Content available at: https://www.ipinnovative.com/open-access-journals

Panacea Journal of Medical Sciences

Journal homepage: https://pjms.in/

Original Research Article

Assessment of the epidemiological overlap among morphologically identical species of entamoeba among the patients attending Calcutta school of tropical medicine

Soumya Biswas[1], Shantanu Mandal[1], Arani Debnandi[1], Abhishek Sengupta[1]*, Tapashi Ghosh[1]

[1]Dept. of Microbiology, Calcutta School of Tropical Medicine, Kolkata, West Bengal, India

Abstract

Introduction: Parasitic infections like Amoebiasis are endemic to most tropical and subtropical regions of developing countries like India. Among Entamoeba species, Entamoeba histolytica, the causative agent for amoebiasis, is highly pathogenic while the other species are considered as non-pathogenic and needs no medical treatment in most of the cases.

Aim & Objective: To estimate the accurate prevalence of infection by different microscopically identical species of Entamoeba in human faecal samples by PCR based identification among patients attending a tertiary care hospital in Kolkata.

Materials and Methods: A total of 1837 stool specimens in this study were collected from patients attending department of Calcutta School of Tropical Medicine for a period of 12 months. Stool samples were screened via microscopy for Entamoeba species identification followed by confirmation by conventional Polymerase chain reaction. Data were analyzed with Graph pad Prism 9.

Results & Discussion: In the study, 91/1837 (4.95%) samples were microscopically positive for Entamoeba cyst or trophozoite and 81/1837 (4.41%) were Polymerase chain reaction positive for Entamoeba. E. moshkovskii infection (38.27%, 31/81) appeared to be the most predominant, followed by E. histolytica (19.75%, 16/81); E. dispar (9.88%, 08/81) and E. poleki (1.23%, 01/81) while 25/81(30.86%) belonged to other Entamoeba species.

Conclusion: The results provide an important data for the clinician about the advantages of PCR over microscopy and also discriminate non-pathogenic Entamoeba species from the pathogenic E. histolytica to avoid unnecessary treatment with anti-amoebic drugs.

Keywords: Entamoeba, Entamoeba histolytica, microscopy, Polymerase Chain reaction

Received: 23-07-2024; **Accepted:** 02-03-2025; **Available Online**: 19-08-2025

1. Introduction

An infection caused by the protozoan *Entamoeba histolytica* is termed as Amoebiasis, or amoebic dysentery. The WHO considered that about 110000 deaths and 50 million people were infected annually with *E. histolytica* worldwide.[1] The carriers of this amoeba infection develop clinical symptoms approximately 4 to 10% within a year. After Malaria and Schistosomiasis, amoebic dysentery is considered as the third leading cause of death from parasitic disease worldwide.[2] *E. histolytica*, *E. dispar*, *E. moshkovskii*, *E. bangladeshi*, *E. poleki*, *E. coli* and *E. hartmanni* are the six species of Entamoeba have been identified to colonize the human gut. *E. histolytica*, *E. dispar*, *E. moshkovskii* are morphologically similar but their biochemical and genetic features are different.[2,3] *Entamoeba histolytica* considered as a pathogenic species that causes amoebic dysentery and other invasive diseases, including amoebic liver abscess, cerebral amoebiasis, genitourinary amoebiasis and respiratory tract infections. Mainly clinical diagnosis of amoebiasis depends on the visualization of parasites by microscopy of saline wet mount or iodine/Lactophenol Cotton Blue (LCB) wet mount preferably after formol-ether concentration technique for many decades because it is simple and cheap.[4-6] *E. histolytica* has required alternative diagnostic methods for differentiation because it is morphologically indistinguishable from non-pathogenic *Entamoeba dispar*, *E. moshkovskii* and the newly described *Entamoeba bangladeshi*.[7] Molecular methods are very helpful to provide accurate and reliable identification of Entamoeba species

