Annals of Parasitology 2024, 70(2), 91–101
doi: 10.17420/ap7002.526
Copyright© 2024 Polish Parasitological Society

Original paper

Detection and molecular identification of *Entamoeba* species in faecal samples from Duhok province, Kurdistan Region, Iraq

Haliz Khalid HASAN[1], Ahmed Basheer MOHAMMED[1], Wijdan Mohammed Salih MERO[1,2]

[1]Department of Biology, College of Science, University of Zakho, Zakho 42002, Kurdistan Region, Iraq
[2]College of Science, Nawroz University, Duhok 42001, Kurdistan Region, Iraq

Corresponding Author: Haliz Khalid Hasan; e-mail: haliz.hasan@stud.uoz.edu.krd

ABSTRACT. The study involved the estimation of the prevalence of *Entamoeba* spp. using microscopy and molecular techniques among symptomatic outpatients from April 2021 to March, 2022. Stool samples were collected from 2592 outpatients with amoebosis symptoms of both sexes and different ages (≤ 1 to 60). Also, 207 stool samples were taken randomly from asymptomatic individuals and examined microscopically to detect infection with *Entamoeba* spp. the positive specimens were used for molecular analysis with positive symptomatic samples targeting the 18S rRNA gene by nested PCR. Microscopically 21.68% (562/2592) were positive, for *Entamoeba* spp. Males showed highest infection rate than females (67.43% vs 32.56%). Ages from 1–10 years showed the highest rate (54.09%), and urban inhabitant had somewhat a higher rate than rural one (58.54% vs 41.45%) which was statistically non-significant (P>0.05). Among asymptomatic individuals, 57% (61/107) were positive for *Entamoeba* spp. Nested PCR analysis yielded 73% positive samples for *Entamoeba* spp. with a fragment size of 897 bp. Three fragment sizes were produced, for *E. histolytica*, *E. dispar* and *E. moshkovskii* which were 439, 174 and 553 bps, respectively. Single infection occurred with, *E. histolytica* in 46%, of symptomatic and 6% of asymptomatic cases, *E. dispar* in 38% of asymptomatic and 10% of symptomatic cases, *E. moshkovskii*, was reported at very low rate among both groups.

Keywords: *Entamoeba* spp., epidemiology, nested PCR, 18S rRNA

Introduction

Amoebosis has a worldwide spread mainly in tropical and subtropical countries. Yearly around 50 million cases were reported, from them 10% turned to invasive disease, causing 40,000–100,000 deaths annually [1,2]. *Entamoeba* genus comprise between 30–50 different species, of which there are 10 accepted species infecting humans, 9 of them live in the intestinal lumen namely: *E. histolytica*, *E. dispar*, *E. moshkovskii*, *E. bangladeshi*, *E. coli*, *E. harmanni*, *E. polecki*, *E. chattoni* and *E. struthionis* while only one species *E. gingivalis* live in the oral cavity. The known pathogenic species among them are *E. histolytica*, *E. moshkowskii* and *E. bangladeshi* all of them are identical morphologically (*Entamoeba* complex), and cannot be differentiated microscopically from each other [3–6]. *Entamoeba dispar*, a non-invasive species, was proposed for the first time by Brumpt but soon discarded and considered as a synonym of *E. histolytica* because at that time (the first half of the 20th century) both species cannot be differentiated on morphological grounds. Until the 1990 decade, it was thought that *E. histolytica* had three forms, „races" or zymodemes: the „minuta". the „non-pathogenic magna" and the „pathogenic magna" races. Evidences were accumulating until [7] proposed the zymodemes were in fact different species. Thus, the „minuta" form corresponded to *E. hartmanni*, the „non-pathogenic magna" form to *E. dispar*, and the „pathogenic magna" form to *E. histolytica*. In 1978, Tanyuksel et al. [8] supported the distinction between *E. histolytica* and other two species which

were isolated from asymptomatic and symptomatic individuals using the isoenzymatic profile. Then the differences between both species were further confirmed using biochemical, immunological and molecular studies [8]. The third species *E. moshkovskii* was first detected by [9] in sewage water in Moscow and was considered as free-living organism until 1991 when Clark and Diamond, using molecular techniques, identified the Laredo strain of *E. histolytica* (an isolate obtained in 1956 and identified as „aberrant" or „atypical" *E. histolytica* due to its ability of growing at room temperature) as in fact *E. moshkovskii* when was isolated from an inhabitant in Laredo, Texas, USA suffering from epigastric pain, intestinal disturbances, weight loss and diarrhea [10]. Later on, this species was isolated from patients and discriminated from other *Entamoeba* species in many countries, such as Bangladesh, Thailand, Australia and India [11–14], respectively. The fourth species *E. bangladeshi* was first identified in Bangladesh in 2010–2011, through the investigation of diarrheal stool positive for *Entamoeba* via microscopy or culture, but they were negative for *E. histolytica*, *E. dispar* and *E. moshkovskii* via PCR, a new species was recognized and called *E. bangladeshi* [15], this species is also considered as a pathogenic species.

Despite to many published studies that distinguished *Entamoeba* spp. using molecular tools worldwide, limited studies have been performed in Iraq in general and in Kurdistan region particularly. The published data in Iraq are those conducted in Al-Najaf by [16] who reported the presence of *E. histolytica* in 24% (25/104) stool samples examined, in Baghdad [17] detected *E. histolytica* at a rate of 7% (7/100) in diarrheal stool of children using multiplex RT-PCR test. While in Al-Qadisiyah [18] recorded infection with *Entamoeba* spp. in 85.18% (92/108) of the examined stool samples constituted, *E. dispar* and *E. dispar* in combination with *E. moshkovskii* in 83.33% (90/108) and 1.85% (2/108) of the samples, respectively, without any case of *E. histolytica*. On the other hand, in Diwaniya [19] proved the presence of the three species of *Entamoeba* in single and mixed infections, with *E. dispar* at the highest rate (72.6%), then *E. histolytica* (41.1%), and *E. moshkovskii* (2.74%). In Erbil province [20] performed microscopical examination of stool sample from asymptomatic healthy individual and recorded *Entamoeba* spp.in 7.4% of the enrolled people. They targeted the 18S rRNA gene using the nested PCR in the microscopically positive samples and reported, *E. histolytica* in 6%, *E. dispar* in 4.3%, and *E. moshkovskii* in 0.3%.

