Am. J. Trop. Med. Hyg., 67(1), 2002, pp. 123–127

AMEBIC INFECTIONS DUE TO THE *ENTAMOEBA HISTOLYTICA–ENTAMOEBA DISPAR* COMPLEX: A STUDY OF THE INCIDENCE IN A REMOTE RURAL AREA OF ECUADOR

SIMONETTA GATTI, GIOVANNI SWIERCZYNSKI, FRANCISCO ROBINSON, MARIELLA ANSELMI, JAVIER CORRALES, JUAN MOREIRA, GREGORIO MONTALVO, ANTONELLA BRUNO, ROBERTA MASERATI, ZENO BISOFFI, AND MASSIMO SCAGLIA

Laboratory of Parasitology, Virology Service, Instituto di Ricovero e Cura a Carattere Scientifico Policlinico San Matteo, Pavia, Italy; Laboratory of Microbiology, Desenzano Hospital, Desenzano-Brescia, Italy; Centre for Community Epidemiology and Tropical Medicine, Esmeraldas, Ecuador; Centre for Tropical Diseases, S. Cuore Hospital, Negrar-Verona, Italy; Public Hospital of Borbòn, Esmeraldas, Ecuador; Department of Infectious Diseases and Infectious Diseases Research Laboratories, University-IRCCS Policlinico San Matteo, Pavia, Italy

Abstract. An epidemiologic field study was conducted in the village of Borbòn in Esmeraldas province in northern Ecuador to compare different parasitologic methods in the diagnosis of infection with the Entamoeba histolytica/Entamoeba dispar complex. The results of two stool antigen detection assays (the Prospect™ Entamoeba histolytica microplate assay and the E. histolytica II™ assay) were compared with isoenzyme characterization of the amebic isolates. Nearly all (176 of 178, 98.9%) subjects were positive for intestinal parasites on direct microscopic examination, and cysts and/or vegetative forms morphologically consistent with the E. histolytica/E .dispar complex were recorded in 48 of 178 cases (27%). Culture in Robinson's medium was positive for amebic stocks in 89 (50%) of the 178 samples tested. Of the 37 isolates successfully stabilized, cloned, and characterized by zymodeme analysis, seven (18.9%) showed isoenzyme patterns of E. histolytica, whereas 26 (70.3%) showed patterns of E. dispar. The remaining four strains were identified as Entamoeba coli (three isolates; 8.1%) and Dientamoeba fragilis (one strain; 2.7%).The immunochromatographic tests showed different degrees of sensitivity and specificity when compared with isoenzyme characterization as the reference technique. The microplate assay, which does not discriminate between E. histolytica and E.dispar, showed a sensitivity of 54.5% and a specificity of 94% for both these amebic species. In contrast, the second-generation E. histolytica II test had a sensitivity of 14.3% and a specificity of 98.4% for E. histolytica sensu stricto. Our survey clearly demonstrated that more specific and sensitive diagnostic tests, such as stool antigen detection assays and isoenzyme analysis, are needed to establish the actual worldwide distribution of E. histolytica and E. dispar.

INTRODUCTION

Amebiasis is still one of the major health problems in tropical and subtropical areas, and is characterized by low socioeconomic status and poor hygiene that favor the indirect fecal-oral transmission of the infection.[1]

With regard to the global prevalence of the infection, the data of Walsh,[2] assessed as 480 million people harboring *Entamoeba histolytica* worldwide, with approximately 40 million developing overt clinical disease and 50–100,000 deaths yearly, did not distinguish between the pathogenic species *E. histolytica* and the non-pathogenic *E. dispar*. In 1925, Brumpt[3] proposed the hypothesis that *E. histolytica* and *E. dispar*, although morphologically indistinguishable, were different species. Sargeaunt,[4] using isoenzyme characterization, showed irrefutable evidence that Brumpt was correct. This information was subsequently supported by the work of Tannich and others[5] and Diamond and Clark.[6] In 1997, the accumulated data enabled a formal re-description of *E. histolytica*, the causative agent of amebiasis, separating it from *E. dispar*, a harmless commensal of the human gut.[7] On the basis of several epidemiologic studies performed worldwide, it was determined that the vast majority of infected humans harbor the non-pathogenic species *E.dispar*, whereas only 10–12% of such subjects may develop illness due to *E. histolytica.*[7] However, information on the geographic distribution of *E. histolytica* and *E. dispar* is still incomplete and unreliable because many surveys have not been carried out using standard procedures, notably those able to discriminate between the two species.[8]

The estimation of the burden of infection with *E. histolytica/E. dispar* is not only important for the endemic tropical and subtropical areas, but also for the developed areas, in the light of the continuous increase in immigration and the number of travelers to developing countries.

