Diagnosis and epidemiology of amoebiasis in India

Joyobrato Nath[1], Baby Singha[2], Jaishree Paul[3] and Sankar Kumar Ghosh[4,5,*]

[1]Department of Zoology, Pandit Deendayal Upadhyaya Adarsha Mahavidyalaya, Karimganj 788 723, India
[2]Department of Zoology, Gurucharan College, Silchar 788 004, India
[3]School of Life Sciences, Jawaharlal Nehru University, New Delhi 110 067, India
[4]Department of Biotechnology, Assam University, Silchar 788 011, India
[5]University of Kalyani, Kalyani, Nadia 741 235, India

It is now well established that *Entamoeba histolytica* was indeed a species complex comprising of pathogenic *E. histolytica* and morphologically indistinguishable non-pathogenic *E. dispar* and *E. moshkovskii*. A greater hindrance is the different and inconsistent use of diagnostic methods in different areas of the world. Though microscopy has poor sensitivity, it seems that till today, many epidemiological studies are either based on microscopy alone or PCR assay carried out on microscopy screened samples or PCR assay performed on a very small sample size and thus fails to figure out the true magnitude of amoebiasis. The present review recommends DNA-based systematic approach like rDNA-based DNA dot blot screening followed by PCR assay to determine the true prevalence rate, suggesting its implication in the large-scale epidemiological study. DNA-based studies from across the world showed that the prevalence rate varies from 0.55% to 69.6% among human populations. The studies indicate that various unhygienic practices like unhygienic toilet facilities, poor living conditions, hand washing habits, etc. HIV infection and mutation in LEPR are among common factors that increase the likelihood of amoebiasis. On the other hand, till today it remains unclear if the *E. histolytica* causing intestinal and extra-intestinal amoebiasis is a similar or different strain.

Keywords: *Entamoeba* complex, microscopy, DNA-based diagnosis and molecular epidemiology, risk factor, strain typing.

AMOEBIASIS as a deadly disease may have been first recognized by Hippocrates (460–377 BC)[1]. Later, Feder Losch in 1875 first identified *Entamoeba histolytica* in human faecal samples and considered it to be associated only with inflammatory process and accordingly named it *Amoeba coli*[2]. Many years later in 1903 *E. histolytica* and *E. coli* were differentiated by Fritz Schaudinn, who documented the taxonomic description with the name of *E. histolytica*[3].

In 1925, *E. histolytica* was differentiated from its closely related species *E. dispar*, which was proposed to be non-pathogenic and found only in asymptomatic carriers[4]. However, it was ignored for 50 years till the biochemical evidence was reported in 1973 (ref. 5). The development of axenic culture medium in 1960 further allowed *in vivo* and *in vitro* studies of *E. histolytica* strain[6]. Its discrimination using isoenzyme electrophoresis confirmed that *E. histolytica* was in fact a species complex consisting of pathogenic and non-pathogenic species[7]. Amoebiasis causes approximately 100,000 deaths annually, placing it second to malaria in terms of mortality worldwide among protozoan parasite infections[8].

The existence of *Entamoeba* complex in the human population has been discussed since 1925, when Emile Brumpt proposed the existence of two morphologically similar species namely pathogenic *E. histolytica* and non-pathogenic *E. dispar*. During the second half of the 20th century, the knowledge of these two species and a third nonpathogenic species *E. moshkovskii* suggested the rapid development of sensitive and specific molecular or immunological-based diagnostic technology[9–11]. Thus, reclassification of *E. histolytica* into *Entamoeba* complex comprising of *E. histolytica*, *E. dispar* and *E. moshkovskii* has further added complexity to the epidemiology of amoebiasis as they cannot be distinguished by microscopy which is the most commonly used clinical diagnostic method (Figure 1).

The aim of the present review is to highlight the current methods of diagnosis for both clinical and epidemiological study. The article also reviews the burden of these morphologically similar and genetically different species of *Entamoeba* to draw a scenario of the prevalence of amoebiasis among the human population. Moreover, in an effort to better understand the relationship between genotype and virulence pattern, a systemic review is necessary to uncover the current methods for *E. histolytica* strain discrimination.

Conventional approaches in amoebiasis diagnosis: their limitations

The main purpose of detection and differentiation of *E. histolytica* from other *Entamoeba* species in stool samples is the detection of the causative agent of amoebiasis.

*For correspondence. (e-mail: drsankarghosh@gmail.com)

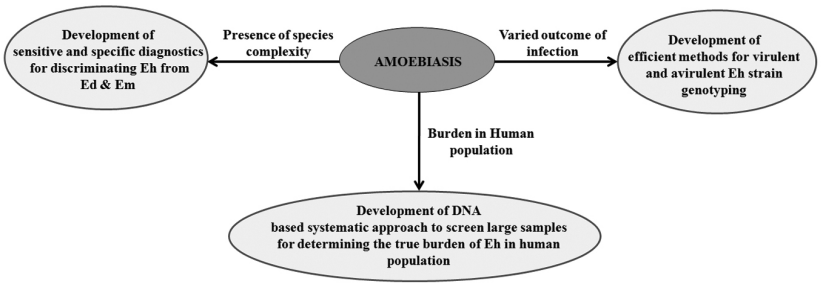

Figure 1. Challenges associated with the amoebiasis from diagnostic and genotyping point of view. Eh = *E. histolytica*; Ed = *E. dispar*; Em = *E. moshkovskii.*