Most studies performed in Duhok province involved microscopic identification or serological tests. This reason encouraged us to perform this study among symptomatic and asymptomatic individuals to identify by genetic analysis the causative agents of diarrhea among these individuals in Duhok city, Kurdistan region of Iraq.

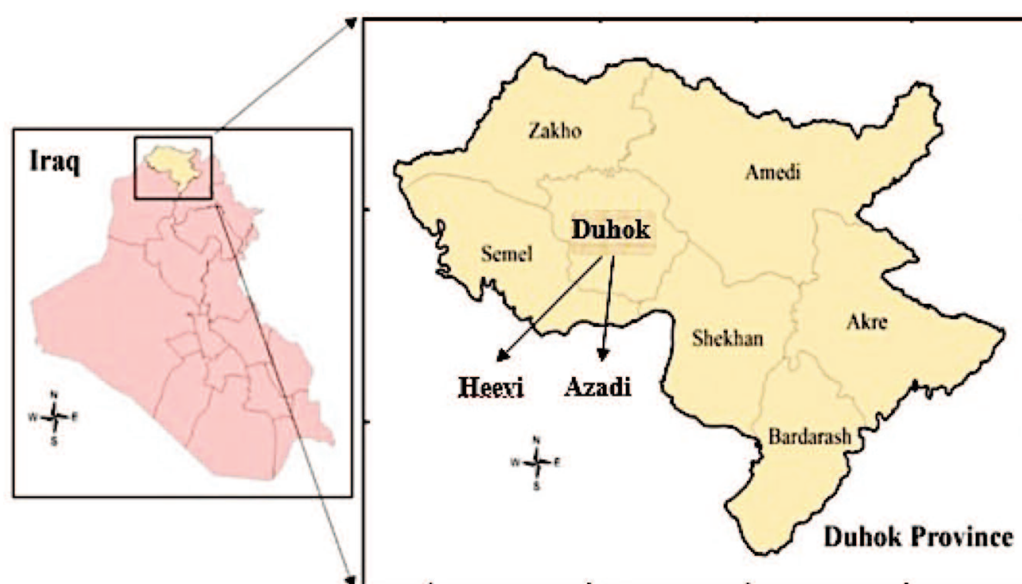

Figure 1. Map showing the location of the Azadi and Heevi Hospitals in Duhok city, Iraq

Materials and Methods

Sample collection

From April 2021 to March 2022, a total of 2592 stool samples were taken from diarrheic outpatients with amoebosis symptoms of both sexes and different ages (≤ l to 60) who visited Azadi teaching hospital and Heevi pediatric hospital in Duhok city (Fig. 1). Furthermore, 207 samples were collected randomly from asymptomatic individuals from both sexes and different ages, the positive specimens from them were used for molecular analysis along with positive symptomatic specimens. Patient informed consent was taken, and for children the consent was taken from their parents. Furthermore, an approval was obtained from the ethical committee of the General Directorate of Health in Duhok (Ethical clearance no. 0652021-2-4) to use the data and the collected specimens in the current study.

About 10 g of fresh stool sample was taken from each enrolled outpatient using a sterile wooden spatula avoiding to contaminate the sample with urine or water. Each sample was placed in a sterile clean disposable plastic container with a close lid, then each sample was labeled with the patient's full information e.g., date, name, gender, age, residence, etc. Each sample was examined macroscopically to determine its consistency, color, odor, presence of blood and mucous, followed by microscopic examination using both wet mount and Zinc sulfate flotation techniques. The results of the stool analysis were recorded either as positive or negative for *E. histolytica* complex depending on the presence of cysts/trophozoites. 100 microscopically positive samples (50 from symptomatic outpatients + 50 from asymptomatic) each about 0.2 g were used for molecular analyses. The 50 symptomatic patient samples were selected according to the abundance of cysts/trophozoites in the examined specimen, presence of mucous or blood and patients complains. While the selection of asymptomatic specimens depends on the abundance of cysts and these samples were free from blood or mucus.

Molecular identification

Molecular identification was conducted in the Molecular Biology Laboratory, Biology Department, Faculty of Science, Zakho University. DNA was extracted, using the instructions provided by the manufacturer of the Presto DNA Stool Extraction Kit (Geneaid, Taiwan), followed by using a low salt buffer to elute the silica membrane spin column and to determine the concentration and purity of the DNA that was recovered.

Amplification of the 18S rRNA gene for the genus *Entamoeba* produced 898 bp, while the second PCR primer targeted *E. dispar*, *E. moshkovskii*, and *E. histolytica* by amplifying 174 bp, 553, and 439 bps, respectively [21]. The first pair of primers E-1 (5'-TAAGATGCACGAGAGCGAAA-3') and E-2 (5'-GTACAAAGGGCAGGGACGTA), were used to amplify 18S rRNA gene. The second pair of nested PCR primers were used for species identification: ED-1 (5'-TCTAATTTCGATTAGA ACTCT-3') and ED-2 (5'- TCCCTACCTATTAGA CATAGC-3'); EM-1 (5'-GAAACCAAGAGTTT CACAAC-3') and EM-2 (5'-CAATATAAGGCTT GGATGAT-3'); EH-1 (5'-AAGCATTGTTTCTAG ATCTGAG-3') and EH-2 (5'-AAGAGGTCTAA CCGAAATTAG-3') [21].

For PCR amplification, the thermal cycler (Applied, Germany) was used with 40 µl reaction volume made up of 20 µl Hot Start Master Mix (containing Taq DNA polymerase 1 unit/10 µl, 2x reaction buffer and enzyme stabilizer) (GeNet Bio, South Korea), 4 µl forward and reverse primers (10 pmoles for each primer), and 4 µl DNA template with 12 µl of deionized distilled water).

