Molecular Study for the Virulence Factor of *Entamoeba spp.* by Gene Sequence Technique

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ABSTRACT

Amoebiasis is characterized as one of the invasive extraintestinal or intestinal infections. The current study used PCR for 296 microscopically positive patients. The molecular study showed that 144 (48.64%) were *Entamoeba* (*E.*) *histolytica*, 91 (30.74%) were *E. dispar* and 52 (17.56%) were *E. moskovski*. The association of *E. histolytica* and *E. dispar* was recorded for 9 (3.04%). The positive samples with PCR have been submitted to detect the virulence factors of *E.histolytica* by PCR and the results showed that 61 samples (42.36 %) were positive with cysteine proteases gene, 50 (34.72%) were positive with amoebapore and 33 (22.91%) were positive with lectin. The gene sequence for cysteine protease, amoebapore, and lectin in local *E. histolytica* human isolates showed the nucleotide alignment similarity and substitution mutations in all of the cysteine protease, amoebapore, and Gal/GalNAc lectin. Phylogenetic tree analysis based on cysteine protease gene partial sequence in local *E. histolytica* human isolates that used for genetic variation analysis showed genetic closed related to NCBI-BLAST *E. histolytica* strain (M27307.1) at total genetic changes (0.0020-0.0050%), while phylogenetic tree analysis based on amoebapore B gene partial sequence in local *E. histolytica* human isolates that used for genetic variation analysis showed genetic closed related to NCBI-BLAST *E. histolytica*, strain (MS30-1047) at total genetic changes (0.20-0.050%). Phylogenetic tree analysis based on Gal/GalNAc lectin gene partial sequence in local *E. histolytica* human isolates that were used for genetic variation analysis showed genetic close related to NCBI-BLAST *E.histolytica* HM-1:IMSS strain (AP023115.1) at total genetic changes (0.0080-0.0020).

Key Words: *Amoebiasis, Entamoeba histolytica, PCR, Cysteine protease*

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INTRODUCTION

Amoebiasis is one of the 3 most common parasitic diseases in the world after malaria and bilharzia that affects more than 50 thousand people worldwide with 100,000 cases of death per year [1].

According to WHO, amoebiasis is the status in which the human body harbors with or without clinical manifestation *Entamoeba* (E.) *histolytica*, a protozoan, pseudopod-forming parasite [2]. Amoebiasis can be asymptomatic or can lead to the development of a severe infection that manifests as amoebic colitis or amoebic liver abscess [3]. The severity of amebiasis is illustrated in several ways. Amebic colitis is one of the leading causes of severe diarrhea worldwide and is among the top 15 causes of diarrhea [4]. In the first two years of life in children living in developing countries, diarrhea remains the 3rd leading cause of death in these countries accounting for 9% of all deaths in children under 5 years of age [5].

Fulminant amebic colitis is an uncommon complication of amebiasis but is associated with high mortality. On average, more than 50% of individuals with severe colitis die [2]. *Entamoeba* is classified as a Category B priority

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biodefense pathogen by the National Institute of Allergy and Infectious Diseases (NIAID) due to its low infectious dose, chlorine resistance, and environmental stability. Properties that can pose a threat of easy spread through food and water contamination [2]. Even in low-incidence settings, these properties of the parasite lead to epidemics among military personnel and the general population. In addition, E. *histolytica* is one of the most common causes of infectious diarrhea in travelers returning from endemic areas [5].

The development of molecular biology techniques has improved our understanding and allowed us to recognize and differentiate E. *histolytica* from non-pathogenic *Entamoeba* species. Among the many species of *Entamoeba* that infect humans. Previous descriptions of amoebiasis have been based on microscopy alone and have not been able to differentiate *E. histolytica* from these twins, which are 3 morphologically identical species. Among these three, *E. moshkovskii* can cause diarrhea, while *E. dispar* and *E. bangladeshi*, newly described, are considered non-pathogenic [4].

There is no vaccine to prevent amoebiasis. Nitroimidazoles are the basic treatment for invasive amoebiasis. Given the toxicity that can be associated with this class of drugs as well as the new resistances already reported, new therapeutic means are needed [6]. In addition, the NIAID has set priorities for drug development for the treatment of Category B pathogens including amoebiasis. For these reasons, a better understanding of the pathogenicity of amoebiasis is needed to improve new treatment tools and prevention [6].