Microscopy-based diagnosis

Traditional diagnosis of amoebiasis relies on microscopy of wet preparation, concentrations and permanently stained smears of faeces. Confusion between *E. histolytica* and the other two non-pathogenic *Entamoeba* species, amoebae like *Endolimax nana* and white blood cells in the faeces frequently results in over-representation[12]. Haematophagous amoebic trophozoite in a stool sample is the foremost key in the diagnosis of *E. histolytica* in wet preparation; however such a finding is rarely seen[13]. The existence of *E. dispar* and *E. moshkovskii* in humans has made the classic diagnosis of *E. histolytica* by microscopy impossible as the three species cannot be discriminated microscopically. As a result, sensitivity and specificity of microscopy are less than 60% and can be perplexed by the false positive result. Moreover, microscopy performed on aspirated material from the amoebic liver abscess (ALA), are often negative for trophozoites[14]. However, comparatively a better sensitivity of 59.4% could be attained when formalin-ether sedimentation and trichrome staining technique were performed together[15]. Recently, in indirect immunofluorescence microscopy, bioconjugated fluorescent silica nanoparticles were used for rapid detection of *E. histolytica*[16]. Colonoscopy followed by microscopy of the material aspirated or scraped from the site of ulcers may enhance the diagnostic capability of colonoscopy for invasive intestinal amoebic colitis.

Culture-based diagnosis

Different culture techniques have been used for amoebiasis diagnosis. Robinson medium[17] and TYSGM-9 medium[18] are the more commonly used media for the xenic cultivation of *E. histolytica*, while TP-S-1, TY I-S-33 and YI-S are the common media in axenic cultivation[14]. However, culture methods are time-consuming, laborious and often unrewarding, with a sensitivity of only about 50% and require a technique for species discrimination[19]. As the culture of *E. histolytica* from clinical samples such as faeces or liver abscesses has a significant false-negative rate, is time-consuming and is technically difficult, it is not accepted for routine clinical diagnosis.

Antigen and antibody-based diagnosis

Isoenzyme analysis, particularly hexokinase, antibody-based detection assays like indirect hemagglutination, immunoelectrophoresis, counterimmunoelectrophoresis, immunodiffusion, complement fixation, indirect immunofluorescence assay, enzyme-linked immunosorbent assay, etc., the heavy subunit of the galactose/N-acetyl-galactosamine inhibitable lectin-based detection assay are used for species discrimination. However, an isoenzyme analysis usually takes one to several weeks as it completely relies on successful amoebae culture, making it unfeasible for use in the routine diagnosis of amoebiasis. Furthermore, the amoebic cultures and therefore isoenzyme analyses are negative for many microscopy positive stool samples[14].

Additionally, the problem with an antibody-based diagnosis is that serum IgG antibodies persist for years, even after successful therapy and/or eradication of the parasite. This limits the usefulness of the test, particularly in endemic areas due to its failure in distinguishing past from current infection. However, from a diagnostic point of view serological tests may be helpful in developed countries, where amoebiasis is uncommon. While most studies have reported excellent sensitivity and specificity of the enzyme-linked immunosorbent assay, there are a number of studies where researchers found both lower than expected sensitivities or specificities and cross-reactivity of ELISA[20–22].

DNA-based diagnosis in molecular epidemiology

In the post-genomic era, new diagnostic tools specific to *E. histolytica* are being exploited by clinicians and researchers to differentially identify and treat patients as well as to estimate the true burden. DNA-based tests for diagnosis of amoebiasis have already become the gold standard by which sensitivity and specificity of other diagnostic techniques are measured. In studies conducted in

Ethiopia and Nicaragua, PCR results showed that *E. histolytica* is a rare finding in patients with diarrhoea[23,24]. DNA probe-based technique[25], PCR-based assays, including gene amplification with specific primers, multiplex PCR, nested PCR, PCR-restriction fragment length polymorphism, real-time PCR, touchdown PCR and microarray[26] have been adapted for accurate detection of *E. histolytica*.

The successful amplification of the signature genomic location of the parasite using PCR depends on the quality of genomic DNA. Modified genomic DNA isolation protocol particularly raising of lysis temperature, addition of the freeze-thaw cycle proved to be useful in extracting DNA from many enteric protozoa in the faeces[27]. PCR is suitable for detection of *E. histolytica* in formalin-fixed, paraffin-embedded samples that are younger than 2 decades, while PCR was unable to detect it in samples older than 3 decades[28]. Additionally, the sensitivity of PCR, particularly in low intensity infection, can be increased by using primers targeting the rDNA plasmid due to its high copy number.

Diagnosis of amoebic liver abscess

Conventional techniques like ultrasound, computer tomography, technectium-99 liver scan and magnetic resonance cannot distinguish amoebic liver abscess (ALA) from pyogenic abscesses or necrotic tumours. Microscopy performed on aspirated material from a ALA is often negative for trophozoites[14]. Serological tests like IHA is not recommended nowadays as it cannot corroborate the present from past infection. Several studies from different parts of the world have recommended TechLab *E. histolytica* II kit and species-specific PCR assay for the definitive diagnosis of extraintestinal ALA[29].

Strain variation and its detection

Besides diagnosis, till today it is not very clear whether or not the intestinal and extra-intestinal amoebiasis is due to infection of similar *E. histolytica* strain. Reports have shown that some *E. histolytica* isolates are more virulent than others and thus it is quite possible that the genotype of the strain influences the outcome of infection[30]. Moreover, as 90% of the infections remain asymptomatic, it further suggests along with a sensitive and specific diagnosis of amoebiasis, development of genotyping technique to identify the virulent strains.

A longitudinal study conducted in South Africa revealed that highly polymorphic, interspersed, short tandem repeats (STR) scattered between tRNA genes were suitable for tracking the transmission of a known strain[31]. A study found one asymptomatic isolate with unique STR patterns in 4 tRNA-linked STR loci from Japanese *E. histolytica* samples[32]. The polymorphic nature of the multiple tandem repeats of serine-rich *E. histolytica* protein (SREHP) and chitinase are also the basis for strain identification. It has been reported that SREHP genotypes of clinical isolates from patients with liver abscesses were unique and distinct from those derived from intestinal amoebic patients[33]. However, some studies observed a non-significant genotypic difference between isolates from diarrhoeic and non-diarrheic samples, but significant presence of profiles unique to a particular geographical area was found[34,35]. A recent study revealed the presence of several different genotypes of *E. histolytica* among patients with liver abscesses and thus it is erroneous to correlate a single genotype with it[36].