The PCR cycling and running parameters were set at an initial denaturation cycle of 95°C for 10 minutes, followed by 30 cycles of 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds, with a final extension of 72°C for 5 minutes. For the second PCR the same cycling and running parameters as the first one was applied, except 35 cycles were used in the first step and the annealing temperature was changed to 52°C. On 1.5% and 2% agarose gels, the PCR products were electrophoresed using a 1X Tris-boric acid-EDTA buffer (TBE) and 0.2 mg/ml ethidium bromide (Sigma-Aldrich, St. Louis, Missouri, USA) with a 100 bp DNA marker ladder (Promega Corp., Madison, Wisconsin, USA).

PCR product sequence analysis

BigDye terminators and an ABI 3730XL sequencer (Macrogen® Corp., Seoul, South Korea) were used in the sequencing of a duplicate sample of each species. Each identified species' nucleotide sequences were deposited at GenBank under the accession numbers of *E. histolytica* (MZ905641 and MZ905500), *E. dispar* (MZ913023 and MZ913017) and *E. moshkovskii* (MZ913026 and MZ913027).

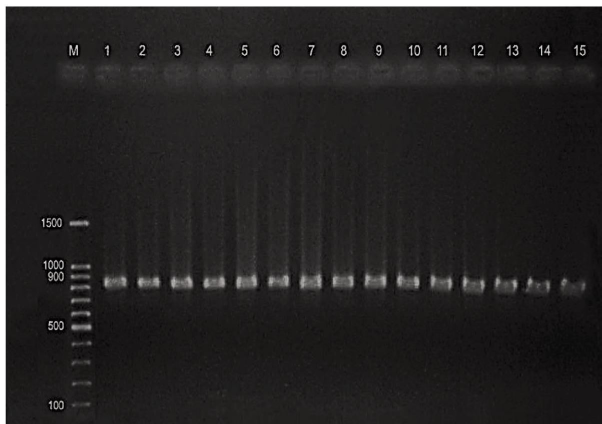

Figure 2. PCR product electrophoresed on a 1.5 percent agarose gel with primers specific to the *Entamoeba* genus. Lane (M) DNA ladder (100–1500 bp); lanes 1–15 898 bp amplicon size

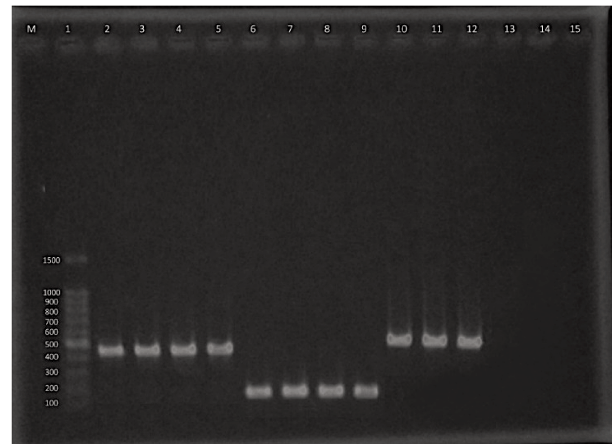

Figure 3. PCR product electrophoresed on a 1.5 percent agarose gel. Lane (M) DNA ladder (100–1500 bp); lanes 1–4 primer specific to the *E. histolytica* 439 bp; lanes 5–8 primer specific to *E. dispar* 174 bp*,* and lanes 9–11 primer specific to *E. moshkovskii* 553 pb

Statistical analysis

The obtained data, that represented as numbers and percentages, were analyzed by SPSS version 25 software. The Chi-square was applied. *P*-value less than 0.05 was considered significant, while higher values were deemed as non-significant.

Results

Microscopic examination and associated factors

The results revealed infection rate of 21.68% (562/2592) with *Entamoeba* spp. among the symptomatic samples examined as illustrated in Table 1. Among the studied factors, male showed a higher rate than females (67.43vs 32.56%), with statistically a highly significantly ($P<0.001$) difference between both sexes. Regarding ages, the ages from 1–10 years showed the highest rate (54.09%) with an inverse relationship between the age and the percentage of infection up to 50 years of age. At ages above 51 the rate was somewhat consistent. Statistically, highly significant ($P<0.000$) difference were recorded among different ages.

According to residency, in this study, the majority (58.54%) of patients infected with *Entamoeba* lived in urban areas, while 41.45% of them lived in rural areas, statistical analysis of the data did not show any significant difference ($P>0.95$) between both groups.

Regarding asymptomatic samples 28.02% (58/207) were positive for *Entamoeba* spp. cysts, and 50 of them were selected for molecular analysis .

Nested PCR

In this study**,** 100 microscopically positive stool samples (50 symptomatic outpatients and 50 asymptomatic individuals) were used for DNA extraction. The concentration of the extracted DNA ranged from 35 to 115 µg/ml, and the purity ranged from 1.7–1.9 measured at A260/A280 nm. Nested PCR results indicated that 73% (73/100) of microscopically positive yielded 898 bps bands for *Entamoeba* spp. using universal primers (E1 and E2) for amplifying 898 of 18S rRNA gene (Fig. 2). The nested PCR produced three fragments with different sizes for *Entamoeba* species using the species-specific primers. For *E. histolytica,* the product size was 439 bp, for *E. dispar* was 174 bp and *E. moshkovskii* was 553 bp (Fig. 3). The overall rate of *Entamoeba* spp. was 73%, comprising from *E. histolytica*, *E. dispar* and *E. moshkovskii* in addition 5 specimens were not identified as they did not produce any band with the three species-specific primers for *Entamoeba* spp. used in this study.

Types of infection

Regarding the types of infections, single infections with *E. histolytica*, *E. dispar*, and *E. moshkovskii* were reported at 26%, 24% and 3%, respectively. While 7% (7/100) of the cases were double infections with *E. histolytica* and *E. dispar*, 2 (4.0%) with *E. histolytica* and *E. moshkovskii* and 6% (6/100) with the three species as shown in Table 2. With regard to each group separately, 46% (23/50) of symptomatic patients harbored *E. histolytica,* 10% (5/50) *E. dispar* and 4% (2/50) *E.*

Table 1. The characteristics of the microscopically examined stool samples of the enrolled outpatients in the study and their relationship with some risk factors (n = 2592)

Characteristics	Total examined	Positive n (%)	*P*-value
Gender			
Male	1890	379 (67.43%)	0.001
Female	702	183 (32.56%)	
Age groups			
1–10	1627	304 (54.09%)	0.000
11–20	287	152 (27.04%)	
21–30	337	57 (10.14%)	
31–40	135	22 (3.91%)	
41–50	71	8 (1.4%)	
51–60	67	11 (1.9%)	
>60	68	8 (1.4%)	
Residential area			
Urban area	1437	329 (58.54%)	0.95
Rural area	1155	233 (41.45%)	
Total	2592	562 (21.69%)	

moshkovskii in single infections. While 14% (7/50) harbored both *E. histolytica* and *E. dispar*, 4% (2/50) *E. histolytica* and *E. moshkovskii*, 12% (6/50) harbored the three species and 10% (5/50) segments of *Entamoeba* genus did not produce any segment with the used 3 species-specific primers.

