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ORIGINAL ARTICLE

Prevalence and Molecular Identification of *Entamoeba* Species Complex in Asymptomatic and Symptomatic Egyptians

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Abstract

Background: ***Entamoeba* (E.) *histolytica*, is a protozoan parasite that causes intestinal and extraintestinal amoebiasis, leading to significant morbidity and mortality in developing nations. *E. histolytica*, *E. dispar*, and *E. moshkovskii*, members of *E.histolytica* complex species, are all morphologically similar.**

Aim of the study: **The current work aimed to utilize molecular techniques for identifying the exact prevalence of *E. histolytica* species complex in an Egyptian population.**

Patients and methods: **The present work was a cross-sectional study done on 133 individuals. A single fecal sample was collected from each individual and examined microscopically before and after concentration. Multiplex PCR was used to molecularly identification of *E. histolytica* complex species among positive stool samples.**

Results: **Intestinal parasite prevalence was 51.1% and *E. histolytica* complex was detected in 30 cases (22.6%). The most prevalent parasite in coproscopically positive samples was *E. dispar* (63.3%). *E. histolytica* and *E. moshkovskii* were detected in 23.4% and 13.3%, respectively. In asymptomatic individuals, a statistically significant correlation was detected between sociodemographic factors and *Entamoeba* species, but in symptomatic individuals, only age categories were statistically significant.**

Conclusion: **The most common *Entamoeba* species found in the examined subjects is *E.dispar*. For the actual prevalence of *E. histolytica*, molecular diagnostics is required which could also prevent overmedication.**

Keywords: *E. histolytica* complex, Egypt, Microscopy, Multiplex PCR

1. Introduction

Amoebiasis is a widespread public health issue, especially in underdeveloped nations. It is one of the most frequent parasitic intestinal illnesses globally, causing thousands of fatalities each year.[1]

Entamoeba histolytica (*E. histolytica*), one of several *Entamoeba* species identified in humans, has long been recognized as a pathogen. It has been determined that only non-invasive infections can be brought on by *E. dispar* and *E. moshkovskii*, whereas *E. histolytica* can induce both invasive and non-invasive illnesses.[2]

A wide variety of clinical symptoms of amoebiasis exist. The infection may be asymptomatic but may also cause invasive extraintestinal disease. The clinical symptoms are variable and may include watery diarrhea, dysentery, persistent stomach pain, tenesmus, and weight loss in certain cases.[3]

In most countries, microscopic examination of wet and stained smears of patient fecal specimens is used for identifying amoebic cysts and trophozoites to diagnose amoebiasis. Despite being a simple and

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Table 1. The used primers in the multiplex PCR.

Primers	Sequence	Expected product size(bp)
Entamoeba Common forward primer (EntaF)	5′-ATG CAC GAG AGC GAA AGC AT-3′	
E. histolytica reverse primer (EhR)	5′-GAT CTA GAA ACA ATG CTT CTC T-3′	167-bp
E.moshkovskii reverse primer (EmR)	5′-TGA CCG GAG CCA GAG ACA T-3′	579-bp
E. dispar reverse primer (EdR)	5′-CAC CAC TTA CTA TCC CTA CC-3′	753-bp

cost-effective diagnostic approach, microscopy is unable to distinguish between the four *Entamoeba* species' cysts and trophozoites, making it impossible to determine the precise *Entamoeba* spp. found in samples. Moreover, to establish a definite diagnosis of infections down to the level of species for useful epidemiological studies and the choice of therapeutic approaches, *Entamoeba* species differentiation in clinical specimens by other techniques is essential.[4]

For the detection of different *Entamoeba* species, molecular methods depended on DNA amplification are particularly sensitive and specific methods.[5,6]

This study used a multiplex PCR (mPCR) assay to give an update on the present epidemiological situation of *Entamoeba* species in asymptomatic and symptomatic Egyptians.

2. Patients and methods

A cross-sectional study was carried out on 133 individuals attending Cairo University Hospital clinics for screening for the parasite as part of a routine check-up or having GIT symptoms in the period from May 2020 to April 2021. The present work was conducted after having the approval of the Ethics Committee of Al-Azhar University.

2.1. Collection and microscopic examination of stool specimens

All stool specimens were collected from GIT symptomatic and asymptomatic individuals in labelled, dry, clean plastic containers. Personal information including age, sex, and clinical history was recorded using a questionnaire.