EhLINEs/EhSINEs together account for about 11.2% of the *E. histolytica* genome. Due to their mobile nature, they may influence the virulence of the parasite by activating or silencing the genes in their vicinity. In a modified AFLP procedure called 'Transposon display', unique banding pattern for each strain of HM-1: IMSS, 200: NIH, HK-9 and Rahman has been generated[25]. Similarly, in a recent study SINE occupancy at different loci was used to classify axenically cultivated *E. histolytica* strains and clinical isolates into distinct genotypes[30]. Comparison of the transcriptome of highly virulent HM-1: IMSS and less virulent Rahman strain highlighted lower expression of 32 genes, including cysteine proteinases, AIG family members and lasting light chains. Thus, one possible explanation for the differential outcome of disease may be that a single gene product is not responsible, but rather multiple pathways[37]. Additionally, small RNA pyrosequencing indicates that small RNA population may contribute differential gene expression between virulent and nonvirulent strains[38].

Approaches in epidemiology of amoebiasis

A greater hindrance is the varied, inconsistent application of existing methods in different areas of the world. A summary of different approaches used for estimating the magnitude of amoebiasis in the human population is shown in Figure 2.

Microscopy-based conventional approach

As sensitivity and specificity of microscopy are poor (generally less than 60%) and can be perplexed by the false positive result, it is not possible to accurately estimate the true burden of amoebiasis in human populations. Despite poor sensitivity and specificity, microscopy-based detection is still used both in clinical laboratories for diagnostic purposes as well as in research laboratories for epidemiological study.

PCR-based conventional approach

These days most epidemiological studies carried out for amoebiasis in developing countries are based either on

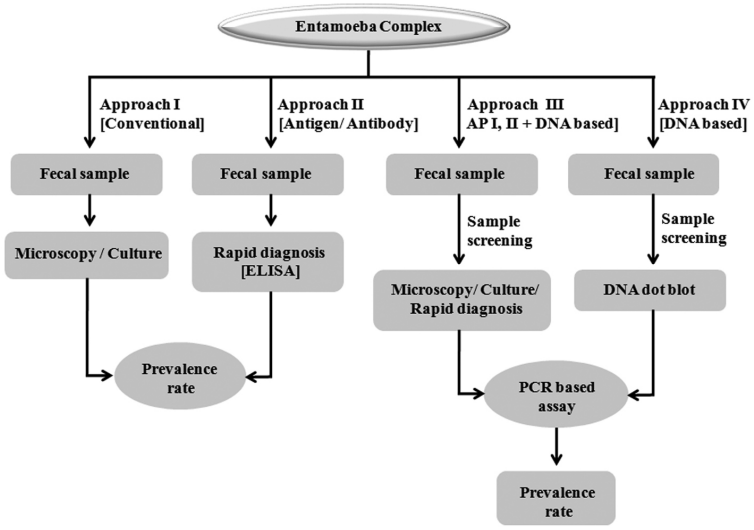

Figure 2. Conventional and molecular approaches used by different workers in the epidemiology of amoebiasis.

PCR assay performed on small sample size or on culture/ microscopy/ELISA screened samples. Both microscopy and culture have poor sensitivity and specificity and ELISA shows cross reactivity[39]. Hence the true magnitude of the disease cannot be determined as the screening tools have low sensitivity and often encounter false negatives. In addition, it is also not possible for developing countries to carry out epidemiological studies on large samples using PCR assay due to the cost of DNA isolation and downstream PCR reaction. Thus, most of the molecular epidemiological studies were based on small sample size.

Integrated, systematic approach

We have reported an entirely DNA-based systematic approach in our previous study where samples were initially screened using a DNA-based dot-blot technique followed by PCR assay for species discrimination. The sensitivity and specificity of the dot-blot analysis were found to be 100% and 99% respectively, suggesting the implication of the approach for large-scale epidemiological study[27]. This approach is best suited for large-scale epidemiological study, particularly in developing countries as it scales down the: (1) cost of a large number of stool kits for isolating genomic DNA and (2) number of subsequent PCR reactions for species discrimination.

Epidemiology of amoebiasis

The true epidemiology of amoebiasis remains unknown because much of the earlier published data are based on microscopy. With the discrimination of *E. histolytica* from *E. dispar*, most individuals who were previously believed to have asymptomatic infection with *E. histolytica* actually carry *E. dispar*. Worldwide PCR-based studies indicate that prevalence of amoebiasis varies greatly in different parts of the world.

Indian scenario

Sporadic studies have been performed in India, but detailed analysis, especially after the re-description of *E. histolytica* and *E. dispar* and with the report of colonization of *E. moshkovskii* in human, has been reported only from a few sites of India. Microscopy-based prevalence rates of 14.8%, 42.0% and 21.8% were also recorded respectively from Karnataka, Himachal Pradesh and Tamil Nadu[40–42].

Mukherjee *et al.*[43] reported a prevalence rate of 3.6% using direct microscopy, PCR, and ELISA amongst the diarrhoeal patients in Kolkata. Prakash *et al.*[44] compared PCR assay and microscopy of stool samples from patients with intestinal and ALA cases. Riboprinting of rRNA genes from amoeba has also been used[45]. Srivastava *et al.*[25] using PCR assay reported a prevalence rate of 8.8% among volunteers, residing in a New Delhi slum. Comparatively a low prevalence rate of 1.7% using small subunit (SSU) rRNA gene-based PCR was reported from Pondicherry[10]. In a comparative study conducted in the same lab, prevalence rate of intestinal amoebiasis was reported around 19.9% using microscopy. However, nested multiplex PCR, confirmed *E. histolytica* in only 12.2% of positive samples[46]. We earlier reported an overall prevalence rate of 13.7% among the Northeast Indian population using an integrated systematic molecular approach[47]. In the current year, a seroprevalence rate of 15.38% was reported among suspected amoebiasis groups from South India[22].