In light of all these elements, we proposed to study the epidemiological profile of intestinal amoebiasis in a sample of 296 Stool samples from patients of Iraqi individuals.

From this total sample, we have to differentiate *Entamoeba* species using multiplex PCR and specify the virulence factors of *Entamoeba* spp. gene by conventional PCR, and sequence the gene responsible for the virulence of *Entamoeba* spp.

MATERIALS AND METHODS

Collection of stool samples

The study area and the sample collection is Thi-Qar (Iraq). All of the medical information about amoebiasis in the Province of Thi-Qar for 12 years (between Jan. 2010 and Dec. 2021) has been obtained from the records of the patients at the Public Health Dept of Health Office of Thi-Qar, which included gender, age, date, and address. Direct smears have been utilized for the diagnosis of patients who have amoebiasis. Molecular studies have been carried out at 2 locations, which are: Mohammed Al-Mosawi and Al-Chebish Hospitals, between Feb. 2021 and Oct. 2021. Those hospitals treat patients of any age. There have been 296 stool samples obtained randomly from patients who have diarrhea, and after that, they have been examined (via a general stool examination) with the use of microscopy to observe the cysts and/or trophozoites in stool. Every one of those 80 samples has been kept in a refrigerator at -20 °C in a stool container.

Macroscopic examination

The stool samples were examined with the naked eye to detect color, appearance, blood, mucus, and smile.

Molecular diagnosis method

Extraction of the genomic DNA

DNA has been obtained from 200 mg stool with the use of a commercial Kit presto™ stool gDNA according to the manufacturer's instructions. For the estimation of the Genomic DNA**,** the Nanodrop spectrophotometer checked the obtained genomic DNA from stool samples. The measurement of the DNA purity through the reading of absorbance at (260 nm\280 nm).

Preparation of the PCR master mixture

The PCR amplification of targeted genes: sequences of the primer that were utilized have been: for EntaF, 5ATG CAC GAG AGC GAA AGC AT3; for EhR, 5GAT CTA GAA ACA ATG CTT CTC T3; for EdR, 5CAC CAC TTA CTA TCC CTA CC3; and the EmR, 5TGA CCG GAG CCA GAG ACA T3. All of the primer sequences have been compared with the sequences in the GenBank. This has shown that the forward primer (Enta-F) sequence has only been found in the *Entamoeba* and that the 3 reverse primer (EdR, EmR, and EhR) sequences have been defined as specific species. Which is why, they're proper for the differentiation of the species. Forward primer in combination with suitable reverse primer produces 573 bp PCR product with the *E. histolytica* DNA, 390 bp PCR product with the E. dispar DNA, and 553 bp product with the *E. moshkovskii* DNA. The reaction of the PCR amplification has been carried out in 51 µl final volume in 0.1 ml PCR tubes with the use of a Px-2 thermal cycler (ThermoHybaid, UK). The conditions of the reaction have been optimized to combine the forward primer (EntaF) with every one of the 3 reverse primers (EdR, EmR, and EhR) in one reaction mix and under similar conditions. The mix of the reaction included 200 M of deoxy-nucleoside triphosphate, 0.1 M of every one of the reverse and forward primers, 6 mM $MgCl_2$, 1 Taq buffer, 0.5 U of the Taq polymerase, and 101 of the extracted samples of the DNA. The amplification of every one of the species-specific DNA fragments started with an initial denaturation at 94 °C for 3 min, which was

succeeded by 30 cycles of 94 °C for 1 minute, 58 °C for 1 minute, and 72 °C for 1min, with a final extension at 72 °C for 7 min. Amplified products have been visualized with the ethidium bromide staining after the electrophoresis on 1.50% of the agarose gel [7].

PCR Product analysis

Electrophoresis of the Agarose gel, the products that were amplified have been electrophoresed in 2.0% of the agarose gel. The application seemed as one band with a 470 bp length.

DNA sequencing

All genes under the study of DNA product with primer f and primer R and results were read according to the BLAST (Basic Local Alignment search tool) and available on the NCBI and determine the types of genetic tree in detection strain in the *E. histolytica*, *E. dispar,* and *E. moshkovskii* and determine of virulence factor genes in *E. histolytica* species by gene sequence.