Microscopic examination for the fecal samples was done by both direct wet mount and formalin-ethyl acetate concentration method.[7]

2.2. DNA extraction and multiplex PCR

Following the manufacturer's recommendations, copro-DNA extraction from stool samples that were positive by microscopic examination for *E. histolytica* species complex was bone by QIAamp Fecal DNA MiniPrep™. Until processing, the extracted DNA was kept at − 20 °C.

Multiplex PCR was used to amplify DNA extracts for detection of *Entamoeba* species complex using rRNA gene small subunit (SSU). [9] The specific - species primers used in this work was shown in (Table 1). The conditions of thermal cycling included initial denaturation at 94 °C for 4 min, 35 cycles consisting of denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min, extension at 72 °C 80 s, and final extension at 72 °C for 7 min The products of PCR were visualized on 1.5% agarose gel that was stained with ethidium bromide on a UV light system.[4,8] The produced PCR expected sizes were demonstrated in Table 1.

2.3. Statistical analysis

The statistical package software SPSS model 26 was used to enter the data (Chicago, IL, USA). Data were collated and the descriptive statistics were defined as quantitative variables by mean and standard deviation and qualitative variables by frequency and percentage. The χ^2 test was used to determine statistical significance, and data were deemed statistically significant if the *P* value was less than 0.05.

3. Results

3.1. Prevalence of intestinal parasites among study populations

The prevalence of intestinal parasites in the study populations according to microscopic examination

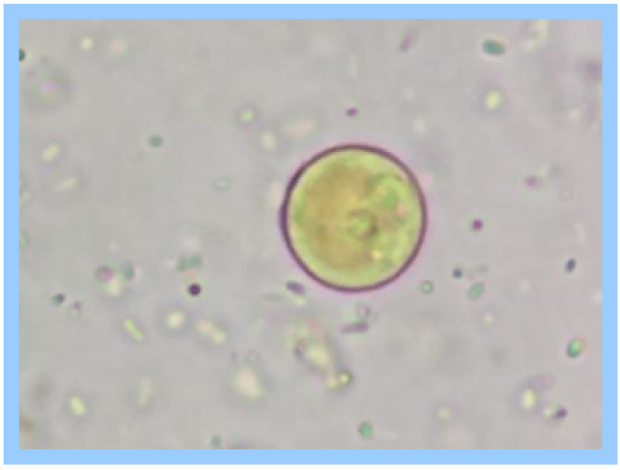

Fig. 1. Entamoeba species complex (iodine stained X100).

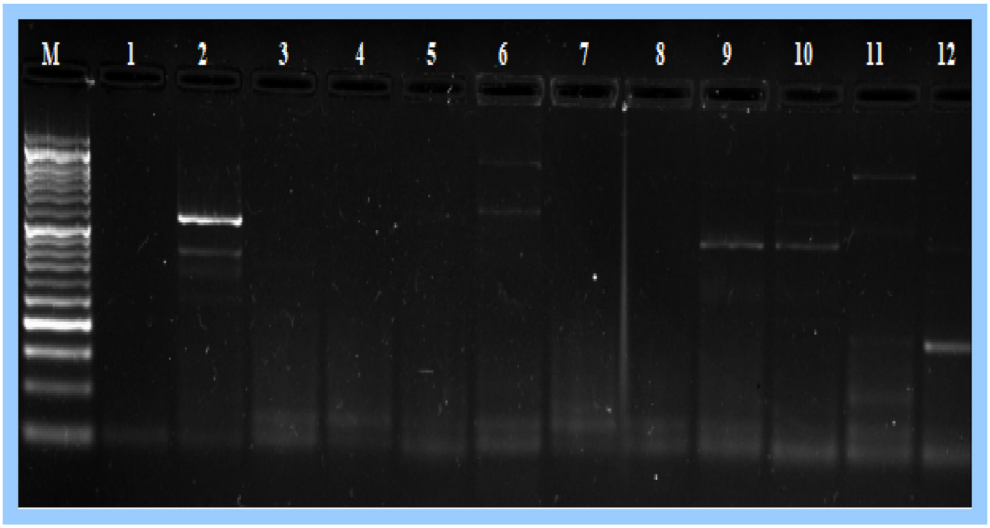

Fig. 2. Gel photo of Entamoeba-species mPCR products. Lane M: a marker of DNA with a molecular weight of 50 bp. Lane 1: control negative specimens. Lanes 2, 9 and10 positive for E. moshkovski specimens at 580 bp. Lanes 6 and 11: positive for E. dispar sample at 752 bp. Lane 12: positive for E. histolytica samples at 166 bp. Lanes 3,4,5,7 and 8: negative samples.