International scenario

In Dhaka (Bangladesh), where diarrhoeal diseases are the leading cause of death in children younger than six years

Table 1. Prevalence rate of *E. histolytica* reported from different parts of the world within last five years

Entamoeba spp.	Prevalence	Technique	Reported from (year)
E. histolytica	1.5%	PCR	Iran (2011)[51]
E. histolytica	14.0%	PCR	Palestine (2011)[52]
E. histolytica	9.1% (Eh), 1.4% (Eh + Ed)	PCR	Malaysia (2012)[53]
E. histolytica	9.0%	PCR	Pakistan (2012)[54]
E. histolytica	10%	PCR	UAE (2013)[21]
E. histolytica	54.5%	Microscopy	Rwanda (2013)[55]
E. histolytica	10.2% (Eh), 3.3% (Eh + Ed)	PCR	Malaysia (2013)[56]
E. histolytica	36.6% and 19.4%	Antigen-based	Brazil (2013)[57]
E. histolytica	4%	PCR	Venezuela (2013)[58]
E. histolytica/dispar	48.0%	Microscopy	Saudi Arabia (2014)[59]
E. histolytica	22.55%	PCR	Cote d'Ivoire (2014)[60]
E. histolytica	0.55%	PCR	Colombia (2015)[61]
E. histolytica	39.4%	Microscopy	Egypt (2015)[62]
E. histolytica	28.2%	ELISA	Mexico (2015)[63]
E. histolytica	1.7%	PCR	Ethiopia (2017)[64]
E. histolytica	23.8%	PCR	Brazil (2016)[39]
E. histolytica/dispar	16.15%	Microscopy	Saudi Arabia (2016)[65]
E. histolytica/dispar	15%	Microscopy	Uganda (2016)[66]
E. histolytica	1.4%	Microscopy	Germany (2016)[67]

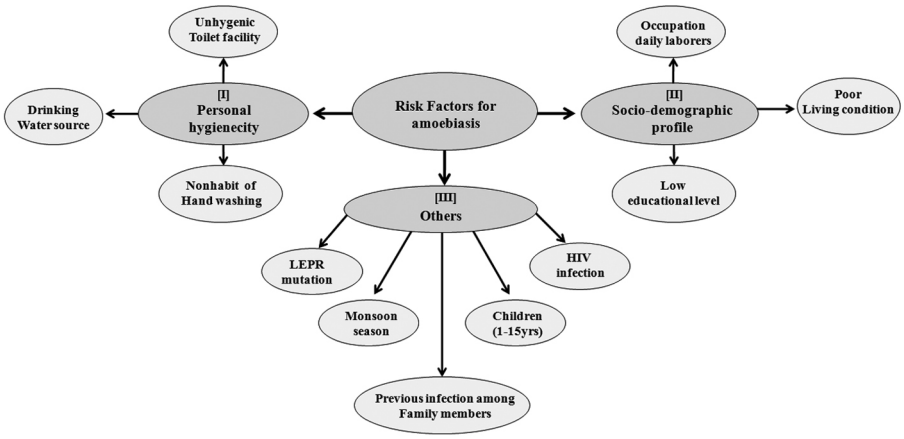

Figure 3. Risk factors and predictors of high *E. histolytica* infection.

of age, 50% of children have serological evidence of amoebiasis by five years of age[48]. A high frequency of *E. histolytica* asymptomatic infection, higher than *E. dispar* infection (13.8% versus 9.6%), was detected by PCR in a rural Mexican community[49]. A much higher prevalence of *E. histolytica* monoinfection of 69.6% and mixed infection of 7.6% was found among children in Gaza, Palestine[50]. Besides PCR, many studies have documented the prevalence rate using ELISA, serological assay, and even direct microscopy as shown in Table 1[51–67]. Thus, it is very difficult to compare the true prevalence of amoebiasis because of the lack of uniformity in diagnostic methods.

Burden of nonpathogenic species in human population

Existence of *E. moshkovskii* in Indian population was reported for the first time from a research hospital in Pondicherry which revealed a higher prevalence of *E. dispar* (8.8%) and *E. moshkovskii* (2.2%)[10]. PCR analysis of microscopically positive samples demonstrated a 1 : 46 ratio of *E. histolytica* to *E. dispar* from Agboville[68]. Much higher *E. moshkovskii* prevalence rates of 13% and 12.8% were reported among HIV seropositive patients of Tanzania and Southern Assam respectively[69,70]. A study conducted among Ethiopian prisoners and primary-school children highlighted 91.4% of the microscopy positive samples as *E. dispar*[23]. Similarly, in Australia, 50% of the microscopy positive faecal samples were found to be *E. moshkovskii* positive in the PCR assay[11]. It is now well established that prevalence of *E. moshkovskii* and *E. dispar* is much higher than that the *E. histolytica* in human population.

Presence of non-pathogenic *E. moshkovskii* has also been reported from countries like Bangladesh, Turkey, India, Iran, Australia, Tanzania, Colombia and Malaysia and they are usually not associated with the disease[47]. Recently much higher prevalence rates of *E. moshkovskii*

(25.4%) and *E. dispar* (23.2%) compared to *E. histolytica* (0.55%) were reported from Colombia[60]. Interestingly, studies from India and Malaysia reported mono-infection of *E. moshkovskii* in symptomatic patients[10,71]. In our earlier study, we have also found few symptomatic participants mono-infected with *E. moshkovskii*. However, further studies in this direction are needed to justify the role of *E. moshkovskii* in gastroenteritis disorders and its virulence.

