PREVALENCE AND SPECIES DISTRIBUTION OF E. HISTOLYTICA AND E. DISPAR IN THE VENDA REGION, LIMPOPO, SOUTH AFRICA

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Abstract. The prevalence and species distribution of Entamoeba histolytica and E. dispar in the Venda region were determined in stool samples collected from public hospitals and primary schools by ELISA and a nested polymerase chain reaction (PCR). E. histolytica was detected in 37/197 (18.8%) and 1/47 (2.1%) samples, whereas 50/197 (25.3%) and 4/47 (8.5%) had E. dispar in the hospitals and schools, respectively. The age groups most infected were 0–2 (33%) years followed by 20–29 years (27%). E. histolytica was significantly associated with diarrhea (77.4% versus 22.6%; \( \chi^2 = 39.48, P < 0.05 \), and with the presence of lactoferrin (85.7% versus 14.2%) in the stools, indicating intestinal inflammation (\( \chi^2 = 29.605, P < 0.05 \)). E. histolytica was found in 5 (16.12%) of the 31 HIV-positive individuals and in 33 (15.5%) of the 213 HIV-negative individuals. E. histolytica infections are common in the Venda region and are associated with diarrhea and intestinal inflammation.

INTRODUCTION

Amebiasis is defined as the infection by Entamoeba histolytica that results in dysentery or amebic abscess. First discovered in 1875 by Fedor A. Lösch, this infectious agent is known to be common in developing areas, although local contamination cases have been described in developed countries such as France and Sweden. It is well known that annually 500 million individuals are infected worldwide, resulting in 100,000 deaths. However, the true distribution of the disease is not clear in most African regions such as the Venda region of South Africa. This has been particularly complicated by the existence of two different species morphologically identical but genetically different namely E. histolytica, which is pathogenic, and E. dispar, which is non-pathogenic. Even with E. histolytica, the majority of infected individuals are asymptomatic; ~10% develop diarrhea or colitis and a smaller subset develop extraintestinal disease mainly amebic liver abscesses. It is thus necessary to differentiate between E. histolytica and E. dispar infections to avoid unnecessary treatment of patients infected with the non-pathogenic E. dispar.

Microscopy remains the main method for the diagnosis of amebiasis and is used in most African countries. However, it cannot differentiate between E. dispar and E. histolytica, and the accuracy of this method in detecting E. histolytica depends heavily on the skills of the technician and has been shown to be less sensitive and less specific compared with other methods such as immunofluorescence (IFA), antigen detection, and polymerase chain reaction (PCR). Serological methods have also been used to distinguish between prior infection with E. histolytica and E. dispar; patients infected with E. dispar do not develop serum anti-amebic antibody titers, but 75–85% of patients with symptomatic E. histolytica infection develop detectable anti-amebic antibodies acutely, and > 90% of patients develop antibodies after they recover from infection. Although different methods provide different results, the usefulness of serological methods for the diagnosis of acute amebiasis in endemic regions is limited; for example, IHA may stay positive for several years, whereas ELISA and amebic gel diffusion (AGD) test may revert to negative after treatment. Thus, new methods based on antigen or DNA detection have been designed for the diagnosis of amebiasis. Molecular methods, such as PCR, have aided in alleviating some of the sensitivity and specificity issues traditionally associated with the detection of protozoan pathogens. A number of PCR-based assays, including gene amplification with specific primers, multiplex PCR, restriction fragment length polymorphism, and real-time PCR, have been developed for the identification of E. histolytica infections. Very few studies in Africa have used molecular methods to detect and differentiate between E. histolytica and E. dispar. There has been very high prevalence of E. histolytica determined by microscopy in different countries, which some authors have described as over diagnosis after molecular methods have failed to detect E. histolytica sensus stricto. However, there is a need to reevaluate the methodology used, by comparing the PCR with other methods such as the antigen detection, which has shown to be very specific for E. histolytica.