was 65 positive samples, 27 (20.3%) *E. histolytica* complex (Figs. 1 and 2), 13 (9.8%) *Giardia intestinalis*, 3 (2.3%) *Cryptosporidium* species, 19 (14.3%) *Blastocystis* species, 1 (0.8%) *E. histolytica* complex and *Giardia intestinalis*, and 2 (1.5%) *E. histolytica* complex and *Blastocystis* species. While 68 (51.1%) samples are negative (Table 2).

Data analysis of variables among the study population revealed that 56 (42.1%) were asymptomatic (control group) and 77 (57.9%) of them had GIT symptoms (patient group). (Table 3).

Table 3. Distribution of GIT symptoms among the study population.

	Frequency	Percent
GIT-Symptoms		
Asymptomatic	56	42.1
Symptomatic	77	57.9
Total	133	100.0

3.2. Correlation between individual's characteristics of amoebiasis among asymptomatic and symptomatic cases

Table 4 presents the correlation between the sociodemographic characteristics of asymptomatic individuals and *Entamoeba* complex species. The infected patients with *E. histolytica*, *E. moshkovskii*, or both *E. histolytica* and *E. dispar* demonstrated a significant association with all studied variables. While Table 5 represents the correlation between the sociodemographic characteristics of symptomatic individuals and *Entamoeba* species, which showed significant correlation only with age group and presence of other intestinal parasites.

4. Discussion

Microscopy is typically employed for protozoa diagnosis in fecal samples and the identification of *Entamoeba* spp. continued to be problematic. When the diagnosis is based solely on microscopic examination, this method, however, failed to distinguish

Table 2. Results of microscopy for detection of prevailing parasites.

			Frequency	Percent
Microscopic examination	Parasites	*E. histolytica* complex	27	20.3
		Giardia intestinalis	13	9.8
		Cryptosporidium species	3	2.3
		Blastocystis species	19	14.3
		E. histolytica species complex and *Giardia intestinalis*	1	0.8
		E. histolytica species complex and *Blastocystis* species	2	1.5
		Total	65	48.9
	No ova and parasites		68	51.1
Total			133	100.0

Table 4. Sociodemographic characteristics of amoebiasis among asymptomatic cases.

	Samples *N* (%)	Positive *N* (%)			
	Variables in Asymptomatic cases	*E.histolytica*	*E.dispar*	*E.moshkovaskii*	*P* value
Age group					
Infants	1 (1.8%)	1 (1.8%)	0 (0.0%)	0 (0.0%)	
Preschool child	1 (1.8%)	1 (1.8%)	0 (0.0%)	0 (0.0%)	
School child	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.0001*
Adolescent	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Young adult	3 (5.4%)	0 (0.0%)	3 (5.4%)	0 (0.0%)	
Middle-aged adult	6 (10.7%)	1(1.8%)	4 (7.1%)	1 (1.8%)	
Old adult	2 (3.6%)	0 (0.0%)	0 (0.0%)	2 (3.6%)	
Sex					
Females	4 (7.1%)	0 (0.0%)	1 (1.8%)	3 (5.3%)	0.034*
Males	9 (16.1%)	2 (3.6%)	7 (12.5%)	0 (0.0%)	
Residence					
Rural	7 (12.5%)	0 (0.0%)	5 (8.9%)	2 (3.6%)	0.034*
Urban	6 (10.7%)	2 (3.6%)	3 (5.3%)	1 (1.8%)	
Intestinal Parasites					
E. complex	12 (21.4%)	2 (3.6%)	7 (12.5%)	3 (5.3%)	
Giardia intestinalis	2 (3.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Cryptosporidium species	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Blastocystis species	7 (12.5%)	0 (0.0%)	0(0.0%)	0 (0.0%)	0.0001*
E.histolytica complex and *Giardia intestinalis*	1(0.8%)	0 (0.0%)	1 (0.8%)	0 (0.0%0	
E. complex and *Blastocystis* species	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Total	56 (100%)		13 (23.2%)		

E. histolytica from the morphologically similar non-pathogenic species like *E. moshkovskii* and *E. dispar* leading to false estimation of the exact prevalence and unneeded use of chemotherapeutic agents.[9,10]

In this work, *Entamoeba* species were identified and *E. histolytica*, *E. dispar*, and *E. moshkovskii* were distinguished using microscopic and molecular methods.

