Entamoeba histolytica and Entamoeba dispar: Epidemiology and Comparison of Diagnostic Methods in a Setting of Nonendemicity

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Recent studies suggest that stool antigen assays are more sensitive and specific than microscopy for the diagnosis of Entamoeba histolytica infection. One hundred twelve patients presenting at 3 centers with symptoms or risk factors of E. histolytica infection were prospectively enrolled in this study to evaluate new diagnostic tests for infections with E. histolytica and Entamoeba dispar. Four ELISA-based stool antigen kits for detecting E. histolytica or E. dispar were blindly compared with stool microscopy. Amebic serology was assessed by indirect hemagglutination. When antigen assays were used as the reference standard, microscopy performed at referral centers was more specific (68.4% vs. 9.5%) but less sensitive (70.4% vs. 92.1%) than microscopy performed in community laboratories. Diagnosis with the E. histolytica test and Merlin Optimun S ELISA indicated that only 3 (4.2%) of 72 coproantigen-positive stools were positive for E. histolytica. Indirect hemagglutination was a good predictor of E. histolytica infection when titers of antibody to ameba were ≥1 : 512.

Infection with Entamoeba histolytica has the potential to cause dysentery and extraintestinal disease, whereas E. dispar is considered to be a harmless commensal [1, 2]. The World Health Organization has recommended that “E. histolytica should be specifically identified and, if present, treated; if only E. dispar is identified, treatment is unnecessary” [3]. The diagnosis of E. histolytica infection has traditionally relied upon microscopic examination of fresh or fixed stool specimens. However, microscopy has several limitations [4–6], most importantly, the inability to distinguish E. histolytica from E. dispar, because they are morphologically identical [7]. Amebic culture with isoenzyme analysis is considered to be a reference standard to differentiate E. histolytica from E. dispar, but this method is not widely available and is not practical for routine diagnostic laboratories [8, 9]. Detection of antibodies to ameba in patient sera by indirect hemagglutination (IHA) has been reported to indicate E. histolytica infection. However, with serological testing it may be difficult to distinguish past from present infection in individuals who emigrate from, or currently reside in, an area of endemicity [4, 10].

Stool antigen assay has been shown to be as sensitive and specific as culture with isoenzyme analysis and to outperform microscopy for the detection of E. histolytica in areas of endemicity [8, 11]. Four ELISA-based stool antigen kits are now commercially available. The Entamoeba test detects the Gal/GalNAc lectin of both E. histolytica and E. dispar, while the E. histolytica test recognizes the lectin of the pathogen alone [8, 11]. The ProSpecT ELISA detects the E. histolytica/E. dispar complex but cannot distinguish pathogen from nonpathogen. The Merlin Optimun S ELISA has been reported to detect the serine-rich protein of E. histolytica alone [12]. These assays provide a reference standard to evaluate new and old diagnostic assays as well as an opportunity to revisit epidemiological questions concerning amebiasis in settings of nonendemicity.

We prospectively collected epidemiological data for and serum and fresh stool samples from patients presenting in an area of nonendemicity who were suspected of having E. histolytica infection with the following objectives: to evaluate new diagnostic test kits for diagnosis of E. histolytica/E. dispar complex infection; to compare microscopy performed at referral centers with that performed in community laboratories for detection of the E. histolytica/E. dispar complex; to determine the proportion of infections caused by E. histolytica in an area of nonendemicity; and to evaluate the clinical utility of serological testing to differentiate E. histolytica from E. dispar in this setting.

