

# Conventional Microscopy Versus Molecular and Immunological Methods in the Diagnosis of Amoebiasis

Dear Editor,

*Entamoeba histolytica*, the causative agent of intestinal amoebiasis affects more than 50 million people worldwide. Amoebiasis is considered to be the most common parasitic infection particularly in the tropics and subtropics.<sup>[1]</sup> It is the second leading cause of the death from parasitic diseases worldwide.<sup>[2]</sup> Humans are the primary reservoir and infection happens to be by ingestion of mature quadri-nucleate cyst through contaminated food and water.<sup>[3]</sup> Treatment and management of infection with *E. histolytica* has been considerably affected since 90% of the infected individuals remain asymptomatic. Clinical diagnosis of amoebiasis also remains elusive in most of the cases due to contrasting illness course in different communities, varied clinical presentations and unavailability of infrastructures in the developing countries.

Difficulty In the diagnosis of amoebiasis is due to the presence of other harmless commensals such as *Entamoeba dispar* as reported by Brumpt in 1925 and other noninvasive amoebae such as *Entamoeba moshkowskyi*, *E. poleki*, *E. coli*, and *E. hartmanni*.<sup>[4-7]</sup>

The laboratory diagnosis of *E. histolytica* currently relies on the direct microscopic identification of the parasite. Other methods of diagnosis include the culture, using Boek and Drbohlav's biphasic amoebic medium, isoenzyme assay using different zymodemes, stool ELISA on monoclonal antibodies to galactose specific adhesin, rapid indirect haemagglutination assay (IHA) to detect serum anti-amoebic antibodies and polymerase chain reaction (PCR) nested multiplex PCR targeting 16S like rRNA gene, real-time PCR, single round PCR, and PCR-RFLP (restriction fragment length polymorphism).<sup>[8-12]</sup>

Of the available diagnostic techniques, the microscopic detection of the morphological forms of the parasite in stool samples is often used in developing countries. Limitation of the microscopic detection is that it is insensitive to differentiate between pathogenic strains of *Entamoeba* from other nonpathogenic amoebae. Diagnosis by culture, though is much sensitive and specific, is laborious and time consuming which may require several weeks. Amoebic culture can also

show false negative results which can be accounted to either delay in processing or probably anti-amoebic therapy prior to stool collection. ELISA using monoclonal antibodies (MAbs) directed against pathogen specific epitopes of the galactose adhesin means to diagnose amoebiasis. Detection of antibodies to *E. histolytica* in patients by using indirect haemagglutination assay (IHA) may fail to distinguish past from present infection.

Results of several studies on detection and differentiation of *E. histolytica*, *E. dispar*, *E. moshkowskyi* and other harmless amoebae in clinical specimen using PCR showed the potential use of molecular methods in the diagnosis of amoebiasis.<sup>[13]</sup> A recent study which involved 218 stool samples has demonstrated the use and role of PCR in differentially diagnosing pathogenic *E. histolytica* (51) from morphologically resembling non-pathogenic *E. dispar* (39),<sup>[14]</sup> which otherwise by conventional microscopy cannot be differentiated. Shih-yu Liang *et al.* in their study have evaluated 130 fecal specimens and showed that molecular methods have 100% specificity towards differential identification of *E. histolytica* and other nonpathogenic amoebae.<sup>[15]</sup> Significance and advantages of DNA based techniques over other methods in identifying the parasites, quantify and provide important information on formulating and implementing the parasite control programs in both human and animal is highlighted in a recent article by Hunt PW.<sup>[14]</sup> Diagnosis of amoebiasis is usually performed on clinical grounds alone in most of the endemic countries having limited resources. Microscopic methods, though are cost-effective require well-trained laboratory personnel. This has remarkably affected the estimates of global prevalence of amoebiasis due to *E. histolytica*. The prevalence and the true epidemiology of amoebiasis are still unclear. Previous studies showing high rates of infection with *E. histolytica* may not be true as studies reported that *E. dispar* is about 10 times more common.<sup>[15]</sup>

The focus should now be on recent developments in the diagnosis and management of amoebiasis. With advance in the laboratory techniques that can differentiate pathogenic *E. histolytica* from other nonpathogenic amoebae studies must be encouraged to estimate the true prevalence of *E. histolytica* infection.

