Entamoeba histolytica and Entamoeba dispar infections as detected by monoclonal antibody in an urban slum in Fortaleza, Northeastern Brazil

Infeccão pela Entamoeba histolytica and Entamoeba dispar detectada através de anticorpos monoclonais nas fezes em uma Comunidade Urbana em Fortaleza, Nordeste do Brasil

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Abstract In this study the authors used the ELISA-based antigen detection tests that distinguish E. histolytica from E. dispar to examine the prevalence of E. histolytica infection in individuals from an urban slum in Fortaleza, Northeastern, Brazil. This test has a sensitivity and specificity that is comparable to PCR and isoenzyme analysis, which is the gold standard. Single stools samples were obtained from 735 individuals. The prevalence of E. histolytica infection was 14.9% (110/735) and 25.4% (187/735) for E. dispar-E. histolytica complex. The most affected age group for E. histolytica /E. histolytica-E. dispar infection was the 1-5 year olds but there was no remarkable decrease with age. There was no significant difference in colonization rates between males and females. The results from this survey demonstrate that E. histolytica is highly prevalent in the Community studied. Furthermore, it offers promise for the antigen detection test as a sensitive and technically simple tool for detecting E. histolytica infection in the field.


Resumo Neste estudo, utilizamos testes de detecção antigênica baseada em ELISA que distinguem entre E. histolytica e E. dispar, para examinar a prevalência das infecções por E. histolytica em indivíduos de uma favela urbana em Fortaleza, no nordeste do Brasil. Esse teste possui sensibilidade e especificidade comparáveis às da PCR e da análise isoenzimática, que é o padrão ouro. Amostras simples de fezes foram obtidas de 735 indivíduos. A prevalência da infecção por E. histolytica foi de 14,9% (110/735) e de 25,4% (187/735) para o complexo E. dispar-E. histolytica. A faixa etária mais acometida por infecções com E. histolytica/E. histolytica-E. dispar foi a de 1-5 anos, mas não houve redução significativa com a idade. Não houve diferença significativa nas taxas de colonização entre homens e mulheres. Os resultados desta pesquisa mostram que E. histolytica é altamente prevalente na comunidade estudada. Além disso, oferecem esperança para o uso do teste de detecção antigênica como um recurso sensível e tecnicamente simples para a detecção de infecção com E. histolytica no campo.


Amebiasis is a significant health problem in developing countries. Entamoeba histolytica infection is responsible for up to 100,000 deaths per year.

Previously E. histolytica had been classified as consisting of pathogenic and nonpathogenic zymodemes. However this parasite is now recognized as a genetically distinct species, the invasive parasite E. histolytica, which is the etiologic agent of amebic colitis and liver abscesses, and the noninvasive parasite Entamoeba dispar which has never been associated with disease.

The diagnosis of amebiasis by microscopic identification of cysts or trophozoites in the stool is time consuming, requires expertise and does not distinguish pathogenic E. histolytica from nonpathogenic E. dispar. It is at best only 60% sensitive. Culture is more sensitive than microscopy, and isoenzyme analysis of cultured amebae enables the differentiation of E. histolytica from E. dispar. However, amebic cultures and isoenzyme analysis require a week to complete and are negative in many microscopy-positive stools.
New approaches to the detection of *E. histolytica* and *E. dispar* are based on *E. histolytica* antigen detection in stool and detection of *E. histolytica*-specific DNA by PCR amplification. Haque and co., compared PCR, antigen detection and isoenzyme analysis for specific detection of *E. histolytica* in fresh stool samples. All three techniques showed excellent correlation. Only the enzyme linked immunosorbent assay, that distinguishes between *E. histolytica* and *E. dispar* antigens, was both rapid and technically simple. These ELISA kits use monoclonal antibodies (Mab) directed against cross-reactive and species-specific epitopes of the Gal/GalNAc lectins from *E. histolytica* and *E. dispar*. The *E. histolytica* Test specifically detects *E. histolytica* while the Entamoeba Test detects the *E. histolytica*-*E. dispar* complex.

The authors have previously shown that amebiasis in Gonçalves Dias and nearby communities is frequent, however the prevalence of *E. histolytica* and *E. dispar* were not well characterized. Therefore an *E. histolytica* antigen detection test was used to examine the prevalence of *E. histolytica* and *E. dispar* within an urban slum in Northeastern Brazil.

