JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1995, p. 2558–2561 Vol. 33, No. 10
0095-1137/95/$04.00+0

Rapid Diagnosis of *Entamoeba* Infection by Using *Entamoeba* and *Entamoeba histolytica* Stool Antigen Detection Kits

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Received 5 April 1995/Returned for modification 23 June 1995/Accepted 11 July 1995

Humans are infected by two morphologically identical species of *Entamoeba*: *Entamoeba histolytica* causes amebic colitis and liver abscess, and *Entamoeba dispar* is noninvasive. Several weeks of culture and isoenzyme (zymodeme) analysis are required to differentiate *E. histolytica* from *E. dispar*. Here we report a field trial of commercial antigen detection kits designed to rapidly detect and differentiate *E. histolytica* from *E. dispar* in stool specimens. Stool specimens from 202 patients with diarrhea were examined for *E. histolytica* and *E. dispar* by microscopy, culture, and antigen detection. Compared with culture, microscopic identification of the *E. histolytica-E. dispar* complex was 60% sensitive and 79% specific, while the screening antigen detection test for the *E. histolytica-E. dispar* complex was 80% sensitive and 99% specific. Differentiation of *E. dispar* from *E. histolytica* by the *E. histolytica*-specific test was 95% sensitive and 93% specific compared with zymodeme analysis. We conclude that the antigen detection test for the *E. histolytica-E. dispar* complex is more sensitive and specific than microscopy and that the *E. histolytica*-specific antigen detection test is as reliable and much more rapid than zymodeme analysis for the differentiation of *E. histolytica* from *E. dispar*.

As early as 1925 Brumpt (4) proposed that two morphologically identical species of *Entamoeba*, both of which produced quadrinucleate cysts measuring 10 μm or more in diameter, infected humans. Brumpt found that only one of the species caused disease in kittens or human volunteers and named the nonpathogenic species *Entamoeba dispar*. These studies languished in the absence of a means of distinguishing the two morphologically identical parasites. It was not until Sargeaunt and colleagues (19) demonstrated in 1978 that isoenzyme typing could be used to distinguish the pathogenic from the nonpathogenic species of *Entamoeba* that the issue was reexamined.

Largely because of the pioneering work of Sargeaunt and colleagues, *Entamoeba histolytica* has recently been redescribed as two distinct species (5, 7–9, 17–20, 22, 23). The pathogenic species *E. histolytica* (formerly called the pathogenic zymodemes of *E. histolytica*) and the nonpathogenic species *E. dispar* (formerly called the nonpathogenic zymodemes of *E. histolytica*) are morphologically identical but can be differentiated by isoenzyme analysis, typing with monoclonal antibodies (MAbs) to surface antigens, and restriction fragment length polymorphisms (for a review, see reference 7). Isoenzyme analysis has been performed on a research basis on more than 3,000 isolates (17). The nonpathogenic isoenzyme pattern of *E. dispar* has never been seen in an isolate from a patient with invasive disease, and only uncommonly has the pathogenic zymodeme pattern of *E. histolytica* been observed in amebae isolated from asymptomatically colonized humans (17–20). Genetic evidence that the pathogenic and nonpathogenic phenotypes of amebae are different species comes from analyses of restriction fragment length polymorphisms, sequences of single-copy genes, and small-subunit rRNA sequences (5, 7–9, 22, 23).

Earlier reports that *E. histolytica* and *E. dispar* could convert in culture (3, 15, 16) were impossible to reconcile with the genetic differences demonstrated between these two species and were recently shown to be artefactual: Clark and Diamond (6) used polymorphisms in the repeat region of the serine-rich antigen to demonstrate that the "converted" isolates of Mirelman and colleagues (15) were identical to standard axenic laboratory strains HM-1:IMSS and 200:NIH, supporting the conclusion that reports of zymodeme conversion represent contamination of nonpathogenic cultures with standard pathogenic strains.