Statistical analyses

Statistical analyses proceeded in every group of this study, descriptive statistics analyzed with the use of the ANOVA have been carried out with the use of the mean and standard errors (SE) with the LSD test for the continuous variables ($P < 0.05$) been determined as significant, and X (P-value) has been considered as significant. Every analysis has been carried out with the SPSS program for Windows (v. 23. /2010).

RESULTS AND DISCUSSION

Epidemiology results

During the 12-year study period (June 2010-December 2021), a total of 98,876 people were infected with amoebiasis. The distribution of patients by age showed that the most represented age bracket is between 21 and 30 with a percentage of 24.38%. The age bracket between 31 and 40 is present with a percentage of 24.07%. The 3rd place is occupied by the age bracket between 11 and 20. and the last least represented is that over 40.

For the distribution according to place of residence, the rural region is slightly superior with 58.96%.

In terms of gender distribution, we noted a slight predominance of women with 50.73%.

The distribution of patients infected during the 12 years of the study is shown in **Figure 1**. We have recorded a small decrease in the number of infected cases since 2010 with a slight increase in 2019 in the number of positive cases.

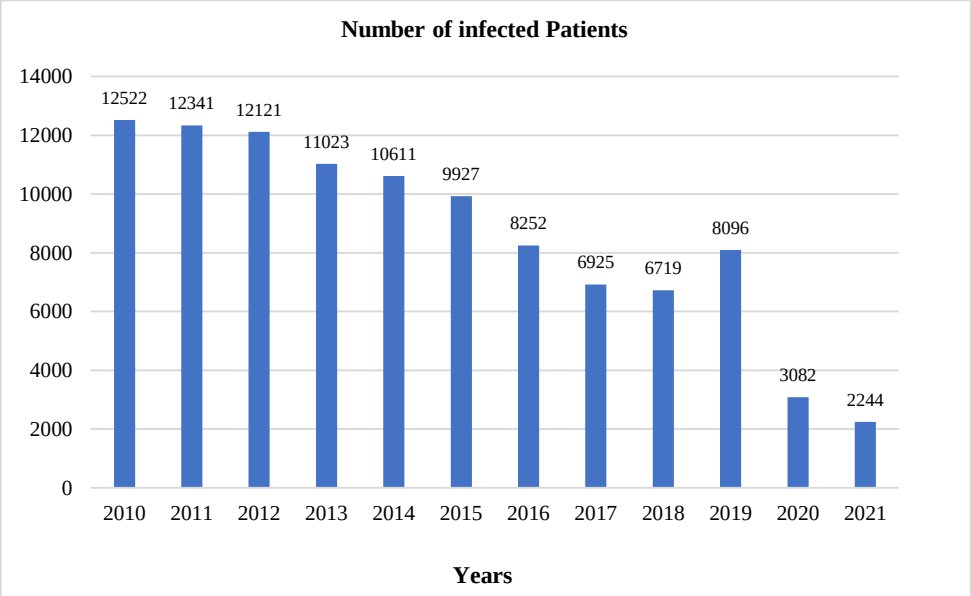


Figure 1. Amoebiasis infections are distributed according to year. Values are presented as numbers.

Microscopic examination results

Microscopic analysis of the 296 samples tested showed the presence of *Entamoeba* cysts or trophozoites. Microscopic examination was performed after the ether sedimentation technique.

Based on microscopic examination results, we classified our patients (n = 296) according to age **(Table 1)**.

Table 1. Number of patients infected with amoebiasis according to age.

Age groups (years)	Number of infected patients	%
< 1-10	98	33.10
11-20	65	21.95
21-30	56	18.91

31-40	45	15.20
> 40	32	10.81
Total	296	99.97

X^2 = 42.074, P-value = 0.00, significant P < 0.05

We also distributed the microscope-positive samples according to the area of residence. We noticed that 51.35% of the patients were of rural origin and 48.64% of them were of urban origin.

The distribution of the positive patients according to gender showed that the male gender is slightly dominant with 51.01% of the cases.

PCR results

PCR according to Entamoeba species

We have performed PCR for the 296 microscopically positive patients. The molecular study showed that *E. histolytica* was the 1st with 48.64% of cases. *E. dispar* was the 2nd with 30.74% of cases and *E. moskovski* the 3rd with 17.56% of cases. The association of *E. hitolytica* and *E. dispar* was recorded for 3.04% of cases.

PCR according to age

The distribution of positive patients after PCR showed that the most affected age group was between 1 and 10 years with a percentage of 42.36%.