Risk factors associated with amoebiasis

People from households with an average socio-economic status generally had a much higher risk of infection. A case-control study conducted among residents of Hanam province in settings where Nhue river waters are intensively used in agriculture suggested that socio-economic and personal hygiene factors determine *E. histolytica* infection[72]. Being an opportunistic parasite, infection rate showed inconsistency between male and female hosts[73]. With regard to host genetic susceptibility, an amino acid substitution in the cytokine receptor homology domain 1 of LEPR is reported to be associated with increased susceptibility to *E. histolytica* infection. Children carrying the allele for arginine (223R) were nearly 4 times more likely to have an infection compared to those homozygous for the ancestral glutamine allele (223Q). An association of this allele was also found with ALA in a cohort of adult patients in Bangladesh and North India[74,75].

Chen *et al.*[76] identified rural areas of the respondents where alcoholism and diabetes were risk factors of amoebic liver abscess in Southeast Taiwan. Zhou *et al.*[77] observed receptive anal sex and sadomasochistic behaviour as the potential predictors of *E. histolytica* infection among men who have sex with men. Studies also indicated low personal hygiene, poor rural background, contact with animals, not washing hands after playing with soil or gardening and presence of already infected family members were important predictors for intestinal protozoan infections including *E. histolytica*[38,47]. The factors that increase the likelihood of amoebiasis as already discussed are highlighted in Figure 3.

A study conducted on antiretroviral-treated HIV/AIDS patients in Ethiopia also reported that unavailability of latrines and lack of handwashing with soap were associated with *E. histolytica*/*dispar* and *Giardia lamblia* infections[78]. Like other opportunistic parasites, *E. histolytica* also takes advantage of a weaker immune system of AIDS patients and the parasite load generally depends on the clinical status of the patients like CD4 T cell count, and ART status[27]. Apart from CD4 cell count and ART, HIV sero-positive men who have sex with men in Taiwan are at increased risk of *E. histolytica* infection and invasive amoebiasis[79]. Though many reported presence of *Entamoeba* species among domestic and wild animals, few studies confirmed a significant association of parasite infection in human and their close contact with domestic animals, especially dogs and cats[72].

Conclusion

A greater hindrance is the varied and inconsistent application of existing methods in different areas of the world. Though rDNA-based species-specific PCR assay is the method of choice, it is difficult to employ PCR assay in large-scale epidemiological studies due to the cost of DNA isolation and PCR assay. Thus, many studies till now, reported the burden of amoebiasis where the diagnosis is based on microscopy alone or PCR assay performed on the microscopically screened sample. This perhaps makes it impossible to compare the true prevalence rate even after ninety years of the Brumpt theory. In this direction, our review suggests either the use of more than one microscopy-based technique or ELISA together as screening assay or entirely rDNA based dot-blot technique as a screening assay. Both these approaches will increase the sensitivity while minimizing the cost associated with genomic DNA isolation and downstream PCR reaction. Our review also highlights that unhygienic toilet facilities, poor living conditions, various unhygienic practices, HIV infection, and mutation in LEPR are among the risk factors and predictors of amoebiasis. To understand the actual dynamics of transmission in populations particularly those in close contact with domestic animals genotyping of *E. histolytica* from humans and animals are highly recommended. In addition, though many developed techniques for strain genotyping are available, it still remains unclear if the *E. histolytica* that causes intestinal and extra-intestinal amoebiasis is a similar or different strain.

1. Kean, B. H., A history of amebiasis. In *Amoebiasis: Human Infection by Entamoeba histolytica* (ed. Ravdin, J. I.), John Wiley, New York, 1988, pp. 1–10.
2. Lesh, F. A., Massive development of amoebas in the large intestine. *Am. J. Trop. Med. Hyg*., 1975, **24**, 383–392.
3. Schaudinn, F., The development of some rhizopoda (preliminary report). *Arbeiten aus dem Kaisherlichen Gesundheitsamte*, 1903, **19**, 547.
4. Brumpt, E., Étude sommaire de l' '*Entamoeba dispar*' n. sp. Amibe à kystes quadrinucléés, parasite de l'homme. *Bull. Acad. Med*., 1925, **94**, 943–952.
5. Martinez-Palomo, A., Gonzalez-Robles, A. and de la Torre, M., Selective agglutination of pathogenic strains of *Entamoeba histolytica* induced con A. *Nat. New Biol.*, 1973, **245**, 186–187.
6. Diamond, L. S., Axenic cultivation of *Entamoeba histolytica*. *Science*, 1961, **134**, 336–337.
7. Sargeaunt, P. G., Williams, J. E. and Grene, J. D., The differentiation of invasive and non-invasive *Entamoeba histolytica* by isoenzyme electrophoresis. *Trans. R. Soc. Trop. Med. Hyg*., 1978, **72**, 519–521.
8. World Health Organization, Amoebiasis. *Weekly Epidemiological Record*, 1997, **72**, 97–99.

