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Genetic Sequencing-Based Analysis of Virulence Determinants in Entamoeba Species

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Abstract

Amoebiasis remains a notable invasive infection affecting both the intestinal and extraintestinal systems. The present investigation aims to explore the epidemiological characteristics of intestinal amoebiasis among a cohort of 296 stool samples collected from Iraqi patients. In this investigation, PCR analysis was performed on samples from 296 individuals who were identified as microscopically positive. Molecular diagnostics revealed the presence of *Entamoeba (E.) histolytica* in 144 cases (48.64%), *E. dispar* in 91 cases (30.74%), and *E. moskovski* in 52 cases (17.56%). Coinfection with *E. histolytica* and *E. dispar* was noted in 9 cases (3.04%). PCR-positive isolates were further analyzed to determine the presence of *E. histolytica* virulence markers, with 61 samples (42.36%) testing positive for the cysteine proteases gene, 50 (34.72%) for the amoebapore gene, and 33 (22.91%) for the lectin gene. Sequencing of these virulence genes—cysteine protease, amoebapore, and Gal/GalNAc lectin—in locally obtained *E. histolytica* strains revealed nucleotide homology as well as mutational substitutions across all three genes. Phylogenetic analysis of the partial sequence of the cysteine protease gene indicated close genetic proximity to the *E. histolytica* strain (M27307.1) listed in the NCBI-BLAST database, with observed genetic variation ranging from 0.0020% to 0.0050%. A similar analysis based on the partial sequence of the amoebapore B gene demonstrated alignment with the *E. histolytica* strain (MS30-1047), with a variation range of 0.020% to 0.050%. In addition, phylogenetic reconstruction using partial sequences of the Gal/GalNAc lectin gene from local isolates revealed a close genetic relationship to the *E. histolytica* HM-1:IMSS strain (AP023115.1), with differences ranging from 0.0080% to 0.0020%.

Keywords: Cysteine protease, Amoebiasis, PCR, *Entamoeba histolytica*

Introduction

Amoebiasis ranks among the top three parasitic infections globally, following malaria and bilharzia, with over 50,000 cases reported worldwide and an estimated annual mortality of 100,000 individuals [1]. The World Health Organization defines amoebiasis as a condition where the human host carries *Entamoeba (E.) histolytica*—an amoeboid protozoan parasite—either symptomatically or asymptomatically [2]. This infection may present without clinical signs or progress into severe pathologies such as amoebic colitis or amoebic liver abscess [3]. The impact of amoebiasis on health is multifaceted. Notably, amoebic colitis is a leading contributor to cases of severe diarrhea across the globe, ranking among the fifteen most common diarrheal illnesses [4]. In developing countries, diarrheal diseases continue to represent a significant threat, particularly within the first two years of life, where they are responsible for approximately 9% of deaths in children under five years old [5].

Though fulminant amoebic colitis is a rare consequence of *E. histolytica* infection, its fatality rate is high, with mortality surpassing 50% among patients with severe

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forms [2]. Due to its low infectious dose, ability to resist chlorine and environmental resilience, *Entamoeba* has been classified by the National Institute of Allergy and Infectious Diseases (NIAID) as a Category B priority pathogen for biodefense. These biological characteristics heighten the risk of widespread transmission through contaminated water and food sources [2]. Even in non-endemic regions, these traits have contributed to localized outbreaks, notably affecting military personnel and civilian populations. Furthermore, *E. histolytica* frequently appears as a causative agent of travel-associated infectious diarrhea among individuals returning from endemic zones [5].

Advances in molecular biology have significantly refined the ability to detect and distinguish *E. histolytica* from non-pathogenic members of the *Entamoeba* genus. Traditionally, microscopic examination could not reliably differentiate between *E. histolytica* and its morphologically indistinguishable counterparts. Of these, *E. moshkovskii* has been linked to diarrheal illness, whereas *E. dispar* and the recently identified *E. bangladeshi* are regarded as non-pathogenic [4].

To date, no vaccine exists for amoebiasis prevention. Treatment primarily involves nitroimidazole-based medications. However, concerns surrounding their toxicity and emerging resistance highlight the necessity for alternative therapeutic options [6]. In response, NIAID has identified amoebiasis as a priority for novel drug development efforts targeting category B agents. Enhancing our understanding of the virulence mechanisms underlying amoebiasis is essential to guide the development of innovative treatments and preventive strategies [6].

Given this context, the present investigation aims to explore the epidemiological characteristics of intestinal amoebiasis among a cohort of 296 stool samples collected from Iraqi patients. The objective includes the application of multiplex PCR for species-level identification of *Entamoeba*, followed by conventional PCR analysis to detect specific virulence-associated genes, and sequencing to characterize the genetic profile of these virulence determinants.

Materials and Methods

Sample Collection

The research was conducted in the Thi-Qar region of Iraq, where medical data on amoebiasis cases spanning from January 2010 to December 2021 were collected from the Public Health Department of Thi-Qar's Health Office. This data included patient information such as age, gender, date, and location. The diagnosis of amoebiasis in patients was made using direct stool smears. Molecular analysis was carried out at two hospitals, Mohammed Al-Mosawi and Al-Chebish, from February to October 2021, treating patients of all ages. A total of 296 stool samples were randomly collected from patients presenting with diarrhea, after which the samples were examined using microscopy to detect cysts and/or trophozoites. These samples were stored at -20 °C in sealed stool containers.

