HOUSEHOLD EPIDEMIOLOGY OF ENTAMOeba HISTOLYTICA INFECTION IN AN URBAN COMMUNITY IN NORTHEASTERN BRAZIL

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Abstract. The natural history of infection with Entamoeba histolytica was studied in 2 slum communities in northeastern Brazil. Twenty-eight index patients colonized with E. histolytica were identified. Three stool specimens from the index patients and their household contacts were gathered over a 45-day period and tested for E. histolytica by means of a specific enzyme-linked immunosorbent assay-based detection kit. The detection kit is an antigen capture assay that has been shown to be highly specific for E. histolytica and does not detect nonpathogenic Entamoeba dispar or other enteric organisms. Blood samples were also collected at the start of the study, at 45 days, and at 6 months and analyzed for E. histolytica-specific antibody. High rates of colonization were seen in the family units. Colonization was self-limited, with 85% of colonized patients clearing their infections within 45 days. Reinfection appeared to be low during this time; however, previous seropositivity did not prevent colonization.

INTRODUCTION

The enteric protozoan parasite Entamoeba histolytica is the etiological agent of amebiasis. Approximately 50 million people are infected worldwide with E. histolytica, with the highest incidences in Central and South America, Africa, and India. Only 10% of infected people develop symptomatic amebiasis; however, this still results in an estimated 70,000 deaths each year.1,2

In a previous study of an urban slum in the state of Ceará in northeastern Brazil, we showed that 10.6% of the sample population was colonized with E. histolytica.3 Colonization was determined by an enzyme-linked immunosorbent assay (ELISA)-based antigen detection system for stool specimens that is specific for E. histolytica and has a sensitivity and specificity that is comparable to polymerase chain reaction and isoenzyme analysis, which is the gold standard.4 Most importantly, this kit allowed us to distinguish asymptomatic carriage of E. histolytica from colonization with Entamoeba dispar, the morphologically similar commensal parasite.

In this study, we used the same ELISA kit to follow the natural history of infection of E. histolytica in 2 urban slums in northeastern Brazil. Index cases of E. histolytica were identified and household contacts were examined. We found high rates of intrafamilial infection. Most people cleared their infections within 45 days. Previous seropositivity to an E. histolytica antigen did not appear to confer protection against colonization.

MATERIALS AND METHODS

Collection of epidemiological data. As part of an ongoing study of the epidemiology of E. histolytica infection in Gonçalves Dias and Parque Universitário, both urban slum communities in Fortaleza, Brazil, we enrolled 28 households in which one household member was identified with E. histolytica infection. The study design was to collect stool samples from each household member at the time of identification of the index case and then again 15 and 45 days later. Sera were collected at Days 1 and 45, and 6 months after the initial identification. Informed consent was obtained in Portuguese after a detailed explanation of the study for all participating household members. The study design and participation of humans were approved by the committee for clinical investigation at the Federal University of Ceará in Fortaleza and the human investigation committee at the University of Virginia. A brief questionnaire was given to each household member to obtain demographic data and information about symptoms such as diarrhea, fever, use of medication, and sanitary conditions. People who were ill at the time of interview were treated. All E. histolytica-positive people were treated with metronidazole at the end of the study.

Enzyme-linked immunosorbent assay for detection of galactose/N-acetyl β-galactosamine lectin in stools. The E. histolytica test was performed according to the manufacturer’s instructions (Tech Lab Inc., Blacksburg, VA). Briefly, coated microtiter wells (provided with the kit) were incubated with 0.1 mL of diluted specimen (stool specimen diluted 1:1 in diluent provided with the kit) and 1 drop of monoclonal antibody enzyme conjugate for 2h at room temperature. The contents of the well strips were then shaken out and washed 4 times in the wash solution. After washing, residual liquid was removed by striking the strip once against a paper towel. Substrate solutions were added and incubated 10 min at room temperature. Intensifier was then added, and after an additional 10 min of incubation, the well strips were read in a microtiter plate reader (Titertek Multiskan; Flow Laboratories, McLean, VA) at 450 nm. A positive result was defined as an optical density reading of ≥0.05 after subtraction of the negative control optic density.