The molecular analysis of asymptomatic specimens, revealed 46% (23/50) positive specimens for *Entamoeba* genus, out of which 6% (3/50) were *E. histolytica*, 38% (19/50) were *E. dispar* and 2% (1/50) were *E. moshkovskii,* without any mixed infections.

Table 3 shows the symptoms experienced by infected outpatients. As indicated in the table, all infected patients with *E. histolytica* suffered from amoebic dysentery, diarrhea, gastroenteritis and abdominal pain. While those infected with *E. dispar* complained from diarrhea and abdominal pain these symptoms might be due to other reasons, as this species is nonpathogenic. The three patients infected with *E. moshkovskii* suffered from diarrhea only. The 7 patients infected with both *E. histolytica* and *E. dispar* suffered from abdominal pain with bloody mucoid diarrhea, and the other 2 infected with both *E. histolytica* and *E. moshkovskii* suffered from only diarrhea. On the other hand, the 6 patients who were infected with the three species complained from abdominal pain and diarrhea and 5 patients with unidentified *Entamoeba* species suffered from diarrhea only.

Discussion

Microscopic examination of infection with Entamoeba species

The accurate identification of *Entamoeba*

Table 2. The nested PCR results of microscopically positive symptomatic and asymptomatic stool specimens

Type of specimens	Total n	Positive n %	*E. h* n %	*E. d* n %	*E. m* n %	*E. h+E.d* n %	*E.h+ E. m* n %	*E.h+E.d +E.m* n %
Symptomatic	50	50 (100%)	23 (46%)	5 (10%)	2 (4%)	7 (14%)	2 (4%)	6 (12%)
Asymptomatic	50	23 (46%)	3 (6%)	19 (38%)	1 (2%)	0	0	0
Total	100	73 (73%)	26 (26%)	24 (24%)	3 (3%)	7 (7%)	2 (2%)	6 (6%)

Table 3. Relationships between the symptoms and *Entamoeba* species identified in 50 nested PCR positive specimens of symptomatic outpatients

Entamoeba species	Positive samples		The main symptoms experienced by patients
	n	%	
E histolytica	23	46%	Amebic dysentery, gastroenteritis, abdominal pain
E. dispar	5	10%	Diarrhea, abdominal pain
E. moshkovskii	2	4%	Diarrhea
E. histolytica + *E. dispar*	7	14%	Bloody mucoid diarrhea, abdominal pain
E. histolytica + *E. moshkovskii*	2	4%	Diarrhea
E. histolytica, *E. dispar*, *E. moshkovskii*	6	12%	Diarrhea, abdominal pain
Unidentified species	5	10%	Diarrhea only

histolytica, that cause amoebosis, is a significant goal to the clinical microbiology laboratories, since, amoebosis is one of the three most common causes of death, it causes 100,000 deaths annually [22]. The four *Entamoeba* spp. are morphologically similar, and can not be distinguished despite to the genetic and pathogenic differences [21]. Generally, *E. histolytica*-like species apart of *E. histolytica* are not involved in extraintestinal damage [11,23]. In order to determine the actual prevalence of *E. histolytica* infection, it is necessary to identify the various *Entamoeba* species present in a specimen. As a result, a large number of people have been treated with anti-amoebic drugs unnecessarily, in addition to providing an imprecise picture of the epidemiology of the organism and the illness because previous amplified data relied solely on microscopy [24].

In this study the prevalence of *Entamoeba* spp. in symptomatic patients was estimated microscopically and positive cases were correlated with gender, age and residence. Furthermore, molecular approaches, using nested PCR were used for the identification and differentiation of *Entamoeba* spp. in addition to estimating the prevalence of *E. histolytica* from *E. dispar* and *E. moshkovskii* among symptomatic and asymptomatic individuals in Duhok province. Furthermore, the association of *Entamoeba* species was linked to the symptoms experienced by infected individuals.

The microscopic examination revealed that 21.68 % of the diarrheic samples were infected with *Entamoeba* species. Variable infection rates with *Entamoeba* spp. using microscopic examination have been reported in Kurdistan region and rest parts of Iraq, in Duhok, a rate of 27.9% was reported among infants and children [25]. In another study in Duhok, a rate of 26.1% was reported among children only [26]. In both of Duhok and Erbil, a rate of 26.1% was reported among children [27]. While among Erbil population a rate of 7.4% was reported among the population [20]. In Samarra, a rate of 12.8% was reported among patients [28]. In Al-Qadisyah province, a rate of 28.8% was reported among anemic patients [29] and in Babel province a rate of 62% was reported among patients. In Mosul city, an infection rate of 63.8% was reported in patients visited hospitals and healthcare centers [30].

This variability in infection rates may be due to environmental, behavioral, socioeconomic conditions such as, the low income, the occupation and the level of parent's education for children and health education of the population, regarding children, majority of them play in contaminated areas, and eat without washing their hands, such risk factors can greatly increase the prevalence of these parasites [31,32].