Materials and Methods

Patients with gastrointestinal symptoms (diarrhea, defined as ≥3 loose [conform to the container] bowel movements per day, ab-
dominal pain, weight loss, or bloody stool) or risk factors for *E. histolytica/E. dispar* complex infection (travel within 6 months, male homosexuality, or immigration from the tropics or subtropics <2 years ago) who presented to 3 Canadian urban medical centers from 1993–1998 were eligible for study inclusion. Subjects were requested to provide fresh stool samples for microscopy and ELISA, and serum samples, and to complete a questionnaire. Serum samples were collected to assess the presence of antibodies to ameba by IHA (Cellognost; Behring Diagnostics, Marburg, Germany). Unpreserved stool samples were independently processed for stool antigen of the *E. histolytica/E. dispers* complex (*Entamoeba* test, Techlab, Blacksburg, VA and ProSpecT test, Alexon-Trend, Ramsey, MN) or for stool antigen of *E. histolytica* (Merlin Optimun S test, Merlin Diagnostika, Bornheim-Hersel, Germany and *E. histolytica* test, Techlab); tests were performed according to the manufacturer’s instructions. Discrepant ELISA results between the *Entamoeba* test and ProSpecT test, both of which detect the *E. histolytica/E. dispar* complex, were resolved by using the *E. histolytica*-specific kits and an additional independent ELISA protocol for *E. dispar* that were described elsewhere [13].

**Results**

During the study period, 112 consecutive patients were enrolled (72 males and 40 females). Many patients (33.9%) were asymptomatic; the chief complaints of symptomatic patients were diarrhea (30.4%) and abdominal pain (23.2%). Forty-three percent of subjects were born in areas where amebiasis is endemic (67% of whom were born in Africa or Asia). Risk factors for North American–born and European-born individuals included travel to an area of endemicity (64.1%), male homosexuality (12.5%), or both (7.8%). Sixty-five percent (73) of 112 participants received drug treatment: of these 73 patients, 76.7% (56) received iodoquinol; 21.9% (16), paromomycin; 5.5% (4), diloxanide furoate; and 9.6% (7), metronidazole (some individuals received combination therapy).

Antigen detection by the *Entamoeba* test and ProSpecT test was used to identify specimens positive for the *E. histolytica* *E. dispar* complex. The 2 tests agreed in 92.9% of the cases, with 65 specimens positive and 39 specimens negative for the *E. histolytica/E. dispar* complex (figure 1). Eight discrepant results were identified (positive by the ProSpecT test and negative by the *Entamoeba* test). These 8 specimens were evaluated by the *E. histolytica*-specific kits (1 of 8 was positive for *E. histolytica* [patient isolate EH36] by both tests) and by an independent ELISA that uses monoclonal antibody 318-28 (provided by D. Mirelman, Weizmann Institute of Science, Rehovot, Israel) directed to the 30-kDa lysine-rich surface antigen of *E. dispar* [13]. This strategy indicated that 7 of the 8 specimens with discrepant results were positive for *E. dispar* antigen and that 1 patient (from whom EH36 was isolated) was coinfected with *E. histolytica* and *E. dispar*.

The performance of antigen detection assays suggests that they may be considered as reference standards for the detection of *E. histolytica* and *E. dispar* [8, 11]. We used the consensus result of 2 ELISAs as a reference standard (discrepant results were excluded) to evaluate microscopy both in the community and in the referral setting. Compared with this standard, microscopy performed in the referral setting was more specific (68.4% vs. 9.5%) but less sensitive (70.4% vs. 92.1%) than microscopy performed at community laboratories (table 1). The positive predictive value of a microscopy result was assessed for 3 high-risk groups on the basis of a previously estimated prevalence of *E. histolytica/E. dispar* infection (immigrants, 2.4%; travelers, 4%; male homosexuals, 27%) [14–16]. Although the positive predictive value for referral centers was greater (5.2%–45.2%) than that obtained for community laboratories (2.4%–27.3%) for all 3 high-risk groups, it remained <50%.

Stool samples obtained from all patients were also blindly analyzed by the *E. histolytica* test and Merlin Optimun S ELISA, which are reported to detect only *E. histolytica*. Both of these assays identified that 3 of 112 patients were positive for *E. histolytica*.