*Corresponding author: Abhishek Sengupta
Email: 0209abhishek@gmail.com

https://doi.org/10.18231/pjms.v.15.i.2.458-464

alternatively. Molecular methods like Polymerase Chain Reaction (PCR), including real-time PCR, are helpful to identify *E. histolytica, E. dispar,* and *E. moshkovskii* in a variety of samples.[8] Microscopy without molecular methods diagnosis of *E. histolytica* is overestimated due epidemiological overlapping with the other morphologically identical species. To prevent unnecessary or inappropriate chemotherapy and reduce the emerging problem of drug resistance in amoebiasis, species differentiation of Entamoeba and accurate diagnosis of individuals at risk is important. The present study was designed to avoid excessive and unnecessary treatment with antiprotozoal drugs for Entamoeba species infected individuals. In this study we are targeting to identify the different species of Entamoeba using PCR based molecular method from human stool samples those are reported as microscopically positive for *E. histolytica*. So we could assess the epidemiological overlap with the morphologically identical species of Entamoeba in amoebiasis and provide accurate diagnostic clues to aid appropriate treatment of amoebiasis.

2. Aim & Objective

To estimate the accurate prevalence of infection by different microscopically identical species of Entamoeba in human faecal samples by PCR based identification among patients attending a tertiary care hospital in Kolkata.

3. Materials and Methods

It is a hospital based observational study. Stool specimens for this study were collected from patients attending in-patients department and out-patients department of Calcutta School of Tropical Medicine for a period of 12 months (1st April 2021to 31st March 2022). 1837 stool samples were included in the study. The study was carried out at Helminthology Unit, Calcutta School of Tropical Medicine, Kolkata. Institutional Ethical Committee clearance was obtained prior to commencement of study.

Inclusion criteria are those who were gave informed consent and the Exclusion criteria is food poisoning, allergic diarrhoea, other established parasitic infestation and those patients who were unwilling to give consent. Data collection was done at the time of receiving sample.

Faecal specimens are collected in the early stages of the diarrhoeal disease (if present), when pathogens are present in the highest number, and preferably before antimicrobial treatment is started, if possible.

Study of freshly passed faecal specimen was done by routine microscopy of saline mount, iodine/LCB mount after formol ether concentration technique (When the number of organism in the stool specimen is low or no ova/ parasite/cyst is found in a direct wet mount, the stool is concentrated whenever possible.) and by molecular technique by DNA isolation followed by PCR amplification.(**Table 1**)

For all faecal sample after examine the concentrate, the remaining sample was used for the isolation of DNA by QIAamp Stool DNA Mini Kit. Subsequently genus specific PCR was done, followed by species specific PCR done to distinguish species.

Data were analyzed with Graph pad Prism 9.

4. Results

1837 stool samples were included in the study and subjected to analysis as per material and methods mentioned earlier. Results obtained from clinical and laboratory examinations have been outlined below.

In our study, 91 samples were microscopically positive for Entamoeba cyst or trophozoite and 81 were PCR positive for Entamoeba. Overall prevalence as per microscopy is 4.95% (91/1837) and by PCR is 4.41% (81/1837). Thus, only 89.01% of the positive samples, resembling Entamoeba by microscopy, were true Entamoeba as confirmed by PCR assay.

In our study more children were found to be positive for Entamoeba infections compared to adults. Higher prevalence rates were recorded in 5-12 years (10.20%), followed by 0-4 years (7.41%), 13-18 years (6.90%), 30-50 years (4.56%), 19-29 years (3.94%) while above 50 years (2.94%) had the least. So school going young children are affected more than the adult.(**Table 2**)

The present study showing out of 81 PCR positive cases, the males were more affected with 57 cases (70.37%) than the females with 24 cases (29.63%). Male to female ratio was 2.37:1. Regarding gender as a risk factor, the prevalence of the four Entamoeba spp. was markedly higher in males in our study.(**Graph 1**)

Association in between lower and lowers middle socioeconomic class and prevalence of Entamoeba infection is significant. In the present study, most of the positive patients were from lower social class (60/81, 74.07%).(**Table 3**)

Higher prevalence rate is noted in patients with low education level/illiterate, 56 cases (56/81, 69.14%) and lower in literate patients, 25 cases (25/81, 30.86%).(**Table 4**)

In addition, amoebiasis can be transmitted orally by drinking water contaminated by Entamoeba cysts. The water source of the patients mainly was the untreated water, 64 cases (64/81, 79.01%), and some of them depended on boiled or filtered water, 17 case (17/81, 20.99%).(**Table 5**)

Table 1: The reference primers set used for Entamoeba Species specific amplification.