With the advent of HIV and AIDS, it has become more important to determine the distribution of E. histolytica and E. dispar among HIV-infected individuals. In Mexico, E. histolytica prevalence of 25.3% in the HIV/AIDS group and 18.5% in the HIV-negative group was described using PCR. Likewise, in Taiwan, persons infected with HIV were at increased risk for invasive amebiasis and exhibited a relatively high frequency of elevated antibody titers and intestinal colonization with E. histolytica. Previous studies in South Africa have been based in the Durban area in the eastern coast of the country, where a prevalence of 10% using the PCR has been described. However, no study to our knowledge has been conducted in the Limpopo Province and particularly in the Venda region. Therefore, the aims of this study were to determine the prevalence and species distribution of E. histolytica and E. dispar by antigen detection and a nested PCR followed by restriction fragment length polymorphism and to correlate these infections with diarrhea, inflammation and HIV infections.

MATERIALS AND METHODS

Ethical clearance. This study was approved by the research and ethical committee of the University of Venda, and the Department of Health and welfare and the Department of Health, Division of Infectious Diseases and International Health, University of Virginia, MR4, Lane Road, Room 3148, Charlottesville, VA 22908. E-mail: guerrant@virginia.edu
education in Polokwane, Limpopo Province, South Africa, before the initiation of the study.

**Study site and sample collection.** The three major hospitals (Tshizidzini, Elim, and Donald Frazer) serving the local population of the Venda region and two primary schools were selected for sample collection. Stool samples were collected from November 2003 to May 2005. At the hospitals, samples were collected from patients with gastrointestinal complaints or with diarrhea according to the health center’s guidelines. Briefly, patients were given a clean pan where they deposited their fecal material, and about 10 g of fecal material was transferred to the collection bottle with the assistance of a nurse. The stool samples were transported without delay to the microbiology laboratory at the University of Venda where preliminary tests were conducted and the stools were kept at −80°C until needed. At the primary schools, the objectives of the study were explained to the parents in a meeting with the authority of the schools who distributed the collection bottles to the pupils whose parents had agreed to the study and signed a consent form. The pupils brought the collection bottles home and with the help of their parents collected the stool in the bottles. The samples were collected the following morning at the schools and transported within 2 hours to the laboratory of microbiology, University of Venda. The samples were further aliquoted in a 1.5-M L epipendorf tube without dilution for diarrheic samples or diluted in sterile saline for non-diarrheic (formed) stools. Demographic information such as age and sex, as well as HIV status, was also collected.

**Antigen detection.** The stool samples were tested for *E. histolytica* using the *E. histolytica* II antigen detection Kit (TechLab, Blacksburg, VA) following the instructions provided by the manufacturer. The optical densities (ODs) were read at 450 nm with a Spectronic ELISA reader (Spectra Max 340, Molecular Devices Co., Sunnyvale, CA), and a sample was positive if the difference of OD between the test and the negative control was > 0.05.

**Entamoeba culture and maintenance.** Two reference strains were used in this study and were a kind gift from Dr. Upinder Singh (Department of Medicine, Division of Infectious Diseases, Stanford, CA). These included *E. histolytica* HM-1:IMSS, which was originally isolated from a patient with colitis in 1967 maintained in TYI-S-33 medium, and *E. dispers* SAW760, which was isolated from an adult human man in England in 1979. *E. histolytica/dispar* were isolated from diarrheic stool samples positive for Entamoeba by microscopy. Briefly, 500 µL of positive samples was inoculated in Locke-egg (LE) medium.11 Cultures were observed under light microscopy for growth and inoculated in fresh media every 3 days until establishment of the culture under subcultures. The isolates were maintained in Robinson medium and TYSGM-9, and the medium was changed every week.1213 All cultures were supplemented with rice starch and antibiotics when necessary.

**Genomic DNA purification.** The genomic DNA was purified from stool samples using the QIAamp DNA Stool Mini Kit from Qiagen (Qiagen GmbH, Hilden, Germany) with some modifications. Briefly, 250 µL of liquid stool or diluted stool material was added to 50 µL of potassium hydroxide and 15 µL of 1 mol/L dithiothreitol and mixed thoroughly. After a 30-minute incubation period at 65°C, 8.2 µL of 25% HCl and 80 µL of 2 mol/L Tris-HCl (pH 8.3) were added to the mixture. After a brief vortexing, the protocol continued with the Qiagen mini kit following the manufacturer’s instructions. For cultures, the DNA was purified by the Phenol chloroform method after precipitation of polysaccharide by cetyltrimethylammonium bromide (CTAB) as described previously.14