9. Ximenez, C. *et al.*, Novelties on amoebiasis: a neglected tropical disease. *J. Glob. Infect. Dis.*, 2011, **3**, 166–174.
10. Parija, S. C. and Khairnar, K., *Entamoeba moshkovskii* and *Entamoeba dispar* associated infections in Pondicherry, India. *J. Health Popul. Nutr.*, 2005, **23**, 292–295.
11. Fotedar, R., Stark, D., Marriott, D., Ellis, J. and Harkness, J., *Entamoeba moshkovskii* infections in Sydney, Australia. *Eur. J. Clin. Microbiol. Infect. Dis.*, 2008, **27**, 133–137.
12. Krogstad, D. J., Spencer Jr, H. C., Healy, G. R., Gleason, N. N., Sexton, D. J. and Herron, C. A., Amebiasis: epidemiologic studies in the United States, 1971–1974. *Ann. Intern. Med.*, 1978, **88**, 89–97.
13. Talamas-Lara, D. *et al.*, Erythrophagocytosis in *Entamoeba histolytica* and *Entamoeba dispar*: a comparative study. *Biomed Res. Int.*, 2014, **2014**, 626259.
14. Fotedar, R., Stark, D., Beebe, N., Marriott, D., Ellis, J. and Harkness, J., Laboratory diagnostic techniques for *Entamoeba* species. *Clin. Microbiol. Rev.*, 2007, **20**, 511–532.
15. Anuar, T. S. *et al.*, Evaluation of formalin-ether sedimentation and trichrome staining techniques: its effectiveness in detecting *Entamoeba histolytica*/*dispar*/*moshkovskii* in stool samples. *J. Microbiol. Methods*, 2013, **92**, 344–348.
16. Hemadi, A. *et al.*, Bioconjugated fluorescent silica nanoparticles for the rapid detection of *Entamoeba histolytica*. *Acta Trop.*, 2015, **145**, 26–30.
17. Robinson, G. L., The laboratory diagnosis of human parasitic amoebae. *Trans. R. Soc. Trop. Med. Hyg.*, 1968, **62**, 285–294.
18. Diamond, L. S., A new liquid medium for xenic cultivation of *Entamoeba histolytica* and other lumen dwelling protozoa. *J. Parasitol.*, 1982, **68**, 958–959.
19. van Hal, S. J., Stark, D. J., Fotedar, R., Marriott, D., Ellis, J. T. and Harkness, J. L., Amoebiasis: current status in Australia. *Med. J. Aust.*, 2007, **186**, 412–416.
20. Stark, D. *et al.*, Comparison of stool antigen detection kits to PCR for diagnosis of amebiasis. *J. Clin. Microbiol.*, 2008, **46**, 1678–1681.
21. Elbakri, A., Samie, A., Ezzedine, S. and Odeh, R. A., Differential detection of *Entamoeba histolytica*, *Entamoeba dispar* and *Entamoeba moshkovskii* in fecal samples by nested PCR in the United Arab Emirates (UAE). *Acta Parasitol.*, 2013, **58**, 185–190.
22. Dhanalakshmi, S. and Parija, S. C., Seroprevalence of *Entamoeba histolytica* from a tertiary care hospital, South India. *Trop. Parasitol.*, 2016, **6**, 78–81.
23. Kebede, A. *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**, 43–48.
24. Leiva, B., Lebbad, M., Winiecka-Krusnell, J., Altamirano, I., Tellez, A. and Linder, E., Overdiagnosis of *Entamoeba histolytica* and *Entamoeba dispar* in Nicaragua: a microscopic, triage parasite panel and PCR study. *Arch. Med. Res.*, 2006, **37**, 529–534.
25. Srivastava, S., Bhattacharya, S. and Paul, J., Species- and strain-specific probes derived from repetitive DNA for distinguishing *Entamoeba histolytica* and *Entamoeba dispar*. *Exp. Parasitol.*, 2005, **110**, 303–308.
26. Nath, J., Ghosh, S. K. and Singha, B., Problem in amoebiasis diagnosis in clinical setting: a review from conventional microscopy to advance molecular based diagnosis. *J. Acad. Indus. Res.*, 2013, **2**, 257–261.
27. Nath, J., Banyal, N., Gautam, D. S., Ghosh, S. K., Singha, B. and Paul, J., Systematic detection and association of *Entamoeba* species in stool samples from selected sites in India. *Epidemiol. Infect.*, 2015, **143**, 108–119.
28. Frickmann, H. *et al.*, Influence of parasite density and sample storage time on the reliability of *Entamoeba histolytica*-specific PCR from formalin-fixed and paraffin-embedded tissues. *Diagn. Mol. Pathol.*, 2013, **22**, 236–244.
