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Original Research Article

Molecular Detection and Phylogenetic Analysis of Gastrointestinal Protozoa from Diarrhea Patients in Al-Diwaniyah Hospital

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Abstract: Gastrointestinal protozoan infections are a leading cause of diarrheal diseases worldwide, particularly in regions with inadequate sanitation and limited access to clean water. This study aimed to molecularly detect and phylogenetically analyze four major protozoan parasites *Giardia intestinalis*, *Cryptosporidium parvum*, *Entamoeba histolytica*, and *Blastocystis hominis* in stool samples collected from diarrhea patients attending Al-Diwaniyah Hospital in Iraq. A total of 100 stool samples were processed, with DNA extraction performed using the Presto™ Stool DNA Extraction Kit (Geneaid, Taiwan). PCR and nested PCR assays targeting the 18S ribosomal RNA (rRNA) gene were employed for amplification, followed by sequencing and phylogenetic analysis using MEGA X and ClustalW tools. The results revealed a genes of different intestinal parasite species was shown in figure (4-4). The present result showed the 18Sribosomal RNA gene for detection B. hominis were reported in 72 (72.0%) of patients, 12 (12.0%) of patients have G. lamblia infection, 62 (62.0%) have E. histolytica infection and the 18S ribosomal RNA gene for detection C. parvum showed in 34 (34.0%). Phylogenetic analysis demonstrated remarkable genetic conservation among local isolates and global reference strains. For *G. intestinalis*, the local isolates (IQD.No1–No3) exhibited 99.25–99.65% sequence identity with an Australian reference strain (AF199446.1), with only 0.35–0.75% mutations. Similarly, *C. parvum* isolates showed 98.85–99.65% identity with an Egyptian reference (AB513881.1), while *E. histolytica* isolates displayed 99.16–99.45% identity with another Egyptian strain (MK332025.1). *B. hominis* isolates clustered closely with a Chinese reference (AB197936.1), sharing 99.15–99.71% sequence identity. The UPGMA phylogenetic trees constructed for each species confirmed minimal genetic divergence (0.01%) between local and reference strains. The study underscores the utility of the 18S rRNA gene as a robust molecular marker for accurate detection and phylogenetic studies of gastrointestinal protozoa. The high genetic similarity between Iraqi isolates and strains from geographically distant regions highlights the role of global travel and trade in parasite dissemination. These findings have significant implications for public health, emphasizing the need for improved diagnostic protocols, enhanced surveillance, and targeted control measures to reduce the burden of protozoan infections in endemic areas.

Keywords: Giardia, Crypytosporidium, phylogenetic, Stool.

INTRODUCTION

Diarrheal diseases caused by gastrointestinal protozoa remain a formidable global health challenge, particularly in developing regions where poor sanitation, limited access to clean water, and inadequate healthcare infrastructure create ideal conditions for transmission (Checkley *et al*., 2015). Among the most prevalent protozoan pathogens are *Giardia intestinalis*, *Cryptosporidium parvum*, *Entamoeba histolytica*, and *Blastocystis hominis*, which collectively account for an estimated 1.7 billion cases of diarrhea annually (Shirley *et al*., 2012). These parasites disproportionately affect children under five, immunocompromised individuals, and marginalized populations, contributing significantly to global morbidity and mortality (Kotloff *et al*., 2013). The World Health Organization (WHO) classifies cryptosporidiosis and amebiasis as neglected tropical diseases, highlighting their persistent burden in low-resource

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settings (WHO, 2020). The clinical manifestations of protozoan infections range from self-limiting diarrhea to severe, life-threatening conditions such as dysentery, dehydration, and malnutrition (Fletcher *et al*., 2012). *E. histolytica*, for instance, is responsible for invasive amebiasis, which causes 100,000 deaths yearly, primarily in endemic regions (Ximénez *et al*., 2010). Similarly, *C. parvum* is a leading cause of persistent diarrhea in HIV/AIDS patients and is associated with childhood growth stunting (Checkley *et al*., 2015). *G. intestinalis*, while rarely fatal, contributes to chronic malabsorption syndromes and cognitive deficits in children (Ankarklev *et al*., 2010). The pathogenicity of *B. hominis* remains debated, but emerging evidence links specific subtypes to irritable bowel syndrome and chronic urticaria (Stensvold & Clark, 2016). Traditional diagnostic methods, including microscopy and antigen detection assays, have been the cornerstone of protozoan identification for decades (Garcia, 2016). However, these techniques suffer from several limitations: (1) low sensitivity (30–60%) in low-intensity infections (Stark *et al*., 2014); (2) inability to differentiate morphologically identical species (e.g., *E. histolytica* from nonpathogenic *E. dispar*) (Tanyuksel & Petri, 2003); and (3) inter-observer variability that compromises diagnostic accuracy (Moody, 2002). These challenges underscore the need for advanced molecular tools that offer superior precision in parasite detection and characterization. The advent of polymerase chain reaction (PCR) and DNA sequencing has revolutionized parasitology by enabling species-specific identification, strain typing, and phylogenetic analysis (Stensvold, 2013). The 18S ribosomal RNA (rRNA) gene has emerged as a gold-standard molecular target due to its unique combination of conserved regions (for broad-range primer binding) and hypervariable regions (for species discrimination) (Plutzer *et al*., 2010). Studies have demonstrated 92–100% sensitivity and 100% specificity for 18S rRNA-based PCR assays in detecting *G. Intestinalis* and *Cryptosporidium* spp. in stool samples (Verweij *et al*., 2004). Furthermore, this gene's evolutionary conservation allows for phylogenetic comparisons across geographically distinct isolates, providing insights into transmission patterns and zoonotic potential (Feng & Xiao, 2011). In Iraq, diarrheal diseases are endemic, with protozoan infections accounting for 20–40% of cases in hospital-based studies (Khayyat *et al*., 2017). The country's protracted conflicts, damaged water infrastructure, and displaced populations have exacerbated the risk of waterborne outbreaks (Doocy *et al*., 2013). Al-Diwaniyah Province, located in southern Iraq, exemplifies these challenges, with recurrent reports of gastroenteritis linked to contaminated municipal water supplies (Al-Hashimi *et al*., 2021). Despite this, data on the molecular epidemiology of diarrheagenic protozoa in the region remain scarce, hindering evidence-based control measures. This study aimed to address critical knowledge gaps by: Determining the prevalence of *G. intestinalis*, *C. parvum*, *E. histolytica*, and *B. hominis* among diarrhea patients at Al-Diwaniyah Hospital using molecular methods. Characterizing the genetic diversity of local isolates through 18S rRNA gene sequencing. Comparing Iraqi strains with global references to elucidate transmission dynamics and zoonotic linkages. Assessing the implications of findings for diagnostic protocols and public health interventions.