**PCR detection of *E. histolytica* and *E. dispers*.** A nested PCR using the primers previously described was used to characterize infection by *E. histolytica* and *E. dispers* from the stools with modifications.15 In the first PCR, primers E1 and E2, which amplified a 0.9-kb fragment of the rRNA gene, were used in a hot-start technique; 18.4 µL of the DNA extracts was mixed with 0.6 µL each of 40 µmol/L solutions of the primers (E-1 and E-2), 2.5 µL of 10× PCR buffer, 4 µL of 25 mmol/L MgCl₂, 0.64 µL of deoxynucleoside triphosphate mix (10 mmol/L each), and 0.4 µL (5 IU/µL) of Taq polymerase. After a first cycle of 15 minutes at 95°C, 50 cycles with denaturation at 92°C for 60 s, annealing at 43°C for 60 s, and extension at 72°C for 90 s were performed.

In the second (nested) PCR, 5 µL of the first PCR product was mixed with 26 µL of water, 1 µL each of 40 µmol/L solutions of the primers (EH-1 and EH-2 for *E. histolytica*; ED-1 and ED-2 for *E. dispers*), 4 µL of 10× PCR buffer, 3.2 µL of 25 mmol/L MgCl₂, 1 µL of deoxynucleoside triphosphate mix, and 0.4 µL of Taq polymerase. PCR was performed as described above, except that the annealing temperature was 62°C. PCR reactions were performed with a Bio-Rad gene cycler. Products were visualized on a 1.3% agarose gel (Invitrogen Life Technologies, Carlsbad, CA) containing ethidium bromide (0.2 µg/mL; Sigma, St. Louis, MO).

**Restriction endonuclease digests.** Ten microliters of the second PCR product was digested with 0.8 µL (10 U/µL) of *DraI* (Biolab) for 60 minutes at 37°C, followed by the addition of 0.4 µL (10 U/µL) of *Sau961* (New England Biolabs, Ipswich, MA) and further incubation at the same temperature for another 90 minutes. The restriction profiles were observed in 2% agarose gel stained with ethidium bromide.

**Fecal leukocyte detection: lactoferrin test.** Stool supernatants were tested according to the manufacturer’s specifications including appropriate kit controls (LEUKO-TEST; Tech Laboratory, Blacksburg, VA). Stool samples were diluted as described by the manufacturer: one drop (50 µL) of stool supernatant was added to 375 µL of diluent yielding a 1:25 dilution. Using the pipette provided with the kit, one drop of the sample and one drop of sensitized latex (lactoferrin antibody–coated latex beads) were mixed and observed for agglutination after 3 minutes. Each test was run in parallel with a negative control as indicated by the manufacturer. Positive controls provided with the test kits were also performed. Agglutination reaction was graded with the unaided eye from 0 (no agglutination) to + (large agglutination with a clear background). The lactoferrin content in the lactoferrin-positive stools samples was quantified using the ELISA method with the IBD scan kit from Techlab (Blacksburg, VA) following the instructions of the manufacturer.

**Statistical analysis.** The results of this study were analyzed using the SPSS software, version 10.1. The χ² test was used to determine the relationship between different results of the patients who provided the stool samples and other parameters such as diarrheal symptoms, sex, age, origin, or lactoferrin test results. The differences were considered significant when the *P* value was < 0.05.
RESULTS

Characteristics of the study population. A total of 244 samples were collected from 244 individuals, among whom 135 (55.33%) were women and 109 (44.67%) were men. One hundred ninety-seven (80.76%) were patients attending different hospitals in the region with gastrointestinal complaints or diarrhea and 47 (19.26%) were apparently healthy individuals (asymptomatic) pupils from two primary schools in the region. The study population was divided into nine age groups of 0–2, 3–5, 5–9, 10–19, 20–29, 30–39, 40–49, 50–59, and > 60 years old. The type of the sample (whether diarrheal or non-diarrheal) was indicated by the physical presentation of the sample at the time of collection. Very soft to liquid specimens were considered to be diarrhea. From the 244 samples, 134 (54.9%) were non-diarrheal, 103 (42.2%) were diarrheal, and 7 (2.9%) were bloody diarrhea. Diarrheal was common in the age groups 0–2 and 2–5 years old and also in the age groups 40–49 and > 60 years old. However, bloody diarrhea was not found in the age group 0–2 years old. Table 1 shows the occurrence of diarrhea in the study population. Among the 197 samples from the hospitals, 31 (15.7%) were from HIV-positive individuals. Diarrhea was more common amongst the HIV-positive group than in the HIV negative (χ² = 12.452, P = 0.002 < 0.05). Among the HIV-positive individuals, 8 (25.8%) were non-diarrheal, 22 (71.0%) were diarrheal, and 1 (3.2%) had bloody diarrhea. Table 1 shows the distribution of the population sample by age group and occurrence of Entamoeba histolytica and E. dispar DNA. Prevalence of Entamoeba histolytica and E. dispar in Venda. Three kinds of infection were detected by PCR from the stool samples: those with E. histolytica alone (without E. dispar), mixed infections containing E. histolytica and E. dispar DNA at the same time, and some with E. dispar alone (without E. histolytica).