Macroscopic Examination

The stool samples underwent visual examination to assess their color, appearance, and the presence of blood, mucus, or any unusual odor.

Molecular Diagnostic Method

Genomic DNA Extraction

DNA was extracted from 200 mg of stool using the PrestoTM stool gDNA Kit, following the manufacturer's instructions. The purity of the extracted genomic DNA was determined using a Nanodrop spectrophotometer by measuring absorbance at 260 nm and 280 nm.

PCR Master Mix Preparation

PCR amplification was performed to target specific genes using the following primer sequences: EntaF (5' ATG CAC GAG AGC GAA AGC AT 3'), EhR (5' GAT CTA GAA ACA ATG CTT CTC T 3'), EdR (5' CAC CAC TTA CTA TCC CTA CC 3'), and EmR (5' TGA CCG GAG CCA GAG ACA T 3'). Sequence analysis showed that the forward primer (Enta-F) is unique to *Entamoeba* species, while the three reverse primers (EdR, EmR, and EhR) are specific to individual species, enabling differentiation among them. The forward primer combined with each reverse primer yields distinct PCR products: 573 bp for *E. histolytica*, 390 bp for *E. dispar*, and 553 bp for E. moshkovskii. The PCR reaction was carried out in a total volume of 51 µl using a Px-2 thermal cycler (ThermoHybaid, UK). The PCR conditions were optimized to combine the forward primer (EntaF) with each reverse primer (EdR, EmR, and EhR) in a single reaction mixture under uniform conditions. The reaction mixture included 200 µM of deoxynucleoside

triphosphates, 0.1 µM of each forward and reverse primer, 6 mM MgCl2, 1x Taq buffer, 0.5 U of Taq polymerase, and 10 µl of extracted DNA. The amplification protocol included an initial denaturation at 94 °C for 3 minutes, followed by 30 cycles of denaturation at 94 °C for 1 minute, annealing at 58 °C for 1 minute, extension at 72 °C for 1 minute, and a final extension at 72 °C for 7 minutes. The PCR products were visualized by ethidium bromide staining after electrophoresis on 1.5% agarose gel [7].

PCR Product Analysis

Electrophoresis was performed using a 2.0% agarose gel to analyze the PCR products. The amplification results appeared as a single band at 470 bp.

DNA Sequencing

The DNA products from the PCR amplification were sequenced using the forward and reverse primers, and the results were analyzed using the BLAST (basic local alignment search tool) on NCBI. The sequencing data were used to identify the genetic profiles of *E. histolytica*, *E. dispar*, and *E. moshkovskii*, and to detect virulence factor genes in *E. histolytica*.

Statistical Analysis

Statistical analysis was performed using descriptive statistics, and ANOVA was used to evaluate continuous variables. The LSD test was applied to determine significant differences ($P < 0.05$). The statistical software SPSS (version 23.0, 2010) was used for all analyses, with a significance threshold set at P-values < 0.05.

Results and Discussion

Epidemiological Findings

Throughout the 12-year study (from June 2010 to December 2021), a total of 98,876 individuals were diagnosed with amoebiasis. The age distribution revealed that the largest group of patients was in the 21-30 years age range, comprising 24.38% of the cases. The 31-40 years age group followed closely with 24.07%, while the 11-20 years age bracket ranked third. The least affected group was those aged over 40 years.

In terms of geographical distribution, patients from rural areas accounted for a higher percentage, with 58.96% of the cases.

Regarding gender, females slightly outnumbered males, with a representation of 50.73%.

The yearly distribution of cases over the 12 years is depicted in **Figure 1**. A slight decline in the number of cases was observed since 2010, although a small increase in positive diagnoses was recorded in 2019.

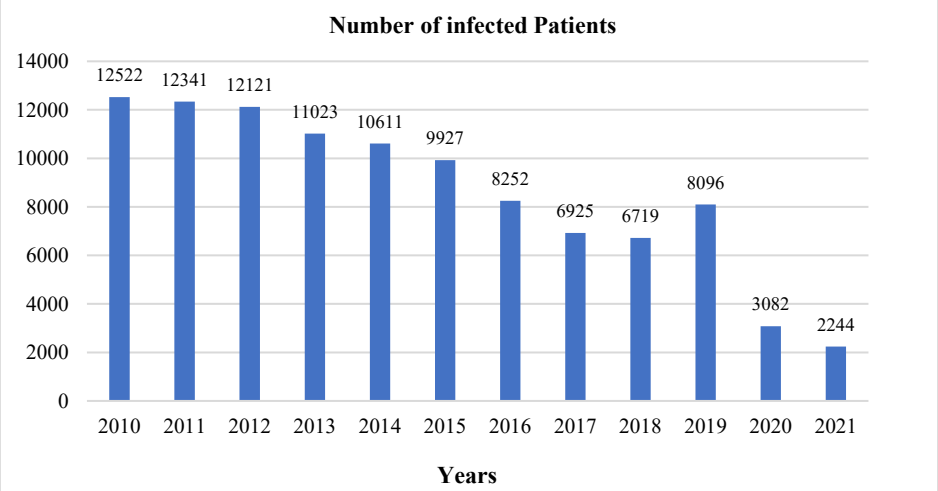


Figure 1. Amoebiasis infections are distributed according to year; values are presented as numbers.