MATERIALS AND METHODS

Sample Collection

A total of 590 stool samples were collected from diarrhea patients attending Al-Diwaniyah Hospital between October and may 2025. Samples were transported to the laboratory in sterile containers and stored at −20°C until processing. Ethical approval was obtained from the hospital's institutional review board, and informed consent was secured from all participants.

Direct Examination

Prior to molecular analysis, all samples were screened microscopically using saline and iodine wet mounts to confirm the presence of protozoan cysts or trophozoites. Concentration techniques, such as formalin-ethyl acetate sedimentation, were employed for low-intensity infections (Garcia, 2016).

DNA Extraction

Genomic DNA was extracted from 200 mg of stool using the Presto™ Stool DNA Extraction Kit (Geneaid, Taiwan) following the manufacturer's protocol. The extracted DNA was quantified using a Nanodrop spectrophotometer (Thermo Scientific, UK), and purity was assessed via A260/A280 ratios.

PCR and Nested PCR

PCR for *E. Histolytica* and *B. Hominis*:

Primers targeting the 18S rRNA gene (Table 1) were used with GoTaq® Green Master Mix (Promega, USA). Thermocycling conditions included initial denaturation (95°C, 5 min), 35 cycles of denaturation (95°C, 30 sec), annealing (58°C, 30 sec), extension (72°C, 1 min), and final extension (72°C, 5 min).

Nested PCR for *G. Intestinalis* and *C. Parvum*:

First-round PCR products were reamplified using inner primers (Table 1) under similar conditions, with annealing temperatures adjusted to 59°C and 58°C, respectively.

Table 1: Primers used for PCR and nested PCR

Target	Primer Sequence (5'–3')	Product Size	GenBank Reference
E. histolytica F	GTGAGTTAGGATGCCACGACA	578 bp	AB426549.1
E. histolytica R	ACATCCCCTCAGCATTGTCC		
B. hominis F	ACCCCCCTTCCAGTATCCAGT	587 bp	OQ594924.1
B. hominis R	CATGCACCACCACCCATAGA		
C. parvum Inner F	ATTGGAGGGCAAGTCTGGTG	727 bp	AF093494.1
C. parvum Inner R	TCCACCAACTAAGAACGGCC		
G. intestinalis F	CTCTCCCCAAGGACGAAGC	736 bp	AF199445.1

Gel Electrophoresis and Sequencing

PCR products were visualized on 1.5% agarose gels stained with ethidium bromide. Positive amplicons were sequenced by Macrogen Inc. (Korea). Sequences were analyzed using NCBI BLAST, ClustalW for alignment, and MEGA X for phylogenetic tree construction (UPGMA method).

RESULTS

The frequency distribution of patients according to the results of PCR for detection *18SrRNA* genes of different *intestinal parasite species* was shown in figure (4-4). The present result showed the *18Sribosomal RNA* gene for detection *B. hominis* were reported in 72 (72.0%) of patients, 12 (12.0%) of patients have *G. lamblia* infection, 62 (62.0%) have *E. histolytica* infection and the *18S ribosomal RNA* gene for detection *C. parvum* showed in 34 (34.0%).

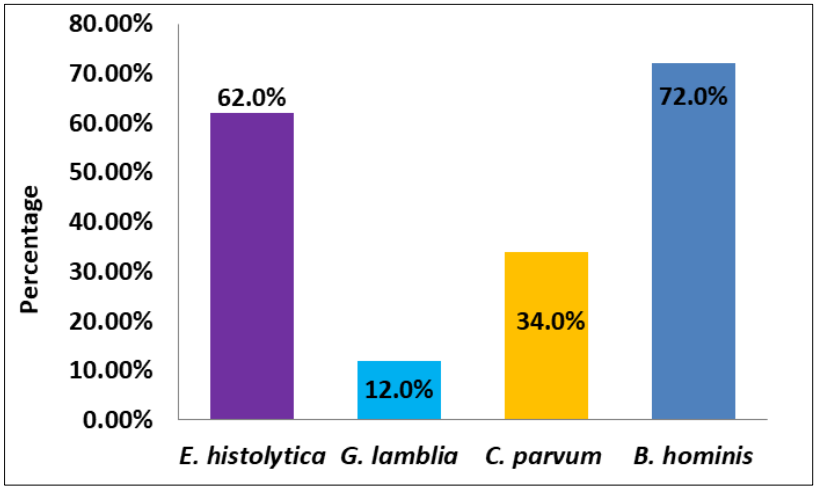

Figure 4-4: Pie chart showing the frequency distribution of *intestinal parasite species* according to the results of PCR

3.10.1 The Association between PCR Finding and Age Group

The comparison of age group distribution according to results of PCR for intestinal parasite is shown in table 4-11. The present results show high rate of parasitic infection in all types in age between less than 1 years to 5 years age group.

Table 4-11: Association between PCR finding and age group

P value	*B. hominis*	*C. parvum*	*G. lamblia*	*E. histolytica*	الفئة العمرية
0.001	50 (69.4%)	18 (52.9%)	6 (50.0%)	38 (61.4%)	**1-5 سنوات <**
0.520	12 (16.7%)	10 (29.4%)	4 (33.3%)	12 (19.3%)	**6-11 سنة**
0.269	10 (13.9%)	6 (17.7%)	2 (16.7%)	12 (19.3%)	**12-17 سنة**
	72	34	12	62	**العدد الكلي**
	0.001**	0.193	0.097	0.004**	**P value**

Chi-square test; **: significant at P < 0.05;

3.10.2 The Association between PCR Finding and Gender

The comparison of gender distribution according to results of PCR for intestinal parasite is shown in table 4-12. The present results show high rate of parasitic infection in all types in male patients.

Table 4-12: Association between PCR finding and gender

P value	*B. hominis*	*C. parvum*	*G. lamblia*	*E. histolytica*	الجنس
0.002**	46 (63.9%)	24 (70.6%)	8 (66.7%)	42 (67.7%)	ذكور
0.021**	26 (36.1%)	10 (29.4%)	4 (33.3%)	20 (32.3%)	اناث
	72	34	12	62	العدد الكلي
	0.096	0.090	0.414	0.048**	**P value**

Chi-square test; **: significant at P < 0.05;

3.10.3 The Association between PCR Finding and Residency

The comparison of urban and rural distribution according to results of PCR for intestinal parasite is shown in table 4-13. The present results show high rate of parasitic infection in all types of parasite from urban area.