E. dispar has never been documented to cause colitis or liver abscess. *E. histolytica* is responsible for all cases of colitis and liver abscess and can also cause asymptomatic colonization. Because *E. dispar* colonization is much more common than *E. histolytica* infection and does not need to be treated, an important emphasis of applied research has been the development of tests that can be used by clinicians to distinguish infections caused by the two amebae.

We previously reported that MAbs against the galactose- and *N*-acetylgalactosamine-inhibitable adherence lectin of *E. histolytica* could be used to distinguish *E. dispar* from *E. histolytica* (12, 13). Epitope 1 and 2 MAbs bind to the lectins of both species, whereas epitope 3 to 6 MAbs recognize only the *E. histolytica* lectin. By using polyclonal antilectin antibodies to capture the lectin and enzyme-conjugated MAb to detect the captured lectin, it was possible to specifically detect *E. histolytica* in the stools of patients with amebic dysentery. Here we

TABLE 1. Comparison of *Entamoeba* ELISA results for fecal specimens with identical results by both microscopy and culture

Entamoeba ELISA result	No. of specimens with the following microscopy and culture result:	
	Positive	Negative
Positive	38	2
Negative	3	104
Total	41	106

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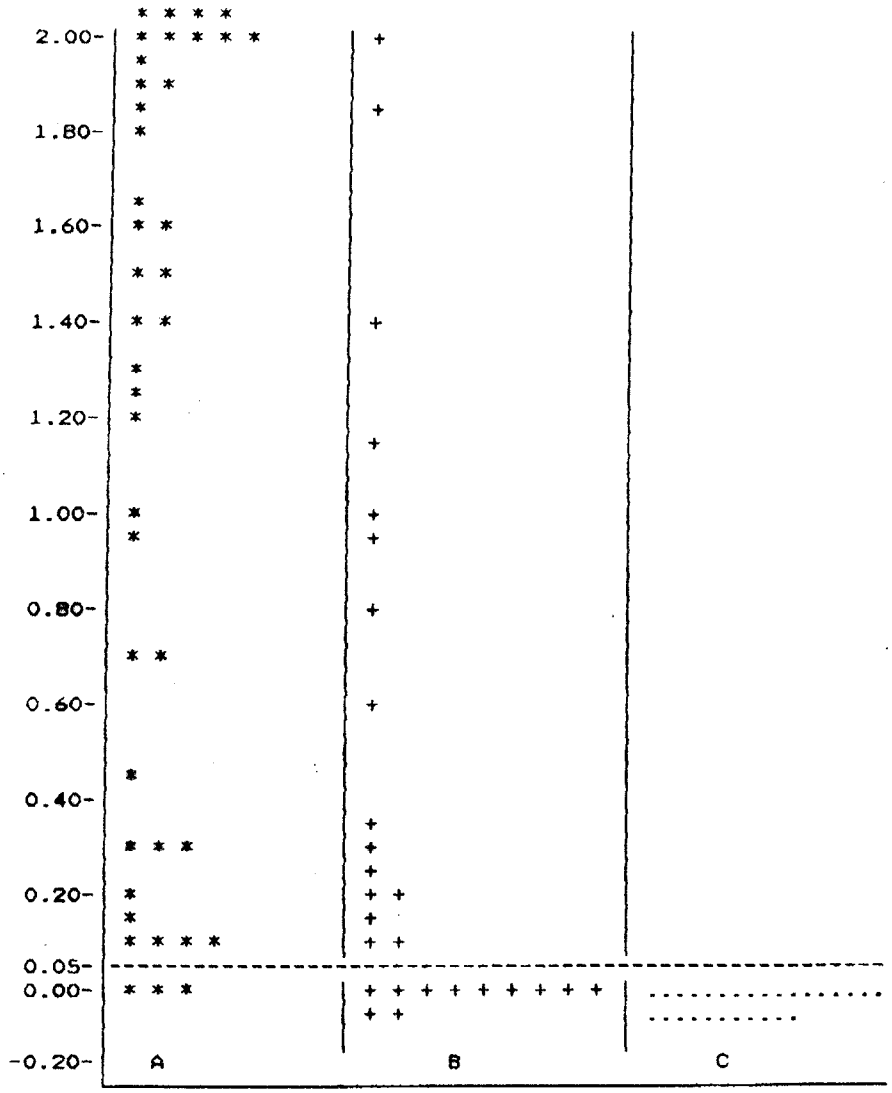

