

Application of the Polymerase Chain Reaction (PCR) in the Epidemiology of *Entamoeba histolytica* and *Entamoeba dispar* Infections

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In this paper, we briefly summarize the latest information on the polymerase chain reaction (PCR) as an epidemiologic tool for *Entamoeba histolytica* and *Entamoeba dispar* infections. This method which employs DNA template directly extracted from formalin fixed stool specimens offers a good promise for an accurate and reliable epidemiology of the two species. The assay is, sensitive enough to detect as few as five cysts in the stool sample, rapid and selectively differentiates *E. histolytica* from *E. dispar* DNA from stool specimens without the need for prior cultivation.

Keywords: Polymerase chain reaction, Epidemiology, *Entamoeba histolytica*, *Entamoeba dispar*

Entamoeba histolytica is now known to consist of two genetically distinct yet morphologically identical species, the invasive parasite, retaining the name *Entamoeba histolytica* Schaudinn, 1903, separating it from the noninvasive parasite, *Entamoeba dispar* Brumpt, 1925 [1]. In view of this recent development in amebiasis research, there is a great need to reassess previously reported prevalence data of *E. histolytica* infections in many parts of the world. Surveys that determine the prevalence of infection by stool examination of parasites measure predominantly *E. dispar*, since this species is more common, while serologic surveys reflect the incidence of *E. histolytica* infection because *E. dispar* does not elicit a positive serologic response in humans [6]. Also, seroepidemiological studies usually reflect seropositivity of samples even years after episodes of amebiasis. These factors therefore, pose a problem on the accuracy of previously reported epidemiologic studies. Furthermore, the frequently quoted global prevalence of *E. histolytica* (500 million) is very misleading [10]. It is more likely that *E. histolytica* is responsi-

ble for only 10% of these infections (50 million) worldwide, while *E. dispar* accounts for the rest [4]. Indeed, the recommendation of the WHO-Pan American Health Organization-United Nations Educational, Scientific, and Cultural Organization to develop improved methods for the specific diagnosis of *E. histolytica* infection is very important for the establishment of accurate prevalence data of *E. histolytica* and *E. dispar* infections worldwide [11].

Diagnosis of intestinal amebiasis based on microscopy is imprecise in view of the morphological similarity of the two species. Amebic culture and isoenzyme analysis, on the other hand, are more sensitive than microscopy, however, are cumbersome and require a week to complete and usually show negative results for many microscopy-positive samples [2]. There is now available an enzyme-linked immunosorbent assay (ELISA) kit that distinguishes between *E. histolytica* and *E. dispar* antigens directly in stool [2]. However, a comparative study on the use of the ELISA and the polymerase chain reaction (PCR) for the detection of the