29. Parija, S. C., Mandal, J. and Ponnambath, D. K., Laboratory methods of identification of *Entamoeba histolytica* and its differentiation from look-alike *Entamoeba* spp. *Trop. Parasitol.*, 2014, **4**, 90–95.
30. Kumari, V. *et al.*, Genomic distribution of SINEs in *Entamoeba histolytica* strains: implication for genotyping. *BMC Genomics*, 2013, **14**, 432.
31. Zaki, M., Reddy, S. G., Jackson, T. F., Ravdin, J. I. and Clark, C. G., Genotyping of *Entamoeba* species in South Africa: diversity, stability, and transmission patterns within families. *J. Infect. Dis.*, 2003, **187**, 1860–1869.
32. Escuetade Cadiz, A., Kobayashi, S., Takeuchi, T., Tachibana, H. and Nozaki, T., Identification of an avirulent *Entamoeba histolytica* strain with unique tRNA linked short tandem repeat markers. *Parasitol. Int.*, 2010, **59**, 75–81.
33. Ayeh-Kumi, P., Ali, I. M., Lockhart, L. A., Gilchrist, C. A. and Petri Jr, W. A., *Entamoeba histolytica*: genetic diversity of clinical isolates from Bangladesh as demonstrated by polymorphisms in the serine-rich gene. *Exp. Parasitol.*, 2001, **99**, 80–88.
34. Samie, A. *et al.*, *Entamoeba histolytica*: genetic diversity of African strains based on the polymorphism of the serine-rich protein gene. *Exp. Parasitol.*, 2008, **118**, 354–361.
35. ElBakri, A., Samie, A., Ezzedine, S. and Odeh, R., Genetic variability of the serine-rich *Entamoeba histolytica* protein gene in clinical isolates from the United Arab Emirates. *Trop. Biomed.*, 2014, **31**, 370–377.
36. Simonishvili, S. *et al.*, *Entamoeba histolytica*: the serine-rich gene polymorphism-based genetic variability of clinical isolates from Georgia. *Exp. Parasitol.*, 2005, **110**, 313–317.
37. Freitas, M. A. R. *et al.*, Differentially expressed genes of virulent and nonvirulent *Entamoeba histolytica* strains identified by suppression subtractive hybridization. *Biomed. Res. Int.*, 2014, **2014**, 285607.
38. Zhang, H., Ehrenkaufer, G. M., Hall, N. and Singh, U., Small RNA pyrosequencing in the protozoan parasite *Entamoeba histolytica* reveals strain-specific small RNAs that target virulence genes. *BMC Genomics*, 2013, **14**, 53.
39. Calegar, D. A. *et al.*, Frequency and molecular characterisation of *Entamoeba histolytica*, *Entamoeba dispar*, *Entamoeba moshkovskii*, and *Entamoeba hartmanni* in the context of water scarcity in northeastern Brazil. *Mem. Inst. Oswaldo Cruz.*, 2016, **111**, 114–119.
40. Shubha, D. and Fatima, F., A coprological survey for assessing intensity of parasitic infection in school children: cross-sectional study. *Trop. Parasitol.*, 2011, **1**, 88–93.
41. Barda, B. *et al.*, Freezing parasites in pre-Himalayan region, Himachal Pradesh: Experience with mini-FLOTAC. *Acta Trop.*, 2013, **130**, 11–16.
42. Dhanabal, J., Selvadoss, P. P. and Muthuswamy, K., Comparative study of the prevalence of intestinal parasites in low socioeconomic areas from South Chennai, India. *J. Parasitol. Res.*, 2014, **2014**, 630968.
43. Mukherjee, A. K., Das, K., Bhattacharya, M. K., Nozaki, T. and Ganguly, S., Trend of *Entamoeba histolytica* infestation in Kolkata. *Gut Pathog.*, 2010, **2**, 12.
44. Prakash, A., Chakraborti, A., Mahajan, R. C. and Ganguly, N. K., *Entamoeba histolytica*: rapid detection of Indian isolates by cysteine proteinase gene-specific polymerase chain reaction. *Exp. Parasitol.*, 2000, **95**, 285–287.
45. Mukhopadhyay, A., Chakraborti, A., Mahajan, R. C. and Ganguly, N. K., *Entamoeba histolytica*: rapid identification and differentiation of Indian isolates by riboprinting. *Exp. Parasitol.*, 2002, **102**, 109–112.
46. Parija, S. C., Garg, A., Pushpa, K., Khairnar, K. and Priya, T., Polymerase chain reaction confirmation of diagnosis of intestinal amebiasis in Puducherry. *Indian J. Gastroenterol.*, 2010, **29**, 140–142.