To determine the true prevalence of amebiasis in a hyperendemic area, such as the western part of South America, an epidemiologic survey using different parasitologic methods for the diagnosis of infection by the *E. histolytica*/*E. dispar* complex was assessed in a rural area of northern Ecuador. The results of two antigen detection assays and three serologic methods (indirect hemagglutination, enzyme immunoassay, and indirect immunofluorescence) were compared with those of isoenzyme characterization of amebic isolates according to Sargeaunt and Williams,[9] which was used as the gold standard technique.

MATERIALS AND METHODS

The study was carried out in Borbòn, a small village of approximately 3,000 inhabitants located in the basin of the Santiago River in Esmeraldas province in the northern coastal area of Ecuador near Colombia (Figure 1). The climate is warm and humid, with a mean temperature of 28°C and a rainfall range of 3,500–4,500 mm. The chief occupations of the population are farming and fishing. The village consists of small masonry and wooden houses, most of which are not supplied with potable water or a bathroom.

The subjects enrolled in the study were school children. After previous meetings with parents and teachers to explain the purpose of the study, fecal and blood samples were collected from each subject. A form for personal (age, sex) and epidemiologic data (race; signs and symptoms, if any; drugs etc.) was completed for all children. The study project was

FIGURE 1. Geographic location of Borbòn village (**arrow**) in northern Ecuador.

approved by the Ecuador Ministry of Health, and by the ethical committees of Borbon Hospital, Ecuador, IRCCS Policlinico San Matteo, Pavia, and Sacro Cuore Hospital, Negrar, Verona, Italy. The informed consent has been signed by the childrens' parents before the study.

The parasitologic protocol consisted of 1) extemporaneous microscopic examination of unfixed fecal samples that were unstained or stained with iodine; 2) Ritchie's fecal concentration of formalin-fixed samples; 3) trichrome staining of polivinyl alcohol-fixed stools; 4) Robinson's *in vitro* culture; 5) screening with two stool antigen detection tests, one specific for pathogenic *E. histolytica* (*E. histolytica* II™; TechLab, Blacksburg, VA) and one that cross-reacts with non-pathogenic *E. dispar* (Prospect™ *Entamoeba histolytica* Microplate Assay; Alexon-Trend, Ramsey, MN); 6) starch gel isoenzyme electrophoresis with four enzymes: glucose phosphate isomerase (GPI, EC 5.3.1.9), hexokinase (HK, EC 2.7.1.1), nicotinamide adenine dinucleotide phosphate (NADPH+):L-malate oxidoreductase (ME, EC 1.1.1.4.0), and phosphoglucomutase (PGM, EC 2.7.5.1); 7) serologic analysis (indirect hemagglutination test [IHA]: Cellognost™ Amoebiasis; Dade, Behring, Marburg, Germany; enzyme immunoassay [EIA]: Amebiasis Serology Microwell ELISA;™ LMD Laboratories, Carlsbad, CA; indirect immunofluorescence test [IIF]: Amoeba-Spot; ™ BioMérieux, Marcy l'Etoile, France).

RESULTS

One hundred seventy-eight students from two elementary schools in the village (84 males [47.2%] and 94 females [52.8%]) with a mean age of 9.5 years (range = 6–16 years) were examined. Most (154; 86.5%) of the children were black; a small percentage was white (15; 8.4%) or Mestizo (9; 5.1%). The majority (158; 88.8%) of the study population did not show any symptoms and only 20 (11.2%) complained irregularly of mild intestinal signs and symptoms (abdominal pain, irregular stool excretion, diarrhea, etc.). Most (112; 62.9%) fecal samples were formed, 32 (18%) were semi-formed, and 29 (16.3%) were soft. Only five (2.8%) subjects had loose stools.