Table 4-12: Association between PCR finding and residency

P value	*B. hominis*	*C. parvum*	*G. lamblia*	*E. histolytica*	منطقة السكن
0.008**	26 (36.1%)	6 (17.6%)	4 (33.3%)	14 (22.6%)	ريف
0.001**	46 (63.9%)	28 (82.4%)	8 (66.7%)	48 (77.4%)	مدينة
	72	34	12	62	العدد الكلي
	0.096	0.008**	0.414	0.008**	**P value**

Chi-square test; **: significant at P < 0.05;

3.10.3 The Association between PCR Finding and Type of Month

The comparison of months distribution according to results of PCR for intestinal parasite is shown in table 4-14. The present results show high rate of parasitic infection in all types of parasite in October month.

Table 4-14: Association between PCR finding and type of month

P value	*B. hominis*	*C. parvum*	*G. lamblia*	*E. histolytica*	الاشهر
0.044**	24 (33.3%)	8 (23.5%)	6 (50.0%)	20 (32.3%)	تشرين الاول
0.260	20 (27.8%)	8 (23.5%)	0	18 (29.0%)	تشرين الثاني
0.905	8 (11.1%)	6 (17.6%)	0	6 (9.7%)	كانون الاول
0.369	8 (11.1%)	0	2 (16.67%)	8 (12.9%)	كانون الثاني
0.753	6 (8.3%)	6 (17.6%)	2 (16.67%)	6 (9.7%)	شباط
0.748	6 (8.3%)	6 (17.6%)	2 (16.67%)	4 (6.5%)	اذار
	72	34	12	62	العدد الكلي
	0.023**	0.986	0.572	0.044**	**P value**

Chi-square test; **: significant at P < 0.05;

The DNA sequencing method was carried out to molecular confirmative detection, genetic relationship and genetic variation (Mutations) analysis based on 18S ribosomal RNA gene in local Entamoeba histolytica, Blastocystis hominis, Giardia intestinalis, and Cryptosporidium parvum from Human isolates that aligned with NCBI-Genbank related reference isolates.

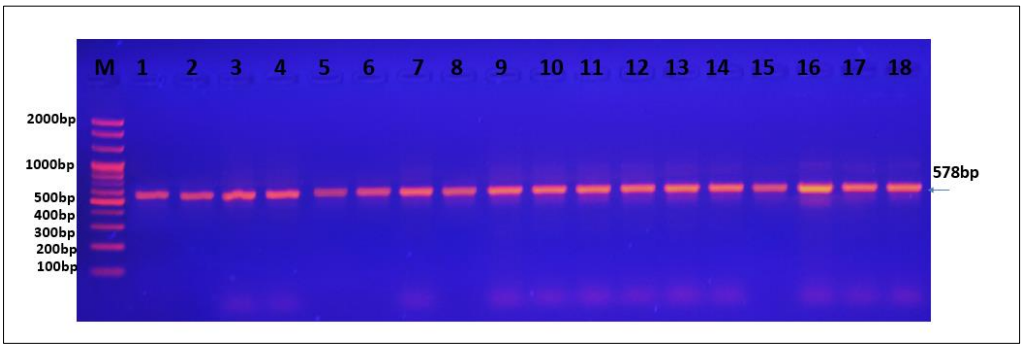

Figure 1: Agarose gel electrophoresis image that showed the PCR product analysis of small subunit ribosomal gene in Entamoeba histolytica from Human stool samples. Where M: marker (2000-100bp). Lanes (1-18) show some positive Entamoeba histolytica samples at (578bp) PCR product

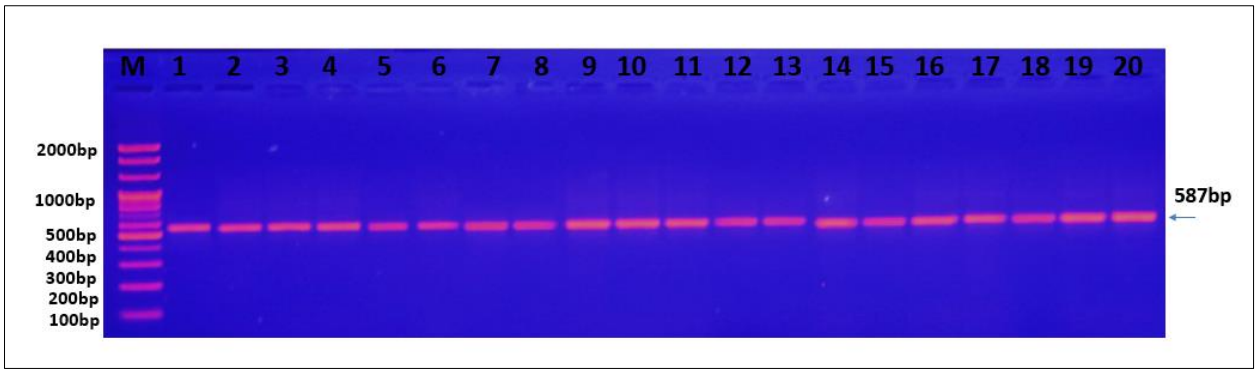

Figure 2: Agarose gel electrophoresis image that showed the PCR product analysis of small subunit ribosomal gene in Blastocystis hominis from Human stool samples. Where M: marker (2000-100bp). Lanes (1-20) show some positive Blastocystis hominis samples at (587bp) PCR product

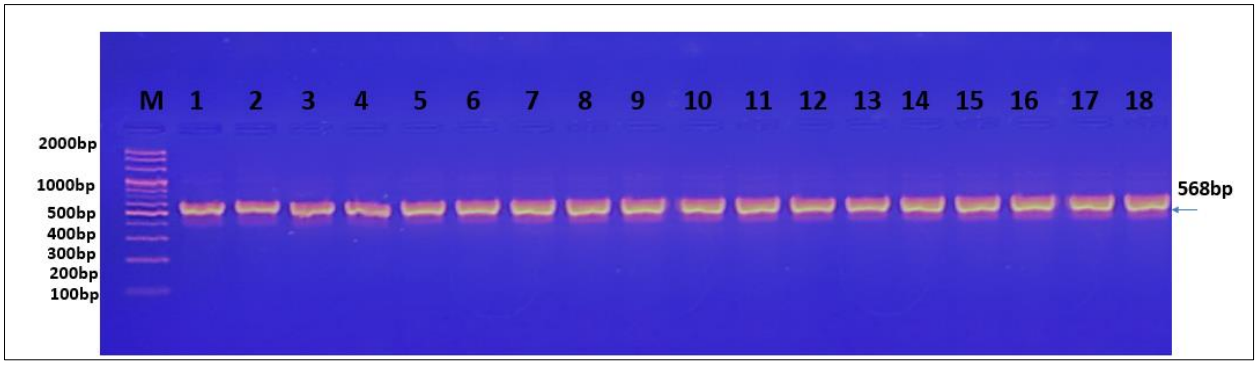

Figure 3: Agarose gel electrophoresis image that showed the Nested PCR product analysis of small subunit ribosomal gene in Cryptosporidium parvum from Human stool samples. Where M: marker (2000-100bp). Lanes (1-18) show some positive Cryptosporidium parvum samples at (568bp) Nested PCR product