PCR according to gender

Similarly for gender, a small male predominance was observed in 51.38% of cases.

PCR according to residential area

According to our results, a very slight non-significant dominance of infection in rural areas was observed at 50.69%.

Frequency of Entamoeba sp. isolated in 40 samples only cysts from symptoms and a symptom individual

The frequency of *Entamoeba* sp. in 40 samples with and without symptoms is presented in **Table 2**.

Table 2. *Entamoeba* species frequency according to the presence and the absence of clinical signs

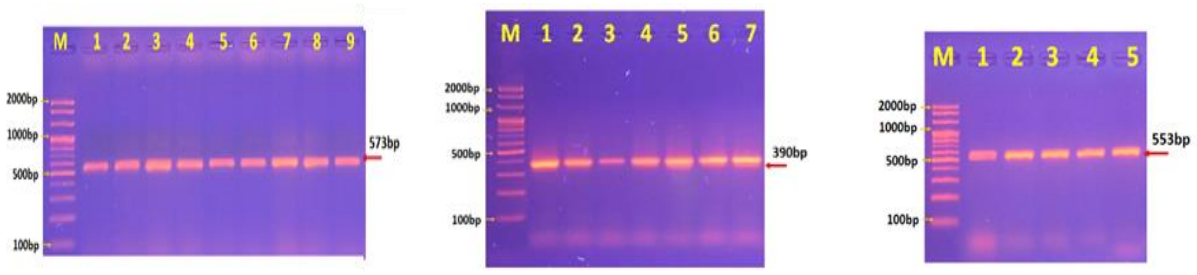
***Entamoeba* cysts**	**With symptoms**		**Without symptoms**		**Total**	
	n	**%**	**n**	**%**	**n**	**%**
E. histolytic	10	33.33	0	0.00	10	25
E. dispar	1	3.33	10	100	17	42.5
E. moskovski	2	6.66	0	0.00	6	15
E. histolytic + *E. dispar*	17	56.66	0	0.00	7	17.5
Total	30	99.98	10	99.98	100	40

X^2=18.039, P value = 0.00043. significant P <0.0

Virulence factor of E. histolytica by PCR

The virulence factors studied in this work are Cytosine protease, Amoebapore, and Gal/lectin. The results of the presence of these virulence factors show that the Cytosine protease is presented in 42.36% (n = 61) of all infected patients, the amoebapore is presented in 34.72% (n = 50) of all infected patients and the Gal/Lectin is presented in 22.91% (n = 33) of cases.

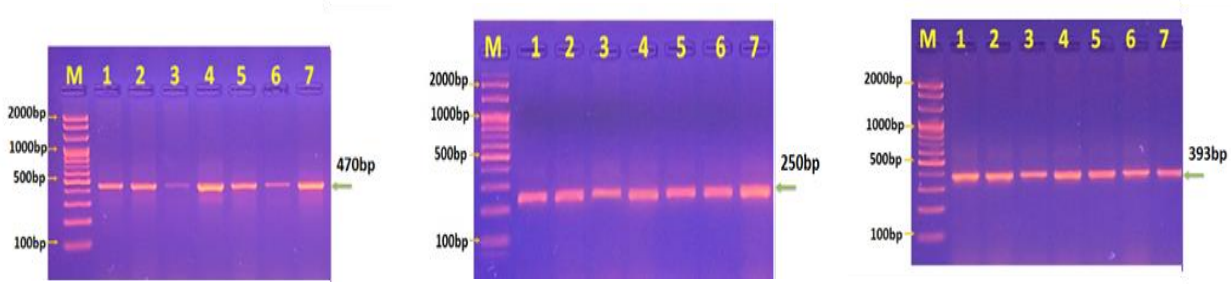
Figure 2 shows the PCR product analysis of a small sub-unit ribosomal RNA gene in *E. histolytica*, *E. dispar*, and *E. moshkovskii*, respectively from Human stool samples.



a) b) c)

Figure 2. Agarose gel electrophoresis of the PCR product analysis of small sub-unit ribosomal RNA gene in *E. histolytica* (a), *E.dispar* (b), *E. moshkovskii*, and (c) from Human stool samples. Lane (M): DNA marker ladder (100-2000 bp).