From the total of 244 samples, E. histolytica DNA was detected in 38 (15.6%) samples, among which 7 (18%) were E. histolytica alone and 31 (82%) were mixed infection with E. histolytica and E. dispar. E. dispar DNA was detected in 54 (22.1%) samples including 23 with E. dispar alone and the 31 samples with mixed infections. There was a significant difference between the occurrence of E. histolytica among patients attending the hospitals and pupils of the primary schools (χ² = 9.160, P = 0.027 < 0.05). From the 47 samples collected from the schools, only 1 (2.1%) contained E. histolytica DNA, whereas 4 (8.5%) had E. dispar DNA. Unlike the samples from the hospitals, there were no mixed infections among the primary school samples. The infection patterns between men and women were not significantly different. From the 135 samples from women, 22 (16.3%) had E. histolytica DNA, with 5 (3.7%) E. histolytica alone and 17 (12.6%) mixed infections. Sixteen (11.9%) had E. dispar DNA. From the 109 stool samples from men, 16 (14.7%) had E. histolytica, with 2 (1.8%) E. histolytica alone and 14 (12.8%) mixed infections. Seven (6.4%) had E. dispar DNA alone. The age groups that were most infected were 0–2 years (33%) followed by 20–29 years (27%). Figure 1 shows the distribution (percentage of positive samples per age group) of E. histolytica and E. dispar infections in the study population.

Entamoeba histolytica was found in 5 (16.1%) of the 31 HIV-positive individuals and in 33 (15.5%) of the 213 HIV-negative individuals. Also, among the 31 HIV-infected patients, 6 (19.3%) had E. dispar, including 4 (12.9%) mixed infections with both E. histolytica and E. dispar. Among the five HIV-positive individuals with E. histolytica, four were women and one was a man. Among the HIV-negative individuals, 15 (13.8%) men and 18 (13.3%) women were infected (χ² = 0.754, P = 0.385). Table 2 shows the Entamoeba species among HIV-negative and HIV-positive individuals.

Entamoeba infections and potential symptoms (diarrhea and inflammation). From the 244 samples, E. histolytica DNA alone was detected in 7 samples, from which 5 (71%) were diarrheal and 2 (29%) were bloody diarrhea. Table 3 shows the occurrence of diarrhea and in relation to Entamoeba infections in the study population. From the 31 mixed infections, 7 (22.6%) were non-diarrheal, 22 (71.0%) were diarrheal, and 2 (6.5%) were bloody diarrhea. Among the E. dispar–positive samples, 14 (60.9%) were non-diarrheal and 9 (39.1%) were diarrheal. Not any of the E. dispar–positive samples were with bloody diarrhea. Table 2 shows the correlations between Entamoeba infections and diarrhea in the samples and indicates that E. histolytica was more associated with diarrhea than non-infected patients (χ² = 39.48, P = 0.05). All the samples were tested for lactoferrin. Amongst the seven samples containing E. histolytica DNA alone (without E. dispar), only one (14.2%) was negative for the lactoferrin test and six (85.71%) were positive.