In the current study, intestinal parasites were detected in 51.1% of the participants, including *Giardia intestinalis* (9.8%), *Blastocystis* species(14.3%), *Cryptosporidium* species (2.3%), *E.histolytica* complex,

Table 5. Sociodemographic characteristics of amoebiasis among symptomatic cases.

Variables in symptomatic cases		Positive *N* (%)			
	Samples *N* (%)	*E.histolytica*	*E.dispar*	*E.moshkovaskii*	*P* value
Age group					
Infants	3 (3.9%)	0 (0.0%)	0(0.0%)	0 (0.0%)	
Preschool child	12 (15.6%)	2(2.6%)	1 (1.8%)	0 (0.0%)	
School child	13 (16.9%)	1 (1.3%)	2(2.6%)	0 (0.0%)	
Adolescent	5 (6.5%)	0 (0.0%)	1(1.8%)	1(1.8%)	0.0001*
Young adult	6 (7.8%)	2 (2.6%)	3(5.4%)	0 (0.0%)	
Middle-aged adult	15 (19.5%)	0(0.0%)	4(5.2%)	0 (0.0%)	
Old adult	6 (7.8%)	0 (0.0%)	0(0.0%)	0 (0.0%)	
Sex					
Females	5(6.5%)	3(3.9%)	1 (1.3%)	1(1.3%)	0.215
Males	12(15.6%)	4 (5.2%)	8(10.4%)	0 (0.0%)	
Residence					
Rural	6 (7.8%)	0 (0.0%)	6 (7.6%)	0 (0.0%)	0.125
Urban	11(14.3%)	5 (6.5%)	5 (6.5%)	1 (1.3%)	
Intestinal Parasites					
E.histolytica complex	15(19.5%)	5 (6.5%)	9(11.7%)	1 (1.3%)	
Giardia intestinalis	11(14.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Cryptosporidium species	3 (3.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0.0001*
Blastocystis species	12(15.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
E.histolytica complex and *Giardia intestinalis*	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
E. complex and *Blastocystis* species	2 (2.6%)	0 (0.0%)	2 (2.6%)	0 (0.0%)	
Total	17(22.07%)		77 (100%)		

and *Giardia intestinalis* (0.8%), and *E.histolytica* complex and *Blastocystis* species (1.5%). Additionally, 22.6% of *Entamoeba* species were detected coproscopically, and after multiplex PCR.

Our results showed that 63.3% of the samples were *E. dispar*-related. The prevalence of *E. dispar* in the current study is comparable to that of the majority of earlier investigations conducted in Egypt by Abozahra et al.[11] who found *E dispar* in 61.8% as well as an Iranian study, which revealed that *E. dispar* made up 54.8% of *Entamoeba* species.[12] On the other hand, a study carried out in the United Arab Emirates reported that *E. histolytica* was more common.[13]

In the present work, when compared to the asymptomatic group, persons with gastrointestinal symptoms had a higher infection rate which coincided with Abd Fadia et al..[14] who showed a higher incidence of infection among symptomatic individuals.

Research from many regions of the world revealed that children had significantly higher levels of intestinal parasite infections including *E. histolytica*.[15–17] On the other hand, the results of this study demonstrated that there was a higher incidence of infection among young and middle-aged adults with non-significant differences among age groups.

In our present study, the distribution among patient residency showed the highest prevalence of *Entamoeba* species in urban areas (12.8%) than in rural areas (9.8%) and this association was highly statistically significant with P value = 0.006. Another study by Mahmood and Bakr.[18] in Erbil City, Northern Iraq showed the highest prevalence rates among urban areas (68.6%) than in rural areas (31.4%) and this association was non statistically significant with P value = 0.322.

4.1. Conclusion

Based on our results, it is recommended to identify *E. histolytica* infection and reassess its prevalence using Multiplex PCR that can distinguish between different *Entamoeba* complex species, to prevent unnecessary therapy and formulate control strategies.

Conflicts of interest

None declared.

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