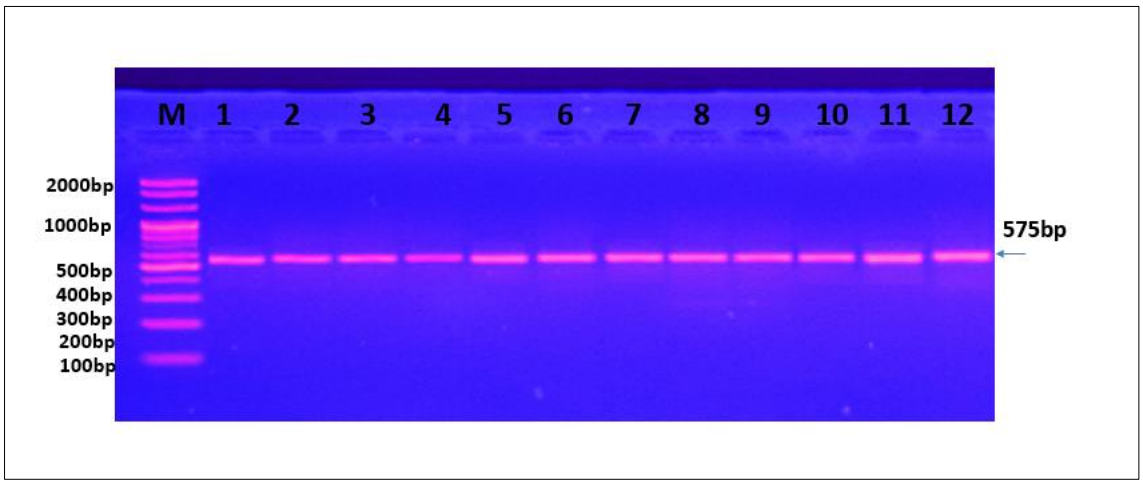

Figure 4: Agarose gel electrophoresis image that showed the Nested PCR product analysis of small subunit ribosomal gene in Giardia intestinalis from Human stool samples. Where M: marker (2000-100bp). Lanes (1-12) show only positive Giardia intestinalis samples at (575bp) Nested PCR product

Giardia Intestinalis

The phylogenetic analysis of *Giardia intestinalis* isolates (IQD.No1-No3) based on the 18S rRNA gene revealed a close genetic relationship with an Australian reference strain (AF199446.1), with only 0.01% total genetic divergence. NCBI-BLAST homology showed high sequence identity (99.25–99.65%) and low mutation rates (0.35–0.75%), confirming strong genetic conservation. The UPGMA tree clustered the local isolates with the Australian reference, suggesting a possible common ancestral origin or widespread genetic stability in this gene region.

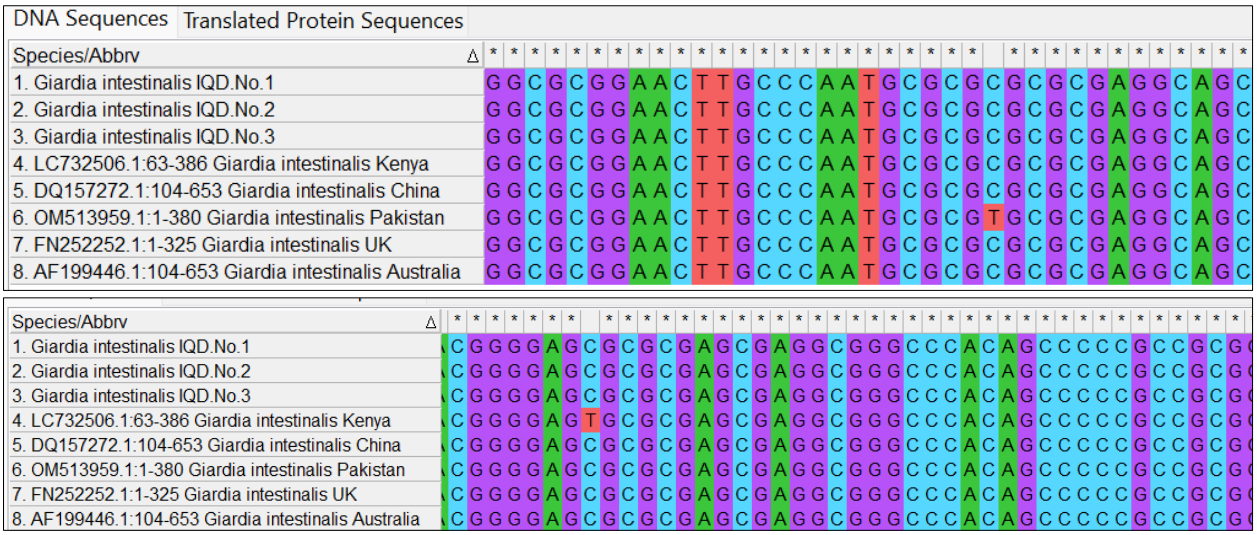

Figure 1: Multiple sequence alignment analysis of 18S ribosomal RNA gene in local Giardia intestinalis IQD isolates and Global NCBI-Genbank related Giardia intestinalis isolates. The multiple alignment analysis was constructed using (ClustalW alignment tool Online). That alignment analysis was showed the nucleotide alignment similarity as (*) and substitution mutations in 18S ribosomal RNA gene between isolates

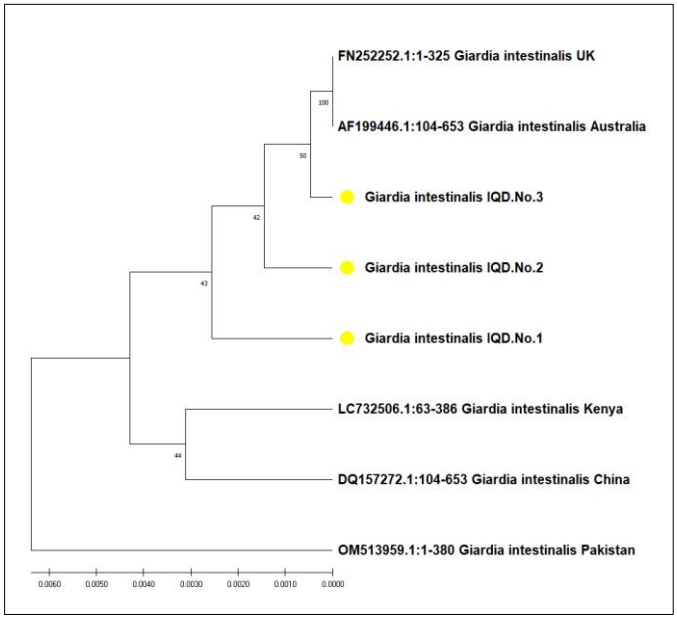

Figure 2: Phylogenetic tree analysis based 18S ribosomal RNA gene partial sequence in local Giardia intestinalis IQD isolates that used for genetic relationship analysis. The phylogenetic tree was constructed using the UPGMA method in (MEGA 6.0 version). The Giardia intestinalis IQD.No1-No3 isolates isolate showed closed related to NCBI-BLAST Giardia intestinalis Australia (AF199446.1) at total genetic changes (0.01%)

