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Species-specific field testing of *Entamoeba* spp. in an area of high endemicity

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Abstract

Entamoeba histolytica has been separated in recent years into 2 morphologically identical species: the apathogenic *E. dispar* and the pathogenic *E. histolytica*, only the latter being pathogenic. Although various laboratory techniques allow discrimination between the 2 species there is a lack of field data about the suitability of available diagnostic tests for use in epidemiological studies and few epidemiological studies using species-specific diagnosis have been performed at community level in endemic areas, especially in sub-Saharan Africa. We conducted a repeated cross-sectional study of 967 schoolchildren in central Côte d'Ivoire to compare and evaluate light microscopy, 2 different antigen detection assays, and one polymerase chain reaction (PCR) assay. Microscopy and a non-specific antigen capture *Entamoeba* enzyme-linked immunosorbent assay (ELISA) were used for the primary screening of all children (time t_0). The prevalence of the *E. histolytica*/*E. dispar* species complex at t_0 was 18·8% by single microscopical examination and 31·4% using the non-specific ELISA. Approximately 2 months after the initial screening, fresh stool specimens were collected on 2 consecutive days (t_1 and t_2) from (i) all the children who were positive by microscopy at t_0 ($n = 182$) and (ii) 155 randomly selected children who were negative at the primary screening. These samples were tested with a second antigen detection ELISA specific for *E. histolytica* ($n = 238$) and with a species-specific PCR assay ($n = 193$). The second and third examinations (t_1 and t_2) revealed an additional 43 infections with the species complex *E. histolytica*/*E. dispar*, so that the cumulative microscopical prevalence for t_1 and t_2 was 27·7%. The overall prevalence of *E. histolytica* by species-specific ELISA antigen detection was low (0·83%), while the prevalence of *E. dispar* was 15%. When analysing only microscopically positive samples by PCR ($n = 129$), the ratio *E. histolytica*:*E. dispar* was very low (1:46), suggesting that the vast majority of *Entamoeba* infections in this area were apathogenic. Both species-specific tests performed well but the ELISA was easier to use for large-scale field screening.

Keywords: amoebiasis, *Entamoeba dispar*, *Entamoeba histolytica*, diagnosis, epidemiology, Côte d'Ivoire

Introduction

It is now generally accepted that there are 2 genetically distinct but morphologically indistinguishable species of *Entamoeba*, *E. histolytica* and *E. dispar* (see DIAMOND & CLARK, 1993). Only *E. histolytica* has the potential to cause dysentery and extra-intestinal disease; *E. dispar* is considered to be a harmless commensal. The World Health Organization has reaffirmed the definition of amoebiasis as infection with *E. histolytica sensu stricto*, with or without clinical manifestation, and recommended that '*E. histolytica* should be specifically identified and, if present, treated; if only *E. dispar* is identified, treatment is unnecessary' (ANONYMOUS, 1997).

The diagnosis of *E. histolytica* has traditionally relied upon microscopical examination of fresh or fixed stool specimens. A number of epidemiological studies had been performed before the clear distinction of the 2 species was established. Because they are microscopically indistinguishable, the validity of studies based on stool surveys only has been questioned. The frequently quoted statistics of 40 million to 50 million people infected and up to 100 000 dying of amoebiasis worldwide are based in large part on these parasitological surveys, and this has led to an unclear picture of the epidemiology of amoebiasis (WALSH, 1986; ANONYMOUS, 1997).

In recent years, several alternative diagnostic methods have been developed for the differentiation of the 2 species: isoenzyme typing (SARGEAUNT *et al.*, 1978, 1987), deoxyribonucleic acid (DNA) probes for use with the polymerase chain reaction (PCR) and restriction endonuclease pattern analysis (TANNICH & BURCHARD, 1991; ROMERO *et al.*, 1992; KATZWINKEL-WLADARSCH *et al.*, 1994; TROLL *et al.*, 1997), and enzyme-linked immunosorbent assay (ELISA) antigen-detection systems based on monoclonal antibodies (ABD-ALLA *et al.*, 1993; HAQUE *et al.*, 1994). Cultivation of amoebae followed by isoenzyme analysis is a standard method of differentiation of *E. histolytica* and *E. dispar*. However, this method is time-consuming, not widely available and essentially impractical for large-scale epidemiological studies. PCR amplification of ribosomal ribonucleic acid (rRNA) genes of *Entamoeba* spp., as well as a new ELISA directed against the Gal/GalNAc lectin (produced by TechLab Inc., Blacksburg, VA, USA) have been reported to approach the sensitivity and specificity of isoenzyme analysis in detecting *E. histolytica* directly in stool samples of symptomatic patients (HAQUE *et al.*, 1995, 1998). However, a comparative study using cultured parasites as the source of DNA and antigen showed that the PCR was more sensitive than antigen detection by ELISA (MIRELMAN *et al.*, 1997).