Regarding gender, the rate of amoebosis was significantly higher ($P<0.001$) in males than in females, this is in line with a study in Nineveh province/Iraq who found higher rate of amoebosis in males than females (49.1% vs 34.6%) [33]. Another study in Mecca/Saudi Arabia also, reported higher rate in males than females (33.3% vs 31.4%) [34], similarly, studies from Yemen (64.0% vs

36.0%) and India (27.1% vs 23.5%) reported higher rates in males than females [35,36]. On the other hand, in some studies higher amoebosis rates were reported among females than in males, such as In Erbil [20] reported a higher prevalence of amoebosis among females than males (58.8% vs 41.4%). In Samarra city [28] also, reported a higher infection rate in females as compared to males (53.6% vs 46.3%). While [37] reported nearly close rates between both males and females (49.6% versus 50.4 %). The disparity in infection rates between males and females could be related to behavioral, immunological and hormonal differences, since androgens leads to excessive recruitments of leukocytes in males thus induce the risk of immune-mediated pathology [38]. Furthermore, males are the working class that have more interaction with the environment, in addition they eat and drink in public locations or from street sellers, increasing their chances of infection [36,39].

Furthermore, infection rates varied by age category, with the highest rate (54.09%) among ages of 1–10 years, then the rate declined with the increase in age up to the age of 50 years. As regards to age also, variable rates have been reported by various researchers, such as [40], in some villages near Baghdad, found the highest infection rates (26.20% and 32.5%) among the ages 1–10 and 13–18 years which is partly in line with the current study. in Kassala/Sudan [41] reported the high rate (51.7%) of infection among the age group 10–19 years. While in Erbil [20] found the highest frequency of infection (13.8%) of *E. histolytica* among the ages 1–4 years and in Thi-Qar [37] reported the highest rate (11.1%) among the ages 5–14 years. The high rate of infection with *Entamoeba* spp. among infants might be due to the feeding method, improper bottle sterilization techniques, or using unsterilized tap water for milk preparation, all of which indicate the significant of hygiene application in controlling amoebosis. As regards to children, they spend more time outside their home playing and most of them eat outside their home, thus are exposed to higher risk factors [42,43].

In the current study, somewhat, a higher rate of infection was reported among urban residents as compared with rural settings (58.54% vs 41.45%) even though the difference between both groups was statistically non-significant. Similarly, in Baghdad/Iraq, higher rates were reported among urban than rural (70% vs 30%) [36,44] also, reported a higher rate of amoebosis were reported among urban than rural inhabitant (28.9% vs 23.8%). While, the present results contradict with those performed in Thi-Qar, Iraq [18,37] they found higher infection rates in residents of rural areas than urban one (9.77 % vs. 7.26% and 69.4% vs. 30.6%) respectively. The increase in infection rates among urban residents may be due to factors like low economic standard, consumption of raw vegetables, eating outside their homes, improper hand wash before meals in addition to poor application of hygiene habits [36].

Identification of Entamoeba species using nested PCR technique

The estimation of the accurate prevalence of *E. histolytica* in stool specimens, require the application of molecular tools, since the microscopic identification of *E. histolytica* is inaccurate and undependable, especially in specimens with morphologically indistinguishable species as *E. histolytica*, *E. dispar*, *E. moshkovskii* and other *Entamoeba* spp., therefore, molecular techniques are the best method that can be used for the identification of such species [45]. The majority of methods used for DNA extraction from stool samples are expensive and time consuming leading to process a small number of samples. For this reason, in the present study Presto Stool DNA extraction Kit, was used, and yielded 100% positive results for *Entamoeba* genus, this kit is proved to be efficient, reliable and is successful for recovering DNA from faecal materials [46]. The 54% of the negative asymptotic samples in the present study might be due to many reasons such as the absence of *Entamoeba* organisms in these samples.

All samples of symptomatic patients produced 898 bp fragments for genus *Entamoeba* (50/50), while 23/50 from asymptomatic individuals were positive. The highest rate among symptomatic samples was with *E. histolytica* accounting for 46% in single infections and contributed in all mixed infections with other species, while *E. dispar* was predominant among asymptomatic samples at a rate of 38%, and *E. histolytica* was found in 6% only of the asymptomatic samples. These finding are partly in line with the study in Thi-Qar [37] they also detected these both species at high rates using nested and real time PCR, but among symptomatic patients even though at lower rates than the current study which were 31.3% for *E. histolytica* and 17.5% for *E. dispar*. In Zakho, Iraq [47] using

nested PCR, recorded 46/50 positive samples for *Entamoeba* genus among internally displaced people, of them 23 contained *E. histolytica*, in 9 cases both *E. histolytica* and *E. dispar,* while the three species were recorded in 7 cases, 5 cases with only *E. dispar* and both *E. dispar* and *E. moshkovskii* in 2 cases. Similarly in Yemen [35] *E. histolytica* was reported at a higher rate than *E. dispar* (20.2% vs 15.7%). Reports from Bangladesh and Madagascar showed higher prevalence of *E. histolytica* in diarrheal stool and other gastrointestinal discomfort among patients as compared to the stool of healthy control individuals [48,49].

The high rate of *E. histolytica* might be attributed to the differences in the socio-economic status, environmental conditions and other risk factors. On the other hand, the present study results contradict with those conducted in Diwanyha province, south-central region of Iraq [19], as he reported higher prevalence of *E. dispar* than *E. histolytica* among symptomatic diarrheal patients. The high rate of *E. dispar* in the specimens of asymptomatic individuals in the current study is in line with the study of [36] who reported a rate of 7.5% among asymptomatic population in India, while *E. histolytica* was found at 5% among the same population.

Amoebosis is an important food and water born disease, and its high prevalence in Iraq could be attributed to poor socioeconomic conditions, low level of hygiene application and contamination of water supply, as it is estimated that nearly 500 tons of sewage are dumped daily into Iraqi rivers, contaminating the water source, particularly in the south of the country, where these rivers are the main sources of water [50].

The differentiation of *Entamoeba* species in stool sample is very important task, because *E. histolytica* causes intestinal and extraintestinal amoebosis, a parasitic disease that causes morbidity and mortality in developing countries and some of the developed one [51,52]. Therefore, *E. histolytica* infections must be differentiated from *E. dispar* and *E. moshkovskii* for therapeutic purposes. According to the available data, the rate of *E. dispar* is around ten folds higher than that of *E. histolytica* globally [51]. As a result, it is necessary to analyze the prevalence in various geographic regions because the local prevalence can vary greatly.