Figure 3 shows the result of PCR of virulence factors cysteine protease, amoebapore, and Gal NAc genes in *E. histolytica* from Human stool samples.



a) b) c)

Figure 3. Agarose gel electrophoresis image that showed the PCR product analysis of virulence factors cysteine protease (a), amoebapore (b), and Gal NAc (c) genes in *E. histolytica* from Human stool samples. Lane (M): DNA marker ladder (100-2000 bp).

Virulence factors of E. histolytica DNA sequence results

The multiple alignment analysis in NCBI-Genbank was constructed using the ClustalW alignment tool. Online. This alignment analysis showed the nucleotide alignment similarity as (*) and substitution mutations in virulence factors amoebapore gene between isolates **(Figures 4 and 5)**.

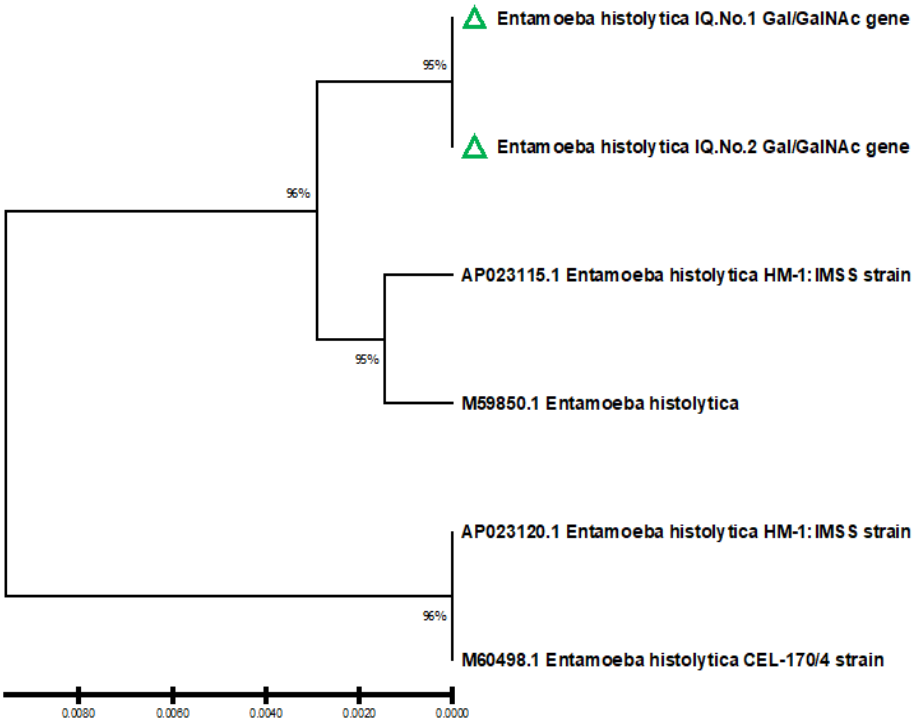


Figure 4. Phylogenetic tree analysis based virulence factors Gal/GalNAc gene partial sequence in local *E. histolytica* IQ isolates that were used for genetic analysis. The phylogenetic tree was constructed using the Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The local *E. histolytica* IQ isolates were shown closely related to NCBI-BLAST *E. histolytica* HM-1:IMSS strain (AP023115.1) at total genetic changes (0.0080-0.0020%).

