COMPARISON OF ANTIGEN-CAPTURE ELISA TO STOOL-CULTURE METHODS FOR THE DETECTION OF ASYMPTOMATIC *ENTAMOEBA* SPECIES INFECTION IN KAFER DAOUD, EGYPT

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Abstract. We performed a prospective field study in the village of Kafer Daoud in Menofia, Egypt to compare the fecal culture method with enzyme linked immuno assay (ELISA) for detection of 170 kDa lectin antigen in feces for diagnosis of asymptomatic *Entamoeba histolytica* and *Entamoeba dispar* infection. All subjects with *E. histolytica* or *E. dispar* infection detected by culture also had positive ELISA for amebic antigen in their feces and an additional 57 *Entamoeba* infections missed by culture were detected by ELISA (*P* < 0.001 compared to culture). The presence of fecal anti-lectin IgA antibodies and serum anti-LC3 (recombinant cysteine-rich lectin protein) IgG antibodies were positive predictors for *E. histolytica* infection (*P* < 0.03). Of interest, infection with *Trichomonas hominis* but not *Blastocystis hominis* was positively associated with *E. histolytica* infection (*P* < 0.05). In conclusion, ELISA for detection of fecal lectin antigen is a more sensitive method than fecal culture for detecting asymptomatic *E. histolytica* infection.

INTRODUCTION

*Entamoeba* species, *histolytica* and *dispar*, infect up to 10% of the world’s population. The highest burden of *Entamoeba* sp. infection occurs in developing countries, with a combined prevalence as high as 25% in Mexico, 38% in the continent of India, and 25% in sub-Saharan and tropical Africa. All *E. dispar* infections and 90% of *E. histolytica* intestinal infections are asymptomatic; *E. histolytica* causes approximately 50 million cases of invasive amebiasis (colitis and liver abscess) and 100,000 deaths per year. Based upon studies utilizing culture of fecal samples for detection, the prevalence of *Entamoeba* sp. infection in an endemic area was estimated at 2.6%. Culture provides up to a four-fold increase in sensitivity compared to microscopy for detection of *E. histolytica* trophozoites or cysts in feces; however, its absolute sensitivity is unknown. Utilizing polyclonal or uncharacterized monoclonal anti-amebic antibodies, ELISA has been successful in detecting *E. histolytica* antigen in stool. However, early reports did not differentiate *E. histolytica* from *E. dispar* infection. Monoclonal antibodies to the galactose-inhibitable adherence lectin 170 kDa subunit of *E. histolytica* have been utilized in ELISA to differentiate *E. histolytica* from *E. dispar*. The epidemiologic studies using Tech lab kits demonstrated lower sensitivity than the gold standard of culture and isoenzyme analysis. Detection of serum lectin antigen by ELISA has been demonstrated to be useful in identifying *E. histolytica* infections. In a prospective field study in a small village in Egypt, we investigated whether stool culture or a monoclonal antibody-based ELISA for detection of fecal-lectin antigen method was more sensitive for diagnosing asymptomatic *E. histolytica* and *E. dispar* infection.

MATERIALS AND METHODS

Study populations. Single serum and stool samples were obtained over a one-month period from each of the 182 randomly selected subjects, two-thirds of whom were women living in Kafer Daoud village located in Menofia, Egypt. Subjects ranged in age from 6 to 60 years and were asymptomatic, without recent history of gastrointestinal complaints or use of amebicidal drugs such as metronidazole. Fecal samples were also collected from 55 patients at the University of Minnesota Hospital, Minneapolis, Minnesota, with no history of parasitic infection and negative fecal microscopy; control sera were obtained previously from 47 healthy employees at the University of Virginia, Charlottesville, Virginia, with no history of amebic infection. *Entamoeba* species, *Blastocystis hominis* and *Trichomonas hominis* were differentiated by morphology on microscopy.

