. Prevalence of infection was similar in both sexes as determined by all three techniques (female-to-male ratios of E. histolytica-E. dispar complex infection of 0.8, 0.8, and 1.0 as determined by microscopy, culture, and antigen detection, respectively). Prevalence of E. histolytica infection was also similar in both sexes as determined by antigen detection (female-to-male ratio = 0.9).

Of the 32 stool samples that were positive for E. histolytica by the antigen detection test, 24 were also tested by the PCR test. The PCR identified 21 as E. histolytica (including three stool samples positive for both E. histolytica and E. dispar), one stool sample as E. dispar, and two stool samples as negative, giving a correlation of 87% for antigen detection.

It was possible to collect both stool and blood samples from 232 children. This subgroup had a higher prevalence of E. histolytica infection than the entire group of 680 children (7.3% versus 4.7%) (Table 2). In this subgroup, 32.7% had serum anti-Gal/GalNAc antibodies (76 of 232), including 40.2% of the females (43 of 107) and 26.4% of the males (33/125), giving a female-to-male ratio of 1.5 (P = 0.02, by uncorrected chi-square test). Antibodies to lectin were most common in children 48–60 months of age (47%). Children four years of age had a significantly higher prevalence of antibodies to lectin than children three years of age and less (P < 0.001, by uncorrected chi-square test) (Table 2).

Entamoeba dispar infection was not associated with the production of antibodies to lectin (33% of the E. dispar-infected children and 31% of the children with no parasite infection had serum antibodies to lectin). In contrast, 100% of the children infected with E. histolytica had serum antibodies to lectin (Figure 1). This serum antibody response to lectin in E. histolytica-infected children included antibodies against the CRD of lectin (94% of the E. histolytica infected children were positive for antibodies to CRD with a mean ± SD OD of 0.59 ± 0.18). Antibodies to lectin and CRD persisted in the sera of 17 children with E. histolytica infection over one year of follow-up: the ratio of acute to con-

### Table 1

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>No.</th>
<th>Microscopy</th>
<th>Culture</th>
<th>E. histolytica</th>
<th>E. dispar</th>
</tr>
</thead>
<tbody>
<tr>
<td>24–35</td>
<td>241</td>
<td>10 (4.1%)</td>
<td>18 (7.4%)</td>
<td>9 (3.7%)</td>
<td>25 (10.3%)</td>
</tr>
<tr>
<td>36–47</td>
<td>229</td>
<td>13 (5.6%)</td>
<td>30 (13.1%)</td>
<td>12 (5.2%)</td>
<td>31 (13.5%)</td>
</tr>
<tr>
<td>48–60</td>
<td>210</td>
<td>10 (4.8%)</td>
<td>23 (10.9%)</td>
<td>11 (5.2%)</td>
<td>30 (14.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>680</td>
<td>33 (4.8%)</td>
<td>71 (10.4%)</td>
<td>32 (4.7%)</td>
<td>86 (12.6%)</td>
</tr>
</tbody>
</table>

1. Fecal smears were examined microscopically for E. histolytica-E. dispar cysts and trophozoites.
2. Fecal specimens were cultured in Robinson’s medium.
3. E. histolytica and E. dispar were detected using Entamoeba and E. histolytica fecal antigen detection tests (TechLab, Inc., Blacksburg, VA).

### Table 2

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>No.</th>
<th>E. histolytica infection detected by</th>
<th>Antigen</th>
<th>Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>24–35</td>
<td>41</td>
<td>1 (2.4%)</td>
<td>5 (12.2%)</td>
<td></td>
</tr>
<tr>
<td>36–47</td>
<td>89</td>
<td>6 (6.7%)</td>
<td>23 (25.8%)</td>
<td></td>
</tr>
<tr>
<td>48–60</td>
<td>102</td>
<td>10 (9.8%)</td>
<td>48 (47.1%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>232</td>
<td>17 (7.3%)</td>
<td>76 (32.7%)</td>
<td></td>
</tr>
</tbody>
</table>
amoeba test detected more E. histolytica study in rural children we made a similar observation. To
in stool specimens than microscopy or culture. In our earlier demonstrated that infection with
the urban slums. Other community-based studies have also E. histolytica.
ied earlier using the same antigen detection tests.
ence of an antibody response against the lectin CRD.
Seventeen E. histolytica-infected children were re-examined for E. histolytica at six and 12 months. Fifteen of sev-
eventeen had no detectable infection at six months (two after having received metronidazole for dysentery), with the re-
maining two children clearing the infection without specific treatment at 12 months.

valescent antibody responses was 0.98 for antibodies to lec-
tin and 0.91 for antibodies to CRD.
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maining two children clearing the infection without specific treatment at 12 months.