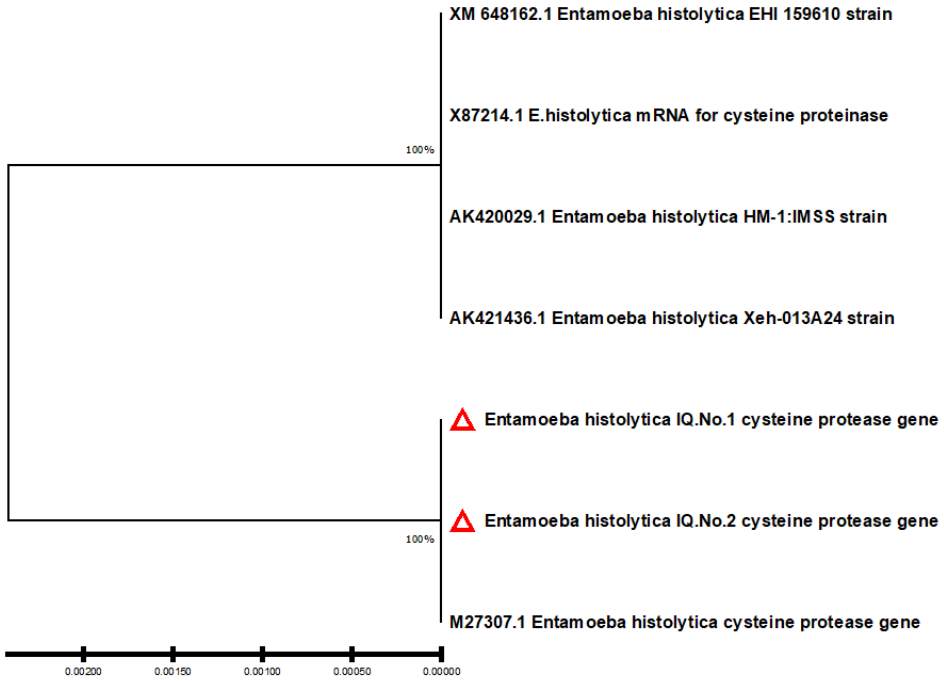


Figure 5. Phylogenetic tree analysis based virulence factors cysteine protease gene partial sequence in local *Entamoeba histolytica* IQ isolates that were used for genetic analysis. The phylogenetic tree was constructed using the Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The local *E histolytica* IQ isolates were shown closely related to NCBI-BLAST *E. histolytica* strain (M27307.1) at total genetic changes (0.0020-0.00050%).

Several infections represent a major public health problem on an international scale, including intestinal parasitic infections. Epidemiological research of different types of intestinal parasitic infections in various regions is often aimed at detecting populations at risk. In the work of Said *et al.* [8], several biological, environmental, socioeconomic, and behavioral factors play an important role in the transmission of intestinal parasitic infections. Other factors such as professional and intellectual levels influence the transmission of the disease and the spread of infection and mortality [7-12].

During our 12-year study period, we included 98,866 amoebic patients (50.73% female and 49.25% male), We did not observe any significant difference between the 2 sexes. This result is consistent with the result reported in Iraq in the work of Al-Damerchi and Al-Ebrahimi [13], and Hamza *et al.* [14].

In Iraq, the COVID-19 pandemic influenced the infection rate especially during the year 2020, as we observed a clear decrease in the number of admitted patients who refused to consult because of the risk of catching the covid 19 virus in hospitals and health centers.

The rate of infection during our work varied according to age with a maximum observed in the 1 to 10 years age group. This can be explained by the daily activities and habits of this age group which play an important role in the risk and degree of exposure of this category to the parasite. Our results are consistent with those reported by Ahmed *et al.* [15] and are also consistent with those of Al-Taei *et al.* [16], Said *et al.* [8], Al-Saqur *et al.* [17] in Iraq and Al-Dalabeeh *et al.* [18] in Jordan.

We also studied the distribution of infection between rural and urban areas. According to our results, we observed a slight difference between the 2 regions. Indeed, there were more infections in rural areas compared to urban areas. Several works have also shown a predominance of infection in rural areas [14].

We studied the monthly distribution of the infection rate. We found variation by month during our study period. Indeed, environmental factors including temperature variation, drinking water pollution, lack of sanitation in some areas as well and dietary habits have a powerful impact on the incidence of this infection in our region.

E. histolytica has morphologically identical twins which are *E. dispar* and *E. moshkovskii*. The latter 2 are not pathogenic and do not require treatment in the vast majority of cases. However, *E. histolytica* is known as the only pathogenic amoeba as it is hematophagous. Hence, the a need to identify and isolate this pathogenic amoeba in the reveal.

In our work, we were able to identify the 3 species, *E. histolytica*, *E. moshkovskii*, and *E. dispar* using

microscopic and molecular methods. The macroscopic identification of trophozoites and cysts allows the isolation of the genus *Entamoeba* in medical laboratories. However, this morphological identification does not allow a correct diagnosis because it is unable to distinguish *E. hitolytica* from other non-pathogenic amoebae.

Therefore, molecular methods were used to identify *Entamoeba* species and to isolate *E. hitolytica* from the stool. To the best of our knowledge, our work is the first in the Kurdistan region of western Iran, which encompassed seven laboratories and was able to identify *Entamoeba* species and assess their prevalence. According to the results of the molecular analysis, all 3 species are present in this region. In other regions of Iran, the prevalence of these amoebae as well as other parasites has decreased significantly recently.