Stool culture. Fecal samples were collected in the morning and kept in ice until transported to Cairo within the next twelve hours. Samples were cultured in Robinson’s Medium as described. After 48 hours, an aspirate of the starch layer was mixed with double strength Lugol’s iodine and examined microscopically. A second reading was performed after 96 hours. Positive cultures were subcultured every 2 or 3 days using fresh slope with starch, 2 drops of peptone, 2 drops of erythromycin and BRS diluted 1 in 4 with pthalate filled up to the bottle neck for incubation at 37°C.

Hexokinase isoenzyme electrophoresis. *Entamoeba* species were differentiated by electrophoretic migration of hexokinase isoenzymes as described by Sargeant.

ELISA for detection of 170 kDa lectin antigen in serum and feces. ELISA for detection of 170 kDa antigen was performed according to the method of Abd-Alla and others. Briefly, 96 well flat-bottomed polystyrene microtiter ELISA plates were coated with monoclonal antibody 3F4, which recognizes lectin epitopes present in both *E. histolytica* and *E. dispar*, or with 8C12 antibodies, which recognizes epitopes found only in *E. histolytica* lectin. Non-reactive sites in the wells were blocked with 1% BSA in coating buffer. Stool samples were mixed in an equal volume of PBS containing 2 mM PMSF (United States Biochemical Corp., Cleveland, OH), serum samples were diluted 1:100 in PBS-Tween 1% BSA. Serum or stool samples were added at 100 μl/well and incubated for 2 hours at room temperature or overnight at 4°C. Alkaline phosphatase conjugated anti-lectin monoclonal antibodies (8A3, which recognizes both *E. histolytica* and *E. dispar* or 1G7, specific for *E. histolytica*) were added at 1:1000 dilution. Developing, reading (Bio-Rad Microplate reader-Benchmark, Hercules, CA) and correction for nonspecific background were performed as described.
ELISA for detection of fecal anti-lectin IgA antibodies. ELISA for the detection of fecal anti-lectin IgA antibodies was performed as described by Abo-El-Maged and others. Brieﬂy, ELISA plates were coated with a puriﬁed recombinant cysteine-rich portion of the lectin’s 170 kDa subunit (LC3) (0.2 µg protein/well) and feces were processed as above except that alkaline phosphatase-conjugated goat anti-human IgA antibodies (Sigma, St, Louis, MO) were added above except that alkaline phosphatase-conjugated goat anti-human IgG antibodies were diluted in PBS-Tween with 1% BSA (1:5,000 for IgG, 1:2,000 for IgA), were utilized as secondary antibodies. Alkaline phosphatase-conjugated goat anti-human IgG antibodies diluted in PBS-Tween containing 1% BSA. Developing, reading and correction of nonspeciﬁc background-binding were performed as described.

ELISA for detection of serum anti-LC3 IgG and IgA antibodies. ELISA for detection of serum anti-LC3 IgG and IgA antibodies were performed as described. Brieﬂy, serum samples were diluted at 1:1,000 for assay of IgG and 1:500 for IgA determination in PBS-Tween containing 1% BSA. Alkaline phosphatase-conjugated goat anti-human IgG antibodies diluted in PBS-Tween with 1% BSA (1:5,000 for IgG and 1:2,000 for IgA), were utilized as secondary antibodies. Developing, reading and correction of nonspeciﬁc background-binding were performed as described.

Statistics. All results were expressed as percent positive and percent negative. Z test (converted to P value) was used to determine the significance of difference. The Z test is deﬁned as: Z = (P1 − P0) cutting PQ (1/NI − 1/N0) where P1 = proportion of positive in group one, P2 = proportion of positive in group two, P = pooled proportional estimate = (X1 + X2) / (N1 + N2), X1 = number of positive in group one, X2 = number of positive in group two, N1 = total number of group one, N2 = total number of group two, Q = (1 − P). In the current study the two samples Z test was used.