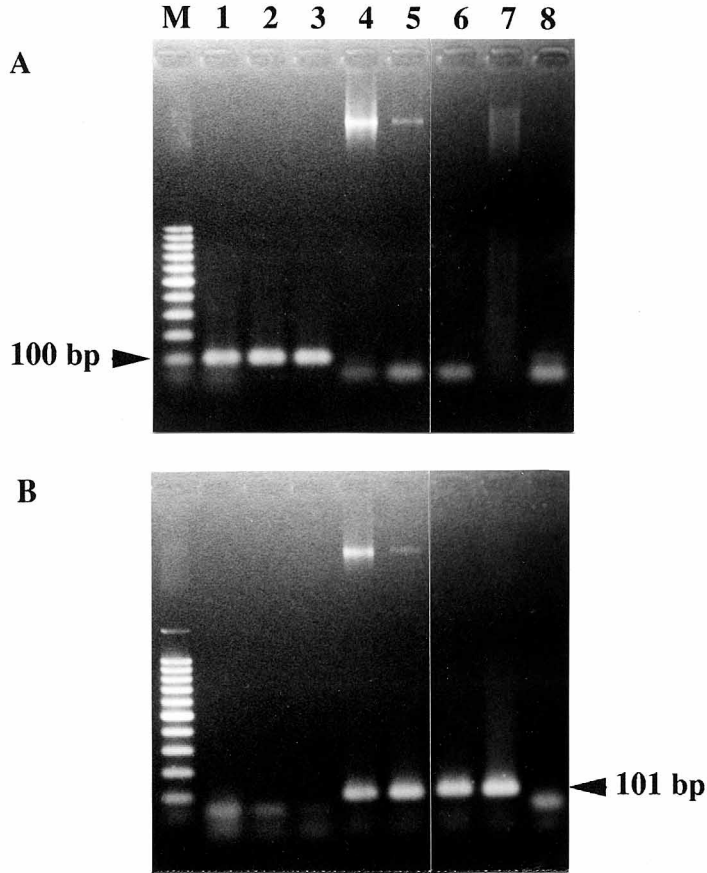


Fig. 1 A, B Agarose gel separation of PCR products amplified by two sets of primers: **A** *Entamoeba histolytica* primers p11 plus p12 and **B** *E. dispar* primers p13 plus p14. (Lane 1 *E. histolytica*-positive control (trophozoites of HK-9), lanes 2-3 *E. histolytica*-positive samples, lane 4 *E. dispar*-positive control (trophozoites of SAW 1734 R, clone AR), lanes 5-7 *E. dispar*-positive samples, lane 8 without template, M DNA size marker - 100 bp ladder). The arrowhead indicates the position and size of PCR products

Table 1 Comparison of microscopy and PCR in differentiating *Entamoeba histolytica*/*E. dispar* and other amebae in formalin-fixed stool specimens

MICROSCOPY	No. of samples	PCR			
		<i>E. histolytica</i>	<i>E. dispar</i>	<i>E. histolytica</i> & Negative <i>E. dispar</i>	
<i>E. histolytica</i> / <i>E. dispar</i>	165	18	147	0	0
Other amebae	151	0	9	0	142
Negative	88	0	0	0	88
TOTAL	404	18	156	0	230

two species indicated that PCR was more advantageous than the ELISA in epidemiologic studies [5]. It is therefore important to assess the usefulness of the PCR in accumulating data on the prevalence of *E. histolytica* and *E. dispar* in the field that are more accurate.

In an effort to find a method that avoids time-consuming culture procedure, we have established the use of PCR using DNA template directly extracted from formalin-fixed stool samples [7,8]. According to Herniou et al. [3], the phosphodiester backbone of the nucleotide chain of the DNA is preserved in the presence of formaldehyde which makes it very useful in recovering DNA even from fixed fecal specimens. Moreover, the use of formalin-fixed stool for DNA extraction is advantageous in terms of safe handling, storage and transportation of samples.

The genomic DNA extraction protocol from stool specimens that we have established primarily consists of five steps: washing with phosphate-buffered saline (PBS), freezing and thawing, detergent treatment (Triton X-100), proteinase K digestion and phenol-chloroform extraction. PCR is carried out using primers specific for *E. histolytica* and *E. dispar* (p11 plus p12 and p13 plus p14, respectively) as previously described [9]. This assay is sensitive enough to detect as few as five cysts in the stool sample, can be performed in one day and selectively differentiates *E. histolytica* from *E. dispar* DNA from stool specimens (Fig. 1) without the need for prior cultivation.

We have applied this method to document the prevalence of the two species in a number of communities in Luzon, the main island of the Philippines [7,8]. Our studies have demonstrated the reliability of the PCR over microscopy in clearly documenting multiple infections among subjects in epidemiologic studies (Table 1). This was observed in view of the fact that coinfection of other amebae-positive samples with *E. dispar* was only detected after PCR analysis. Clearly, the PCR-based detection of *E. histolytica* and *E. dispar* directly from stools has potential application for epidemiologic studies. An additional benefit is that it can be used to monitor the efficacy of treatment, which is the limitation of serologic tests because of the persistence of the antibody response even after successful treatment.

Moreover, the use of the PCR in detecting *E. dispar* among cyst carriers can facilitate longitudinal studies to determine the nature of this nonpathogenic species in the hosts.

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