**DISCUSSION**

The major conclusions of this study are that asymptomatic E. histolytica infection is common in this urban population of children less than five years old and that it carries a risk of the future development of invasive amebiasis. The presence of an antibody response against the lectin CRD in children with noninvasive E. histolytica infection is interesting in light of our observation that these antibodies passively transfer immunity in an animal model of the disease. Prospective studies will be required to evaluate the role of anti-lectin and anti-CRD antibody responses in preventing human invasive infection with E. histolytica.

In this study, we differentiated E. histolytica from E. dispar infection by antigen detection and found that the overall prevalence of E. histolytica infection by stool antigen detection was approximately 4.7%. The prevalence of E. histolytica infection in the urban slum preschool children was at least four times higher than in the rural preschool children that we studied earlier using the same antigen detection tests.

This could be due to the poor hygienic conditions and inadequate sanitation in the urban slum promoting fecal-oral transmission of cysts. Colonization with E. dispar was three times more frequent than with E. histolytica in asymptomatic children from the urban slums. Other community-based studies have also demonstrated that infection with E. dispar is more common than infection with E. histolytica.

Both microscopy and culture underestimated the prevalence of E. histolytica-E. dispar complex infection. The Entamoeba test detected more E. histolytica-E. dispar infections in stool specimens than microscopy or culture. In our earlier study in rural children we made a similar observation. To resolve discrepant results, in this study we tested 24 of 32 stool specimens that were positive for E. histolytica infection by antigen detection with a PCR test and found excellent correlation. Thus, most of the additional cases of infection detected by the antigen-detection tests were true positive results.

The cumulative level of exposure in preschool children to E. histolytica was measured by the presence of serum antibodies specific for the Gal/GalNAc lectin. We found that approximately 33% were positive for antibodies to lectin. Similar rates of sera positive for antibodies to lectin have been observed in poor individuals in Brazil and South Af-
rica and for total antibodies to E. histolytica in preschool children in Bangladesh. We also observed a higher sero-
prevalence in females than in males, although prevalence of stool infection was similar in both sexes as determined by all techniques used in this study. Similar observations were made in a study from Brazil.

The anti-lectin seropositivity rate was much higher than the rate of current E. histolytica infection because antibodies were demonstrated to persist for at least one year in our study. Antibodies against CRD remained high in these children more than the one-year period of follow-up, whereas in an adult population in South Africa treated for amebic liver abscess, levels of antibody to CRD decreased over a similar time period. The persistence of anti-CRD antibodies may be due to the lack of treatment or to continual exposure to the parasite in this urban slum population.

Antibodies to lectin were present in the sera of all (17 of 17) children who were colonized with E. histolytica at the time of serum collection, as has been seen previously in a smaller number of individuals in South Africa. In contrast, children infected with E. dispar at the time of serum collection did not have higher rates of seropositivity than uninfected children. Thus, antibodies to lectin are a marker of E. histolytica infection, but not E. dispar infection. The lack of an anti-lectin antibody response in children with E. dispar infection is likely a reflection of the noninvasive character of E. dispar. Interestingly, in northeastern Brazil E. histolytica infection frequently does not result in detectable serum antibodies to lectin, suggesting fundamental differences in the parasite and or host in this region.

Our results have demonstrated that preschool children in the urban slum that we studied are at great risk of acquiring E. histolytica infection, with almost half infected by five years of age. Many infections with E. histolytica may be asymptomatic and cleared spontaneously. However, more information is required about the likelihood of asymptomatic infection progressing to invasive disease, and the role of anti-lectin antibody responses in limiting progression. Now that diagnostic tests that rapidly detect intestinal E. histolytica infection and differentiate E. histolytica from E. dispar are available, study of the epidemiology and immunology of E. histolytica infection in different parts of the world is likely to lead to rational control strategies.

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![Figure 1. Serum antibodies against Entamoeba histolytica lectin. Sera from children were tested at a 1:1,000 dilution for antibodies against the E. histolytica galactose/N-acetyl-D-galactosamine lectin. The presence or absence of E. histolytica or E. dispar infection in the children at the time of serum collection is shown.](image-url)
tralia, Bangladesh, Belgium, Canada, China, Germany, Japan, the Netherlands, Norway, Republic of Korea, Saudi Arabia, Sweden, Switzerland, the United Kingdom, and the United States; international organizations include the Arab Gulf Fund, the Asian Development Bank, the International Atomic Energy Centre, the United Nations Children’s Fund (UNICEF), the United Nations Development Program (UNDP), the United Nations Population Fund (UNFPA), and the World Health Organization (WHO); and private foundations include the Child Health Foundation, the Ford Foundation, the Rockefeller Foundation, and the Sasakawa Foundation; and private organizations include American Express Bank, Bayer AG, CARE, Family Health International, Helen Keller International, the Johns Hopkins University, Procter and Gamble, Rand, Sandoz, Swiss the Red Cross, and the University of California at Davis.

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