The results of direct examination of the fecal samples were positive in 168 subjects (94.4%). Ritchie's concentration showed positive results in 147 of 155 children examined (94.8%) (in 23 cases the concentration was not done). Trichrome staining identified cystic or vegetative protozoan forms in 158 of 178 cases (88.8%). When the results of the three direct methods were combined, nearly all (176 of 178; 98.9%) were positive. Most (162 of 176; 92.1%) of the children with intestinal infections harbored more than one parasitic species. Forty-nine (27.8%) had only protozoa, 10 (5.7%) had helminths but no protozoa, and 103 (58.6%) subjects were infected by both.

Comparisons of the identification of the *E. histolytica*/*E. dispar* complex by microscopy, antigen detection tests, and culture with electrophoresis in starch gels are shown in Table 1. Cystic and/or vegetative forms morphologically attributable to *E. histolytica*/*E. dispar* (by at least one of the three direct methods performed) were identified by microscopy in 48 of 178 cases (27%).

In vitro culture in Robinson's medium revealed the presence of amebae in 89 of 178 samples examined (50%). Thirty-seven (41.6%) of 89 amebic isolates were morphologically consistent with the *E. histolytica/E. dispar* complex; they were cloned and subjected to electrophoresis in starch gels. Seven (18.9%) of the typed isolates were *E. histolytica,* whereas 26 (70.3%) were identified as *E. dispar* with different zymodemes (Table 2). Three stocks (8.1%) showed the isoenzyme pattern of *Entamoeba coli* and one (2.7%) showed the pattern of *Dientamoeba fragilis* (Table 2).

Of the 56 specimens identified as *E. histolytica*/*E. dispar* by microscopy and/or culture with electrophoresis, 22 were positive by both methods. Eleven were positive only by culture

TABLE 1

Results of antigen detection kits for *Entamoeba histolytica* (Eh) and *E. dispar* (Ed) compared with microscopy (extemporaneous examination of unfixed samples, Ritchie's fecal concentration, and trichrome stain) and culture with zymodeme identification by starch gel electrophoresis*

	Microscopy positive (at least one of the three methods)				Microscopy negative (all methods)				
	Culture positive				Culture positive				
Method	Eh	Ed	Other†	Culture negative	Eh	Ed	Other‡	Culture negative	Total
Alexon+ Techlab+	1	0	0	0	0	0	0	1	2
Alexon+ Techlab–	3	11	2	9	0	3	0	6	34
Alexon– Techlab+	0	1	0	0	0	0	1	1	3
Alexon– Techlab–	1	5	1	14	2	6	0	110	139
Total	5	17	3	23	2	9	1	118	178

* Only *E. histolytica/E. dispar* are considered in this analysis. Samples with any other protozoa are considered negative.

† Samples in this column were compatible with Eh/Ed by microscopy, but electrophoresis indicated *Entamoeba coli* (2 samples) and *Dientamoeba fragilis* (1 sample).

‡ Sample compatible with Eh/Ed by microscopy, but electrophoresis indicated *E. coli.*

TABLE 2
Entamoeba histolytica and *E. dispar* zymodeme distribution*

Zymodeme	*E. histolytica* (7 isolates)	*E. dispar* (26 isolates)
I		6 (23.1%)
II	5 (71.4%)	
VI	1 (14.3%)	
VIII		1 (3.8%)
IX		4 (15.4%)
X		2 (7.7%)
XIII	1 (14.3%)	
XVI		2 (7.7%)
XVII		7 (26.9%)
XVIII		4 (15.4%)

* Other typed strains: *Entamoeba coli* (3 isolates; 8.1%); *Dientamoeba fragilis* (1 isolate; 2.7%).

and 23 only by microscopy. Four microscopy-positive, culture-positive samples were excluded by this analysis because the electrophoretic study showed that they were *E. coli* (three isolates) and *D. fragilis* (one isolate) (Table 1). In the 22 stool samples positive for the *E. histolytica*/ *E.dispar* complex by both microscopy and culture, Alexon-Trend test confirmed an amebic infection in 15 specimens (68.2%), whereas the Techlab test showed positive results for only two subjects (9%).