As regards to the symptoms associated with each species, most (46%) of the patients infected with *E. histolytica* and *E. histolytica* in combination with *E. dispar* and *E. moshkovskii* suffered from dysentery or diarrhea, gastroenteritis and abdominal pain. Similarly, in India [36] stated that *E. histolytica* was observed in the majority of the GI discomfort patients and in all those with liver abscess. While [53] reported that about 10-20% of people infected with *E. histolytic* complained of diarrhea which occasionally mixed with blood, abdominal pain and vomiting, and the disease was severe in very young ages, in older individuals and those with weak immune status. He further added that the risk factors associated with increasing the severity of the disease and mortality are, young age, immunity status, malignancy, pregnancy, malnutrition, alcoholism, and those taking corticosteroids.

Also, the majority of patients with *E. dispar* and *E. moshkovskii* infection in this study were symptomatic they suffered from abdominal pain and diarrhea, even though these species were reported to be nonpathogenic, these symptoms might be due to the presence of other microorganism or any other reason. To ascertain this observation more studies are required as pointed out in India [54] they stated that dysentery was connected to both *E. dispar* and *E. moshkovskii* infection in an Indian investigation, and as a result, more research with controller and patient groups is needed to prove the true pathogenic potential of *E. dispar* and *E. moshkovskii* [54].

In conclusions, based on the analysis of epidemiological data, it is found that amoebosis is endemic in this region and it cause a significant health burden but it lacks effective control measures. Therefore, it is important to consider these factors during the application of control measures that will minimize the chances of infection. Furthermore, specific diagnostic measures are mandatory for precise diagnosis of the pathogenic and nonpathogenic to reduce the use of anti-amoebic medications. Hence, our study substantiates the validity of employing molecular identification techniques for the detection of both pathogenic and non-pathogenic strains of *Entamoeba*, to facilitate the accurate prescription of suitable medications.

Acknowledgements

The authors thank the Biology Department, Faculty of Science, Zakho University, Kurdistan region/Iraq, for providing some research facilities,

and to the laboratory team of Azadi and Heevi hospitals for providing the stool samples and using participants data.

References

[1] WHO/PAHO/UNESCO report. 1997. A consultation with experts on amoebiasis. Mexico City, Mexico 28–29 January. *Epidemiological Bulletin* 8(1): 13–14.

[2] Baxt L.A., Singh U. 2008**.** New insights into *Entamoeba histolytica* pathogenesis. *Current Opinion in Infectious Diseases* 21(5): 489–494. doi:10.1097/QCO.0b013e32830ce75f

[3] Ximénez C., Morán P., Rojas L., Valadez A., Gómez A. 2009. Reassessment of the epidemiology of amebiasis: state of the art. *Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases* 9(6): 1023–1032. doi:10.1016/j.meegid.2009.06.008

[4] Mulinge E., Mbae C., Ngugi B., Irungu T., Matey E., Kariuki S. 2021**.** *Entamoeba* species infection in patients seeking treatment for diarrhea and abdominal discomfort in Mukuru informal settlement in Nairobi, Kenya. *Food and Waterborne Parasitology* 23: e00122. doi:10.1016/j.fawpar.2021.e00122

[5] Brumpt E. 1928**.** Differentiation of human intestinal amoebae with four nucleated cysts. *Transactions of The Royal Society of Tropical Medicine and Hygiene* 22(2): 115–124. doi:10.1016/S0035-9203(28)90002-4

[6] Diamond L.S., Clark C.G. 1993. A redescription of *Entamoeba histolytica* Schaudinn, 1903 (emended Walker, 1911) separating it from *Entamoeba dispar* Brumpt, 1925. *The Journal of Eukaryotic Microbiology* 40(3): 340–344. doi:10.1111/j.1550-7408.1993.tb04926.x

[7] Sargeaunt P.G., Williams J.E., Grene J.D. 1978. The differentiation of invasive and non-invasive *Entamoeba histolytica* by isoenzyme electrophoresis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 72(5): 519–521. doi:10.1016/0035-9203(78)90174-8

[8] Tanyuksel M., Petri W.A.Jr. 2003. Laboratory diagnosis of amoebiasis. *Clinical Microbiology Reviews* 16(4): 713–729. doi:10.1128/CMR.16.4.713-729.2003

[9] Tshalaia L. 1941. A species of *Entamoeba* detected in a sewage. *Meditsinskaya Parazitologiya i Parazitarnye Bolezni* 10(2): 244–252.

[10] Clark C.G., Diamond L.S. 1991. The Laredo strain and other '*Entamoeba histolytica* like' amoebae are *Entamoeba moshkovskii. Molecular and Biochemical Parasitology* 46(1): 11–18. doi:10.1016/0166-6851(91)90194-b

[11] Ali I.K., Hossain M.B., Roy S., Ayeh-Kumi P.F., Petri W.A.Jr., Haque R., Clark C.G. 2003. *Entamoeba moshkovskii* infections in children, Bangladesh. *Emerging Infectious Diseases* 9(5): 580–584. doi:10.3201/eid0905.020548

[12] Hamzah Z., Petmitr S., Mungthin M., Leelayoova S., Chavalitshewinkoon-Petmitr P. 2006. Differential detection of *Entamoeba histolytica*, *Entamoeba dispar*, and *Entamoeba moshkovskii* by a single-round PCR assay. *Journal of Clinical Microbiology* 44(9): 3196–3200. doi:10.1128/JCM.00778-06

[13] Fotedar R., Stark D., Beebe N., Marriott D., Ellis J., Harkness J. 2007. PCR detection of *Entamoeba histolytica*, *Entamoeba dispar*, and *Entamoeba moshkovskii* in stool samples from Sydney, Australia. *Journal of Clinical Microbiology* 45(3): 1035–1037. doi:10.1128/JCM.02144-06

[14] Philips S.A., Manochitra K., Padukone S., Parija S.C. 2018. Detection of *Entamoeba s*pecies: a comparative analysis of nested multiplex PCR and recombinase polymerase amplification. *International Journal of Current Microbiology and Applied Sciences* 7(03): 1803–1808. doi:10.20546/ijcmas.2018.703.212

[15] Royer T.L., Gilchrist C., Kibir M., Arju T., Ralston K.S., Hjaque R., Clark C.G., Petri W.A.Jr. 2012. *Entamoeba bangladeshi* nov. sp., Bangladesh. *Emerging Infectious Diseases* 18(9): 1543–1545. doi:10.3201/eid1809.120122

[16] Al-Hameedawi J.J.Y. 2014. Molecular identification of *Entamoeba histolytica* parasite by using actin and amebapores-A genes. *Kufa Journal for Nursing Sciences* 4: 48–55.