Name	Code	Primer Sequence	Amplicons Size
Entamoeba histolytica	EH-1(F) EH-2(R)	TGCATGTGTAAGTATAAAGACCAAG GAATGAATTGGCCATTTTGTACT	162 bp
Entamoeba disper	ED-1(F) ED-2(R)	AGTACAAAGTGGCCAATTTATGTAAG GATTTTACTCAACTCTAGAGTTATGTG	374 bp
Entamoeba moshkovskii	EM-1(F) EM-2(R)	CCACTCTCTTCACGGGGAGT CCTTCAAGAAGTGGAGTTAACCA	305 bp
Entamoeba poleki	EP-1(F) EP-2(R)	GGATAACTCTTGTTAATTGCAGAGC TCGATTCTATTAATTATCGTCACTACC	343 bp

Table 2: Showing distribution of positive Entamoeba cases according to age

Age	Total number of sample examined	PCR Positive	Prevalence	95% CI	P value
0-4 Years	27	2	7.41%	0.010-0.245	0.445
5-12 Years	49	5	10.20%	0.040-0.222	0.045
13-18 Years	116	8	6.90%	0.033-0.132	0.178
19-29 Years	482	19	3.94%	0.025-0.061	0.561
30-50 Years	789	36	4.56%	0.033-0.063	0.781
Above 50 Years	374	11	2.94%	0.016-0.053	0.121

Table 3: Table showing association of Entamoeba infection with Socio-economic class*

Socio-economic class	Total number of sample examined	PCR			
		Positive	Prevalence	95% CI	P value
I (Upper)	151	1	0.66%	0.0001-0.040	0.019
II (Upper middle)	312	8	2.56%	0.012-0.051	0.081
III (Middle)	390	12	3.08 %	0.017-0.054	0.101
IV (Lower middle)	563	33	5.86%	0.041-0.081	0.044
V (Lower)	421	27	6.41%	0.044-0.092	0.022

*****According to modified BG Prasad scale 2021.

Table 4: Showing association of entamoeba infection with literacy status

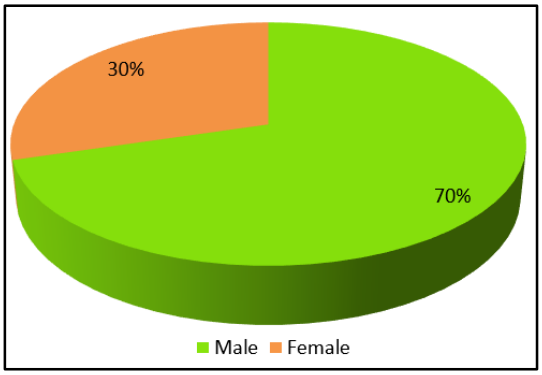
Educational status	Total number of sample examined	PCR			
		Positive	Prevalence	95% CI	P value
Illiterate	1057	56	5.30%	0.041-0.068	0.031
Literate	780	25	3.21%	0.022-0.047	

Table 5: Showing association of entamoeba infection with drinking water

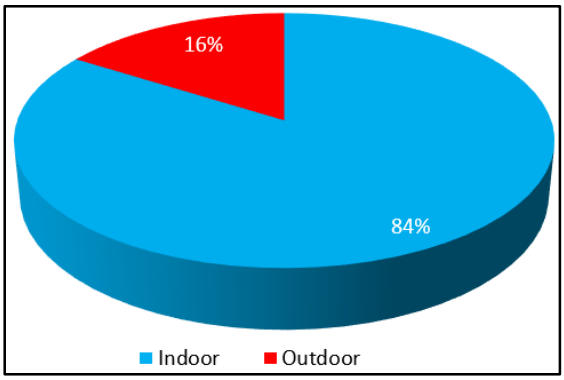
Drinking Water	Total number of sample examined	PCR			
		Positive	Prevalence	95% CI	P value
Untreated	1253	64	5.11%	0.040-0.065	0.033
Filtered/ Boiled	584	17	2.91%	0.018-0.047	