Microscopic Analysis Results

A total of 296 samples were analyzed under a microscope, revealing the presence of *Entamoeba* cysts or trophozoites. The ether sedimentation method was utilized for this examination. Following the microscopic analysis, the patients were categorized by age, as shown in **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

Upon categorizing the microscope-positive samples by geographic origin, it was observed that 51.35% of patients hailed from rural areas, while 48.64% were from urban regions.

Regarding gender, a slight male predominance was noted, with males comprising 51.01% of the total cases.

PCR Results for Entamoeba Species

A PCR analysis was conducted on all 296 microscopically positive samples. The molecular examination revealed that *E. histolytica* was the most prevalent species, accounting for 48.64% of the cases. *E. dispar* followed with 30.74%, while *E. moskovskii* represented 17.56% of the infections. An association between *E. histolytica* and *E. dispar* was observed in 3.04% of the cases.

PCR Results by Age

The PCR analysis indicated that the most affected age group was children aged 1 to 10 years, representing 42.36% of the positive cases.

PCR Results by Gender

For gender distribution, there was a slight male predominance with 51.38% of the positive cases being male.

PCR Results by Residential Area

Our findings showed a marginal and statistically insignificant predominance of infection in rural areas, with 50.69% of the cases occurring in these regions.

Frequency of Entamoeba Species in Symptomatic and Asymptomatic Samples

The frequency of *Entamoeba* species detected in 40 samples, both symptomatic and asymptomatic, is detailed in **Table 2**.

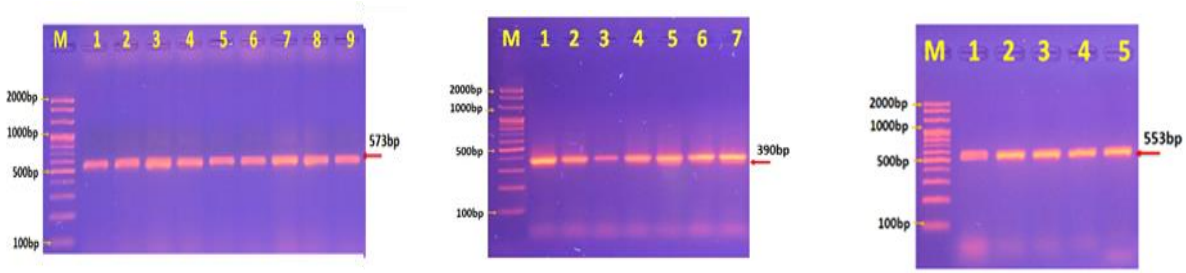
Table 2. *Entamoeba* species frequency according to the presence and the absence of clinical signs

***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 Factors of E. histolytica Detected by PCR

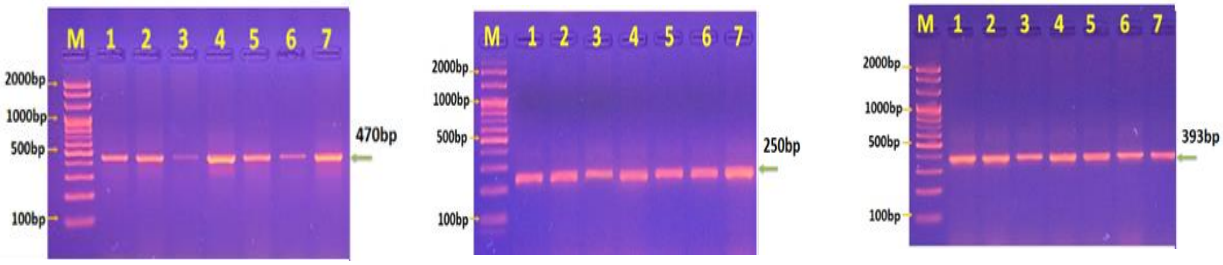
In this study, the virulence factors under investigation were cysteine protease, amoebapore, and Gal/lectin. The findings revealed that cysteine protease was detected in 42.36% (n = 61) of the infected individuals, amoebapore was present in 34.72% (n = 50), and Gal/lectin was found in 22.91% (n = 33) of the cases. Figure 2 illustrates the PCR analysis of the small subunit ribosomal RNA gene from human stool samples, identifying *E. histolytica*, *E. dispar*, and *E. moshkovskii*.



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 illustrates the PCR analysis results for the virulence factors, amoebapore, cysteine protease, and GalNAc genes, detected in *E. histolytica* samples obtained from human stool.



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 nucleotide sequence alignment analysis of the virulence factors in *E. histolytica* was performed using the ClustalW tool available on NCBI-GenBank. The results highlighted specific nucleotide similarities and identified substitution mutations in the amoebapore gene across various isolates (**Figures 4** and **5**).