To estimate the sensitivity of both methods, we assumed culture with zymodeme identification as the only reliable golden standard for true-positive sample. Of the seven samples with a positive culture for *E. histolytica sensu stricto*, the TechLab test result was positive for only one sample, corresponding to a sensitivity of 14.2% (1 of 7). Of the 33 samples positive for the *E. histolytica*/*E. dispar* complex (seven *E. histolytica* and 26 *E. dispar*), the Alexon-Trend test (which does not discriminate between the two species) result was positive in 18 cases, giving a sensitivity of 54.5%.

As a proxy for isoenzyme characterization, we tested 118 samples negative for *E. histolytica*/*E. dispar* by both microscopy and culture: the four samples with a positive microscopy result in culture, but not confirmed by zymodeme analysis, were excluded for this purpose. Of the 118 samples negative for *E. histolytica*/*E. dispar* by both microscopy and culture, Alexon-Trend test result was positive for seven specimens and the TechLab test result was positive for two specimens, giving a specificity of 94% and 98.3%, respectively. Thus, the Alexon-Trend test had a sensitivity of 54.5% and a specificity of 94%, while the TechLab test had a sensitivity of 14.2% and a specificity of 98.3% if matched with culture and microscopy.

Microscopy was positive (with at least one method) for 22 of the 33 culture-positive samples and for 18 of the 141 culture-negative specimens, giving a sensitivity of 66.6% and a specificity of 83.7% (the four samples positive by culture but not confirmed by zymodeme identification were excluded from the analysis).

In testing the concordance between direct antigen detection methods and electrophoretic characterization (Table 3), we found that only in one of seven cases (14.3%) was there correspondence between the specific test for *E. histolytica* (TechLab) and proven *E. histolytica* strains, whereas a positive result was also observed in two other cases, one with a strain of *E. coli* and another with a strain of *E. dispar* zymodeme XVIII. The Alexon-Trend test showed a correlation with typed *E. histolytica* strains in four of seven cases (57.1%) and with typed *E. dispar* in 14 of 26 cases (53.8%). No correlation was found between antigen detection tests and cases with high *E. histolytica* serotiters that were negative in *in vitro* cultures.

A serologic study was performed on 175 of 178 samples and showed negative serotiters for *E. histolytica* in 37 children (21.1%), border-line values in 25 (14.3%), and positive titers in most of them (113; 64.6%). Among the seropositive subjects, 75.2% (85) showed medium or high serotiters with at least two of the three methods used.

Of the 37 subjects with positive *in vitro* cultures, 26 (70.3%) showed significant anti-*E. histolytica* serotiters. All seven cases with positive *E. histolytica* isolation showed high serologic values with all three techniques carried out.

Children found to be infected with *E. histolytica* were treated as follows: two with mild diarrhea and discontinuous abdominal pain were treated with metronidazole (30 mg/kg/day given orally three times a day for 10 days); the other five asymptomatic cyst passers were treated with paromomycin (35 mg/kg/day orally, three times a day for seven days). The results of the parasitologic follow-up (coproparasitology and *in vitro* culture) carried out seven days later were negative in all cases.

DISCUSSION

In this survey, the use of two direct assays for detection of fecal lectin antigen to identify *E. histolytica* and *E. dispar* infections in a highly endemic area of South America was compared with stool culture and zymodeme analysis (as reference technique). Results were also compared with direct microscopy to prior and after a concentration procedure, trichrome staining, and serologic assays using different methods (IHA, ELISA, and IIF).

A high incidence of intestinal parasitic infections was observed (176 of 178 subjects positive for parasites; 98.9%). Most (162 of 176, 92.1%) individuals harbored more than one

TABLE 3
Results of antigen detection tests for *Entamoeba histolytica* (enzyme-linked immunosorbent assay [ELISA] TechLab) and for *E. histolytica/E. dispar* complex (ELISA Alexon) and correlation with electrophoresis for *Entamoeba* species characterization*

	ELISA T + (n = 5)	ELISA A + (n = 36)	Antigen – (n = 139)
E. histolytica positive strains (7)	1 (14.3%)†	4 (57.1%)†	3 (42.8%)
E. dispar positive strains (26)	1 (3.8%)	14 (53.8%)	11 (42.3%)
Entamoeba coli positive strains (3)	1 (33.3%)	1 (33.3%)	1 (33.3%)
Dientamoeba fragilis positive strain (1)	0	0	1 (100%)
Fecal culture negative or not subjected to EF (141)	2 (2/141)	17 (17/141)	123 (123/141)