Given the current paucity of reliable data on the worldwide distribution of *E. histolytica* and *E. dispar*, it is critically important to arrive at better estimates of the burden of *E. histolytica* infection by using improved diagnostic tests. In sub-Saharan Africa only a few epidemiological surveys using species identification by isoenzyme analysis have been conducted, in South Africa (JACKSON *et al.*, 1982; GATHIRAM & JACKSON, 1985, 1987) and Ethiopia (GATTI *et al.*, 1998). Information is crucial for the clinical and public health management of the disease, at both national and global levels. Moreover, adequate diagnostic methods and epidemiological information are urgently needed in view of the recent developments towards an amoebiasis vaccine (STANLEY, 1997; HUSTON & PETRI, 1998). Obtaining further species-specific prevalence data on amoebiasis and the development of field testing strategies are therefore of high priority.

Based on previous work in Côte d'Ivoire revealing a high prevalence of the *E. histolytica*/*E. dispar* complex (UTZINGER *et al.*, 1999), the objectives of the present study were (i) to assess the prevalence of the complex in children in Côte d'Ivoire using a newly available

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ELISA and repeated microscopy, (ii) to identify *E. histolytica* specifically using an established PCR assay (TROLL *et al.*, 1997) and an antigen detection ELISA test, and (iii) to compare the diagnostic performance of these tests.

Materials and Methods

Study area and population surveyed

The study was carried out near the town of Agboville in the south of Côte d'Ivoire between March and June 2000. All schoolchildren attending standards 4–6 at 8 randomly selected primary schools in the Agboville school district were enrolled. The objectives of the study were discussed with the school directors and, after obtaining their consent, the sex and age of the children were recorded. The day before the first survey, children were issued with a small plastic container and asked to return the containers with a small portion of their morning stool. After stool collection, children were issued with a new container for the following sample collection. In total 3 stool samples were collected, at time t_0 at the beginning of the study, and at times t_1 and t_2, approximately 2 months after t_0 (Fig. 1).

Identification of E. histolytica/E. dispar *complex infections*

The stool specimens were taken to the laboratory in Abidjan within 3 h after collection. Before being further processed, each specimen was examined macroscopically for consistency with special emphasis on liquid specimens and on blood visible by eye.

A total of 967 stool samples was collected at time t_0, and a 1–2 g portion was preserved in sodium acetate–