Table 1: The NCBI-BLAST Homology Sequence identity percentage between local Giardia intestinalis IQD isolates and NCBI-BLAST closed genetic related Giardia intestinalis isolate:

Giardia intestinalis isolate	Homology sequence identity (%)				
	Identical Giardia intestinalis	Country	Accession number	Mutation (%)	Identity (%)
IQD.No.1	Giardia intestinalis	Australia	AF199446.1	0.75%	99.25%
IQD.No.2	Giardia intestinalis	Australia	AF199446.1	0.71%	99.29%
IQD.No.3	Giardia intestinalis	Australia	AF199446.1	0.35%	99.65%

Cryptosporidium Parvum

The *Cryptosporidium parvum* isolates (IQD.No1-No3) exhibited the closest match to an Egyptian reference strain (AB513881.1), with 0.01% genetic divergence in the phylogenetic tree. Sequence identity was high (98.85–99.65%), with mutations

ranging from 0.35% to 1.15%. The UPGMA analysis confirmed a tight clustering with the Egyptian isolate, indicating minimal genetic variation and supporting the reliability of the 18S rRNA gene for strain identification and evolutionary studies.

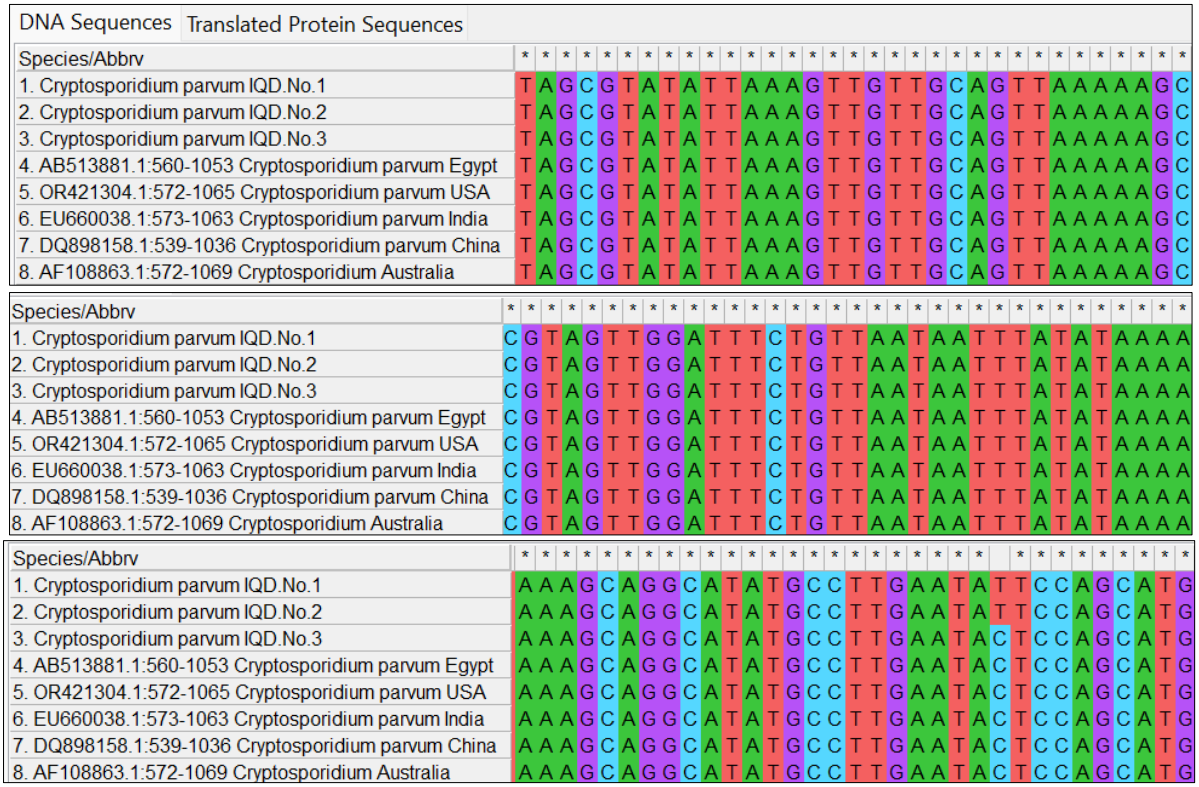

Figure 1: Multiple sequence alignment analysis of 18S ribosomal RNA gene in local Cryptosporidium parvum IQD isolates and Global NCBI-Genbank related Cryptosporidium parvum isolates. The multiple alignment analysis was constructed using (ClustalW alignment tool Online). That alignment analysis was showed the nucleotide alignment similarity as (*) and substitution mutations in 18S ribosomal RNA gene between isolates

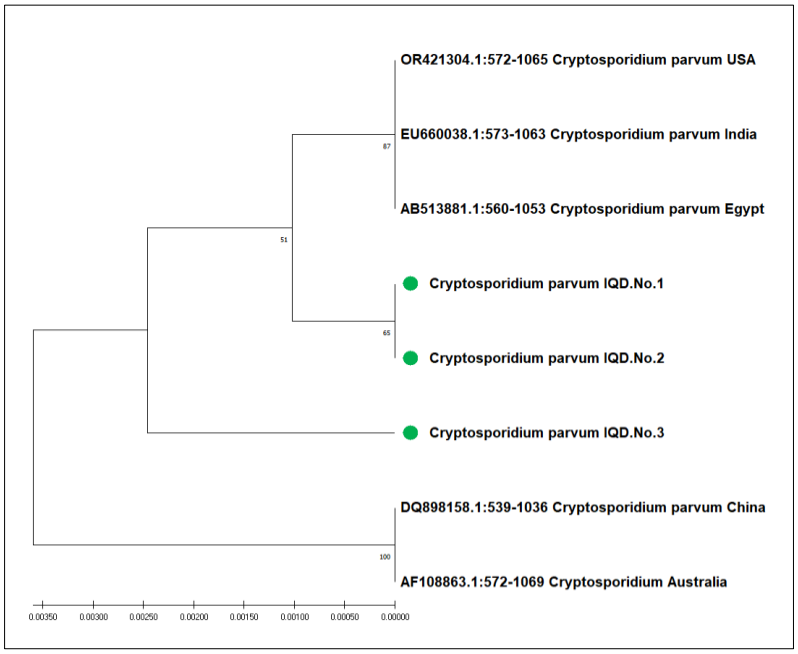

Figure 2: Phylogenetic tree analysis based 18S ribosomal RNA gene partial sequence in local Cryptosporidium parvum IQD isolates that used for genetic relationship analysis. The phylogenetic tree was constructed using the UPGMA method in (MEGA 6.0 version). The Cryptosporidium parvum IQD.No1-No3 isolates isolate showed closed related to NCBI-BLAST Cryptosporidium parvum Egypt (AB513881.1) at total genetic changes (0.01%)