FIG. 1. Distribution of *Entamoeba* ELISA results obtained with stool samples positive for *Entamoeba* species by microscopy or culture. (A) Microscopy positive, culture positive; (B) microscopy negative, culture positive; (C) microscopy positive, culture negative. The results are the optical density of the sample after subtraction of the optical density of the negative control sample. Positive results by the ELISA were defined as an optical density of >0.05 after subtraction of the optical density of the negative control sample.

report the first field test of commercial antigen detection kits based on these antilectin antibodies, including a comparison of antigen detection with standard microscopic identification and stool culture identification of the parasite.

MATERIALS AND METHODS

Specimens. Stool samples were collected from patients with diarrhea seen in 1994 at the International Centre for Diarrheal Disease Research, Dhaka, Bangladesh. Single stool samples were obtained from 96 consecutive patients diagnosed with *E. histolytica-E. dispar* complex infection (by microscopy or culture, or both) and 106 consecutive patients negative by microscopy and culture for *Entamoeba* infection and were examined with the antigen detection kits. Stools were cultured for *Entamoeba* species in Robinson's medium, and zymodeme analyses were performed as described previously (12).

Antigen detection. The *Entamoeba* test (designed to detect but not differentiate the antigens of *E. histolytica* and *E. dispar* in stool specimens) and the *E. histolytica* test (designed to detect specifically *E. histolytica* in stool specimens) were performed according to the manufacturer's instructions (TechLab, Inc., Blacksburg, Va.). Briefly, assay microtiter wells (provided with the kit) were incubated with 0.1 ml of diluted specimen (stool specimen diluted 1:1 in diluent provided with the kit) and 1 drop of MAb-enzyme conjugate for 2 h at room temperature. The contents of the well strips were then shaken out and were washed four times in the wash solution. After washing, residual liquid was removed by striking the strip once against a paper towel, substrate solutions were added, and the strip was incubated at room temperature for 10 min. Intensifier was then added, and after an additional 10 min of incubation the well strips were read in a microtiter plate reader (Titertek Multiskan; Flow Laboratories, McLean, Va.) at 450 nm. A positive result was defined as an optical density reading of >0.05 after subtraction of the negative control optical density. Sensitivity was calculated as the number of true positives/(number of true positives + number of false negatives); specificity was calculated as the number of true negatives/(number of true negatives + number of false positives).

RESULTS AND DISCUSSION

Patients diagnosed with *Entamoeba* infection by microscopy or culture, or both, were more likely to have visible blood in their stools (19 versus 1.8%), were less likely to have liquid stools (50 versus 71%) and were, on average, older (74 versus 54% ≥6 years old) than patients with diarrhea attributed to other causes (data not shown).