[17] Hussein R.A., Al-Mayah Q.S., Merdaw M.A.Z., Al-Bashier N.T., Al-Abbas A.A., Jasem I.A. 2015. Evaluation of multiplex real-time PCR and ELISA in detection of intestinal protozoan parasites from children with diarrheal disease. *International Journal of Advanced Research* 3(9): 782–788.

[18] Khalaf M.S., Rashid S.A. 2018. Molecular study of *Entamoeba dispar* and *Entamoeba moshkovskii* isolated from amoeboid dysetery in comparison with *Entamoeba histolytica* infection. *Journal of Pharmaceutical Sciences and Research* 10(9): 2129–2133.

[19] Dasheesh T. 2016. Molecular identification of some species of *Entamoeba* isolated from patients with diarrhea in Afak City/Al-Qadisiyah governorate using real-time PCR technique. *International Journal of Recent Scientific Research* 7(5): 11207–11211.

[20] Faqe Mahmood S.A., Mustafa H.B. 2020. Molecular identification and prevalence of *Entamoeba histolytica*, *Entamoeba dispar* and *Entamoeba moshkovskii* in Erbil City. Northern Iraq. *Polish Journal of Microbiology* 69(3): 1–10. doi:10.33073/pjm-2020-028

[21] Khairnar K., Parija S.C. 2007. A novel nested

multiplex polymerase chain reaction (PCR) assay for differential detection of *Entamoeba histolytica, E. moshkovskii* and *E. dispar* DNA in stool samples. *BMC Microbiology* 7: 47. doi:10.1186/1471-2180-7-47

[22] Kellerman R.D., Rakel D., KUSM-W Medical Practice Association. 2022. Conn's current therapy. Elsevier Health Sciences.

[23] Anuar T.S., Al-Mekhlafi H.M., Abdul Ghani M.K., Abu Bakar E., Azreen S.N., Salleh F.M., Ghazali N., Bernadus M., Moktar N. 2012. Molecular epidemiology of amoebiasis in Malaysia: highlighting the different risk factors of *Entamoeba histolytica* and *Entamoeba dispar* infections among Orang Asli communities. *International Journal for Parasitology* 42(13–14): 1165–1175. doi:10.1016/j.ijpara.2012.10.003

[24] Kebede A., Verweij J.J., Petros B., Polderman A.M. 2004. Short communication: misleading microscopy in amoebiasis. *Tropical Medicine and International Health* 9(5): 651–652. doi:10.1111/j.1365-3156.2004. 01236.x

[25] Badry A.H., Jameel A.Y., Mero W.M. 2014. Pathogenic microorganisms associated with diarrhea in infants and children in Duhok Province, Kurdistan Region/Iraq. *Science Journal of University of Zakho* 2: 266–275. doi:10.25271/2014.2.2.201

[26] Hussein J.N., Meerkhan A.A. 2019. The incidence of intestinal parasites among children in Hivi Pediatric Hospital, Duhok, Iraq. *Science Journal of University of Zakho* 7(1): 1–4. doi:10.25271/sjuoz.2019.7.1.571

[27] Yilmaz H., Abdullah A.M. 2017. Prevalence of intestinal parasites (*Entamoeba* species and *Giardia lamblia*) in Duhok and Erbil Cities, Northern Iraq. *Journal of Microbiology and Experimentation* 4(6): 119–122. doi:10.15406/jmen.2017.04.00132

[28] Bazzaz A.A., Shakir O.M., Alabbasy R.H. 2017. Prevalence of two gastrointestinal parasites *Entamoeba histolytica* and *Giardia lamblia* within Samarra city, Iraq. *Advances in Bioscience and Biotechnology* 8(11): 399–410. doi:10.4236/abb.2017.811029

[29] Alomashi G.B., Al-Shabbani A.H. 2019. Prevalence of intestinal parasitic infestation in anemic patients attended to Al-Diwaniyah Teaching Hospital at Al-Qadisiyah Province/Iraq. *International Journal of Pharmaceutical Quality Assurance* 10(3): 60–65. doi:10.25258/ijpqa.10.3.22

[30] Ahmed N.M., Al-Niaeemi B.H., Dawood M.H. 2020. Epidemiological and clinical aspects about the endemic intestinal parasites in reviewers of Mosul General hospitals and healthcare centers/Nineveh governorate-Iraq. *EurAsian Journal of BioSciences* 14: 6037–6043.

[31] Yassin M.M., Shubair M.E., al-Hindi A.I., Jadallah S.Y. 1999. Prevalence of intestinal parasites among school children in Gaza City, Gaza Strip. *Journal of the Egyptian Society of Parasitology* 29(2): 365–373.

[32] Coulibaly J.T., Fürst T., Silué K.D., Knopp S., Hauri D., Ouattara M., Utzinger J., N'Goran E.K. 2012. Intestinal parasitic infections in schoolchildren in different settings of Côte d'Ivoire: effect of diagnostic approach and implications for control. *Parasites and Vectors* 5: 135. doi:10.1186/1756-3305-5-135

[33] Al-Naimi S.H.S. 2001. Epidemiological study of parasites that cause diarrhea in the province of Nineveh, to estimate the level globulinsimmune serum albumin in patients with certain intestinal parasites, Master Thesis, College of Science, University of Mosul, Iraq.

[34] Al-Harthi S.A., Jamjoom M.B. 2007. Enteroparasitic occurrence in stools from residents in Southwestern region of Saudi Arabia before and during Umrah season. *Saudi Medical Journal* 28(3): 386–389.