![FIGURE 1. Prevalence of E. histolytica and E. dispar including mixed infections in the different age groups among patients attending hospitals and primary schools pupils.](image-url)
Nine (29%) stool samples with mixed infections tested negative for lactoferrin, whereas 22 (71%) were positive at different levels. Among the 23 samples positive with only *E. dispar* DNA (without *E. histolytica*), 14 (60.9%) were negative, which indicates a significant difference with *E. histolytica* ($\chi^2 = 29.605, P = 0.003$). Among the samples negative for *E. histolytica* and *E. dispar*, 101 (50.2%) were negative for lactoferrin. We also quantified lactoferrin in positive samples using IBD Scan (Techlab), and the results of the IBD scan corresponded with those of the Leukotest. Samples considered to have elevated lactoferrin content are those with lactoferrin concentrations > 7.25 µg/mL. The lactoferrin content of the *E. histolytica*-positive samples varied between 8.73 µg/mL for the weak positives and 23.3 µg/mL for the high positives, with three samples containing > 20 µg/mL among the seven samples positive for *E. histolytica* alone. From the eight samples positive for *E. dispar* alone, only one had a lactoferrin content > 20 µg/mL. Thus, *E. histolytica* was significantly associated with inflammation ($\chi^2 = 29.605, P < 0.05$) followed by mixed infections. *E. dispers* was not associated with inflammation. Table 4 shows the correlations between lactoferrin level and Entamoeba infection.

**Comparison of antigen detection and PCR.** Two different methods were used for the detection of *E. histolytica* from stool samples: the Techlab *Entamoeba histolytica* II antigen detection ELISA and PCR. Figure 2 shows the agarose gel obtained after restriction of the nested PCR product for the identification and differentiation of *E. histolytica* and *E. dispers*. The ELISA test detected 25 (10.2%) positive from the 244 samples, whereas the PCR reaction detected *E. histolytica* in a total of 38 (15.5%), including mixed infections that constituted 82% of all the *E. histolytica*-positive samples. No sample containing *E. dispers* was detected by the ELISA test, which detected 18 mixed infections but could not detect *E. histolytica* in 13 mixed infections. One sample was positive for the ELISA test but was negative for the PCR method, and one sample containing *E. histolytica* alone as determined by PCR could not be detected by ELISA. Table 5 shows the different results obtained by PCR and the antigen detection ELISA test. Compared with the PCR test, the antigen detection had a specificity of 96% and a sensitivity of 63.1%.

**DISCUSSION**

The objectives of this study were to determine the prevalence of *E. histolytica* in two different population groups in the Venda region, to compare the relative occurrence of *E. histolytica* and *E. dispers*, and to correlate this with the production of diarrhea and inflammation in the study population and among HIV patients. The existence of two species morphologically identical but genetically different was suggested as early as 1925 by Brumpt. However, it was not until 1993 that enough biochemical, immunologic, and genetic data were gathered to re-classify *E. histolytica* into two separate species: *E. histolytica*, which can invade the gut mucosa and cause diarrhea and extra-intestinal disease, and *E. dispers*, which causes only asymptomatic colonization.16 Worldwide, diarrheal diseases are a leading cause of pediatric morbidity and mortality, with 1.5 billion episodes and 1.5–2.5 million deaths estimated to occur annually among children < 5 years of age.17 Most important is the effect this diarrhea could have on

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**TABLE 2**

Distribution of Entamoeba infections by sex and HIV status

<table>
<thead>
<tr>
<th>Entamoeba PCR</th>
<th>Sex</th>
<th>HIV status: number of cases and percent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Entamoeba negative</td>
<td>86 (40.3%)</td>
<td>11 (35.5%)</td>
</tr>
<tr>
<td></td>
<td>73 (34.2%)</td>
<td>13 (41.9%)</td>
</tr>
<tr>
<td></td>
<td>159 (74.6%)</td>
<td>24 (77.4%)</td>
</tr>
<tr>
<td></td>
<td>183 (75%)</td>
<td></td>
</tr>
<tr>
<td><em>E. histolytica</em> positive*</td>
<td>15 (7%)</td>
<td>1 (3.2%)</td>
</tr>
<tr>
<td></td>
<td>33 (15.5%)</td>
<td>5 (16.1%)</td>
</tr>
<tr>
<td></td>
<td>38 (15.6%)</td>
<td></td>
</tr>
<tr>
<td><em>E. dispers</em> positive*</td>
<td>20 (9.4%)</td>
<td>1 (3.2%)</td>
</tr>
<tr>
<td></td>
<td>213 (100%)</td>
<td>31 (100%)</td>
</tr>
<tr>
<td></td>
<td>244 (100%)</td>
<td></td>
</tr>
</tbody>
</table>

* Including the mixed infections.