Of the 96 specimens that were positive for *Entamoeba* spe-

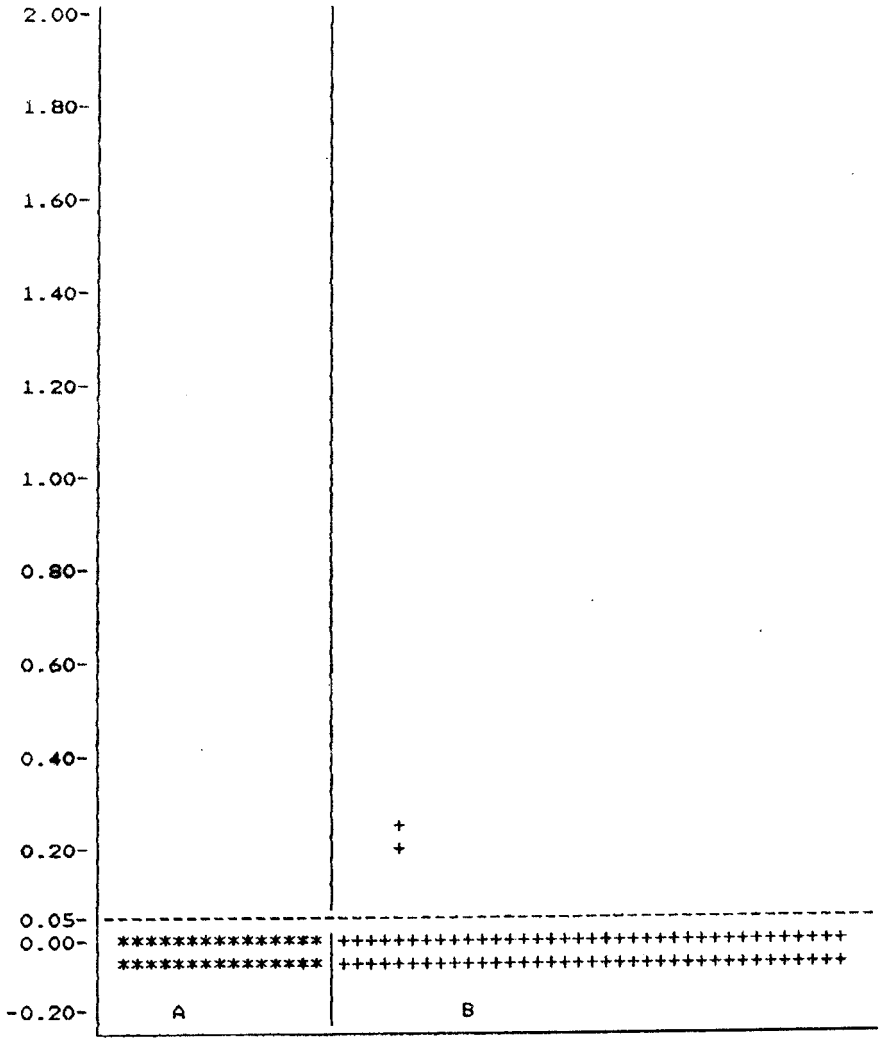

FIG. 2. Distribution of *Entamoeba* ELISA results obtained with *Entamoeba*-negative stool samples that were positive for other parasites or that were parasite-free. (A) *Entamoeba*-negative stool samples positive for other parasites; (B) *Entamoeba*-negative stool samples free of parasites. The results are the optical density of the sample after subtraction of the optical density of the negative control sample. Positive results of the ELISA were defined as an optical density of >0.05 after subtraction of the optical density of the negative control sample.

cies by culture or microscopy, 41 were positive by both techniques, 27 were positive only by culture, and 28 were positive by microscopy but negative by culture. For the 41 specimens that were positive for *Entamoeba* species by both microscopy and culture, the *Entamoeba* enzyme-linked immunosorbent assay (ELISA) was positive for 38 specimens, for a sensitivity of 93%. Of the 106 specimens that were negative for *Entamoeba* species by both microscopy and culture, the *Entamoeba* ELISA was positive for 2 specimens, for a specificity of 98% (Table 1; Fig. 1 and 2). Thus, for patients who have been diagnosed with

TABLE 2. Comparison of microscopy and *Entamoeba* ELISA results with culture results

Test and result	No. of specimens with the following culture results:	
	Positive	Negative
Microscopy		
Positive	41	28
Negative	27	106
Entamoeba ELISA		
Positive	54	2
Negative	14	132

TABLE 3. Some microscopic characteristics of stool specimens from patients infected with *E. histolytica* and *E. dispar* (diagnosed by *Entamoeba* and *E. histolytica* tests)

Characteristic	No. (%) of specimens	
	E. histolytica[a]	*E. dispar*[b]
Visible blood in stool	14 (58)	1 (3.3)
Erythrocyte positive	17 (70)	4 (13.3)
Ingested erythrocytes	13 (68)	3 (15.7)
2+ or above *E. histolytica-E. dispar*	14 (73)	2 (10.5)

[a] $n = 24$ (microscopy positive, culture positive, $n = 19$; microscopy negative, culture positive, $n = 5$).