The prevalence of *E. histoltyica* is higher than *E. moshkovskii* and *E. dispar*, according to WHO/PAHO/UNESCO and MFU (1997).

Our results showed that 33.33% of the cases had only *E. histolytica* in 10 samples and 17 samples in association with *E. dispar*. Our results are similar to many works [19-21].

Other work in southwest Iran found similar results yet these areas were often dominated by *E. hitolytica*.

The majority of *E. histolytica* cases may be *E. dispar* which is not pathogenic, according to a 1997 WHO report (1997). However, it has also been shown that patients without symptoms can be infected with *E. histolytica* [22, 23].

Clinically, Persistent abdominal pain and diarrhea with or without mucus and/or blood were recorded in all cases of E. histolytica infection (mixed or single infections) with a prevalence of 0.36%. This observation is in agreement with studies in Africa [24] and Pakistan [25] which showed that *E. histolytica* often causes digestive manifestations in patients.

According to Espinosa *et al*. [26], Dvorak *et al*. [27], *E. dispar* is generally considered a non-virulent species in vivo. However, several other observations have shown that E. dispar can be a pathogenic amoeba. Indeed, Shibayama *et al*. [28] and Herbinger *et al*. [29], showed that the majority of E. dispar isolates from travelers in Brazil have been pathogenic and can cause amoebic liver abscesses in vivo.

In our study, out of 30 patients, only 7 were infected with *E. dispar*. Among the 7, only 3 cases, a PID including gastric pain was present.

As for *E. moshkovskii*, Diamond *et al*. proposed the idea that *E. moshkovskii* may also be a virulent species. However, this contradicts the results of our work since only one patient infected with *E. moshkovskii* presented gastric signs combining persistent diarrhea and abdominal pain.

Four similar surveys conducted in Tunisia, Australia, Bangladesh, and Malaysia have also demonstrated that people can be considered true hosts for this species [19-21].

In addition, investigations by Fotedar *et al*., Khairnar *et al*., and Parija *et al*., linked *E. moshkovskii* infection to GAD in Australia and India [21]. In Malaysia, Anuar *et al*. showed the need for further research to establish a true link between GAD and *E. moshkovskii* and to discover the potential pathogenicity of this species [19].

It is important to mention that in our work we did not confirm the presence of other non-infectious or infectious diseases associated with diarrhea and gastroenteritis such as bacterial and viral infections and this is due to the low number of positive cases on the one hand and financial constraints on the other hand. Thus, we are not able to support the link between clinical symptoms and *Entamoeba* complex infection; further research in this area is needed.

In summary, in our work, we were able to isolate *E. dispar*, *E. histolytica*, and *E. moshkovskii* in Kurdistan province, especially from Gastrointestinal disease (GID) patients. According to PCR results, *E. histolytica* is more common compared to *E. moshkovskii* and *E. dispar*. Only a few cases of *E. moshkovskii* have been documented in Iraq. In our work, a single isolate of this amoeba was found for the first time. In general, it was found that GID symptoms can be related to *E. dispar* and *E. moshkovskii.*

In our work, the cysteine proteinase gene was chosen for the study of *E. histolytica* virulence. This gene represents one of three genes (EhCp2, EhCP1, and Ehcp5) accounting for approximately 90% of the active genes in *E. histolytica*.

Studies have shown that cysteine proteinases play a key role in the penetration of *E. histolytica* into the mucosa. This penetration is essential in the pathogenesis of amoebiasis. By crossing this mucosal and epithelial barrier, the proteolytic enzymes released by the parasite facilitate its adhesion to tissues [26].

To study the virulence of this gene, we opted for a molecular study. The results revealed the presence of the CP1 gene in 144 samples. Our results are consistent with those of Tannich *et al*. [30] and Tawfik *et al*. [31].

The search for the Amebopore gene was also one of the results of our work. We found the Amebopore gene in *E. histolytica* in 50 out of 144 samples with a percentage of 34.72%. In addition, trophozoites possess amoebapores that are always present in their cytoplasm. Several studies reported that *E.histolytica* can induce necrosis and apoptosis in eukaryotic cells [30, 32-34]. The lectin gal gene was found in 144 of 33 samples, with a percentage of 22.91%, which is consistent in terms of genetic

presence with the results of some studies. Several studies showed the role of this gene in *E. histolytica* and stated that the purpose of the cell-killing target is dependent on the adhesion of the parasite cell because it has a gene that is responsible for the encryption of Lectin Gal \ Nac [23, 35-39].