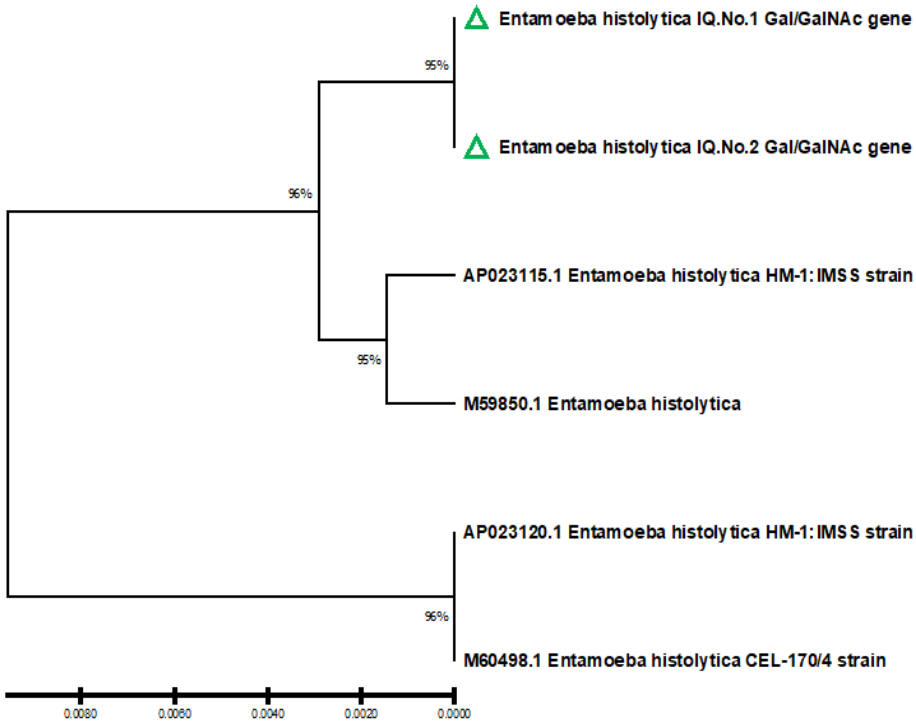


Figure 4. The phylogenetic tree analysis is based on the partial Gal/GalNAc gene sequence from local *E. histolytica* IQ isolates used for genetic analysis; the tree was constructed using the unweighted pair group method with arithmetic mean (UPGMA) in MEGA 6.0; the local *E. histolytica* IQ isolates were closely related to the NCBI-BLAST *E. histolytica* HM-1:IMSS strain (AP023115.1), with genetic variations ranging from 0.0080% to 0.0020%.

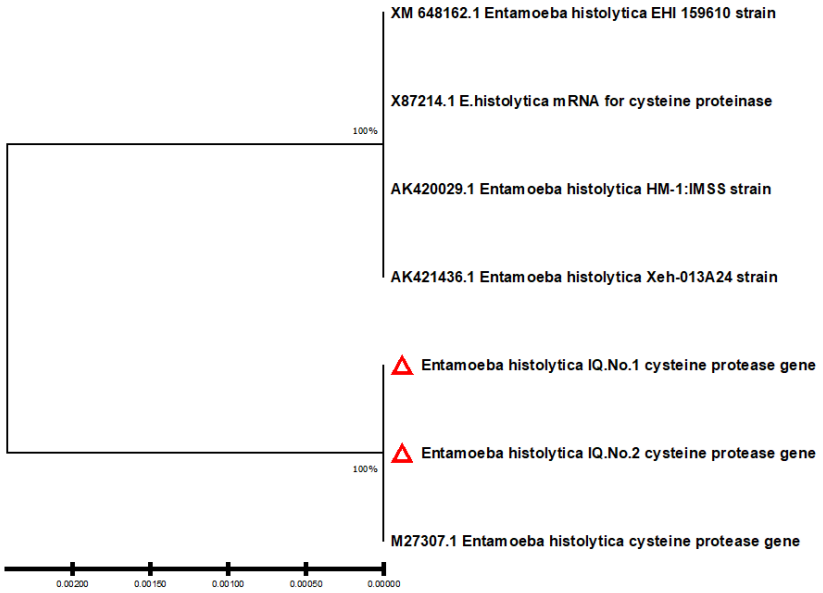


Figure 5. Phylogenetic tree analysis based on the virulence factors cysteine protease gene partial sequence in local *Entamoeba histolytica* IQ isolates used for genetic analysis; the tree was constructed using the unweighted pair group method with arithmetic mean (UPGMA) in MEGA 6.0 software; the local *E. histolytica* IQ isolates were closely related to the NCBI-BLAST *E. histolytica* strain (M27307.1), with genetic variations ranging from 0.00050% to 0.0020%.

Global public health faces considerable challenges due to intestinal parasitic infections, which require thorough epidemiological studies to identify populations at greater risk. Saida [8] highlighted that the transmission of these infections is influenced by a combination of biological, environmental, socioeconomic, and behavioral factors. In addition, professional and educational backgrounds can significantly affect the spread and mortality rates associated with these infections [7-12].

In our study spanning 12 years, we analyzed 98,866 amoebiasis cases, with 50.73% of the patients being female and 49.25% male. We found no notable gender-based differences, consistent with previous studies by Al-Damerchi and Al-Ebrahimi [13] and Hamza *et al.* [14] in Iraq.