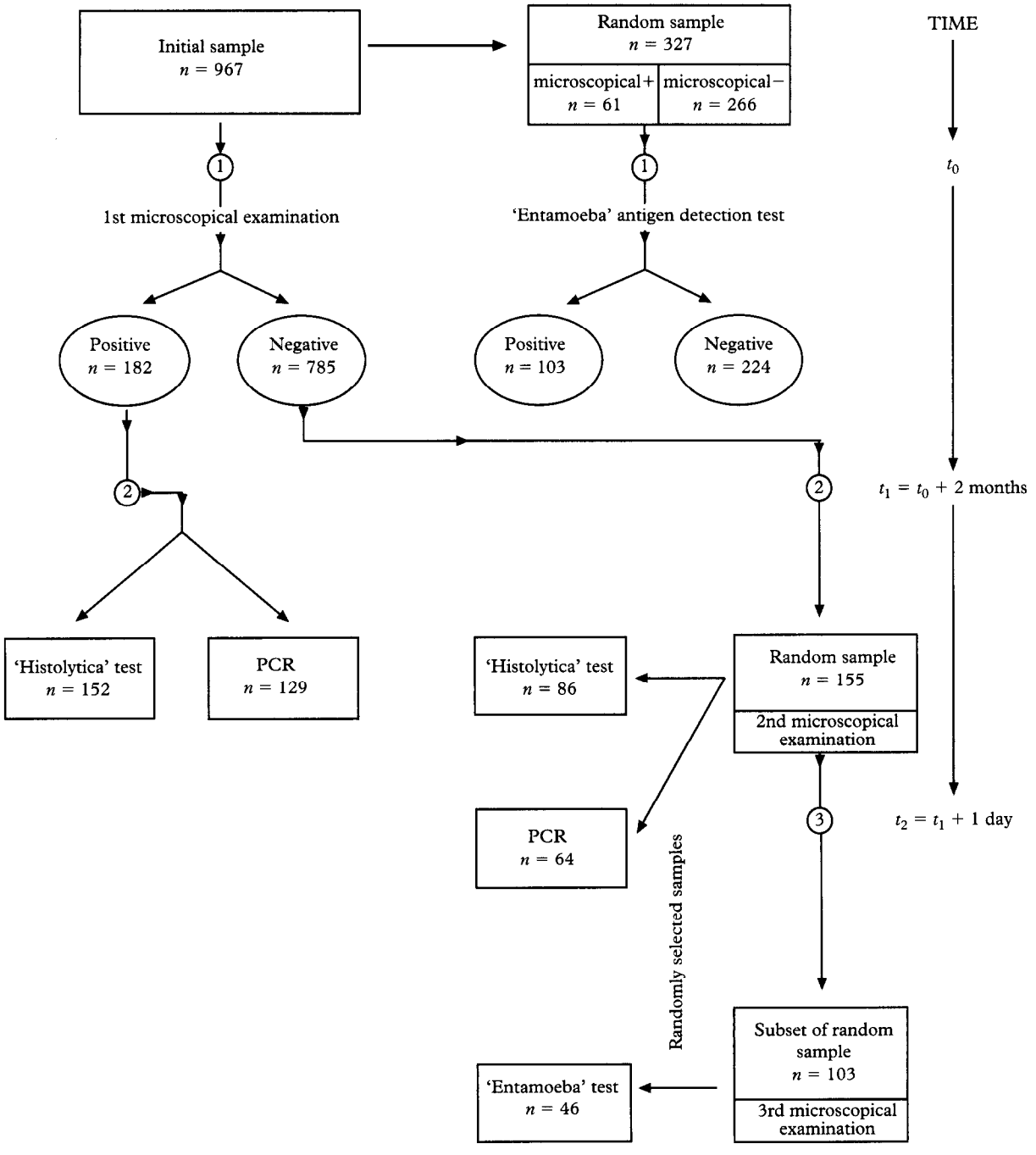

Fig. 1. Screening procedure for 967 schoolchildren in Côte d'Ivoire for detection of the *Entamoeba histolytica/E. dispar* complex and specific identification of the parasites. Numbers in circles indicate collection of fresh stool specimens.

acetic acid–formalin (SAF). The samples were examined after processing according to MARTI & ESCHER (1990) by light microscopy within 6 weeks (Fig. 1). All samples containing *E. histolytica/E. dispar* and other protists and helminths were recorded semi-quantitatively by distinguishing between 3 levels: 1+ = 1–5 parasites seen per slide; 2+ = 1–2 parasites seen per microscope field; 3+ = more than 2 parasites per microscope field. Microscopical examination was done by one of 3 skilled and experienced laboratory technicians. Slides containing cysts or trophozoites of *E. histolytica/E. dispar* were systematically checked and confirmed by a second technician.

A single Kato–Katz thick smear was also prepared from the stool samples collected at t_0, according to KATZ *et al.* (1972). The slides were examined by light microscopy and all helminth eggs were identified and recorded quantitatively.

Approximately 2 months after the first screening, 155 fresh stool samples were collected from a random selection of previously microscopically negative children on 2 consecutive days (times t_1 and t_2), processed as described above and examined by light microscopy during the following 3 weeks. These 2 repeat examinations allowed the comparison of repeated microscopical examinations with the 'Entamoeba' ELISA. The results of examinations at t_0 and t_1/t_2 were not pooled for analysis because of the risk of new infections occurring during the 2 months interval between t_0 and t_1/t_2.

Antigen detection ELISA

A randomly selected sample of 327 specimens of the initial 967 stool samples was tested at t_0 with the 'Entamoeba' antigen capture ELISA, to detect the Gal/GalNAc lectin of both *E. histolytica* and *E. dispar*. Additionally, and for comparison with repeated microscopical samples, 46 of the initially microscopically negative specimens were also tested at t_1/t_2 by the 'Entamoeba' ELISA. The tests were performed according to the manufacturer's instructions (TechLab Inc.) and the optical density was measured with an automatic photometer at 450 nm.

Species-specific testing

Species-specific diagnosis was done with the aid of (i) a PCR assay developed by TROLL *et al.* (1997) and (ii) the 'Histolytica' ELISA antigen capture test (TechLab Inc.) using stool specimens of children found to be infected with *E. histolytica/E. dispar* at the first microscopical examination (t_0) and samples from randomly selected microscopically negative children (Fig. 1). Due to problems in tracing children and also sometimes to insufficient material in the samples, the numbers of specimens tested by the 2 species-specific tests differed. The PCR targets rRNA genes of both *E. histolytica* and *E. dispar* in 2 separate reactions and therefore allows positive identification of both species. By contrast, the 'Histolytica' antigen detection ELISA is specific only for *E. histolytica* adhesin.