[35] Al-Areeqi M.A., Sady H., Al-Mekhlafi H.M., Anuar T.S., Al-Adhroey A.H., Atroosh W.M., Dawaki S., Elyana F.N., Nasr N.A., Ithoi I., Lau Y.L., Surin J. 2017. First molecular epidemiology of *Entamoeba histolytica*, *E. dispar* and *E. moshkovskii* infections in Yemen: different species-specific associated risk factors. *Tropical Medicine and International Health* 22(4): 493–504. doi:10.1111/tmi.12848

[36] Singh A., BanerjeeI T., Khan U., Shukla S.K. 2021. Epidemiology of clinically relevant *Entamoeba* spp. (*E. histolytica/dispar/moshkovskii/bangladeshi*): a cross sectional study from North India. *PLoS Neglected Tropical Diseases* 15(9): e0009762. doi:10.1371/journal.pntd.0009762

[37] Flaih M.H., Khazaal R.M., Kadhim M.K., Hussein K.R., Alhamadani F. 2021. The epidemiology of amoebiasis in Thi-Qar Province, Iraq (2015–2020): differentiation of *Entamoeba histolytica* and *Entamoeba dispar* using nested and real-time polymerase chain reaction. *Epidemiology and Health* 43: e2021034. doi:10.4178/epih.e2021034

[38] Sellau J., Groneberg M., Fehling H., Thye T., Hoenow S., Marggraff C., Weskamm M., Hansen C., Stanelle-Bertram S., Kuehl S., Noll J., Wolf V., Metwally N.G., Hagen S.H., Dorn C., Wernecke J., Ittrich H., Tannich E., Jacobs T., Bruchhaus I., Altfeld M., Lotter H. 2020. Androgens predispose males to monocyte-mediated immunopathology by inducing the expression of leukocyte recruitment factor CXCL 1. *Nature Communications* 11(1): 3459. doi:10.1038/s41467-020-17260-y

[39] Tasawar Z., Kausar S., Lashari M.H. 2010. Prevalence of *Entamoeba histolytica* in humans. *Pakistan Journal of Pharmaceutical Sciences* 23(3): 344–348.

[40] Kadir M.A., Rasheed S.A. 2008. Prevalence of some parasitic helminths among slaughtered ruminants in Kirkuk slaughter house, Kirkuk, Iraq. *Iraqi Journal of Veterinary Sciences* 22(2): 81–85.

doi:10.33899/ijvs.2008.5722

[41] Mohamed M.M., Ahmed A.I., Salah E.T. 2009. Frequency of intestinal parasitic infections among displaced children in Kassala Town. *Khartoum Medical Journal* 2(1): 175–177.

[42] Naous A., Naja Z., Zaatari N., Kamel R., Rajab M. 2013. Intestinal amebiasis: a concerning cause of acute gastroenteritis among hospitalized lebanese children. *North American Journal of Medical Sciences* 5(12): 689–698. doi:10.4103/1947-2714.123253

[43] Hassan A.O., Mero W.M. 2020. Prevalence of intestinal parasites among displaced people living in displacement camps in Duhok Province/Iraq. *The Internet Journal of Microbiology* 17(1): 1–8. doi:10.5580/IJMB.55008

[44] Al-Janabi M.A., Altikrity I.A. 2014. The spread of *Entamoeba histolytica* infection among children attended the Central Child Hospital in Baghdad city. *Iraqi Journal of Science* 55 (3A): 979–985.

[45] Al-Dalabeeh E.A., Irshaid F.I., Roy S., Ali I.K., Al-Shudifat A.M. 2020. Identification of *Entamoeba histolytica* in patients with suspected amebiasis in Jordan using PCR-based assays. *Pakistan Journal of Biological Sciences* 23(2): 166–172. doi:10.3923/pjbs.2020.166.172

[46] Zebardast N., Haghighi A., Yeganeh F., Seyyed Tabaei, S.J., Gharavi M.J., Fallahi S., Lasjerdi Z., Salehi N., Taghipour N., Kohansal C., Naderi F. 2014. Application of multiplex PCR for detection and differentiation of *Entamoeba histolytica*, *Entamoeba dispar* and *Entamoeba moshkovskii*. *Iranian Journal of Parasitology* 9(4): 466–473.

[47] Alberfkani M.I. 2021. Prevalence and molecular identification of some parasites and pyoderma associated with scabies among displaced people in Zakho Camps, Kurdistan Region, Iraq. PhD Thesis, Faculty of Science, University of Zakho, Iraq.

[48] Haque R., Mondal D., Karim, A., Molla, I.H., Rahim A., Faruque A.S., Ahmad N., Kirkpatrick B.D., Houpt E., Snider C., Petri W.A.Jr. 2009. Prospective case-control study of the association between common enteric protozoal parasites and diarrhea in Bangladesh. *Clinical Infectious Diseases* 48(9): 1191–1197. doi:10.1086/597580

[49] Randremanana R., Randrianirina, F., Gousseff M., Dubois N., Razafindratsimandresy R., Hariniana E.R., Garin B., Randriamanantena A., Rakotonirina H.C., Ramparany L. Ramarokoto C.E., Rakotomanana F., Ratsitorahina M., Rajatonirina S., Talarmin A., Richard V. 2012. Case-control study of the etiology of infant diarrheal disease in 14 districts in Madagascar. *PloS One* 7(9): e44533. doi:10.1371/journal.pone.0044533

[50] Korzeniewski K. 2006. The epidemiological situation in Iraq. *Przegląd Epidemiologiczny* 60(4): 845–855.

[51] Petri W.A.Jr., Haque, R., Lyerly D., Vines R.R. 2000. Estimating the impact of amoebiasis on health. *Parasitology Today* 16(8): 320–321. doi:10.1016/s0169-4758(00)01730-0

[52] Ohnishi K.Y., Kato A., Imamura M., Fukayama T., Tsunoda Y., Sakaue M., Sakamoto M., Sagara H. 2004. Present characteristics of symptomatic *Entamoeba histolytica* infection in the big cities of Japan. *Epidemiology and Infection* 132(1): 57–60. doi:10.1017/s0950268803001389

[53] Stanley S.L.Jr. 2003. Amoebiasis. *Lancet* 361(9362): 1025–1034. doi:10.1016/S0140-6736(03)12830-9

[54] Parija S.C., Khairnar K. 2005. *Entamoeba moshkovskii* and *Entamoeba dispar*-associated infections in Pondicherry, India. *Journal of Health, Population, and Nutrition* 23(3): 292–295.

Received 04 July 2023
Accepted 10 July 2024