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LETTER TO THE EDITOR

Entamoeba histolytica AND *Entamoeba dispar* IN THE NORTHEAST BRAZIL

Sir,

There was for many years a controversy concerning the relationship between the presence of protozoa *Entamoeba histolytica* and clinical symptoms. This species was easily identified by optical microscopy from *Entamoeba coli* in faecal samples. The latter species was definitely assumed not being pathogenic. However, among the strains of *E. histolytica* some were recognized as non-pathogenic. In 1970 decade isoenzymes electrophoretic patterns approaches were reported to distinguish these strains, namely, invasive and non-invasive strains of *E. histolytica*[18]. This concept brought back the proposal of BRUMPT[5] who described the non-pathogenic ameba as *Entamoeba dispar*. CLARK & DIAMOND[6] referred *E. histolytica sensu lato* as both species: the pathogenic species (*E. histolytica sensu stricto*) and the non-pathogenic species (*E. dispar*). Recently, molecular biology tools have been used to solve this polemic issue. Analysis of the gene encoding for a small subunit ribosomal RNA supported the existence of the two species[11]. On the basis of this rRNA data, two primers were designed to produce polymerase chain reaction amplification from both *E. histolytica* and *E. dispar*. Primer specificity for the two ameba was assessed by theoretical and experimental data base of eukaryotic and prokaryotic DNAs. The amplified stretch encompasses a polimorphic *Dde* I restriction site which allowed, after cleavage of the fragment, *E. histolytica* and *E. dispar* to be distinguished. The reliability of this identification method was assessed comparing the results with those based on classic isoenzyme analysis. Furthermore, molecular biology of the hexokinase isoenzyme pattern was capable to distinguish pathogenic *E. histolytica* from non-pathogenic *E. dispar*[14]. In that study it was also observed that the four obtained bands in the isoenzyme patterns of these two forms of *Entamoeba* were correlated to four different cDNAs and that the four recombinant hexokinases produced in *Escherichia coli* comigrated with their natural counterparts. Molecular biology approaches have been recently used as an adjunct to microscopy, immunoassays and isoenzyme analysis in the diagnosis of amebic diseases[3, 17]. However, immunoassays have been widely employed in the laboratorial routine. Gel diffusion precipitation test (GDP) was considered by some researchers[10, 15, 21] to be one of the most reliable serological tests for diagnosis of amebiasis, since it is well correlated with the clinical course of symptomatic amebiasis. Enzyme-linked immunosorbent assay (ELISA) is also a tool for serodiagnostic method. Many antigens have been reported as specific for diagnosis of amebiasis such as *E. histolytica* trophozoite antigens, HM-1 IMSS[13, 23], pathogen-specific epitopes of the galactose adhesin of *E. histolytica*[7], single recombinant *E. histolytica* antigen, P1-EIA[8] and antigenic 170-Kda subunit of the amebal Gal/GalNAc-lectin[19].

The controversial issue on amebic disease in the Northeast Brazil has been studied in the Laboratório de Imunopatologia Keizo Asami (LIKA) inside of a Brazilian and Japanese joint venture supported by the Japan International Cooperation Agency (JICA).

The prevalence of *E. histolytica* in developing countries is often assumed to be high, frequently without supporting data[13]. In Brazil, studies of *E. histolytica* among low income population have shown a difference among the North, Northeast and South regions. From 1988 to 1994, LIKA performed studies related to the prevalence of *E. histolytica* in Brazilian cities of North (Belém and Manaus), Northeast (Recife, Jaboatão, Bodocó, Palmares, Glória do Goitá and São Luiz) and South (São Paulo) regions. The used methodology varied since traditional serological test, such as GDP, and zymodemes to molecular biology, using genomic DNA amplification. The evaluated strains included both sex and age varying from newborn to adults. The results showed that the Amazon region (North) presented both *E. histolytica* and *E. dispar* with higher prevalence for *E. histolytica* while in the Northeast the *E. dispar* predominated[1, 2, 12, 13, 20].

Contradictory to these findings, BRAGA et al.[4] studying the level of exposure of the Gonçalves Dias community in Fortaleza, Northeast of Brazil, to *E. histolytica*, reported the presence of serum antibodies specific for the Gal/GalNAc lectin. The results of this study demonstrated that this community in the Northeast Brazil is highly endemic for *E. histolytica* with infections rate similar to other developing nations.

In spite of BRAGA's results, different from those found in LIKA studies, the Northeast region seems to have a diverging parasitologic profile concerning the presence of *E. histolytica* and *E. dispar*. An attempt to identify cysts of *E. histolytica* and *E. dispar* in human stools, the application of recombinant DNA technology brings new horizons into the understanding of this protozoan parasite and its disease. The molecular biology techniques are fast, safe and outstanding approaches to overcame doubts and to answer questions about the occurrence of *E. histolytica* or *E. dispar* in the Northeast of Brazil. Polymerase chain reaction (PCR), random amplified polymorphic DNA (RAPD) and other methods have been used to identify and compare strains of Entamoeba[9, 16, 22]. The Entamoeba characterization in the Northeast region of Brazil will be completely elucidated through the molecular biology techniques. For such reasons specific primers[17] will be used, in studies at LIKA, to amplify the genetic material of both *E. histolytica* and *E. dispar*, searching for a complete identification of this protozoa in Northeast region of Brazil.

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