PCR assay

After initial concentration in SAF fixative the resulting pellets were washed 3 times with distilled water, transferred to 1·5 mL Eppendorf tubes, and suspended in 250 µL digestion buffer (100 mM Tris, pH 8·0; 25 mM ethylenediaminetetraacetic acid). The samples were then frozen and transported to Switzerland for further analysis. Subsequent analysis of the samples was done according to TROLL *et al.* (1993), with the following modifications: (i) DNA was purified after extraction using the QIAamp tissue mini-kit (Qiagen AG, Switzerland) in order to eliminate PCR inhibitors; (ii) the purified DNA was used without dilution in the PCR; (iii) deoxythymidine triphosphate was used instead of deoxyuridine triphosphate; (iv) restriction endonuclease (*Dra*I) digests were performed on only a small number of *E. dispar* samples, in particular those showing faint bands, in order to confirm the identity of the amplified fragment.

'Histolytica' antigen detection test

In this assay *E. histolytica* is specifically identified due to differences in the Gal/GalNAc lectin between *E. histolytica* and *E. dispar* (see HAQUE *et al.*, 1995). The tests were performed according to the manufacturer's instructions (TechLab Inc.).

Chemotherapy

Treatment for intestinal helminth and protozoan parasites was given to all children found to be infected according to the Côte d'Ivoire national guidelines. Medication and doses used were approved by the Chief Medical Officer of the Agboville Health Department and administered by a pharmacist of the local Health Department. *E. histolytica/E. dispar* and *Giardia duodenalis* infections were treated with 3 daily doses of metronidazole (10 mg/kg bodyweight) for 8 d. Infections with *Schistosoma mansoni* were treated with a single dose of praziquantel (40 mg/kg bodyweight) and all other helminths (i.e. *Ascaris lumbricoides*, *Trichuris trichiura*, hookworms) with a single dose of albendazole.

All results of the microscopical examination and all data from the other diagnostic assays were double-entered into the Excel™ 1997 software package. After transfer to the Foxpro™ 2.6 database, frequency and consistency tests were carried out. Analysis of associations and diagnostic performance calculations were carried out with the STATA™ 7.0 statistics package (Stata Corporation, College Station, Texas, USA).

Results

Parasitology and non-specific ELISA

Complete results for the first microscopical examination were obtained for 967 children (90% of the enrolled children). The median age was 12 years with a range from 8 to 16 years. There were significantly more boys ($n = 589$) than girls ($n = 378$) ($z = 38{\cdot}8$, $P < 0{\cdot}001$). Macroscopic stool examination revealed 574 liquid/unformed specimens (59%). Only 5 children presented specimens containing macroscopically detectable blood, of which only one was subsequently shown to harbour *E. histolytica/E. dispar* by microscopy. There was no association between liquid/unformed specimens and infection with *E. histolytica/E. dispar* (odds ratio = 1·13, 95% confidence interval (CI) 0·82–1·56).

The point prevalence of *E. histolytica/E. dispar* for the single microscopical examination at t_0 was 18·8% (182/967; 95% CI 16·4–21·4). Most of the children infected with *E. histolytica/E. dispar* showed infection levels of 1+ (83%). The predominant protozoan parasite was *E. coli* with a prevalence of 63·4%. Prevalences for all protozoa are presented in Table 1.

The combined results from the single Kato–Katz thick smear and the first microscopical examination showed that hookworms and *S. mansoni* were the commonest helminths (Table 1).

The non-specific 'Entamoeba' antigen detection ELISA was performed on a total of 327 specimens randomly selected from the initial 967 (Fig. 1). The parasitological prevalence of *E. histolytica/E. dispar* infections within this sample based on a single microscopical examination was 18·7% (61/327), which is close to the prevalence of 18·8% found in the overall sample of 967 children. The 'Entamoeba' ELISA detected 103 positive cases, giving a higher prevalence rate of 31·5% (103/327; 95% CI 26·5–36·8).