* ELISA T = TechLab Test; ELISA A = Alexon Test; EF = isoenzyme electrophoresis.
† One case of *E. histolytica* positive for both tests.

parasitic species and 103 of 176 children (58.6%) had both protozoa and helminths in their fecal samples. This high detection rate is clearly related to poor sanitation, nutrition, use of contaminated water, and domestic animal promiscuity.[8,10]

Among the subjects enrolled in the present study, as in other groups tested in tropical and subtropical areas where amebiasis is considered to be endemic or hyperendemic,[8,10,11] infection with *E. dispar* occurs more frequently than with *E. histolytica*. In our survey, most of the *E. dispar* isolates belonged to zymodemes I and XVII. Zymodeme I is the most commonly encountered throughout the world,[12] and zymodeme XVII has been observed in other studies carried out in South America[13] and Canada.[14] This demonstrates that the zymodeme distribution may be influenced by geographic factors.[8,15] Isoenzyme analysis identified seven *E. histolytica* isolates; five of seven strains (71.4%) belonged to zymodeme II, the pathogenic zymodeme with a worldwide distribution.[12]

Most surveys of this type rely on examination of one stool sample because of the difficulty in collecting more specimens. Using 3–5 samples/subject might increase the percentage of positive microscopic results.[8,16,17]

The kits used to identify the amebic galactose-inhibitable lectin in stool samples showed different degrees in sensitivity and specificity when compared with the isoenzyme assay. The Alexon-Trend test detects the specific antigen of both pathogenic *E. histolytica* and nonpathogenic *E. dispar*. In our survey, it showed a sensitivity of 54.5% and a specificity of 94.0%, which are comparable to those observed in other studies.[18,19] The second-generation TechLab test, a monoclonal antibody–based ELISA specific for *E. histolytica* galactose-inhibitable lectin, showed a sensitivity of 14.2% and a specificity of 98.3%. The sensitivity of both techniques was lower than the sensitivity of direct microscopic examination (66.0%), when compared with Robinson's culture and zymodeme identification (as the gold standard).

The specificity of both direct antigen detection techniques was higher than that of microscopy (83.7%). Although we realize that our results should be confirmed with a larger number of fecal samples, the sensitivity of both techniques was far from being satisfactory in our study. Conversely, the specificity was quite high and may even be underestimated, since we did not have a reliable gold standard for true-negative results. The sensitivities of both microscopy and culture are also not optimal, and some of the samples that we classified as false-positive with the Alexon-Trend and/or Techlab tests might well be true-positive (false-negative by microscopy and culture).

With regard to sensitivity, the clear discrepancy between our data and those previously reported[10,16,20] could be due to the fact that the assays recognize the specific antigens on the surface of the vegetative forms only, which are generally identified in diarrheal stools during an acute amebic infection, and not in the cystic stage of the parasite, which are detected in formed feces. In fact, the majority of subjects we examined did not experience diarrhea, but had formed or semi-formed stools with only immature or mature cystic forms microscopically identified.

The stool antigen detection kits are promising, and may be suitable for use as reference standards if used in patients with acute diarrhea or with extraintestinal localizations.[20] However, people harboring *E. histolytica* may show a wide range of conditions. In particular, most subjects living in endemic areas are often asymptomatic cyst passers and represent, from an epidemiologic point of view, the most neglected category of infected subjects.[8,10,21–23] In these cases, based on our results, the sensitivity of the antigen detection kits needs to be increased. *In vitro* culture and isoenzyme analysis are 100% specific, although their sensitivity is more difficult to assess. Unfortunately, they are difficult to perform and time-consuming. Several biomolecular techniques are available, based on the amplification of different rRNA genes of the two relative species,[18,24,25] but most of them cannot be performed in field studies because they require expensive technical support, chemicals, and well-trained personnel.

In our study, significant anti-amebic serotiters were detected in all subjects with proven *E. histolytica* intestinal infection. However, high antibody levels were also found in *E. dispar*-positive subjects and in most culture-negative cases. This finding agrees with those in other epidemiologic surveys carried out in endemic countries, such as Mexico,[10] where the high prevalence of specific antibodies in the general population implies current or past asymptomatic infection with *E. histolytica.* It is important to note that the serologic response is as marked in asymptomatic carriers as it is in patients with invasive amebiasis, suggesting that anti-amebic serotiters could be an important predictor of protection in these populations.