**TABLE 3**

Correlation between Entamoeba infections and diarrhea in the study population

<table>
<thead>
<tr>
<th>Diarrheal symptoms</th>
<th>Entamoeba PCR: number of cases and percent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Entamoeba negative</td>
</tr>
<tr>
<td>Non-diarrheic</td>
<td>113 (61.7%)</td>
</tr>
<tr>
<td>Diarrheic</td>
<td>67 (36.6%)</td>
</tr>
<tr>
<td>Bloody diarrhea</td>
<td>3 (1.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>183 (100%)</td>
</tr>
</tbody>
</table>
the physical and cognitive development of these children later in their lives.\textsuperscript{20} In our study population, there were more women than men as seen in the general South African population, where there are 100 women for 86 men in the age group 18–60 years.\textsuperscript{16} Diarrhea is a major cause of morbidity; patients attending the hospitals had a diarrhea rate of 45.1\%, with 2.9\% containing blood. This rate is comparable with one found in India, where acute watery diarrhea was most common (58.9\%), followed by dysentery (24.2\%) and persistent diarrhea (16.9\%).\textsuperscript{19} The most affected in our study were children <2 years of age, in whom 70\% of the stools were diarrheal.

According to the South African Department of Health, the HIV prevalence in the general population is 10.8\% for all South Africans >2 years of age in 2005.\textsuperscript{21} Among those between 15 and 49 years old, the estimated HIV prevalence was 16.2\% in 2005.

Women were more affected (13.3\%) than men (8.2\%). In the Limpopo Province, the prevalence in the whole population was 8\%. In our study, 15.7\% of the patients visiting the hospitals were positive for HIV. This is closer to the national prevalence for individuals between 15 and 49 years of age. These rates are still high compared with countries from other parts of the African continent such as Mali (1.9\%), but are comparable with the rates in other countries in the Southern African sub-region such as Malawi (14.2\%) and Zambia (16.5\%).\textsuperscript{19} It is well known that chronic diarrhea is one of the major AIDS-defining illnesses in World Health Organization (WHO) classification and occurs in 60–90\% of HIV-infected patients in Africa. In a Swiss Cohort Study, diarrhea was found to be an independent predictor of poor survival among patients with HIV and AIDS.\textsuperscript{22,23} In our study, diarrhea was very common and was present in 74.2\% of fecal specimens submitted from cases in the HIV population and is thus in agreement with data from previous studies. The causes of these diarrhea symptoms are diverse, and \textit{E. histolytica} has been shown to be one of the causes of infectious diarrhea in HIV-positive and HIV-negative individuals worldwide.

After the reclassification of \textit{Entamoeba histolytica}, the epidemiology of amebiasis needed to be redefined by the use of methods that are able to differentiate between \textit{E. histolytica} and \textit{E. dispar}. Thus, different PCR methods have been developed with variable efficiencies. A nested PCR previously described has been successfully used to differentiate between \textit{E. histolytica} and \textit{E. dispar}.\textsuperscript{19,24,25} Using the same method; we were able to differentiate between \textit{E. histolytica} from \textit{E. dispar} in samples collected from patients visiting public hospitals with gastrointestinal complaints or diarrhea and pupils attending public primary schools in the Venda region. \textit{E. histolytica} was found both in the hospital and in the schools. However, \textit{E. histolytica} was less common among primary school children between 5 and 15 years of age. These findings underscore the potential role of \textit{E. histolytica} in morbidity in the study area because the association between \textit{E. histolytica} infections and diarrhea was statistically significant ($P < 0.05$). Similar results have been found in other countries around the world such as Thailand.\textsuperscript{26}

During the past decade, molecular methods have been used to determine the true distribution of \textit{E. histolytica} and \textit{E. dispar} around the world. Infection rates and species variability (ratio between the occurrence of \textit{E. histolytica} and \textit{E. dispar}) varied tremendously from one region to the other. In Italy, more patients were found to be infected with \textit{E. dispar} (8.3\%) than \textit{E. histolytica} (5.6\%) using PCR assays.\textsuperscript{27} In Sweden, amebiasis is a notifiable disease, and 400–500 cases are reported annually to the Swedish Institute for Infectious Disease Control (SMI). PCR analysis showed that 165 (79.7\%) patients were positive for \textit{E. dispar}, whereas only 10 (4.8\%) patients were positive for \textit{E. histolytica}.\textsuperscript{28} In contrast, higher rates of \textit{E. histolytica} infections was found in Mexico compared with \textit{E. dispar} infections (13.8\% versus 9.6\%) using PCR.\textsuperscript{29} Similarly, in the Philippines, 74 cases (65.48\%) were positive for \textit{E. histolytica} and 6 cases (5.30\%) were positive