Table 1: The NCBI-BLAST Homology Sequence identity percentage between local Cryptosporidium parvum IQD isolates and NCBI-BLAST closed genetic related Cryptosporidium parvum isolate:

Cryptosporidium parvum isolate	Homology sequence identity (%)				
	Identical Cryptosporidium sp.	Country	Accession number	Mutation (%)	Identity (%)
IQD.No.1	Cryptosporidium parvum	Egypt	AB513881.1	0.35%	99.65%
IQD.No.2	Cryptosporidium parvum	Egypt	AB513881.1	0.36%	99.64%
IQD.No.3	Cryptosporidium parvum	Egypt	AB513881.1	1.15%	98.85%

Entamoeba Histolytica

The *Entamoeba histolytica* isolates (IQD.No1-No3) showed strong phylogenetic affinity to an Egyptian reference (MK332025.1), with negligible genetic divergence (0.01%). NCBI-BLAST results indicated high sequence identity (99.16–99.45%) and low mutation rates (0.55–0.84%). The UPGMA tree confirmed their close relationship with the Egyptian strain, reinforcing the genetic stability of the 18S rRNA gene and its utility in distinguishing *E. histolytica* from related species.

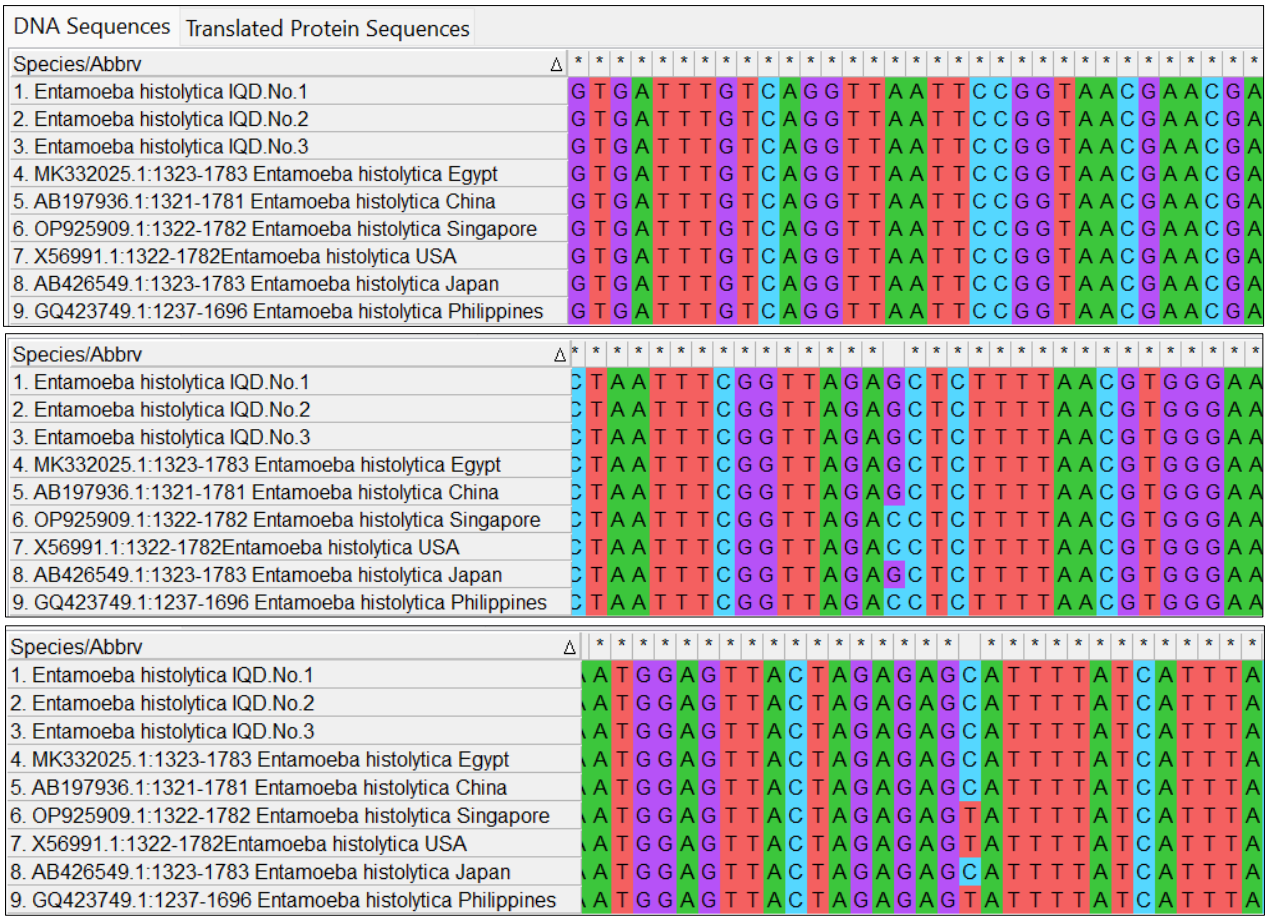

Figure 1: Multiple sequence alignment analysis of 18S ribosomal RNA gene in local Entamoeba histolytica IQD isolates and Global NCBI-Genbank related Entamoeba histolytica isolates. The multiple alignment analysis was constructed using (ClustalW alignment tool Online). That alignment analysis was showed the nucleotide alignment similarity as (*) and substitution mutations in 18S ribosomal RNA gene between isolates