In conclusion, our investigation clearly demonstrates that diagnostic methods more specific and sensitive than direct microscopy, such as stool antigen detection assays, isoenzyme analysis, and polymerase chain reaction–based tests, are needed to establish the true distribution of *E. histolytica* and *E. dispar,* and to determine the prevalence of asymptomatic carriers about which there is little current information. Finally, the biochemical identification of the different zymodemes of *Entamoeba* in any population leads to a better understanding of its epidemiologic status.

Acknowledgments: We are very grateful to Dr. W. A. Petri, Jr. (University of Virginia Health System, Charlottesville, VA) and TechLab, Inc. (Blacksburg, VA) for providing the diagnostic kits, and to Dr. P. G. Sargeaunt (London School of Hygiene and Tropical Medicine) for the suggestions and the careful revision of the English text. We also acknowledge the valuable and excellent technical contributions of Maria Gobbo (Laboratory of Parasitology and Tropical Medicine, S. Cuore Hospital, Negrar-Verona, Italy) and Anna Maria Bernuzzi and Claudia Cevini (Laboratory of Parasitology, Virology Service, IRCCSS, Matteo, Pavia, Italy).

Financial support: This work was supported by "Ricerca Corrente 2001", IRCCS Policlinico San Matteo (Pavia, Italy); MURST 2001-2002, the Italian Ministry of University and Scientific Research (Rome, Italy); the Italian Ministry of Foreign Affairs, Department of Cooperation (Rome, Italy) through the program MLAL/2347-Esmeraldas, Ecuador, executed by "Movimento Laici America Latina."

Authors' addresses: Simonetta Gatti, Parasitology Laboratory, Virology Service, IRCCS Policlinico San Matteo, V.le Taramelli, 5, 27100 Pavia, Italy, Telephone: 39-0382-502698, Fax: 39-0382-423320. Giovanni Swierczynski, Microbiology Laboratory, Desenzano Hospital, Desenzano (BS), Italy, Telephone and Fax: 39-0309-913994. Francisco Robinson, Juan Moreira, and Gregorio Montalvo, Centre for Community Epidemiology and Tropical Medicine, Esmeraldas, Ecuador, Telephone: 593-6-722-035, Fax: 593-6-722-039. Javier Corrales, Public Hospital of Borbòn, Esmeraldas, Ecuador, Telephone: 593-6-722-035, Fax: 593-6-722-039. Mariella Anselmi and Zeno Bisoffi, Centre for Tropical Diseases, S. Cuore Hospital, Negrar (VR), Italy, Telephone: 39-0456-013326, Fax: 39-0456-0113252. Antonella Bruno, Roberta Maserati, and Massimo Scaglia, Department of Infectious

Diseases and Infectious Diseases Research Laboratories, University-IRCCS Policlinico San Matteo, V.le Taramelli, 5, 27100 Pavia, Italy, Telephone: 39-0382-502698, Fax: 39-0382-423320.