RESULTS

Stool culture and isoenzyme determination. Fifteen of 182 Egyptian subjects studied (8.2%) had a positive stool culture for Entamoeba species. Of these, by zymodeme determination eight were found to be E. dispar, ﬁve were E. histolytica and two were of indeterminate species. Twenty three (12.6%) of the 182 subjects had a positive microscopy for amebic cysts; other parasites found included Giardia lamblia (1), Entrobius vermiculari (7), Ascaris lumbricoid (2), Ancylostom duodenale (2), Hymenolypus nana (1), Trichurus trichiura (1), and Schistosoma mansoni (3).

ELISA for detection of fecal 170 kDa lectin antigen. As summarized in Table 1, 100% of stool samples positive by culture for E. histolytica or E. dispar species were correctly identiﬁed and differentiatered by ELISA used to detect fecal lectin antigen. In the two positive cultures whose species were not identiﬁable by zymodeme analysis, we found that up to 100 trophozoites per well from these cultures were not reactive in the ELISA. Of 23 fecal samples positive for microscopy, 84% were positive for fecal lectin antigen detection (41% E. histolytica, 43% E. dispar). Of the 17 infected with other parasites detected by microscopy, only 3 had positive lectin antigen ELISA tests. As reclient in Table 1, additional E. histolytica and E. dispar infections were detected by ELISA, (P < 0.001) compared to stool culture.

Correlation of infection by E. histolytica and E. dispar infections with detection of anti-amebic antibodies and lectin antigenemia. Entamoeba histolytica-infected subjects were more likely to have fecal anti-lectin IgA antibodies and serum anti-LC3 IgG antibodies, in comparison to uninfected controls and or those with E. dispar infection; Table 2, (P < 0.004) and (P < 0.02) respectively. No differences were found, in regard to anti-amebic antibody responses between E. dispar infected subjects and uninfected subjects. Entamoeba histolytica infection, as diagnosed by fecal antigen detection was more frequently associated with the presence of lectin antigenemia (P < 0.017).

Correlation of infection by Blastocystis hominis and Trichomonas hominis with E. histolytica and E. dispar infection. Entamoeba dispar infection was positively associated with B. hominis (Table 3, P = 0.014, but not T. hominis infection (P = 0.412). In contrast, E. histolytica infection was associated with infection by T. hominis (P = 0.021). Experimentally, up to 100 T. hominis or B. hominis trophozoites per well were not recognized in the lectin-capture ELISA.

DISCUSSION

This study compares the use of stool culture and zymodeme analysis to performance of ELISA for fecal lectin antigen to detect E. histolytica and E. dispar infections in a highly endemic area. Results were correlated with fecal microscopy, ELISA for detection of serum 170 kDa lectin

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<tr>
<th>Table 1</th>
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<tr>
<td>ELISA for detection of Entamoeba histolytica (E.h.) and Entamoeba dispar (E.d.) 170 kDa lectin antigen, correlation with culture for Entamoeba species</td>
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<tr>
<th></th>
<th>E.h. culture (+) (n = 5)</th>
<th>E.d. culture (+) (n = 8)</th>
<th>Fecal culture (–) (n = 167)</th>
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<tbody>
<tr>
<td>E.h. antigen (+)</td>
<td>5 (100%)</td>
<td>0</td>
<td>34 (34/167)</td>
</tr>
<tr>
<td>E.d. antigen (+)</td>
<td>0</td>
<td>8 (100%)</td>
<td>36 (36/167)</td>
</tr>
<tr>
<td>Antigen (–) (n = 99)</td>
<td>0</td>
<td>97 (97/167)</td>
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* = positive; – = negative.