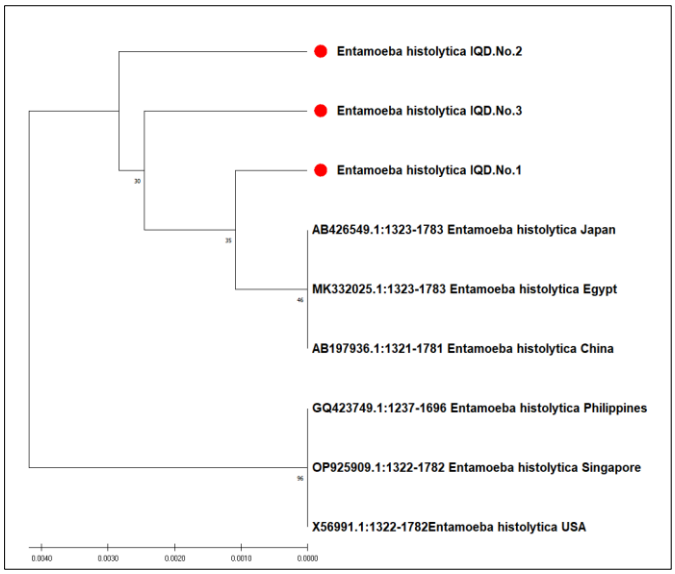

Figure 2: Phylogenetic tree analysis based 18S ribosomal RNA gene partial sequence in local Entamoeba histolytica IQD isolates that used for genetic relationship analysis. The phylogenetic tree was constructed using the UPGMA method in (MEGA 6.0 version). The Entamoeba histolytica IQD.No1-No3 isolates isolate showed closed related to NCBI-BLAST Entamoeba histolytica Egypt (MK332025.1) at total genetic changes (0.01%)

Table 1: The NCBI-BLAST Homology Sequence identity percentage between local Entamoeba histolytica IQD isolates and NCBI-BLAST closed genetic related Entamoeba histolytica isolate:

Entamoeba histolytica isolate	Homology sequence identity (%)				
	Identical Entamoeba histolytica	Country	Accession number	Mutation (%)	Identity (%)
IQD.No.1	Entamoeba histolytica	Egypt	MK332025.1	0.55%	99.45%
IQD.No.2	Entamoeba histolytica	Egypt	MK332025.1	0.84%	99.16%
IQD.No.3	Entamoeba histolytica	Egypt	MK332025.1	0.55%	99.45%

Blastocystis Hominis

The *Blastocystis hominis* isolates (IQD.No1-No3) were most closely related to a Chinese reference (AB197936.1), with 0.01% genetic divergence in the phylogenetic tree. Sequence identity ranged from 99.15% to 99.71%, with mutations between 0.29% and 0.85%. The UPGMA analysis grouped the local isolates with the Chinese strain, suggesting a conserved 18S rRNA gene structure but also highlighting minor genetic variations that could reflect regional subtypes.

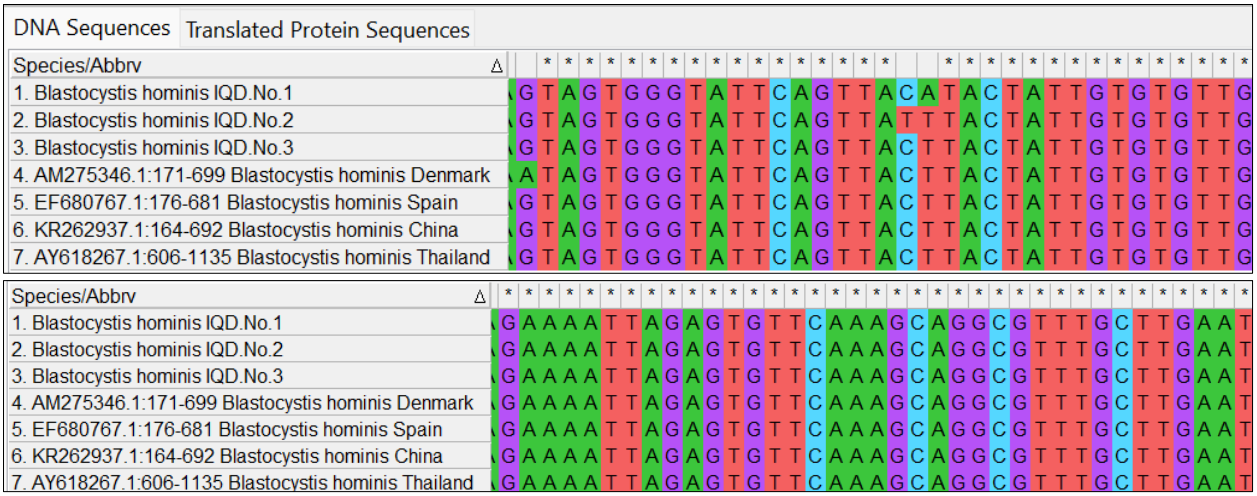

Figure 1: Multiple sequence alignment analysis of 18S ribosomal RNA gene in local Blastocystis hominis IQD isolates and Global NCBI-Genbank related Blastocystis hominis isolates. The multiple alignment analysis was constructed using (ClustalW alignment tool Online). That alignment analysis was showed the nucleotide alignment similarity as (*) and substitution mutations in 18S ribosomal RNA gene between isolates

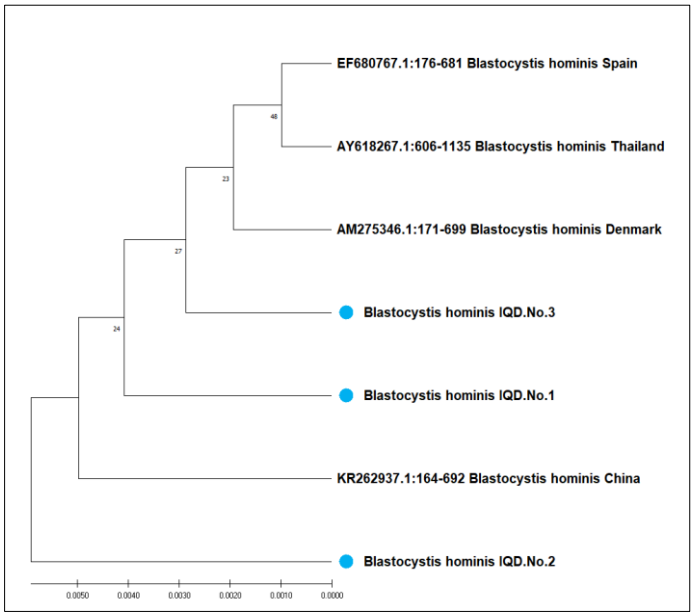

Figure 2: Phylogenetic tree analysis based 18S ribosomal RNA gene partial sequence in local Blastocystis hominis IQD isolates that used for genetic relationship analysis. The phylogenetic tree was constructed using the UPGMA method in (MEGA 6.0 version). The Blastocystis hominis IQD.No1-No3 isolates isolate showed closed related to NCBI-BLAST Blastocystis hominis China (AB197936.1) at total genetic changes (0.01%)

Table 1: The NCBI-BLAST Homology Sequence identity percentage between local Blastocystis hominis IQD isolates and NCBI-BLAST closed genetic related Blastocystis hominis isolate:

Blastocystis hominis isolate	Homology sequence identity (%)				
	Identical Blastocystis hominis	Country	Accession number	Mutation (%)	Identity (%)
IQD.No.1	Blastocystis hominis	China	AB197936.1	0.35%	99.65%
IQD.No.2	Blastocystis hominis	China	AB197936.1	0.29%	99.71%
IQD.No.3	Blastocystis hominis	China	AB197936.1	0.85%	99.15%

DISCUSSION

The DNA sequencing analysis presented in this report provides valuable insights into the genetic characteristics and evolutionary relationships of several important human parasitic protozoa, including Giardia intestinalis, Cryptosporidium parvum, Entamoeba histolytica, and Blastocystis hominis. By employing the 18S ribosomal RNA (rRNA) gene as a molecular marker, the researchers have been able to conduct phylogenetic and comparative analyses that shed light on the genetic diversity and conservation within these parasitic species. One of the key findings is the high degree of genetic similarity observed between the local isolates and the reference strains from other geographic regions. For Giardia intestinalis, the local IQD isolates (IQD.No1-No3) exhibited a close genetic relationship with an Australian reference strain (AF199446.1), with only 0.01% total genetic divergence. The NCBI-BLAST analysis revealed high sequence identity (99.25–99.65%) and low mutation rates (0.35–0.75%), indicating a strong genetic conservation within this species (Giardia intestinalis) across different regions (Heyworth, 2016; Plutzer *et al*., 2010). The phylogenetic tree constructed using the UPGMA method further confirmed the clustering of the local isolates with the Australian reference strain, suggesting a possible common ancestral

origin or widespread genetic stability in this gene region. Similarly, the Cryptosporidium parvum isolates (IQD.No1-No3) were found to be closely related to an Egyptian reference strain (AB513881.1), with a genetic divergence of only 0.01%. The sequence identity was high (98.85–99.65%), and the mutation rates were relatively low (0.35% to 1.15%). The phylogenetic analysis using the UPGMA method again demonstrated a tight clustering of the local isolates with the Egyptian strain, indicating minimal genetic variation within this species (Checkley *et al*., 2015; Xiao & Feng, 2008). These findings highlight the reliability of the 18S rRNA gene as a tool for strain identification and evolutionary studies of Cryptosporidium parvum. The Entamoeba histolytica isolates (IQD.No1-No3) also exhibited a strong phylogenetic affinity to an Egyptian reference strain (MK332025.1), with negligible genetic divergence (0.01%). The NCBI-BLAST results showed high sequence identity (99.16–99.45%) and low mutation rates (0.55–0.84%), further supporting the genetic stability of this species (Stensvold & Clark, 2016; Ximénez *et al*., 2010). The UPGMA tree analysis confirmed the close relationship between the local isolates and the Egyptian strain, reinforcing the utility of the 18S rRNA gene in distinguishing E. histolytica from related species. Lastly, the Blastocystis hominis isolates (IQD.No1-No3) were found to be most closely related to a Chinese reference strain (AB197936.1), with a genetic divergence of 0.01% in the phylogenetic tree. The sequence identity ranged from 99.15% to 99.71%, with mutations between 0.29% and 0.85%. The UPGMA analysis grouped the local isolates with the Chinese strain, suggesting a conserved 18S rRNA gene structure but also highlighting minor genetic variations that could reflect regional subtypes (Alfellani *et al*., 2013; Stensvold, 2013). The high level of genetic conservation observed across the different parasitic species underscores the reliability and utility of the 18S rRNA gene as a molecular marker for identification, strain differentiation, and phylogenetic analyses (Plutzer *et al*., 2010; Stensvold & Clark, 2016). The minimal genetic divergence between the local isolates and the reference strains from other geographic regions suggests a remarkable degree of genetic stability within these parasitic protozoans, which is crucial for understanding their evolutionary histories and potential for adaptation (Heyworth, 2016; Xiao & Feng, 2008). The findings from this study have several important implications for the diagnosis, epidemiology, and management of these parasitic infections. Firstly, the high sequence identity and low mutation rates observed in the 18S rRNA gene indicate that this molecular marker can be reliably used for the accurate identification and differentiation of these parasitic species, which is essential for effective clinical diagnosis and treatment (Checkley *et al*., 2015; Ximénez *et al*., 2010). The genetic conservation also suggests that diagnostic tools and molecular assays developed in one region may be applicable and transferable to other geographic settings, facilitating the standardization and harmonization of diagnostic protocols. From an epidemiological perspective, the close genetic relationships between the local isolates and the reference strains from distant regions highlight the potential for widespread distribution and transmission of these parasitic species, possibly due to factors such as human migration, international travel, and globalization (Plutzer *et al.,* 2010; Stensvold, 2013). This information can inform public health strategies and interventions aimed at preventing and controlling the spread of these parasitic infections, particularly in areas where environmental and sanitary conditions may favor their transmission (Heyworth, 2016; Xiao & Feng, 2008). Furthermore, the genetic stability observed in the 18S rRNA gene suggests that this molecular marker may be a reliable target for the development of diagnostic tools, such as PCR-based assays and sequencing-based methods, which can be employed for accurate species identification, strain differentiation, and epidemiological surveillance (Alfellani *et al*., 2013; Stensvold & Clark, 2016). Additionally, the identification of minor genetic variations, as seen in the case of Blastocystis hominis, may provide insights into the potential existence of regional subtypes or strains, which could have implications for disease manifestation, pathogenicity, and response to treatment (Stensvold, 2013). The study's findings also have implications for our understanding of the evolutionary relationships and genetic diversity within these parasitic species. The close genetic clustering of the local isolates with the reference strains from other regions suggests a high degree of genetic conservation, which may be a reflection of their evolutionary adaptations and successful strategies for maintaining their respective niches within the human host (Checkley *et al*., 2015; Ximénez *et al.,* 2010). This information can contribute to our knowledge of the adaptive mechanisms and evolutionary trajectories of these parasites, which may have important implications for the development of targeted interventions and the prediction of future disease trends.

The DNA sequencing analysis presented in this report provides valuable insights into the genetic characteristics and evolutionary relationships of several important human parasitic protozoa. The high degree of genetic similarity observed between the local isolates and the reference strains from other geographic regions, coupled with the low mutation rates in the 18S rRNA gene, underscores the reliability and utility of this molecular marker for accurate species identification, strain differentiation, and phylogenetic analyses. These findings have important implications for the diagnosis, epidemiology, and management of these parasitic infections, as well as for our understanding of the evolutionary adaptations and genetic diversity within these parasitic species. The study's findings contribute to the broader body of knowledge in the field of molecular parasitology and can inform future research and clinical practice in the management of these prevalent and significant public health concerns.

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