REFERENCES

1. Ravdin JI, 1995. Amebiasis. *Clin Infect Dis 20:* 1453–1466.
2. Walsh JA, 1988. Prevalence of *Entamoeba histolytica* infection. Ravdin JI, ed. *Amebiasis: Human Infection by Entamoeba histolytica*. New York: John Wiley & Sons, 93–105.
3. Brumpt E, 1925. Etude sommaire de l'*Entamoeba dispar* n.sp. Amibe à kystes quadrinuclées, parasite de l'homme. *Bull Acad Med 94:* 943–952.
4. Sargeaunt PG, 1992. *Entamoeba histolytica* is a complex of two species. *Trans R Soc Trop Med Hyg 86:* 348.
5. Tannich E, Horstmann RD, Knobloch J, Arnold HH, 1989. Genomic DNA differences between pathogenic and non pathogenic *Entamoeba histolytica. Proc Natl Acad Sci USA 86:* 5118–5122.
6. Diamond LS, Clark CG, 1993. A redescritpion of *Entamoeba histolytica* Schaudinn, 1903 (emended Walker, 1911), separating it from *Entamoeba dispar* Brumpt, 1925. *J Eukaryot Microbiol 40:* 340–344.
7. World Health Organization, 1997. Amoebiasis. *Wkly Epidemiol Rec 72:* 97–98.
8. Jackson TFHG, 2000. Epidemiology. Ravdin JI, ed. *Amebiasis*. London: Imperial College Press, 47–63.
9. Sargeaunt PG, Williams JE, 1978. Electrophoretic isoenzyme patterns of *Entamoeba histolytica* and *Entamoeba coli. Trans R Soc Trop Med Hyg 72:* 164–166.
10. Petri WA, Singh U, 1999. Diagnosis and managements of amebiasis. *Clin Infect Dis 29:* 1117–1125.
11. Ravdin JI, 2000. Current controversies. Ravdin JI, ed. *Amebiasis*. London: Imperial College Press, 163–170.
12. Sargeaunt PG, 1988. Zymodemes of *Entamoeba histolytica*. Ravdin JI, ed. *Amebiasis: Human Infection by Entamoeba histolytica*. New York: John Wiley & Sons, 370–387.
13. Nozaki T, Da Silva A, Okuzawa E, Nagalhaes M, Tateno S, Takeuchi T, 1990. Zymodemes of *Entamoeba histolytica* isolated in the Amazon and north-east of Brazil. *Trans R Soc Trop Med Hyg 84:* 387–388.
14. Proctor EM, Wong Q, Yang J, Keystone JS, 1987. The electrophoretic isoenzyme patterns of strains of *Entamoeba histolytica* isolated in two major cities in Canada. *Am J Trop Med Hyg 37:* 296–301.
15. Sargeaunt PG, Baveja UK, Nanda R, Anand BS, 1984. Influence of geographical factors in the distribution of pathogenic zymodemes of *Entamoeba histolytica*: identification of zymodeme XIV in India. *Trans R Soc Trop Med Hyg 78:* 96–101.
16. Haque R, Neville LM, Hahn P, Petri WA Jr, 1995. Rapid diagnosis of *Entamoeba* infection by using *Entamoeba* and *Entamoeba histolytica* stool antigen detection kits. *J Clin Microbiol 33*: 2558–2561.
17. Petri WA Jr, 1996. Recent advances in amebiasis. *Crit Rev Clin Lab Sci 33:* 1–37.
18. Mirelman D, Nuchamowitz Y, Stolarsky T, 1997. Comparison of use of enzyme-linked immunosorbent assay-based kits and PCR amplification of rRNA genes for simultaneous detection of *Entamoeba histolytica* and *E.dispar. J Clin Microbiol 35:* 2405–2407.
19. Pillai DR, Keyston JS, Sheppard DC, Maclean JD, Macperson DW, Kain KC, 1999. *Entamoeba histolytica* and *Entamoeba dispar*: epidemiology and comparison of diagnostic methods in a setting of nonendemicity. *Clin Infect Dis 29:* 1315–1318.
20. Haque R, Mollah NU, Ali IKM, Alam K, Eubanks A, Lyelrly D, Petri WA Jr, 2000. Diagnosis of amebic liver abscess and intestinal infection with the TechLab *Entamoeba histolytica* II antigen detection and antibody tests. *J Clin Microbiol 38:* 3235–3239.
21. Gatti S, Cevini C, Bruno A, Novati S, Scaglia M, 1995. Transmission of *Entamoeba histolytica* within a family complex. *Trans R Soc Trop Med Hyg 89:* 403–405.
22. Gatti S, Mahdi R, Bruno A, Cevini C, Scaglia M, 1998. A survey of amoebic infection in the Wonji area of Central Ethiopia. *Ann Trop Med Parasitol 92:* 173–179.
23. Clark CG, 1988. Amoebic disease. *Entamoeba dispar*, an organism reborn. *Trans R Soc Trop Med Hyg 92:* 361–364.
24. Clark CG, Diamond LS, 1991. Ribosomal RNA genes of "pathogenic" and "non-pathogenic" *Entamoeba histolytica* are distinct. *Mol Biochem Parasitol 49:* 297–302.
25. Novati S, Sironi M, Granata S, Bruno A, Gatti S, Scaglia M, 1996. Direct sequencing of the PCR amplified SSU rRNA gene of *Entamoeba dispar* and the design of primers for rapid differentiation from *Entamoeba histolytica. Parasitology 112:* 363–369.