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<th>Table 2</th>
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<td>Anti-amebic antibody response in subjects with asymptomatic Entamoeba histolytica (E.h.) and Entamoeba dispar (E.d.) infection</td>
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<tr>
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<th>Fecal IgA (+) (n = 18)</th>
<th>Serum IgA (+) (n = 13)</th>
<th>Serum IgG (+) (n = 18)</th>
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<tbody>
<tr>
<td>E.h. antigen (n = 39)</td>
<td>11 (28.2%)</td>
<td>4 (10.3%)</td>
<td>8 (20.5%)</td>
</tr>
<tr>
<td>E.d. antigen (n = 44)</td>
<td>1 (2.3%)</td>
<td>2 (4.5%)</td>
<td>1 (2.3%)</td>
</tr>
<tr>
<td>Antigen (–) (n = 97)</td>
<td>6 (6.2%)</td>
<td>7 (7.2%)</td>
<td>9 (9.3%)</td>
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Stool was screened for anti-170 kDa IgA antibodies and sera for anti-LC3 IgG and IgA antibody response. Fecal IgA and serum IgG antibodies were signiﬁcant in E. histolytica but not in E. dispar (P = 0.002 and 0.018, respectively). No significant changes were observed in serum IgA antibody response (P = 0.533).
antigen, serum and mucosal anti-amebic antibody responses, and by culture, with co-infection by *B. hominis* and *T. hominis*.

The prevalence of *E. histolytica* and *E. dispar* infection as determined by stool culture was in agreement with reports from another endemic area. It is unclear why culture has such low sensitivity, compared to ELISA for fecal lectin antigen detection. The high specificity of the monoclonal antibodies used in the antigen-capture ELISA was clearly demonstrated. All positive cultures for *E. histolytica* or *E. dispar* were correctly identified and differentiated by the ELISA. Moreover, the two positive cultures for non-*histolytica* and non-*dispar* trophozoites (hexokinase isoenzyme electrophoresis revealed three bands) were negative by ELISA.

Using ELISA to detect fecal lectin antigen we found that *E. histolytica* or *E. dispar* infection were almost ten-fold more prevalent than estimated by fecal culture. The overall prevalence of *E. dispar* infection might actually be higher than reported, as the monoclonal antibodies used are unable to detect mixed infections in subjects positive for *E. histolytica* fecal antigen. The current study confirmed that *E. histolytica*, but not *E. dispar*, infection induces serum anti-amebic antibody responses, as reported in many studies worldwide.

The Kafer Daoud villagers are considered to be at high risk for gastrointestinal parasitic infections due to the absence of proper sanitary disposal, their continuous contact with domestic animals, their having pets who lack veterinary care, and a high density of flies year around. *Blastocystis hominis* is a common human parasite residing in the colon. Many reports support a role for *B. hominis* as a potential pathogen causing diarrhea, abdominal discomfort, anorexia, and flatulence. However, it is controversial whether *B. hominis* is a pathogen; this is especially of concern in immunocompromised individuals.

The prevalence of *B. hominis* has been found to be as high as 61.8% in a riverside community, 51.8% of children in Chile, 54% of villagers in New Guinea, and 49% of residents in a suburban community in Venezuela. In contrast, *B. hominis* was found in only 11.5% of asymptomatic subjects in the United States and 0.5% of asymptomatic subjects in Japan. Risk factors for infection include travel to the tropics, infection among other family members, and contact with pets. The high prevalence of asymptomatic *B. hominis* infection (52.2%) among the villagers in this study is consistent with reports from various parts of the world.

Trichomonas hominis is a nonpathogenic protozoan parasite that resides in the colon and small intestine; it is rarely associated with diarrhea and has been found outside its normal habitat in the respiratory tract, meningitis following GI fistula, and in esophageal intramural pseudodiverticulosis. The significance of co-infection between *E. histolytica* and *T. hominis* and between *E. dispar* and *B. hominis* is unknown, but could be related to shared risk factors for infection and microbial interference in the gut. Importantly, the monoclonal antibodies used to detect *Entamoeba* sp. did not crossreact in ELISA with *T. hominis* or *B. hominis*. In addition, anti-*B. hominis* monoclonal antibodies are known to be nonreactive with *E. histolytica* antigen.

In conclusion, we found that ELISA for detection of fecal lectin antigen with a sensitivity method than a single stool culture for diagnosis of asymptomatic *Entamoeba* species infection, indicating that asymptomatic *E. histolytica* infection is more common than previously appreciated. The finding of co-infection with nonpathogenic intestinal protozoans may provide further clues to the epidemiology of *Entamoeba* species infection and microbial interactions in the intestinal lumen.

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