**MATERIAL AND METHODS**

Subjects enrolled in this study were from Gonçalves Dias, a community of 1900 inhabitants, located in an urban slum (favela) in Fortaleza, Brazil and nearby slum communities. After a detailed explanation of the study, informed consent was obtained in Portuguese for all subjects that participated in the study. The use of human subjects was approved by Committee for Clinical Investigation at the Federal University of Ceará, and at the University of Virginia. Each subject was questioned for symptoms of intestinal/extraintestinal amebiasis, dysentery, fever and history of drug ingestion.

**Collection of samples.** A single fresh stool specimen was collected from each person without fixative, stored at 2-8°C and tested with the Entamoeba Test and *E. histolytica* Test kits within 24h. The specimens were stored at-20°C if the tests could not be performed within 24h. Microscopic stool examination was realized by direct wet mount of merthiolate-iodine-formaldehyde preserved stools by sedimentation method as described by Hoffman.

**Enzyme-linked immunosorbent assay for detection of Gal/GalNAc lectin in stools.** The Entamoeba Test (designed to detect but not differentiate *E. histolytica* and *E. dispar* in stool specimens) and Entamoeba histolytica Test (designed to specifically detect *E. histolytica* in stool) were performed according to the manufacturer’s instructions (Tech Lab, Inc., Blacksburg, VA). Briefly, coated microtiter wells (provided with the kit) were incubated with 0.1ml of diluted specimen (stool specimen diluted 1:1 in diluent provided with the kit) and 1 drop of Mab-enzyme conjugate for 2h at room temperature. After washing, residual liquid was removed by striking the strip once against a paper towel, substrate solutions were added, and incubated for 10 min at room temperature. Intensifier was then added, and after an additional 10 minutes of incubation the well strips were read in a microtiter plate reader (Titertek Multiskan; Flow Laboratories, McLean, VA) at 450nm. Positive results were defined as an optical density reading of > 0.05 after subtraction of the negative control optic density. Statistical difference was analyzed using the X² (chi-square) test.

**RESULTS**

During the study period from 1996 to 1998, stool samples from 599 individuals were collected, and examined for parasites by microscopy and for *E. histolytica*-*E. dispar* complex and *E. histolytica* by ELISA. An additional 136 stools were examined for *E. histolytica*-*E. dispar* complex and *E. histolytica* by antigen detection kit only. The mean age of the subjects was 17 years (1 year to 80 years): 43.5% (320/735) were males and 56.5% (416/735) females. According to the questionnaires all individuals were asymptomatic.

The overall colonization rate with various parasites by microscopy was 52% (312/599) with 50.6% (158/312) males and 49.3% (154/312) females. Table 1 displays the prevalence rates of infection by individual parasites species in the population sample. Among parasitized individuals 43.9% (137/312) had mixed infections with more than one parasite species. *Ascaris lumbricoides* was the most prevalent parasite at 32.4% (194/599). Very few cases of *Giardia lamblia* infection were found (2.8%; 17/599). *E. dispar*-*E. histolytica* complex was detected by microscopy in 7.5% (45/599) of the stools, with a mean of 9.5 years of age (range 6-67 years) with 37.8% (17/45) males, and 62.2% (28/45) females. *Entamoeba coli* infection was found in 15.8% (95/599).
The prevalence of *E. dispar-E. histolytica* complex detected by antigen detection kit was 25.4% (187/735), mean age 27 years (range 1-72), with 48.1% (90/187) males and 51.9% (97/187) females. The prevalence of *E. histolytica* infection was 14.9% (110/735) with a mean age of 9 years (range 3-64), 51% (57/110) males and 48.2% (53/110) females. Figure 1 shows age-related rates of colonization with *E. histolytica* and *E. dispar-E. histolytica* complex.

### DISCUSSION

Infection with *E. histolytica* is a severe health problem in many tropical and subtropical areas of the world, especially in developing countries. Most epidemiological studies of *E. histolytica* infection were performed before the distinction of two separate species, *E. dispar* and *E. histolytica*, was established. This has raised the question of the validity of these studies. There is a clear need to perform epidemiological studies in amebiasis which distinguish the two species of *Entamoeba* before one can state the true prevalence and impact of *E. histolytica*.