Stool specimens of 155 children who were negative at t_0 were collected again at t_1. At t_2, one day later, only 103 of them were present. These later examinations revealed an additional 43 infections with the *E. histoly-*

Table 1. Prevalence of intestinal protozoa among 967 schoolchildren in Côte d'Ivoire screened by direct stool microscopy[a] and intestinal helminths among 885 children screened by direct microscopy[a] and a single Kato–Katz thick smear

Intestinal parasite	No. of infected children	Prevalence (%)[b]
Protozoa		
Entamoeba histolytica/Entamoeba dispar	182	18·8 (16·4–21·4)
Entamoeba coli	613	63·4 (60·6–66·8)
Giardia duodenalis	77	8·0 (6·3–9·9)
Endolimax nana	447	46·2 (43·3–49·7)
Chilomastix mesnili	98	10·1 (8·3–12·3)
Entamoeba hartmanni	126	13·0 (11·0–15·4)
Iodamoeba buetschlii	85	8·8 (7·1–10·8)
Blastocystis hominis	81	8·4 (6·7–10·4)
Helminths		
Schistosoma mansoni	189	21·4 (18·7–24·2)
Hookworm	271	30·6 (27·6–33·8)
Ascaris lumbricoides	78	8·8 (7·0–10·9)
Trichuris trichiura	85	9·6 (7·7–11·7)

[a]Using sodium acetate–acetic acid–formalin fixation (MARTI & ESCHER, 1993).
[b]95% Confidence interval in parentheses.

tica/E. dispar species complex; the cumulative prevalence for t_1 and t_2 was 27·7%.

Species-specific testing

152 and 129 samples which were positive at t_0 were tested with the specific 'Histolytica' ELISA and the PCR, respectively (Fig. 1). The set of samples tested by the 2 assays did not overlap completely due to problems in sample preparation. The 'Histolytica test' detected 3/152 *E. histolytica* infections (2·0%; 95% CI 0·7–5·6) and the PCR test 2/129 (1·6%; 95% CI 0·4–5·5). *E. dispar* was detected by the PCR in 93 of the 129 samples tested (72·1%; 95% CI 63·8–79·1). 34 of the 129 samples found to be positive by microscopy were negative by PCR (26·4%; 95% CI 19·5–34·6). Based on PCR results with the microscopically positive samples, the species ratio *E. histolytica:E. dispar* was *c.* 1:46. No additional *E. histolytica* case was detected by the 'Histolytica' test or by the PCR when testing the microscopically negative samples.

Performance of diagnostic tests: non-specific tests

Using isoenzyme analysis as the reference test, the 'Entamoeba' antigen detection assay has been shown to be more sensitive (80% vs. 60%) and more specific (99% vs. 79%) than microscopy (HAQUE *et al.*, 1995). We therefore used the results of the 'Entamoeba' test to evaluate single and repeated microscopical examination (Table 2). Single microscopical examination of a stool sample collected at t_0 was moderately sensitive (68·9%; 95% CI 56·4–79·1) and specific (77·1%; 95% CI 71·7–81·7). Repeated microscopical examination on 2 consecutive days at t_1 and t_2 improved sensitivity (77·8%; 95% CI 54·8–91·0) and specificity (89·3%; 95% CI 72·8–96·3), although this was measured on a much smaller sample ($n = 46$ compared with $n = 327$), which is reflected in the much wider 95% confidence intervals. If we had taken single microscopy as reference standard, the sensitivity of the 'Entamoeba' test would have been only 40·8% (95% CI 31·8–50·4) and the specificity would have been 91·5% (95% CI 87·1–94·5). With repeated microscopical examination as reference at t_1 and t_2, the sensitivity of the 'Entamoeba' test would have been 82·4% (95% CI 59·0–93·8) and the specificity would have been 86·2 % (95% CI 69·4–94·5), again on a much smaller sample.

We also compared the 'Entamoeba' ELISA with the PCR, combining the PCR results for *E. histolytica* and *E. dispar*. In total, 101 specimens were examined by both PCR and the 'Entamoeba' test. PCR and the 'Entamoeba' test detected 75 and 53 *E. histolytica/E. dispar* infections, respectively (Fig. 2). The agreement

Table 2. Sensitivity and specificity of one and two consecutive microscopical stool examinations for the detection of the *Entamoeba histolytica/E. dispar* complex compared with the non-species-specific enzyme-linked immunosorbent assay

	ELISA[a]		
Microscopy	Positive	Negative	Total
Single examination[b]			
Positive	42	61	103
Negative	19	205	224
Total	61	266	327
Two examinations[b,c]			
Positive	14	3	17
Negative	4	25	29
Total	18	28	46

[a]Enzyme-linked immunosorbent assay for *Entamoeba* sp.
[b]Using sodium acetate–acetic acid–formalin fixation (MARTI & ESCHER, 1993).
[c]On consecutive days, approximately 2 months after the single examination.