The COVID-19 pandemic had a notable impact on infection rates, especially in 2020. Many patients refrained from visiting healthcare facilities due to concerns about contracting the virus, leading to a decline in reported cases.

The infection rate in our study varied with age, with the highest prevalence observed among children aged one to ten years. This is likely due to the increased exposure risk associated with their daily activities. These results align with findings by Ahmed *et al.* [15], Al-Taei [16], Saida *et al.* [8], Al-Saqur *et al.* [17] in Iraq, and Al-Dalabeeh *et al.* [18] in Jordan.

We also examined the infection rates in rural and urban areas, observing a slight increase in rural regions. This supports other studies that report higher infection rates in rural areas compared to urban settings [14].

We analyzed the infection rate every month and observed that the numbers fluctuated over time during our study. Factors such as environmental conditions, temperature shifts, water contamination, inadequate sanitation in specific regions, and dietary habits significantly impacted the occurrence of infections in the region.

Entamoeba histolytica shares close morphological characteristics with two non-pathogenic species, *E. moshkovskii* and *E. dispar*. While these two species rarely cause harm and typically don't require treatment, *E. histolytica* is the only pathogenic form, known for its hematophagous behavior. This highlights the importance of accurately distinguishing and isolating *E. histolytica* in clinical settings.

In our research, we successfully identified all three species—*E. histolytica*, *E. dispar*, and *E. moshkovskii*—using both microscopic and molecular techniques. Although the macroscopic identification of trophozoites and cysts helps identify the genus *Entamoeba*, it does not differentiate *E. histolytica* from other non-pathogenic amoebae. To address this limitation, we utilized molecular methods to correctly identify and isolate *E. histolytica* from stool samples. This study, which involved seven laboratories in the Kurdistan region of western Iran, represents the first extensive molecular survey of *Entamoeba* species in the region.

Our findings revealed the presence of all three species in the region. In comparison, the prevalence of these amoebae and other parasites has notably decreased in other parts of Iran in recent years. The frequency of *E. histolytica* infections was higher than that of *E. moshkovskii* and *E. dispar*, aligning with data from WHO/PAHO/UNESCO and MFU (1997). Specifically, 33.33% of cases were solely *E. histolytica*, and 17 cases involved co-infection with *E. dispar*. These findings are consistent with prior studies [19-21].

Similar results were reported in southwestern Iran, where *E. histolytica* was the dominant species. It is worth noting that many cases previously identified as *E. histolytica* might have been caused by *E. dispar*, a non-pathogenic species, as highlighted in the WHO report (1997). Despite this, *E. histolytica* has been detected in asymptomatic individuals [22, 23].

All patients infected with *E. histolytica*, regardless of whether the infection was singular or mixed, exhibited persistent abdominal pain and diarrhea, sometimes accompanied by blood and/or mucus, accounting for 0.36% of the total cases. These findings are consistent with studies from Africa [24] and Pakistan [25], where *E. histolytica* was frequently associated with gastrointestinal symptoms.

Although *E. dispar* is generally regarded as non-virulent in vivo by researchers such as Espinosa *et al.* [26] and Dvorak *et al.* [27], some studies, including those by Shibayama *et al.* [28] and Herbinger *et al.* [29], have displayed that *E. dispar* can be pathogenic under certain conditions. In particular, strains from travelers in Brazil were linked to amoebic liver abscesses and other severe conditions.

In our study, 7 out of thirty patients infected with *E. dispar* showed symptoms, and 3 of these cases included pelvic inflammatory disease (PID) along with gastric pain. As for *E. moshkovskii*, despite suggestions by Diamond et al. that it may also be pathogenic, our findings indicated that only 1 patient infected with E. moshkovskii experienced gastrointestinal symptoms, such as persistent diarrhea and abdominal pain.

Studies carried out in Tunisia, Bangladesh, Australia, and Malaysia have also demonstrated that *E. moshkovskii* can infect humans, reinforcing the idea that humans serve as true hosts for this species [19-21].

The studies by Fotedar *et al.*, Khairnar *et al.*, and Parija *et al*. have highlighted a connection between *E. moshkovskii* infections and gastrointestinal disorders (GAD) in both Australia and India [21]. In Malaysia, Anuar et al. stressed the need for continued investigation to fully understand the possible relationship between *E. moshkovskii* and GAD, as well as to assess its pathogenic potential [19].

It is worth noting that our study did not explore other possible infections associated with gastrointestinal issues, such as viral or bacterial causes, due to the limited number of positive cases and financial limitations. Consequently, we are unable to definitively link clinical symptoms to infections within the *Entamoeba* complex, and further studies are needed to clarify these connections.

In conclusion, our research successfully isolated *E. histolytica*, *E. dispar*, and *E. moshkovskii* from the Kurdistan region, particularly from patients suffering from gastrointestinal diseases. Our PCR findings revealed *E. histolytica* as the most commonly detected species, followed by *E. moshkovskii* and *E. dispar*. Cases of *E. moshkovskii* are rare in Iraq, and our study presents the first reported instance of this species. Notably, we observed gastrointestinal symptoms in patients infected with both *E. dispar* and *E. moshkovskii*.