Table 6: Showing association of Entamoebal infection with Diarrhoea and HIV

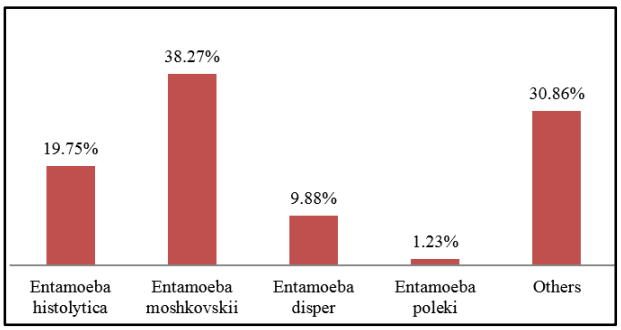
Entamoeba species	**HIV**					**NON HIV**				
Diarrhea	**N**	**n**	**%**	**95%CI**	**P value**	**N**	**n**	**%**	**95% CI**	**P value**
	72	21	29.17%	0.199-0.406	0.0001	410	23	5.61%	0.037-0.083	0.179
Non diarrhea	**N**	**n**	**%**	**95% CI**	**P value**	**N**	**n**	**%**	**95% CI**	**P value**
	114	5	4.39%	0.016-0.101	0.990	1241	32	2.58%	0.018-0.036	0.0001



Graph 1: Pie diagram showing distribution of Entamoeba positive cases according to sex



Graph 2: Pie diagram showing distribution of Entamoeba positive cases according to location



Graph 3: Bar diagram showing prevalence of different species of entamoeba

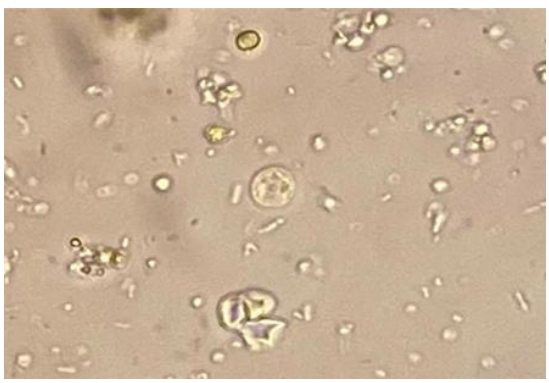


Figure 1: Stool wet mount showing cyst of Entamoeba spp.

Out of 81 PCR positive cases, the patient admitted at In-patient Department was more affected with 68 cases (83.95%) than the patient attending Out-patient Department with 13 cases (16.05%) with an Indoor to Outdoor ratio of 5.23:1.(**Graph 2**)

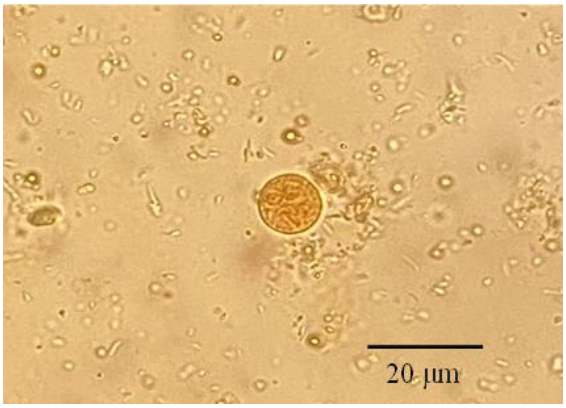


Figure 2: Stool Iodine wet mount showing cyst of Entamoeba spp.