### Table 1 - Prevalence of protozoan parasites in stool.

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Prevalence(%)</th>
<th>Positive(ri)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ascaris lumbricoides</em></td>
<td>32.7</td>
<td>196</td>
</tr>
<tr>
<td><em>Entamoeba coli</em></td>
<td>15.8</td>
<td>95</td>
</tr>
<tr>
<td><em>Trichuris trichiura</em></td>
<td>12.3</td>
<td>74</td>
</tr>
<tr>
<td><em>E. histolytica-E. dispar complex</em></td>
<td>7.7</td>
<td>46</td>
</tr>
<tr>
<td><em>Enterobius vermiculares</em></td>
<td>2.8</td>
<td>17</td>
</tr>
<tr>
<td><em>Endolimax nana</em></td>
<td>2.3</td>
<td>14</td>
</tr>
<tr>
<td><em>Giardia lamblia</em></td>
<td>2.8</td>
<td>17</td>
</tr>
<tr>
<td><em>Hymenolepis nana</em></td>
<td>2.5</td>
<td>15</td>
</tr>
<tr>
<td><em>Strongyloides stercoralis</em></td>
<td>2.3</td>
<td>14</td>
</tr>
<tr>
<td><em>Iodoamoeba buetschlii</em></td>
<td>2.0</td>
<td>12</td>
</tr>
<tr>
<td><em>Ancylostoma</em></td>
<td>2.0</td>
<td>12</td>
</tr>
<tr>
<td><em>Chilomastix mesnili</em></td>
<td>0.3</td>
<td>2</td>
</tr>
</tbody>
</table>

*a* Total of 599 samples were tested  
*b* Commensal organism

![Figure 1](image-url)
In this study, antigen detection was used to distinguish *E. histolytica* from *E. dispar*. This test fulfills the requirements for use in epidemiologic investigations with large samples, as it is easy to use, rapid, and has high sensitivity and specificity. In the present survey of 735 individuals, using an ELISA-based antigen detection kit on stools, approximately 25% were *E. histolytica*-*E. dispar* complex positive, and 15% *E. histolytica* positive. These results show that infection by *E. dispar*-*E. histolytica* complex and *E. histolytica* is endemic in this region of Northeastern Brazil. This is consistent with the authors' previous observation that the overall seropositivity for anti-*E. histolytica* Gal/GalNAc lectin antibodies was 24.7%.

Studies of *E. histolytica* infection in Brazil among low-income populations have shown a difference between the North, Northeast, and South regions. Zymodeme analyses indicated that in the Amazon region (North) both *E. histolytica* and *E. dispar* were found with higher prevalence for *E. histolytica* while in Pernambuco in Northeastern Brazil *E. dispar* predominated. The methodologies that were used to detect *Entamoeba* species varied including serologic tests, zymodeme analysis, and PCR.

Microscopic examinations were performed in 599 stools and revealed the presence of other intestinal protozoans as well as helmintic parasites in approximately 52% of the individuals. *E. histolytica*-*E. dispar* complex were detected in 8% of the stools by microscopy. It was not an objective of this study to compare the detection of *E. histolytica*-*E. dispar* complex by antigen detection test with microscopy, however Haque and col. have shown that microscopy is 37% sensitive and antigen detection test is 94% sensitive when compared with culture.

As shown in Figure 1, the prevalence of *E. histolytica/ E. dispers* and *E. histolytica* was particularly high in the 1-5 year-old age group, but with no remarkable decrease with age. These results suggest a continuous exposure to the parasite throughout life and if immunity exists it does not protect well against colonization. There was no difference in colonization rates between males and females.

In this study we did not identify any significant association of infection by *E. dispers*-*E. histolytica* or *E. histolytica* with diarrhea or other intestinal disorders. This observation is consistent with longitudinal studies of individuals carrying *E. histolytica*, where little or no risk of intestinal disease was found and most individuals cleared their infections within six months.

All individuals with *E. histolytica* positive stools were asymptomatic. Repeated exposure to *E. histolytica* with the development of partial immunity to this parasite could explain the low rate of symptoms despite a high rate of infection by *E. histolytica*. Other possibility is that there may be restricted invasiveness of some strains of *E. histolytica*. Also, since this study was a one-time sampling it was not possible to ascertain for how long the colonized subjects remained asymptomatic.

In conclusion, the present study has demonstrated that *E. histolytica* and *E. dispers* infections are very frequent in this slum-dwelling population sample from Fortaleza Northeastern Brazil. Prospective cohort studies are needed to clearly define the epidemiology of *E. histolytica* infection.

REFERENCES