Enzyme-linked immunosorbent assay serologic evaluation for detection of serum anti-lectin antibodies. The ELISA used was modified from Ravdin et al. The wells of 96-well microtiter plates (polystyrene ELISA plates; Nunc Immuno plate; Nunc Inc., Naperville, IL) were coated with purified E. histolytica galactose/N-acetyl β-galactosamine (Gal/GalNac) lectin by overnight incubation of 0.1 μg of lectin in 100 μL of coating buffer (0.015 M Na₂CO₃, 0.01% NaN₃, pH 9.6) per well at 4°C. Wells were washed 3 times with phosphate-buffered saline (PBS)-Tween 20, 1% bovine serum albumin (BSA), and incubated with 1% BSA in PBS for 2 hr at room temperature to prevent nonspecific binding to the polystyrene. Test sera were added at a 1:1,000 dilution.
TABLE 1

Summary of the study population demographics

<table>
<thead>
<tr>
<th>Participant characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of participants</td>
<td>161</td>
</tr>
<tr>
<td>Index patients</td>
<td>28</td>
</tr>
<tr>
<td>Household contacts</td>
<td>133</td>
</tr>
<tr>
<td>No. of households</td>
<td>28</td>
</tr>
<tr>
<td>Median size (range)</td>
<td>5 (3–16)</td>
</tr>
<tr>
<td>Treated water</td>
<td>25</td>
</tr>
<tr>
<td>Toilets</td>
<td>21</td>
</tr>
<tr>
<td>Median age (range)</td>
<td></td>
</tr>
<tr>
<td>Index patients</td>
<td>11 years (2–56 years)</td>
</tr>
<tr>
<td>Household contacts</td>
<td>12 years (2 months–75 years)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Men and boys</td>
<td>77 (48%)</td>
</tr>
<tr>
<td>Women and girls</td>
<td>84 (52%)</td>
</tr>
<tr>
<td>Diarrhea*</td>
<td></td>
</tr>
<tr>
<td>Stool negative for E. histolytica</td>
<td>11/40 (27%)</td>
</tr>
<tr>
<td>Stool positive for E. histolytica</td>
<td>33/109 (30%)</td>
</tr>
</tbody>
</table>

* Diarrhea is defined as one or more episodes.

** RESULTS **

** Description of study population.** During the study period, August 1996 to October 1998, 28 households with index cases were identified by ELISA of stool specimens and enrolled in the study. Eight households were located in the Gonçalves Dias community, and 20 were in Parque Universitário. Both communities have ~2,000 inhabitants and are located in Fortaleza, Ceará, Brazil. A summary of the study population characteristics is shown in Table 1. Twenty-one out of the 28 households had indoor toilets and 25 had treated water; however, the water is not considered potable. There is little seasonal variation in this region of Brazil. During this study’s collection years, there was a drought, so there was virtually no rainy season. The median household size was 5 members (range, 3–16 members). The median age of the 28 index patients was 11 years (range, 2–56 years), whereas the median age of the 133 family contacts was 12 years (range, 2 months–75 years). Forty (including index patients) of the 161 total household members (25%) were 5 years or younger, and 87 (54%) were 14 years or younger.

** Study compliance.** A diagram of the study compliance is shown in Figure 1. Of the 161 participants, 121 (75%) submitted 3 stool specimens, 21 (13%) submitted 2 stool specimens, 8 (5%) submitted 1 stool specimen, and 11 (7%) submitted no stool specimens. Three serum collections were performed on 82 of 161 (51%) people participating in the study, 30 (19%) had 2 serum collections, 18 (11%) had only 1 serum collection, and 31 (19%) had no serum collections. A total data
Natural history of infection. In 27 of 28 households, at least 1 additional case of *E. histolytica* infection was identified by ELISA examination of stool. Of the 149 households members with at least one stool examination, 109 (73%) (Figure 1A) were infected with *E. histolytica* at Days 1, 15, or 45. Twenty-five percent of the positive stools were from children aged <5 years.

To examine the duration of infection, shedding of *E. histolytica* antigen was analyzed in the 121 people who provided 3 stool specimens (Table 2). On Day 1, 57 of 121 (47%) of the samples were positive for *E. histolytica*. By Day 15, 31 of the 57 stool-positive participants (54%) were positive, and by Day 45, 11 of 57 (19%) were positive for *E. histolytica* antigen. On Day 15, 27 new stool-positive patients were detected. By Day 45, only 7 of these 27 stool-positive participants (26%) were still positive. There were 12 new stool-positive people at Day 45. In total, 66 of 84 (85%) of the stool-positive participants at Days 1 or 15 became negative in 30–45 days. Four people may have been reinfections: they were stool antigen-positive at the start of the study, negative at Day 15, then positive again at Day 45. Twenty-five of the 121 (21%) participants never had an *E. histolytica* antigen-positive stool.

Thirty percent (33 of 109) of the total of *E. histolytica* stool-positive patients reported at least one episode of diarrhea at some time during the study. The diarrhea ranged 1–6 days in duration. There was no attempt to identify other pathogens that may have also been the cause of the diarrhea. During the entire study, bloody diarrhea was reported in 3 people, all members of the same family. The median age of people with *E. histolytica* infection and diarrhea was 7 years (range, 10 months–74 years). Thirty-five percent were aged <5 years. The median age of people without diarrhea was 15 years. A total of 11 cases of diarrhea were reported among 40 stool-negative people (27%). People who reported an episode of diarrhea were found in only 9 of the 28 families.