For our investigation into the virulence of *E. histolytica*, we focused on the cysteine proteinase gene. This gene is one of three, including EhCp2, EhCP1, and EhCp5, that are responsible for approximately 90% of the active virulence factors in *E. histolytica*. Research has shown that cysteine proteinases are crucial for the parasite's ability to penetrate the intestinal mucosa, a key step in amoebiasis pathogenesis. These enzymes facilitate the parasite's adhesion to host tissues [26].

Through molecular analysis, we identified the CP1 gene in 144 of our samples. This finding is consistent with the results from Tannich *et al.* [30] and Tawfik *et al.* [31]. Additionally, we detected the presence of the amoebapore gene in fifty out of the 144 samples, or 34.72%. Amoebapores are consistently present in the cytoplasm of *E. histolytica* trophozoites, and studies have shown that the parasite can induce necrosis and apoptosis in host cells [30, 32-34]. We also detected the lectin gal gene in 33 of the 144 samples, corresponding to 22.91%, which aligns with previous research. The lectin gene plays a significant role in the virulence of *E. histolytica*, as it aids in the adhesion of the parasite to host cells, a critical factor in the pathogenesis of amoebiasis [23, 35-39].

In this investigation, we examined the surface antigens of *E. histolytica* strains isolated from patients suffering from diarrhea. The analysis involved DNA probes to identify variations in the rRNA of these strains. The sequencing results indicated that there are notable differences in the gene structure between pathogenic and nonpathogenic strains, supporting the potential utility of the 18S rRNA probe for detection purposes [40].

Amoebiasis caused by *E. histolytica* remains a significant global health issue, leading to symptoms such as diarrhea, dysentery, abdominal discomfort, fever, and dehydration. The spread of infection is largely influenced by environmental conditions, geography, and the role of host carriers [41]. In a follow-up study, we analyzed 296 stool samples, all identified with *E. histolytica* infection via microscopy, and subjected them to PCR testing. Of the samples, 144 (48.64%) were PCR-positive for *E. histolytica*, confirming the infection with the 18S rRNA gene. Additionally, the cysteine proteinase gene was detected in 61 (42.36%) samples, the amoebapore gene in 50 (34.72%) samples, and the Gal/lectin gene in 33 (22.91%) samples. Research indicates that prolonged cultivation of *E. histolytica* in axenic conditions results in reduced virulence [42]. The primary mechanism for parasite adherence involves its surface lectin binding to Gal/GalNAc residues. This binding, along with the activity of virulence factors like amoebapore, lectin, and proteases, facilitates cellular adhesion and tissue damage.

We constructed a phylogenetic tree based on genetic data using the Tamura-Nei model and Maximum Likelihood method in Mega X software. Our analysis revealed that the local *E. histolytica* human isolates (No. 1 and No. 2) share a close genetic relationship with the NCBI-BLAST *E. histolytica* IQ strain MS30-1047 (AY-956434.2), showing genetic differences ranging from 0.20% to 0.05%. *E. histolytica* is known to interact with mammalian cells, and amoebapore acts as a cytolytic agent, promoting cell lysis through its rapid action on target cells such as macrophages and lymphocytes. While this process may not render the parasite highly lethal, it remains a critical mechanism for identifying *E. histolytica*.

Further phylogenetic analysis of local *E. histolytica* isolates, using the partial sequence of the amoebapore B

gene, confirmed the genetic similarity of isolates No. 1 and No. 2 with the NCBI-BLAST *E. histolytica* IQ strain (M2-2730.1). The observed genetic variations were between 0.0020% and 0.00050%.

Notably, *E. histolytica* harbors eight genes coding for cysteine proteases, which are crucial for its pathogenic behavior. In our study, we also analyzed genetic variation by constructing a phylogenetic tree based on partial sequencing of the cysteine protease gene in local isolates. Again, the Tamura-Nei model and the Maximum Likelihood method were used in Mega X to construct the phylogenetic tree. The genetic analysis showed that the local isolates (No. 1 and No. 2) closely resemble the NCBI-BLAST *E. histolytica* HM-1:IMSS strain (AP023115.1), with genetic variations between 0.0080% and 0.0020%. The presence of *E. histolytica* in stool samples was confirmed using a PCR-based molecular method, which is known for varying sensitivity depending on sample processing techniques and materials used.

Conclusion

Our research has demonstrated that intestinal amoebiasis is particularly prevalent among children aged 1 to 10 years. Additionally, we found that the light microscopy method does not provide the ability to distinguish between *E. dispar*, E. moshkovskii, and *E. histolytica*, as they appear morphologically indistinguishable. Phylogenetic analysis revealed that the local *E. histolytica* isolates were most closely related to GenBank isolate AB002794.1, with a 98.30% genetic similarity. In comparison, the local *E. dispar* isolate showed a 99.23% identity with KT825981.1, while the local *E. moshkovskii* isolates had a 99.65% match with OP537199.1 from GenBank.