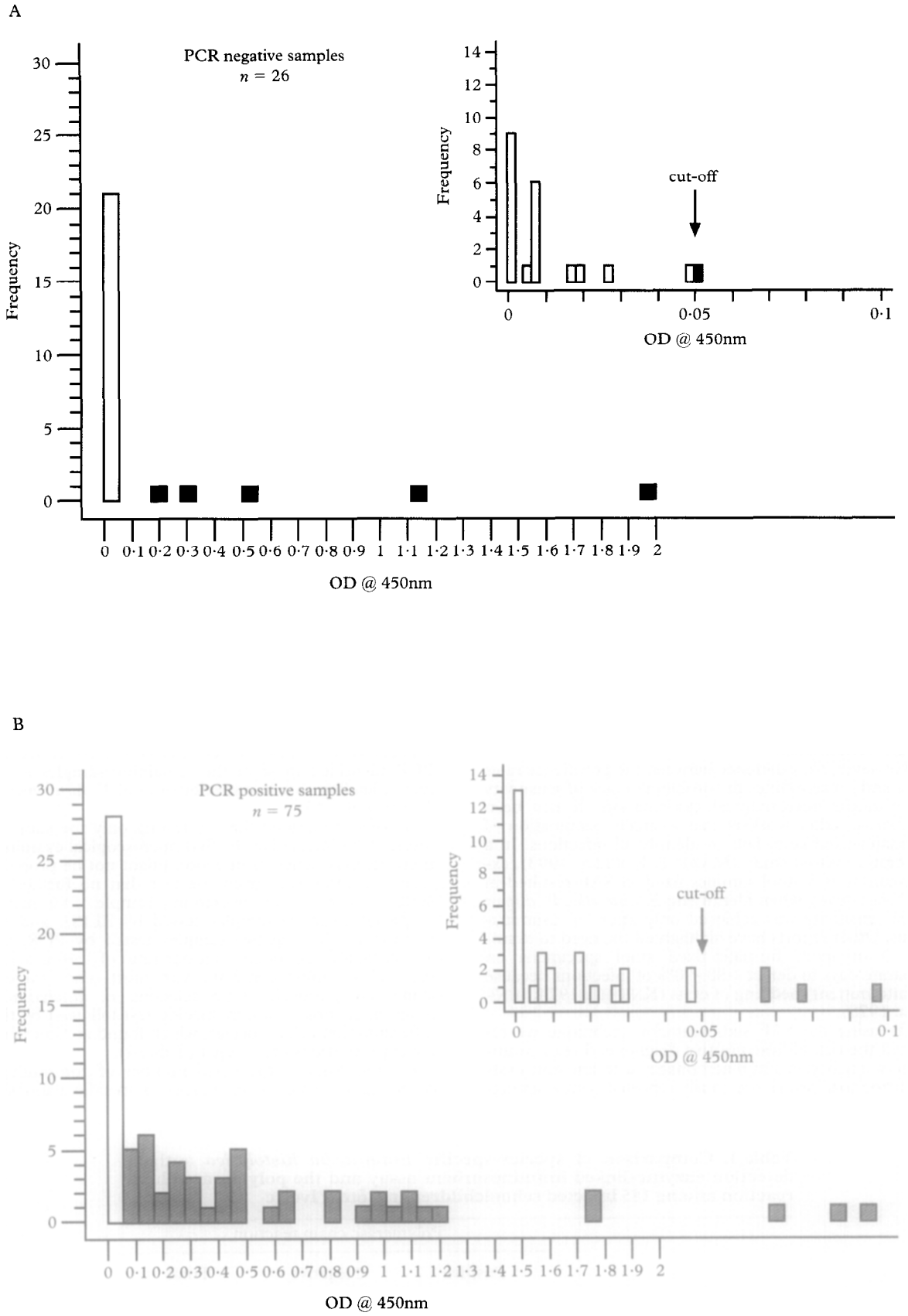

Fig. 2. Optical density (OD) values for the *Entamoeba* enzyme-linked immunosorbent assay (ELISA) of stool specimens tested previously by the polymerase chain reaction (PCR): (A) 26 PCR-negative samples; (B) 75 PCR-positive samples. The small inserts show the same data for a narrower OD range and the OD positivity cut-off value of the *Entamoeba* ELISA (OD = 0·05); white bars indicate concordance between the PCR and ELISA results, shaded bars indicate discordance.

of the 2 tests in detecting positives was only 46·5% (95% CI 36·5%–56·7%).