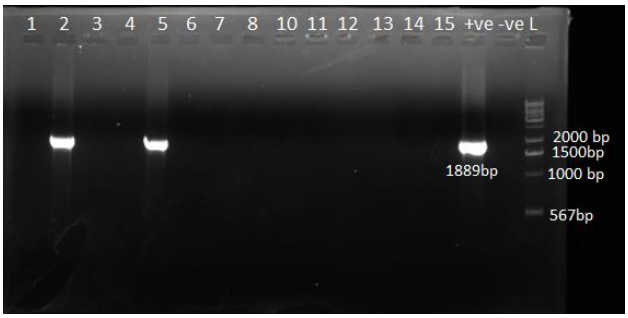


Figure 3: Entamoeba Genus specific PCR assay; *1: E. hitolytica 1889bp, 2: E. dispar 1892bp, 3:E. moshkovskii 1889bp, 4: E. nuttali 1889bp, 5. E. Bangladeshi 1892bp, 6. E. invadens 1910bp, 7. E. coli 2046bp, 8. E. chattoni 1808bp, 9. E. polecki 1803bp*

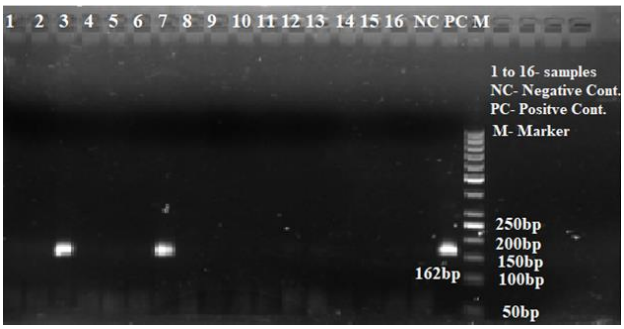


Figure 4: Detection of *Entamoeba histolytica* in stool samples using *E. histolytica* specific primer.Lane M 50 bp marker; Lane PC- *E. histolytica* genomic DNA as positive control; Lane- 3,7 stool genomic DNA positive for *E. histolytica*; Lane- 1, 2, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16 stool genomic DNA negative for *E. histolytica*; Lane- NC no template control.

In our study we have shown the prevalence of Entamoeba species-13.99%, in diarrheal as well as non-diarrheal asymptomatic HIV patients.(**Table 6**)

Out of the 81 PCR positive cases, 31 cases (38.27%) were due to *E. moshkovskii*, 16 cases (19.75%) were due to *E. histolytica*, 8 cases (9.88%) were due to *E. dispar*, only 1

cases(1.23%) were due to *E. poleki* and other Entamoeba occupying 25 cases (30.86%) identified in PCR.(**Graph 3**)

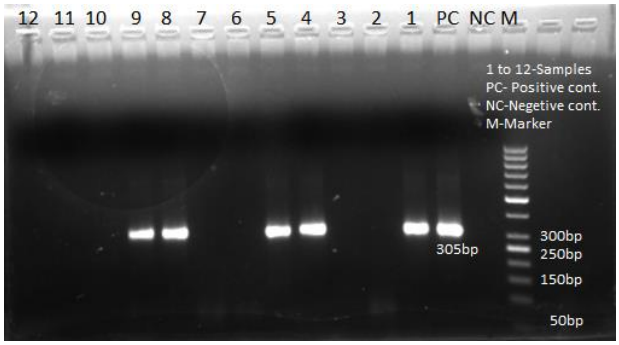


Figure 5: Detection of *Entamoeba moshkovskii* in stool samples using *E. moshkovskii* specific primer. Lane M 50 bp marker; Lane PC- *E. moshkovskii* genomic DNA as positive control; Lane- 1 , 4, 5, 8, 9 stool genomic DNA positive for *E. moshkovskii*; Lane- 2, 3, 6, 7, 10, 11, 12, stool genomic DNA negative for *E. moshkovskii*; Lane- NC no template control