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Conflict of Interest: None

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Ethics Statement: None

References

1. Costa AO, Gomes MA, Rocha OA, Silva EF. Pathogenicity of *Entamoeba dispar* under xenic and monoxenic cultivation compared to a virulent *E. histolytica*. Rev Inst Med Trop Sao Paulo. 2006;48:245-50.
2. Kantor M, Abrantes A, Estevez A, Schiller A, Torrent J, Gascon J, et al. *Entamoeba histolytica*: updates in clinical manifestation, pathogenesis, and vaccine development. Can J Gastroenterol Hepatol. 2018;8:6. doi:10.1155/2018/4601420
3. Ghosh S, Jay Padalia Shannon M. Tissue destruction caused by *Entamoeba histolytica* parasite: cell death, inflammation, invasion, and the gut microbiome. Curr Clin Microbiol Rep. 2019;6(1):51-7.
4. Debbie-Ann TS, Farr L, Watanabe K, Moonah S. A review of the global burden, new diagnostics, and current therapeutics for amebiasis. Open Forum Infect Dis. 2018;5(7):161.
5. Abdelaal Hegazi M, Ahmed Patel T, Salama El-Deek B. Prevalence and characters of *Entamoeba histolytica* infection in Saudi infants and children admitted with diarrhea at 2 main hospitals at South Jeddah: a re-emerging serious infection with unusual presentation. Braz J Infect Dis. 2013;17:32-40.
6. Chavez-Tapia NC, Hernandez-Calleros J, Tellez-Avila FI, Torre A, Uribe M. Image-guided percutaneous procedure plus metronidazole versus metronidazole alone for uncomplicated amoebic liver abscess. Cochrane Database Syst Rev. 2019;21(1):CD004886.
7. Al Saqur IM, Al-Warid HS, Albahadely HS. The prevalence of Giardia lamblia and *Entamoeba histolytica/dispar* among Iraqi provinces. Karbala Int J Mod Sci. 2017;3(2):93-6.
8. Saida LA. Epidemiological study of intestinal parasites among the population of Erbil city, Kurdistan, Iraq. J Raparin Univ. 2016;3:75-86.
9. Haque R, Mollah NU, Ali IK, Alam K, Eubanks A, Lyerly D, et al. Diagnosis of amebic liver abscess and intestinal infection with the Tech Lab *Entamoeba histolytica* II antigen detection and antibody tests. J Clin Microbiol. 2000;38(9):3235-9.
10. Anaya-Velázquez F, Padilla-Vaca F. Virulence of *Entamoeba histolytica*: a challenge for human health research. Future Microbiol. 2011;6(3):255-8.
11. Andersson Y, De Jong B. An outbreak of giardiasis and amoebiasis at a ski resort in Sweden. Water Sci Technol. 1989;21(3):143-6.
12. Coulibaly JT, Fürst T, Silué KD, Knopp S, Hauri D, Ouattara M, et al. Intestinal parasitic infections in schoolchildren in different settings of Côte d'Ivoire:

effect of diagnostic approach and implications for control. Parasit Vectors. 2012;5(1):1-1.
13. Al-Damerchi AT, Al-Ebrahimi HN. Detection of major virulence factor of *Entamoeba histolytica* by using PCR technique AL. Qadisryah Med J. 2016;12(21):36-45.
14. Hamza DM, Malaa SF, Alaaraji KK. Real-time-PCR assay based on phosphoglycerate kinase gene for detection of *Entamoeba histolytica* Trophozoites in stool samples in Holy Karbala, Iraq. Med Leg Update. 2021;21(1):1656-64.
15. Ahmed NM, Al-Niaeemi BH, Dawood MH. Epidemiological and clinical aspects about the endemic intestinal parasites in reviewers of Mosul General hospitals and healthcare centers/Nineveh governorate-Iraq. Eurasian J Biosci. 2020;14(2):6037-43.
16. Al-Taei AH. The prevalence of intestinal parasite among the attending peoples to Al-Hashimyah hospitals for seven years, Babylon province, Iraq. InJournal of Physics: Conference Series 2019 Sep 1 (Vol. 1294, No. 6, p. 062022). IOP Publishing.
17. Al-Saqur IM, Al-Warid HS, Al-Qaisi AQ, Al-Bahadely HS. Prevalence of gastrointestinal parasites in Iraq during 2015. InAIP Conference Proceedings 2020 Dec 4 (Vol. 2290, No. 1). AIP Publishing.
18. Al-Dalabeeh EA, Irshaid FI, Roy S, Ali IK, Al-Shudifat AM. Identification of *Entamoeba histolytica* in patients with suspected amebiasis in Jordan using PCR-based assays. Pak J Biol Sci. 2020;23(2):166-72.
19. Anuar TS, Al-Mekhlafi HM, Abdul Ghani MK, Azreen SN, Salleh FM, Ghazali N, et al. Different clinical outcomes of *Entamoeba histolytica* in Malaysia: does genetic diversity exist? Korean J Parasitol. 2013;51(2):231-6. doi:10.3347/kjp.2013.51.2.231
20. Ali IK. Intestinal amebae. Clin Lab Med. 2015;35(2):393-422. doi:10.1016/j.cll.2015.02.009
21. Petri WA, Singh U. Diagnosis and management of amebiasis. Clin Infect Dis. 1999;29(5):1117-25.
22. Rivera WL, Tachibana H, Kanbara H. Field study on the distribution of *Entamoeba histolytica* and *Entamoeba dispar* in the northern Philippines as detected by the polymerase chain reaction. Am J Trop Med Hyg. 1998;59(6):916-21. doi:10.4269/ajtmh.1998.59.916
23. Phiri JS, Katebe RC, Mzyece CC, Shaba P, Halwindi H. Characterization of biosolids and evaluating the effectiveness of plastic-covered sun drying beds as a biosolids stabilization method in Lusaka, Zambia. Int J Recycl Org Waste Agric. 2014;3:1-10.
24. Sami A, Obi LA, Bessong PO, Stroup S, Houpt E, Guerrant RL. Prevalence and species distribution of *E. histolytica* and *E. dispar* in the Venda region, Limpopo, South Africa. Am J Trop Med Hyg. 2006;75(3):565-71.
25. Yakoob J, Abbas Z, Beg MA, Naz S, Khan R, Jafri W. *Entamoeba* species associated with chronic diarrhea in Pakistan. Epidemiol Infect. 2012;140(2):323-8.
26. Espinosa MC, Castañón GG, Martínez-Palomo A. In vivo pathogenesis of *Entamoeba dispar*. Arch Med Res. 1997;28:204-6.
27. Dvorak JA, Kobayashi S, Nozaki T, Takeuchi T, Matsubara C. Induction of permeability changes and death of vertebrate cells is modulated by the virulence of *Entamoeba* spp. isolates. Parasitol Int. 2003;52(2):169-73.
28. Shibayama M, Dolabella SS, Edward FS, Tsutsumi V. A Brazilian species of *Entamoeba dispar* (ADO) produces amoebic liver abscess in hamsters. Ann Hepatol. 2007;6(2):117-8.
29. Herbinger KH, Fleischmann E, Weber C, Perona P, Löscher T, Bretzel G. Epidemiological, clinical, and diagnostic data on intestinal infections with *Entamoeba histolytica* and *Entamoeba dispar* among returning travelers. Infection. 2011;39:527-35.
30. Tannich E, Nickel R, Buss H, Horsttmann RD. Mapping and partial sequencing of the genes coding for two different cysteine proteinases in pathogenic *Entamoeba histolytica*. Mol Biochem Parasitol. 1992;54(1):109-11.
31. Tawfik A, El-Zamel T, Herrawy A, El-Taweel G. Fate of parasites and pathogenic bacteria in an anaerobic hybrid reactor followed by downflow hanging sponge system treating domestic wastewater. Environ Sci Pollut Res Int. 2015;22:12235-45.
32. Andra J, Herbst R, Leippe M. Amoebapores, archaic effectors peptides of protozoan origin, are discharged into phagosomes and kill bacteria by permeabilizing their membranes. Dev Comp Immunol. 2003;27(4):291-304.