Performance of diagnostic tests: species-specific tests

185 specimens were tested with both the 'Histolytica' antigen detection ELISA and the PCR. The microscopical prevalence of the *E. histolytica/E. dispar* complex in this sample was 76·8% (142/185; 95% CI 70·2–82·3). Both specific assays detected only a small number of *E. histolytica* infections; 3 by the specific antigen detection ELISA and 2 by the PCR (Table 3). Of the 3 *E. histolytica* cases detected by ELISA, the PCR result agreed in one case and identified the remaining 2 specimens as containing *E. dispar*. The remaining positive sample detected by PCR was positive for both *E. histolytica* and *E. dispar* but was found to be negative by the 'Histolytica' antigen detection ELISA. As a quality control measure, the samples found to be positive for *E. histolytica* by either PCR ($n = 2$) or 'Histolytica' ELISA ($n = 3$) were re-examined by PCR by an independent investigator. The second examination confirmed all the results. Using the PCR as reference standard, the specific antigen detection ELISA exhibited an overall test efficiency (proportion of true positives and true negatives) of 98·4% (95% CI 95·3–99·4).

Discussion

The high endemicity of *E. histolytica/E. dispar* in Côte d'Ivoire was confirmed by the present study with schoolchildren, who had an infection prevalence of 18·8% derived from a single microscopical examination and one of 31·4% with the 'Entamoeba' ELISA. The second and third microscopical examinations performed revealed a considerable number of additional infections but we did not consider these cumulatively because we could not exclude reinfections in the period of 2 months between the first and the second specimen collections. These prevalence estimates are conservative because of the probability of missing cases with a single examination.

However, the difference between the prevalence rates at t_1 and t_2 exemplifies the problem of lack of sensitivity of a single microscopical examination. It has been shown by other workers that a single examination of formalin-fixed stool fails to identify all infections. In a laboratory-based study (MARTI & KOELLA, 1993), examination of 3 stool samples fixed in SAF resulted in 92% sensitivity when identifying *E. histolytica/E. dispar*; 98% sensitivity was achieved only after >4 examinations. Other reports have highlighted the need to examine 3 or more formalin-fixed stool specimens on separate days to detect >80–90% of infections because of intermittent shedding of cysts (KNIGHT, 1974; JUNIPER, 1978).

By using the SAF sedimentation technique we reduced the risk of false positive diagnoses due to confusion of leucocytes and macrophages with amoebic cysts and trophozoites, as previously reported with examination of direct smears (KNIGHT, 1974). However, misidentification of other protozoa as *E. histolytica/E. dispar* cysts may have influenced the specificity of our detection. The risk of confounding immature *E. coli* cysts with those of *E. histolytica/E. dispar* is real, especially when differential morphological features such as chromatoid bodies are absent. This issue is of particular importance since both *E. coli* and *E. histolytica/E. dispar* are generally found in the same areas with poor hygienic conditions, and *E. coli* is usually the more prevalent species.

Other field-based studies in West Africa, relying on single examinations of formalin–ether concentrated stool specimens, also found high prevalences of *E. histolytica/E. dispar* (52·3% and 37%; BRAY & HARRIS, 1977 and UTZINGER *et al.*, 1999, respectively). In contrast, community-based surveys in other endemic areas, using similar diagnostic methods, reported substantially lower prevalence rates. A study in South Africa examined 800 specimens and found the prevalence of the species complex to be only 2·8% (GATHIRAM & JACKSON, 1985). In south-east Asia and in Mexico, *E. histolytica/E. dispar* infection rates of 12·4% and 8·1% have been reported (ACUNA-SOTO *et al.*, 1993; RIVERA *et al.*, 1998). In numerous other studies, prevalences of *E. histolytica/E. dispar* were mostly based on examination of direct smears and/or the surveys were hospital-based rather than community-based, and therefore cannot be compared easily with our findings.

This is the first community-based study looking at species-specific prevalence of *E. histolytica* and *E. dispar* in sub-Saharan Africa using diagnostic methods other than isoenzyme analysis. *E. histolytica* infections were assessed separately by a PCR assay and the 'Histolytica' ELISA antigen detection test developed by Techlab Inc. Among specimens shown microscopically to contain *E. histolytica/E. dispar*, both species-specific tests detected a surprisingly low number of *E. histolytica* infections (2 by the PCR assay, including one sample with both *E. histolytica* and *E. dispar*, and 3 by the non-specific ELISA antigen detection test). As expected, PCR identified most of the remaining samples as *E. dispar*, leading to a low species ratio of *E. histolytica* to *E. dispar* of 1:46.