Serologic analysis. Of the 161 participants, 130 (81%) had at least one serum specimen available for analysis (Figure 1B). Of these 130 people, 83 (64%) were positive by ELISA of sera. Of these 83 sera-positive patients, 60 (72%) had *E. histolytica*-positive stools at some time during the study. To further explore the relationship between Gal/GalNAc lectin seropositivity and stool positivity, the data from the 81 people with complete data were analyzed (Table 3). On Day 1 of the study, 23 people were seropositive and stool positive. A total of 24 people were seropositive and stool negative at Day 1. In this group, 15 of 24 (63%) became stool positive at Days 15 or 45. There were 19 people who were seronegative and stool negative at Day 1. Ten of 19 (53%) became positive at Days 15 or 45. In addition, 15 people were seronegative but stool positive at Day 1. Therefore, it appeared that previous seropositivity to the Gal/GalNAc lectin did not appreciably prevent reinfection.

The seropositivity of the 4 people who represented apparent reinfections at the start of the study was split: 2 were seropositive and 2 were seronegative. At the 45-day and 6-month collections, only one person was found to be seropositive. The single person who remained seropositive was the only one of the 4 who reported having diarrhea at some point during the study.

The seropositivity among the different groups at 6 months varied (Table 3). People who were seropositive at Day 1 tended to remain seropositive (31 of 47, 66%). Seronegative people at Day 1 were less likely to be seropositive at 6 months (5 of 34, 15%). Seronegative patients were found in 19 of the 23 families that were represented in the complete data set.

DISCUSSION

Infection with *E. histolytica* is common in inhabitants of developing countries; it predominantly affects people with poor socioeconomic conditions, nonhygienic practices, and malnutrition. Previously, we had shown that in a slum community in northeastern Brazil, 20% of the sample population was colonized with *E. histolytica* or *E. dispar* and 10.6% was colonized with *E. histolytica* alone. In this study, we sought to determine the transmission of *E. histolytica* in households and to study the natural history of infection.

Analysis of our data suggests that *E. histolytica* is highly infective within a family setting. In 27 of the 28 households in this study, at least one additional case of *E. histolytica* infection was identified within families. Overall, 73% of the household contacts were also colonized with *E. histolytica*. An infected family member appears to be an important risk factor for amebiasis. Higher rates of infection have been observed in contacts of patients with amebic liver abscess or with amebic dysentery, or in asymptomatic carriers compared with controls. A study in Mexico also found that 40% of contacts of *E. histolytica* and *E. dispar* carriers were also infected.

Despite the high rate of people infected with *E. histolytica*, symptomatic clinical disease was uncommon. Thirty percent of people infected with *E. histolytica* reported an
In this study, 85% participants cleared the infection by 30–45 days. In the time frame of our study, reinfection appeared to be uncommon. Four patients were positive at Day 1, negative at Day 15, and positive at Day 45; these may represent reinfection. Gathiram and Jackson\(^8\) found that asymptomatic carriers of \(E.\) histolytica cleared their infections within a year. Nanda and others\(^5\) had similar results. Our study suggests that reinfection is possible. Sixty-three percent of seropositive people who were initially stool negative and may play a role in eliminating the parasite and confer protective immunity to \(E.\) histolytica.

This is supported by our earlier studies in Brazil, which showed no correlation between Gal/GalNAc lectin seropositivity and colonization and also indicated that colonization rates remain fairly constant throughout life.\(^3\) Gathiram and Jackson’s\(^8\) study may have also observed reinfection. The zymodemes of \(E.\) histolytica cultured from positive stools collected at 6 months were different than the original stool isolates. A study in 1991 suggested that colonization rates were lower in seropositive people.\(^13\) In these studies, detection of \(E.\) histolytica was by microscopy. This technique has been shown to be insensitive and does not distinguish between \(E.\) histolytica and \(E.\) dispers. Other studies have shown a good correlation between \(E.\) histolytica infection and seropositivity.\(^2,5,10,13\) These differences may reflect parasite or host differences. Some strains of amebae may be more invasive and thus elicit a stronger immune response. Host differences may be relevant because we found that seropositive people tended to remain seropositive, whereas the seronegative population tended to remain negative, regardless of whether they had been colonized with \(E.\) histolytica.

It remains to be determined if invasive disease will elicit a longer-term protective immune response. The eventual clearance of the parasite and at least some delay in reinfecion suggests some immune processes are operating. Secretory immunoglobulin A has been detected in infected people and may play a role in eliminating the parasite and conferring short-term protection against reinfection.\(^14\) Long-term prospective studies of both host and parasite will help further define the nature of protective immunity to \(E.\) histolytica.

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