33. König C, Honecker B, Wilson IW, Gareth D. Neil Hall NH, Roeder T, et al. Taxon-specific proteins of the pathogenic *Entamoeba* Species *E. histolytica* and *E. nuttalli*. Front Cell Infect Microbiol. 2021;11:641472.
34. Leippe M, Andra J, Nickel R, Tannich E, Muller-Eberhard HJ. Amoebapores, a family of membranolytic peptides from cytoplasmic granules of *Entamoeba histolytica*: isolation, primary structure, and pore formation in bacterial cytoplasmic membranes. Mol Microbiol. 1994;14(5):895-904.
35. Ravdin I, Guerrant RL. Role of adherence in cytopathogenic mechanisms of *Entamoeba histolytica*. Study with mammalian tissue cultures and human red blood cells. J Clin Invest. 1981;68(5):1305-13.
36. Ravdin JI, Guerrant RL. A review of the parasite cellular mechanisms involved in the pathogenesis of amebiasis. Rev Infect Dis. 1982;4(6):1185-207.
37. Ravdin JI, Stauffer WM. *Entamoeba histolytica* (amebiasis). Mandell, Douglas, and Bennett's principles and practice of infectious diseases. Philadelphia PA. 2005; Churchill Livingstone.
38. Petri J, WA, Singh U. Enteric amoebiasis. In: Guerrant, R.L.; Walker, D.H.; Weller, P.F. Tropical infectious diseases: principles, pathogens, and practice. 2nd ed. Philadelphia: Churchill Livingstone; 2006. p. 967-83.
39. Murphy BM, Chen JZ, Rolo M, Eldam M, Jordan L, Sivananthan SJ, et al. Intranasal delivery of a synthetic *Entamoeba histolytica* vaccine containing adjuvant (LecA + GLA-3 M-052 liposomes): In vitro characterization. Int J Pharm. 2022;626:122141.
40. Noll J, Helk E, Fehling H, Bernin H, Marggraff C, Jacobs T, et al. IL-23 prevents IL-13-dependent tissue repair associated with Ly6C(lo) monocytes in *Entamoeba histolytica*-induced liver damage. J Hepatol. 2016;64(5):1147-57.
41. Ouattara M, N'guéssan NA, Yapi A, N'goran EK. Prevalence and spatial distribution of *Entamoeba histolytica/dispar* and Giardia lamblia among school children in Agboville area (Côte d'Ivoire). PLoS Negl Trop Dis. 2010;4(1):e574. doi:10.1371/journal.pntd.0000574
42. Mattern CF, Keister DB. Experimental amebiasis. I. Pathogenicity of axenically cultured *Entamoeba histolytica* in the brain of the newborn mouse. Am J Trop Med Hyg. 1977;26(3):393-401.