### Table 4

Lactoferrin presence in the stool samples containing Entamoeba DNA

<table>
<thead>
<tr>
<th>Lactoferrin content</th>
<th>Entamoeba negative</th>
<th>\textit{E. histolytica}</th>
<th>Mixed: \textit{E. histolytica} and \textit{E. dispar}</th>
<th>\textit{E. dispar}</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>101 (55.2%)</td>
<td>1 (14.3%)</td>
<td>9 (29.0%)</td>
<td>14 (60.9%)</td>
<td>125 (51.2%)</td>
</tr>
<tr>
<td>Weak</td>
<td>34 (18.6%)</td>
<td>1 (14.3%)</td>
<td>6 (19.4%)</td>
<td>6 (26.1%)</td>
<td>47 (19.3%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>20 (10.9%)</td>
<td>1 (14.3%)</td>
<td>4 (12.9%)</td>
<td>2 (8.7%)</td>
<td>27 (11.1%)</td>
</tr>
<tr>
<td>Elevated</td>
<td>18 (9.8%)</td>
<td>1 (14.3%)</td>
<td>5 (16.1%)</td>
<td>0</td>
<td>24 (9.8%)</td>
</tr>
<tr>
<td>High</td>
<td>10 (5.5%)</td>
<td>3 (42.9%)</td>
<td>7 (22.6%)</td>
<td>1 (4.3%)</td>
<td>21 (8.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>183 (100.0%)</td>
<td>7 (100.0%)</td>
<td>31 (100.0%)</td>
<td>23 (100.0%)</td>
<td>244 (100.0%)</td>
</tr>
</tbody>
</table>

The lactoferrin test was conducted by the Leuco test of Teclab and confirmed by the IBD CHEK ELISA detection of lactoferrin.

### Table 5

Comparative detection of \textit{E. histolytica} from stool samples by antigen detection and PCR

<table>
<thead>
<tr>
<th>ELISA results</th>
<th>Entamoeba negative</th>
<th>\textit{E. histolytica} only</th>
<th>Mixed: \textit{E. histolytica} and \textit{E. dispar}</th>
<th>\textit{E. dispar} only</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>182</td>
<td>1</td>
<td>13</td>
<td>23</td>
<td>219</td>
</tr>
<tr>
<td>Positive</td>
<td>1</td>
<td>6</td>
<td>18</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>183</td>
<td>7</td>
<td>31</td>
<td>23</td>
<td>244</td>
</tr>
</tbody>
</table>
for *E. dispar* in a mental institution.30 In the Gaza strip, Pal-