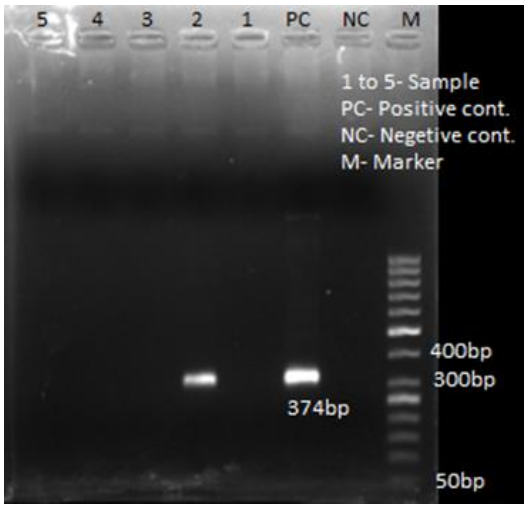


Figure 6: Detection of *Entamoeba dispar* in stool samples using *E. dispar* specific primer. Lane M 50 bp marker; Lane PC- *E. dispar* genomic DNA as positive control; Lane- 2, stool genomic DNA positive for *E. dispar*; Lane- 1, 3, 4, 5, stool genomic DNA negative for *dispar*; Lane- NC no template control

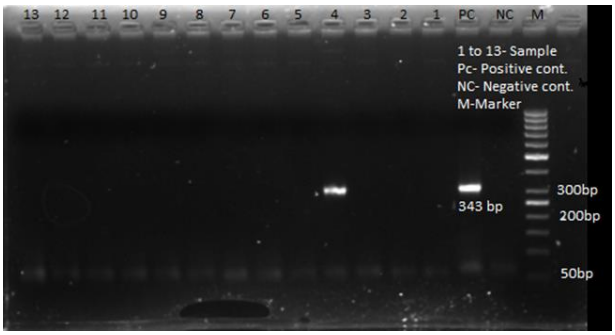


Figure 7: Detection of *Entamoeba poleki* in stool samples using *E. poleki* specific primer. Lane M 50 bp marker; Lane PC- *E. poleki* genomic DNA as positive control; Lane- 4 stool genomic DNA positive for *E. poleki*; Lane- 1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13 stool genomic DNA negative for *poleki*; Lane- NC no template control

5. Discussion

In our study, the overall prevalence of Entamoeba infection as per microscopy is 4.95% (91/1837) and by PCR is 4.41% (81/1837) similar results was observed in studies by Anitha et al.[9] (3%) in South India for confirmation of microscopy findings by PCR for the final diagnosis of Entamoeba infection.

Regarding the age distribution of positive cases, more children were found to be positive for Entamoeba infections compared to adults. The highest prevalence rates are recorded in 5-12 years (10.20%), while above 50 years (2.94%) had the least. Showed high prevalence of 31.0% among younger children between age group of 6-10 years whereas within age groups of 11-15 years and >16 years showed least level of infection, which is similar to our study finding .The both results suggests a correlation with the habits of pre-school age group children and Entamoeba infection.

Among 81 PCR positive cases, the males were more affected with 57cases (70.37%) than the females with 24 cases (29.63%). In Yemen, similar results reported by Al-Areeqi et al. for *E. moshkovskii;* however, in males and females the prevalence rate was similar for both *E. histolytica* and *E. dispar*. Conversely, Ozgumus and Efe in Turkey reported higher prevalence rates of *E. histolytica* infection in females.[10,11]

There is a significant association between lower and lower middle socioeconomic class and prevalence of Entamoeba infection. In our present study, most of the positive patients were from lower social class (60/81, 74.07%), lower income leads to poor living conditions and poor sanitary habits aggravating their wards growth and parasitic infections.[12]

Higher prevalence rate is noted in patients with low education level/illiterate, 56 cases (56/81, 69.14%) and lower in literate patients, 25 cases (25/81, 30.86%). The prevalence of infection correlated with patients with low education level reported by Al-Areeqi et al.; this could be because health-related knowledge and awareness about parasitic intestinal infections and the ways of their transmission is less in non-educated or low educated individuals compared with highly educated individuals.[10,11]

In addition, amoebiasis can be transmitted orally by drinking water contaminated by Entamoeba cysts. The water source of the patients mainly was the untreated water, 64 cases (64/81, 79.01%), and some of them depended on boiled or filtered water, 17 case (17/81, 20.99%). Thus, the water supply pollution can be as a potential source of infection.