[b] $n = 30$ (microscopy positive, culture negative, $n = 19$; microscopy negative, culture positive, $n = 11$).

Entamoeba infection by culture and microscopy, the ELISA for *Entamoeba* species is 93% sensitive and 98% specific.

For the 68 specimens that were positive for *Entamoeba* by culture, the *Entamoeba* ELISA was positive for 54 specimens, for a sensitivity of 80%. Of the 134 specimens that were negative for *Entamoeba* species by culture, the *Entamoeba* ELISA was positive for 2 specimens, for a specificity of 99% (Table 2). In comparison, microscopy was negative for 27 of the 68 culture-positive specimens and positive for 28 of the 134 specimens that were culture negative, for a sensitivity of 60% and a specificity of 79%. Thus, the *Entamoeba* ELISA is more sensitive and specific than microscopy when compared with culture as the "gold standard."

For the 52 culture-positive specimens in which *Entamoeba* could be divided into the species *E. histolytica* and *E. dispar* by zymodeme analysis, the *E. histolytica*-specific ELISA correctly identified 21 of 22 *E. histolytica* infections and 28 of 30 *E. dispar* infections, for a sensitivity of 95% and a specificity of 93%. Thus, the *E. histolytica*-specific ELISA is a sensitive and specific means for the rapid differentiation of *E. histolytica* from *E. dispar* in stool specimens.

Stool specimens from patients diagnosed with *E. histolytica* infection by ELISA were much more likely to have visible blood and trophozoites containing ingested erythrocytes than specimens from patients diagnosed with *E. dispar* infection (58 versus 3.3%) (Table 3). Thus, as expected, signs of dysentery were most common in patients diagnosed with infection with the disease-causing species *E. histolytica.*

Microscopy was apparently a less reliable means of identifying *Entamoeba* species than either culture or antigen detection. None of the samples that were positive by microscopy but negative by culture were positive by the antigen detection tests (Fig. 1), consistent with at least some of the microscopy-positive, culture-negative specimens representing false-positive microscopy results. Other investigators have noted similar problems with the microscopic identification of *Entamoeba* species (14, 17).

In conclusion, the field trial described here demonstrated that the *Entamoeba* and *E. histolytica* test kits are more sensitive and specific than microscopic identification of the parasite in the stools of patients with diarrhea. The kits offer the significant advantage of the rapid differentiation of the disease-causing species *E. histolytica* from the noninvasive parasite *E. dispar*. Other antigen detection- or PCR-based assays have been reported for *E. histolytica*, but the kits used in the present study are the first to reach commercial production (1, 10, 11–13). It remains to be determined whether analysis of more than one stool specimen per patient will significantly improve the sensitivity of detection. The test kits remain less sensitive than culture (which is a research but not clinical tool), but they clearly appear to be superior to morphologic identification.

ACKNOWLEDGMENTS

We thank Md. Ali Arshad Meah for secretarial assistance.

This research was supported by the World Health Organization Programme of Intestinal Parasitic Infections, National Institutes of Health grant AI-26649, the Center for Innovative Technology of the Commonwealth of Virginia, and the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). ICDDR,B is supported by countries and agencies which share its concern for the health problems of developing countries. Current donors include the aid agencies of the governments of Australia, Bangladesh, Belgium, Canada, China, Germany, Japan, The Netherlands, Norway, Republic of Korea, Saudi Arabia, Sweden, Switzerland, the United Kingdom, and the United States; international organizations including the Arab Gulf Fund, Asian Development Bank, International Atomic Energy Centre, the United Nations Children's Fund, the United Nations Development Programme, the United Nations Population Fund, and the World Health Organization; private foundations including Child Health Foundation, Ford Foundation, Population Council, Rockefeller Foundation, and the Sasakawa Foundation; and private organizations including American Express Bank, Bayer AG, CARE, Family Health International, Helen Keller International, the Johns Hopkins University, Procter Gamble, RAND, SANDOZ, Swiss Red Cross, the University of California at Davis, and others.

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