In sequencing the strain separated in the current study from patients with diarrhea have different surface antigens to distinguish through DNA probe, to investigate whether these stains have differences in their rRNA. The sequence that used results shows that the gene of the pathogenic strain differs from the nonpathogenic one level and suggests that the 18SrRNA probe could be of value to detection studies [40].

E. histolytica is currently one of the major health issues and the primary cause of amoebiasis. This disease's symptoms include diarrhea or dysentery, fever, abdominal pain, and dehydration. Climatic and geographical factors, along with the carrier host, are the primary causes of infection [41]. In the subsequent study, 296 stool samples that had been found to have *E. histolytica* infection by microscopic examination were subjected to PCR identification. The results revealed that 144 (48.64%) of the examined stool samples had produced positive results with PCR using the parasite's 18s rRNA gene. The total number of positive PCR samples submitted for virulence factor gene identification revealed that each of the 61 (42.36%) gave positive results with cysteine proteinase gene, 50 (34.72) gave positive results for amoebapore, and 33 (22.91) gave positive results for Gal/lectin gene. Long-term axenic raising decreases the virulence of *E. histolytica* [42]. Adherence of the parasite occurs majorly through surface Gal/GalNAc lectin that connects to uncover the end Gal/GalNAc. Virulence factors include the adhesion and cytolytic occur procedures attachment to three types of molecules (amoebapore, lectin, and proteases). The Tamura-Nei model tree approach and Maximum Likelihood method were used to build the phylogenetic tree in the Mega X version. According to Petri *et al*. the local *E. histolytica* Human isolates (No. 1 and No. 2) have been genetically related to the NCBI BLAST *E. histolytica* lQ strain MS30-1047 (AY-956434.2) at total genetic (0.20-0.050%) alterations. *E. histolytica* is associated with mammalian cells in vivo, amoebapore is a rapid cytolytic case that is appropriate and causes lumps, surface blisters, and lysis of the target cell. This case includes polymorphonuclear, macrophage, leukocytes, and lymphocytes, and the parasite is not dangerous; its identification is incident to the lysis of the target cell by T-lymphocytes. Phylogenetic tree study using genetic variation analysis of local *E. histolytica* Human isolates depending on amoebapore B gene partial sequence. The Tamura-Nei model tree approach and Maximum Likelihood method were used to build the phylogenetic tree in the Mega X version. The local *E. histolytica* human isolates No. 1 and No. 2 were found to have a genetic connection to the NCBI-BLAST *E. histolytica* lQ strain (M2-2730.1) with total genetic (0.0020-0.00050%) alterations. *E. histolytica* is known to have 8 genes that code for cysteine proteases, and these cysteine proteases are known to play a crucial role with important pathogenic agents. Genetic variation analysis was conducted using a phylogenetic tree depending on the partial sequencing of the cysteine protease gene in the local Human isolates of *E. histolytica*. The Tamura-Nei model tree approach and Maximum Likelihood method were used to build the phylogenetic tree in the Mega X version. The local *E. histolytica* Human isolates No. 1 and No. 2 were found to have genetically close ties to the NCBI-BLAST *E. histolytica* HM-1:IMSS strain (AP023115.1) with total genetic (0.0080-0.0020%) alterations. The identification of *E. histolytica* in the stool samples of the clinical samples has been evaluated using a PCR-based molecular detection technique. The materials utilized and the sample processing techniques used determine how sensitive these methods are.

CONCLUSION

In conclusion, our study has shown firstly that intestinal amebiosis is predominant, especially in the 1-10 year age group. Secondly, the light microscopy diagnostic method cannot differentiate between *E. dispar*, *E. Moskovskii*, and *E. histolytica*, as they are morphologically comparable. Phylogenetically, the analysis showed that the greatest identity of the local *E. histolytica* isolates 98.30% with isolate AB002794.1 in GenBank. Furthermore, phylogenetic analysis showed that the greatest identity of the local *E. dispar* isolate *dispar* was 99.23% with isolate KT825981.1 in the GenBank. Finally, our phylogenetic analysis showed that the greatest identity of the local *E. Moskovskii* isolates 99.65% with isolate OP537199.1 in the GenBank.

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Ethics statement: None

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