In March and August 2007 in Jeddah a cross-sectional study was undertaken between at two major public hospitals,

and *E. histolytica / E. dispar* was detected at a prevalence of 8.3% inpatient and 5.9% outpatient.[13] A quite similar results is found in our study with that study (prevalence of Entamoeba infection 4.59% inpatient and 3.66% outpatient department)

In this study we have shown the prevalence of Entamoeba species-13.99%, in diarrheal as well as non-diarrheal asymptomatic HIV patients. Varying prevalence rate of 1.6% to 10.8% has been noted in HIV seropositive individuals in India, it is slight higher in our findings.[14-16] So HIV associated with diarrhoea act as a risk factor for amoebiasis.

E. histolytica was detected in 19.75% of faecal samples, which was lower than those reported by Calegar et al. (23.8%) but higher than those reported by Blessmann et al. (11.2%), Rania Abozahra et al. (14.7%) and Khairnar et al. (7.4%).[11,17,18] But our study, *E. moshkovskii* was detected in 38.27% of faecal samples, which was higher than those reported by Parija et al. (5.26%), Rania Abozahra et al. (11.8%), and El Bakri et al. (2.5%).[11,19,20] Our results are similar to the findings by Ali and co-worker, Ali et al., 2003.[21]

Our study also revealed a higher prevalence of *E. moshkovskii* (1.69%) and lower prevalence of *E. dispar* (0.44%) compared to *E. histolytica* (0.87%).The prevalence sequence is same those reported by Nath et al. in Assam.[22]

6. Conclusion

In this multivariate analysis, factor that independently influenced Entamoeba infection included sources of drinking water, literacy status, economy status, HIV seropositivity, and patients having diarrhoea. Entamoeba infection found to be responsible for most diarrhoea condition especially among children and HIV associated with diarrhoea act as a risk factor for amoebiasis.

In addition, the findings revealed the prevalence of amoebic infections caused by mostly nonpathogenic Entamoeba species, *E. dispar*, *E. moshkovskii* and *E. poleki*, along with the pathogenic *E. histolytica*.

Hence the diagnosis of amoebiasis should be confirmed by PCR to avoid redundant treatment of numerous individuals with antiamoebic drugs which would increase the development of resistant parasitic strains and obtain proper and accurate epidemiological data concerning the organism.

7. Source of Funding

None.

8. Conflict of Interest

None.