47. Nath, J., Ghosh, S. K., Singha, B. and Paul, J., Molecular epidemiology of amoebiasis: a cross-sectional study among North East Indian population. *PLoS Negl. Trop. Dis*., 2015, **9**, e0004225.
48. Haque, R., Ali, I. K. M. and Petri Jr, W. A., Prevalence and immune response of *Entamoeba histolytica* infection in preschool children in Bangladesh. *Am. J. Trop. Med. Hyg*., 1999, **60**, 1031–1034.
49. Ramos, F. *et al.*, High prevalence rate of *Entamoeba histolytica* asymptomatic infection in a rural Mexican community. *Am. J. Trop. Med. Hyg*., 2005, **73**, 87–91.
50. Al-Hindi, A. *et al., Entamoeba histolytica* or *Entamoeba dispar* among children in Gaza, Gaza Strip? *J. Egypt. Soc. Parasitol*., 2005, **35**, 59–68.
51. Pestehchian, N., Nazary, M., Haghighi, A., Salehi, M. and Yosefi, H., Frequency of *Entamoeba histolytica* and *Entamoeba dispar* prevalence among patients with gastrointestinal complaints in Chelgerd city, southwest of Iran. *J. Res. Med. Sci*., 2011, **16**, 1436–1440.
52. Hussein, A. S., Prevalence of intestinal parasites among school children in northern districts of West Bank-Palestine. *Trop. Med. Int. Health*, 2011, **16**, 240–244.
53. Ngui, R. *et al.*, Differentiating *Entamoeba histolytica*, *Entamoeba dispar* and *Entamoeba moshkovskii* using nested polymerase chain reaction (PCR) in rural communities in Malaysia. *Parasit. Vectors*, 2012, **5**, 187.
54. Yakoob, J., Abbas, Z., Beg, M. A., Naz, S., Khan, R. and Jafri, W., *Entamoeba* species associated with chronic diarrhoea in Pakistan. *Epidemiol. Infect*., 2012, **140**, 323–328.
55. Emile, N., Bosco, N. J. and Karine, B., Prevalence of intestinal parasitic infections and associated risk factors among Kigali institute of education students in Kigali, Rwanda. *Trop. Biomed.*, 2013, **30**, 718–726.
56. Lau, Y. L., Anthony, C., Fakhrurrazi, S. A., Ibrahim, J., Ithoi, I. and Mahmud, R., Real-time PCR assay in differentiating *Entamoeba histolytica*, *Entamoeba dispar*, and *Entamoeba moshkovskii* infections in Orang Asli settlements in Malaysia. *Parasit. Vectors*, 2013, **6**, 250.
57. Santos, R. V., Nunes Jda, S., Camargo, J. A., Rocha, E. M., Fontes, G. and Camargo, L. M., High occurrence of *Entamoeba histolytica* in the municipalities of Ariquemes and Monte Negro, State of Rondônia, Western Amazonia, Brazil. *Rev. Inst. Med. Trop. Sao. Paulo*., 2013, **55**, 193–196.
58. Bracho Mora, A., Rivero de Rodriguez, Z., Arraiz, N., Villalobos, R. and Urdaneta, H., Detection of *Entamoeba histolytica* and *Entamoeba dispar* by PCR in children, less than five years of age with diarrhea, in Maracaibo, Venezuela. A preliminary study. *Invest. Clin*., 2013, **54**, 373–381.
59. Al-Malki, J. S., Factors associated with high prevalence of *Entamaeba histolytica*/*dispar* infection among children in Jeddah, KSA. *American-Eurasian J. Agric. Environ. Sci*., 2014, **14**, 50–56.
60. Koffi, M., N'Djeti, M., Konan, T. and Dje, Y., Molecular characterization of intestinal protozoan parasites from children facing diarrheal disease and associated risk factors in Yamoussoukro, Côte d'Ivoire. *Afr. J. Environ. Sci. Technol.*, 2014, **8**, 178–184.
61. Lopez, M. C. *et al.*, Molecular epidemiology of *Entamoeba*: First description of *Entamoeba moshkovskii* in a rural area from Central Colombia. *PLoS ONE*, 2015, **10**, e0140302.
62. Sabah, A. A. and Temsah, A. G., Prevalence of some gastrointestinal parasites in diabetic patients in tanta city, Gharbia Governorate, Egypt. *J. Egypt. Soc. Parasitol*., 2015, **45**, 681–684.
63. Alvarado-Esquivel, C., Hernandez-Tinoco, J., Francisco Sanchez-Anguiano, L., RamosNevarez, A., Margarita Cerrillo-Soto, S. and Alberto Guido-Arreola, C., Serosurvey of *Entamoeba histolytica* exposure among Tepehuanos population in Durango, Mexico. *Int. J. Biomed. Sci.*, 2015, **11**, 61–66.
64. Yimer, M., Zenebe, Y., Mulu, W., Abera, B. and Saugar, J. M., Molecular prevalence of *Entamoeba histolytica*/*dispar* infection among patients attending four health centres in northwest Ethiopia. *Trop Doct*., 2017, **47**, 11–15.
65. Hassen Amer, O., Ashankyty, I. M. and Haouas, N. A., Prevalence of intestinal parasite infections among patients in local public hospitals of Hail, Northwestern Saudi Arabia. *Asian Pac. J. Trop. Med*., 2016, **9**, 44–48.
66. Fuhrimann, S. *et al.*, Risk of intestinal parasitic infections in people with different exposures to wastewater and fecal sludge in Kampala, Uganda: a crosssectional study. *PLoS Negl. Trop. Dis.*, 2016, **10**, e0004469.
67. Heudorf, U., Karathana, M., Krackhardt, B., Huber, M., Raupp, P. and Zinn, C., Surveillance for parasites in unaccompanied minor refugees migrating to Germany in 2015. *GMS Hyg. Infect. Control*, 2016, **11**; doi:10.3205/dgkh000265.
68. Heckendorn, F. *et al.*, Species-specific field testing of *Entamoeba* spp. in an area of high endemicity. *Trans. R. Soc. Trop. Med. Hyg*., 2002, **96**, 521–528.
69. Beck, D. L., Dogan, N., Maro, V., Sam, N. E., Shao, J. and Houpt, E. R., High prevalence of *Entamoeba moshkovskii* in a Tanzanian HIV population. *Acta Trop*., 2008, **107**, 48–49.
70. Nath, J., Ghosh, S. K., Bhattacharjee, P., Paul, J. and Singha, B., High prevalence of *Entamoeba moshkovskii* infection in HIV seropositive patients of Barak Valley, Assam, India. *BMC Infect. Dis*., 2014, **14**, P1.
71. Anuar. T. S., *et al.*, First molecular identification of *Entamoeba moshkovskii* in Malaysia. *Parasitol*., 2013, **139**, 1521–1525.
72. Pham Duc, P., Nguyen-Viet, H., Hattendorf, J., Zinsstag, J., Dac Cam, P. and Odermatt, P., Risk factors for *Entamoeba histolytica* infection in an agricultural community in Hanam province, Vietnam. *Parasit. Vectors*, 2011, **4**, 102.
73. Karambaigi, F., Abdi, J. and Sayehmiri, K., Prevalence of *Entamoeba histolytica* in Iran during 1988 to 2009: Systematic review and meta-analyses. *Afr. J. Microbiol. Res*., 2012, **6**, 3944–3947.
74. Duggal, P., *et al.*, A mutation in the leptin receptor is associated with *Entamoeba histolytica* infection in children. *J. Clin. Invest*., 2011, **121**, 1191–1198.
75. Verma, A. K., Ahuja, V. and Paul, J., The trend in distribution of Q223R mutation of leptin receptor gene in amoebic liver abscess patients from North India: a prospective study. *Biomed Res. Int*., 2014, **2014**, 847132.
76. Chen, H. L., Bair, M. J., Lin, I. T., Wu, C. H. and Lee, Y. K., Clinical manifestations and risk factors of amebic liver abscess in Southeast Taiwan compared with other regions of Taiwan. *Am. J. Trop. Med. Hyg*., 2013, **89**, 1214–1218.
77. Zhou, F. *et al.*, Seroprevalence of *Entamoeba histolytica* infection among Chinese men who have sex with men. *PLoS Negl. Trop. Dis*., 2013, **7**, e2232.
78. Mahmud, M. A., Bezabih, A. M. and Gebru, R. B., Risk factors for intestinal parasitosis among antiretroviral treated HIV/AIDS patients in Ethiopia. *Int. J. STD AIDS*, 2014, **25**, 778–784.
79. Adamu, H., Wegayehu, T. and Petros, B., High prevalence of diarrhoegenic intestinal parasite infections among non-ART HIV patients in Fitche Hospital, Ethiopia. *PLoS ONE*, 2013, **8**, e72634.

Received 3 July 2016; revised accepted 20 February 2018

doi: 10.18520/cs/v114/i10/2045-2052