**Table 1.** Sensitivity and specificity for detection of *Entamoeba histolytica/Entamoeba dispar* complex as determined by microscopy performed in referral centers and in community laboratories, compared with consensus result of *Entamoeba* test and ProSpecT ELISA.

<table>
<thead>
<tr>
<th>Microscopy</th>
<th>No. of tests with indicated result</th>
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<th>Sensitivity, %</th>
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<td>Positive</td>
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<tr>
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<td>21</td>
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</table>

* Entamoeba test and ProSpecT test.

**Figure 1.** Distribution of results from ELISAs of stool specimens from 112 patients with symptoms or risk factors for *Entamoeba histolytica* infection. Absorbances determined by *Entamoeba* test and ProSpecT test were plotted for each stool sample. Absorbances of >0.050 after subtracting negative control were considered positive for each test.
for *E. histolytica* antigen (patient isolates EH36, EH42, and EH77; table 2). Two of these individuals had invasive disease (amebic liver abscess and dysentery). For patient isolate EH36, results of the Entamoeba test and *E. histolytica* test were internally discordant, suggesting that the respective monoclonal antibodies may have different sensitivities.

One hundred two consenting patients were also assessed serologically for antibodies to ameba by IHA. Antiserum specimens from 12 (11.8%) of the 102 were positive (>1:64) for antibodies. The 3 individuals who were positive for *E. histolytica* antigen had the highest titers (>1:512) by IHA. A further 7 seropositive individuals were positive for *E. dispar* alone by antigen detection (table 2). The remaining 2 seropositive patients had no detectable Entamoeba coproantigen by all ELISAs. With use of *E. histolytica* antigen detection as the reference standard, serological titers recommended as positive by the manufacturer (>1:64) were 100% sensitive and 90.9% specific for detection of *E. histolytica*; at a titer of >1:512, serology was 100% sensitive and 100% specific.

### Discussion

Recent studies in areas of endemicity have suggested that antigen detection assays may be an alternative strategy to identify *E. histolytica* infection. This is the first study to blindly evaluate all available stool antigen ELISA kits, microscopy, and serology for diagnosis of *E. histolytica*.*E. dispar* complex infection in a setting of nonendemicity. The results of the Entamoeba test and ProSpecT test to detect *E. histolytica*.*E. dispar* complex infection in this setting demonstrated excellent agreement (>90%). However, our results suggest that the ProSpecT test may be more sensitive than the Entamoeba test for detection of the *E. histolytica*.*E. dispar* complex in stool and are in agreement with results of earlier reconstitution experiments with these tests [13].

Both *E. histolytica*–specific tests detected that only 3 of 112 samples were positive. Of note, the 3 individuals from whom these specimens were obtained had the highest titers by IHA (>1:512), and 2 had clinical evidence of invasive disease. The remaining seropositive patients (all born or were residents for >6 months in areas of endemicity) either had *E. dispar* infections or were not infected as determined by the ELISAs. Previous studies indicate that titers of antibodies to ameba may remain elevated for many years and confound attempts to distinguish current from past infections [4, 17]. In this study, the use of amebic serology to detect *E. histolytica* infection was nonspecific at titers of <1:512.

The clinical implications of this study are significant since only 3 (4.6%) of 65 unequivocally coproantigen-positive stool samples (positive by >2 assays) were positive for *E. histolytica* by ELISA; the remainder were identified as *E. dispar*–positive. Therefore, most patients identified with *E. histolytical/E. dispar* complex infection by microscopy in this setting of nonendemicity received unnecessary therapy. Use of an *E. histolytica* test would allow for a specific diagnosis and obviate the need for unnecessary chemotherapy with its attendant costs, risk of side effects, danger of drug resistance, and potential mistreatment of another disease. Although our investigation and previous studies suggest that the rate of false-positive results for serology (>1:64) is 10%–20%, higher serological titers (>1:512) may help identify *E. histolytica*–infected patients [4, 17].

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### References