estine, *E. histolytica* was identified by PCR in 64 (69.6%) of

the samples and that of *E. dispar* in 21 (22.8%).31

In the African continent, few studies have used molecular

methods such as PCR to determine the prevalence and spe-

cies distribution of *E. histolytica* and *E. dispar*. Using samples

from Kilimanjaro hospitals, the prevalence of histolytica/

dispar was 0.8% and 7.4%, respectively, when ELISA was

used as the gold standard, indicating that *E. dispers* infection

was 14.5 times more prevalent than *E. histolytica* infection.32

In Ethiopia, *E. dispers* DNA was identified in 195 (91.5%) of

the 213 samples checked by PCR, and no *E. histolytica* DNA

was detected using real-time PCR.3 However, in the same

study, laboratory technicians had noted observing he-

matophagous Entamoeba cells in those samples. Similarly, in

Ghana, only one *E. histolytica* case and a considerably higher

prevalence of *E. dispers* (82.8%) was found using *E. his-

tolytica*– and *E. dispers*–specific DNA amplification in real-

time PCR.33 In a previous study in Zimbabwe, a prevalence of

10% for *E. histolytica* and 33% for *E. dispers* was found.14 In

a semi-rural population in the Durban area, South Africa, a

prevalence of 10% for *E. histolytica* was reported in 1985.34

However, since then, no other study has indicated the preva-

lence of *E. histolytica* in other parts of South Africa. Recent

reports note that data are inadequate to indicate true preva-

lence and incidence, and further studies are needed to deter-

mine the burden of infection and disease in Africa.35 In this

study, we found a rate of 15.5% for *E. histolytica*, which is

higher than the rate found in Durban by Gathiram and Jack-

son.36 This can be explained by the fact that our population

was potentially ill and thus had a higher risk of being infected,

which was not the case in the group without diarrhea in whom

there was no mixed infections and only one asymptomatic

case of *E. histolytica* was found. One advantage of this study

was the use of two different methods, which allowed com-

parison and confirmation of cases of *E. histolytica* infections.

We also studied the ability of two different methods to
detect *E. histolytica* infections. The antigen detection test

from Techlab has previously been shown to be suitable for the
diagnosis of amebiasis in endemic areas.36 In this study,

ELISA had a high specificity. It should be noted that samples

positive for PCR and negative with the ELISA test were gen-

erally mixed infections with *E. histolytica* and *E. dispers*. This

might have a hindering effect on the ability of the ELISA test
to detect these samples and might also be related to the

pathogenicity or virulence of the strains involved. It has been

indicated elsewhere that, when both organisms are present in

an individual, *E. dispers* generally outgrows *E. histolytica*.

However, because *E. dispers* is non-pathogenic, the result of

the infection will probably be asymptomatic. Mixed infections

have also been described in Mexico, where 13% of individuals

were found harboring *E. histolytica* and *E. dispers* at the same
time, particularly among HIV-positive individuals.8

The mechanisms of disease production after an infection by

*E. histolytica* are not fully understood. Most *E. histolytica*

infections remain asymptomatic. However, other studies have

suggested that amebic colitis may be encountered during

colonoscopic examination, even in subjects who are asym-

tomatic.37 *E. histolytica* has also been associated with travel-
er’s diarrhea. In a study in Sweden, when the patients were

divided into immigrants and travelers, the percentages with *E.

histolytica* were 3.8% and 9.5%, respectively.38 In invasive

amebiasis, white blood cells can be present in the stool, and

in severe cases, pus can be visible, but fecal leukocyte numbers

are generally not as high as in shigellosis.39 Indeed, virulent *E.

histolytica* can destroy neutrophils on contact, inducing inflam-
mation but showing only pyknotic leukocytes in the stools.40,41

Such a process would be expected to cause evidence of inflam-

mation (i.e., lactoferrin) even without morphologically

clear polymorphonuclear cells (PMNs) in the stool. Inflam-
mation occurs most often, and previous studies have shown

that fecal lactoferrin was the best way to indicate the presence

of PMN in stool samples. In our study, 85.7% of samples with

*E. histolytica* DNA were positive for lactoferrin, with 43% of
cases presenting with high levels of lactoferrin, whereas *E.

dispar–positive samples had only one (4.3%) case with a high

lactoferrin level. This further confirms the pathogenic differ-

eence between the two species. When we excluded other de-
tected organisms, the association of *E. histolytica* with diarr-

hea and with lactoferrin was even stronger. Other studies

have indicated low levels of lactoferrin with *E. histolytica* and

*S. hematobium* infections compared with shigellosis and other

urinary tract infections.42 However, *E. histolytica* infections

had not been ascertained by a specific test such as PCR.

In conclusion, *E histolytica* seems to be common in the

Venda region of South Africa. Mixed infections were espe-

cially frequent as opposed to other areas in the world such as

Japan.23 *E. dispers* was less associated with diarrhea or fecal

lactoferrin and occurred more often than *E. histolytica* in the
general population. Fecal lactoferrin may provide a useful

indicator of acute invasive *E. histolytica* infections and could

be used as screening test for inflammatory diarrhea including

*E. histolytica* in the Venda region, considering its simplicity.

This study also shows the susceptibility of women infected

with HIV to *E. histolytica*, which is also commonly seen in

men with or without HIV. The study of genetic and antigenic

profiles will shed more light on the pathogenicity of this im-

portant protozoal infection and provide insight into improved

control measures such as improved water and sanitation and

vaccine and drug development.

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