Clinicians and microbiologists must focus on specific diagnosis of *E. histolytica* infection by employing the advanced diagnostic tools, thereby avoiding unnecessary and unwarranted chemotherapy.

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## References

- Nuran D, Gonal A, Mehmet S, Babur C, Kanik A, Emekdas G. Detection of *Entamoeba histolytica*/*Entamoeba dispar* in stool specimens by using enzyme-linked immunosorbent assay. *Mem Inst Oswaldo Cruz* 2004;99:769-72.
- Trol H, Marti H, Weiss N. Simple differential detection of *Entamoeba histolytica* and *Entamoeba dispar* in fresh stool specimens by sodium acetat-acetic acid-formalin concentration and PCR. *J Clin Microbiol* 1997;35:1701-5.
- Orozco E. Pathogenesis in amebiasis. *Infect Agents Dis* 1992;1:19-21.
- Kebede A, Verweij JJ, Petros B, Polderman AM. Short communication: Misleading microscopy in amoebiasis. *Trop Med Int Health* 2004;9:615-652.
- Brumpt E. Differentiation of human intestinal amoebae with four-nucleated cysts. *Trans R Soc Trop Med Hyg* 1928;22:101-14.
- Clark CG, Diamond LS. The Laredo strain and other 'Entamoeba histolytica like' amoebae are *Entamoeba mshkovskii*. *Mol Biochem Parasitol* 1991;46:11-8.
- Pariya SC, Rao RS. Stool culture as a diagnostic aid in the detection of *Entamoeba histolytica* in the faecal specimens. *Indian J Pathol Microbiol* 1995;38:359-63.
- Haq R, Kress K, Wood S, Jackson TF, Lyerly D, Wilkins T, *et al*. Diagnosis of pathogenic *Entamoeba histolytica* infection using a stool ELISA based on monoclonal antibodies to the galactose-specific adhesin. *J Infect Dis* 1993;167:247-9.
- Haq R, Ali IK, Akther S, Petri WA Jr. Comparison of PCR, isoenzyme analysis, and antigen detection for diagnosis of *Entamoeba histolytica* infection. *J Clin Microbiol* 1998;36:449-52.
- Caballero-Salcedo A, Viveros-Rogel M, Salvatierra B, Tapia-Conyer R, Sepulveda-Amor J, Gutierrez G, *et al*. Seroepidemiology of amoebiasis in Mexico. *Am J Trop Med Hyg* 1994;50:412-9.
- Kebede A, Verweij JJ, Endeshaw T, Messele T, Tasew G, Petros B, *et al*. The use of real-time PCR to identify *Entamoeba histolytica* and *E. dispar* infections in prisoners and primary-school children in Ethiopia. *Ann Trop Med Parasitol* 2004;98:43-8.
- Liang SY, Hsia KT, Chan YH, Fan CK, Jiang DD, Landt O, *et al*. Evaluation of a New Single-Tube Multiprobe Real-Time PCR for Diagnosis of *Entamoeba histolytica* and *Entamoeba dispar*. *J Parasitol* 2010;96:793-7.
- Liang SY, Chan YH, Hsia KT, Lee JL, Kuo MC, Hwa KY, *et al*. Development of loop-mediated isothermal amplification assay for detection of *Entamoeba histolytica*. *J Clin Microbiol* 2009;47:1892-5.
- Hunt PW. Molecular diagnosis of infections and resistance in veterinary and human parasites. *Vet Parasitol* 2011;180:12-46. Available from: <http://www.sciencedirect.com/science/article/pii/S0304401711003803>.
- Mirelman D, Nuchamowitz Y, Stolarsky T. Comparison of use of enzyme linked immunosorbent assay-based kits and PCR amplification of RNA genes for simultaneous detection of *Entamoeba histolytica* and *E. dispar*. *J Clin Microbiol* 1997;35:2405-7.

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