References

1. Garcia. Diagnostic Medical Parasitology, 6th edn. John Wiley & Sons, Inc., 2016:552
2. Fotedar R, Stark D, Beebe N, Marriott D, Ellis J, Harkness J. Laboratory diagnostic techniques for Entamoeba species. *Clin Microbiol Rev*. 2007;20(3):511–32.
3. López MC, León CM, Fonseca J, Reyes P, Moncada L, Olivera MJ, et al. Molecular Epidemiology of Entamoeba: First Description of Entamoeba moshkovskii in a Rural Area from Central Colombia. *PLoS One*. 2015;10(10):e0140302.
4. López MC, León CM, Fonseca J, Patricia R, Ligia M, Mario O, et al. Molecular epidemiology of entamoeba: first description of entamoeba moshkovskii in a rural area from central Colombia. *PLoS One*. 2015;10(10):e0140302
5. Ngui R, Angal L, Fakhrurrazi SA. Differentiating Entamoeba histolytica, Entamoeba dispar and Entamoeba moshkovskii using nested polymerase chain reaction (PCR) in rural communities in Malaysia. *Parasit Vectors*. 2012;5:187
6. Santos HL, Bandea R, Martins LA, de Macedo HW, Peralta RH, Peralta JM. Differential identification of Entamoeba spp. based on the analysis of 18S rRNA. *Parasitol Res*. 2010;106(4):883–8.
7. Parija SC, Mandal J, Ponnambath DK. Laboratory methods of identification of Entamoeba histolytica and its differentiation from look-alike Entamoeba spp. *Trop Parasitol*. 2014;4(2):90–5.
8. Gomes, Thiago & Garcia, Mariana & Cunha, Flavia & Macedo, Heloisa & Peralta, José Mauro & Peralta, Regina. Differential Diagnosis of Entamoeba spp. in Clinical Stool Samples Using SYBR Green Real-Time Polymerase Chain Reaction. *ScientificWorldJournal*. 2014:645084.
9. Anitha G, Mugunthan M, Nonika R, Gopika J. Trends of Entamoeba histolytica Infections in a Tertiary Care Hospital of South India - A Three Year Perspective Study. *Acta Microbiologica Sinica*. 2020;3(10):38–41.
10. Al-Areeqi AM, Sady H, Al-Mekhlaf MH, Anuar ST, Al-Adhroey HA, Atroosh MW. First molecular epidemiology of Entamoeba histolytica, E. dispar and E. moshkovskii infections in Yemen: diferent species-specifc associated risk factors. *Trop Med Int Health*. 2017;22(4):493–504.
11. Abozahra R, Mokhles M. Baraka K. Prevalence and Molecular Differentiation of Entamoeba histolytica, Entamoeba dispar, Entamoeba moshkovskii, and Entamoeba hartmanni in Egypt. *Acta Parasit.* 2020;65(4):929–35.
12. Nema K, Bankwar S, Dhanvijay V, Kumar A. Intestinal parasitic infections and demographic status of school children in Bhopal region of Central India. *J Pharm Biol Sci*. 2014;9(5):83–7.
13. Al-Braiken FA. Is intestinal parasitic infection still a public health concern among Saudi children? *Saudi Med J*. 2008;29(11):1630–5.
14. Nath J, Ghosh SK, Bhattacharjee P, Paul J, Singha B. High prevalence of Entamoeba moshkovskii infection in HIV seropositive patients of Barak Valley, Assam, India. *BMC Infect Dis*. 2014;14(3):1.
15. Mohandas K, Sehgal R, Sud A, Malla N. Prevalence of intestinal parasitic pathogens in HIV-seropositive individuals in Northern India. *Jpn J Infect Dis*. 2002;55(3):83–4.
16. Nath J, Banyal N, Gautam DS, Ghosh SK, Singha B, Paul J. Systematic detection and association of Entamoeba species in stool samples from selected sites in India. *Epidemiol. Infect*. 2015;143(1):108–19.
17. Calegar AD, Nunes CB, Monteiro LJK, dos Santos PJ, Toma K, Gomes FT. Frequency and molecular characterization of Entamoeba histolytica, Entamoeba dispar, Entamoeba moshkovskii, and Entamoeba hartmanni in the context of water scarcity in northeastern Brazil. *Mem Inst Oswaldo Cruz Rio de Janeiro*. 2016;111(2):114–9.
18. Khairnar K, Parija SC. A novel nested multiplex polymerase chain reaction (PCR) assay for diferential detection of Entamoeba histolytica, E. moshkovskii and E. dispar DNA in stool samples. *BMC Microbiol*. 2007;7:47.
19. Parija CS, Garg A, Pushpa K, Khairnar K, Gastroenterol JPT. Polymerase chain reaction confrmation of diagnosis of intestinal

amebiasis in Puducherry. *Indian J Gastroenterol*. 2010;29(4):140–2.

20. El-Bakri A, Samie A, Ezzedine S, Abu Odeh R .Diferential 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(2):185–190.
21. Ali IK, Hossain MB, Roy S, Ayeh-Kumi PF, Petri WA, Haque R, et al. Entamoeba moshkovskii infections in children, Bangladesh. *Emerg Infect Dis*. 2003;9(5):580–4.
22. Nath J, Ghosh SK, Bhattacharjee P. High prevalence of Entamoeba moshkovskii infection in HIV seropositive patients of Barak Valley, Assam, India. *BMC Infect Dis.* 2014;14(Suppl 3).

Cite this article: Biswas S, Mandal S, Debnandi A, Sengupta A, Ghosh T. Assessment of the epidemiological overlap among morphologically identical species of entamoeba among the patients attending Calcutta school of tropical medicine. *Panacea J Med Sci*. 2025;15(2):458-464.