It could be argued that by testing only the subjects found to be infected at the first microscopical examination, the prevalence of infection might not be properly reflected. However, given the fact that no further *E. histolytica* case was discovered in a sample of 86 microscopically negative samples tested by ELISA and 64 microscopically negative samples tested by PCR, we are reasonably confident that our ratio of 1:46 is accurate. This finding contrasts with most other surveys using the approach of pre-selecting *E. histolytica/E. dispar* infections by a non-specific test followed by the differentiation of the species, which found considerably lower proportions of *E. dispar* (Table 4).

On the basis of the small number of community-based studies which involved species-specific diagnosis,

Table 3. Comparison of species-specific *Entamoeba histolytica* antigen detection enzyme-linked immunosorbent assay and the polymerase chain reaction among 185 infected schoolchildren in Côte d'Ivoire

	Polymerase chain reaction		
	Positive	Negative	Total
Species-specific ELISA[a]			
Positive	1	2	3
Negative	1[b]	181	182
Total	2	183	185

[a] Enzyme-linked immunosorbent assay.
[b] Specimen positive for both *Entamoeba histolytica* and *E. dispar*.

Table 4. Reported prevalence rates and ratios for *Entamoeba histolytica* and *E. dispar* in amoebiasis endemic areas

Area	Reference	Diagnostic method[a]	Prevalence (%)[b]	Species ratio[c]
Turkey	JETTER *et al.*, 1997	PCR	31·6 (73/231)	*E. dispar* only
South Africa	JACKSON *et al.*, 1982	Isoenzymes	10·0 (139/1381)	1:10
Mexico	ACUNA-SOTO *et al.*, 1993	PCR	12·4 (25/201)	Note[d]
Philippines	RIVERA *et al.*, 1998	PCR	8·1 (152/1872)	1:8·5
Bangladesh	HAQUE *et al.*, 1997	ELISA	8·0 (79/987)	1:8
Bangladesh	HAQUE *et al.*, 1998	ELISA	17·4 (118/680)	1:2·7
Côte d'Ivoire	This study	ELISA & PCR	31·5 (103/327)	1:46

[a]ELISA = enzyme-linked immunosorbent assay; PCR = polymerase chain reaction.
[b]*E. histolytica*/*E. dispar* complex.
[c]*E. histolytica*:*E. dispar*.
[d]56% of all individuals infected harboured mixed infections with *E. histolytica* and *E. dispar*.

it appears that the species ratio *E. histolytica*:*E. dispar* varies considerably. Whether this observation reflects small-scale rather than large-scale geographical differences in the distribution of *E. histolytica* cannot be answered at this time. In order to increase the epidemiological understanding of amoebiasis, this issue needs urgently to be further investigated.

The 'Entamoeba' test and the PCR assay showed only limited agreement in detecting infections (46·5%). PCR detected a substantially higher number of *E. histolytica*/*E. dispar* infections than the 'Entamoeba test'. Possibly the additional cases detected by PCR reflected the low sensitivity of a single examination using the other test. However, technical problems with the PCR technique cannot simply be excluded. This technique is potentially prone to cross-contamination due to its power to amplify minute traces of target DNA. To test for possible contamination, our samples were retested twice by PCR by an independent investigator using additional controls, but we could not find a problem with the assay. However, contamination can occur between the field and the laboratory during sample handling or DNA preparation and this cannot be traced later.

When the PCR assay was taken as the standard, the 'Histolytica' ELISA antigen detection test showed high efficiency (98·4%). However, the 2 tests disagreed with 3 samples which were positive for *E. histolytica* by either PCR or ELISA (Table 3). In contrast, another study also using the TechLab specific ELISA and a PCR assay for the diagnosis of *E. histolytica* in stool samples, found a correlation of 84% between the 2 tests (HAQUE *et al.*, 1998). Our findings concerning *E. histolytica* infections are difficult to interpret because of the small number of infections. Despite a rather large initial sample size we failed to detect many such infections because of the unexpectedly low *E. histolytica*/*E. dispar* ratio.

In conclusion, this study produced valuable data in relation to the occurrence of *E. dispar* and *E. histolytica* in communities in sub-Saharan Africa. While the prevalence of the species complex was high, the prevalence of *E. histolytica* was low. This might explain the paradox of the low amount of amoebiasis morbidity reported by health services on the African continent despite a high apparent microscopical infection rate. Field testing with the new generation of ELISA kits proved to be clearly superior to microscopy. Because of the small number of *E. histolytica* cases in our sample the intended comparison of test performance between the 'Histolytica' ELISA and the PCR did not result is a clear conclusion about which test is superior. However, unlike the PCR, the ELISA proved to be easy to use in the field and, if its high diagnostic performance is confirmed, it might well become the standard technique for large-scale field screening.

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Announcement

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