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Molecular analysis of *Giardia lamblia* using small subunit ribosomal RNA gene isolated from food handlers' people in Erbil city

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Cheman Hasan Hamid[1*] **Hawri Mustafa Bakr**[2]

Abstract

Background and objective: *Giardia lamblia* is the intestinal flagellated protozoan parasite causes giardiasis, a gastrointestinal illness, by living and multiplying in the small intestine of humans and other mammals. *Giardiasis* is most commonly transmitted in underdeveloped nations due to a lack of infrastructure for sanitation and hygiene education, as well as the fact that affected individuals often consume tainted food and drink that has mature cysts. The purpose of this research was to identify human giardiasis among food handlers as well as to perform sequencing, and phylogenetic analysis of small subunit ribosomal RNA (ssurRNA) gene.

Methods: A total of 540 fecal samples from food handlers were screened at the central laboratory in Erbil City, including both symptoms and asymptomatic individuals. General stool microscopy is the gold standard for primary diagnosis using saline and iodine wet mount technique. Molecular testing was carried out for samples that tested positive for *Giardia lamblia,* nucleic acid extracted from stool samples using specialize kit for that purpose. A 550 bp fragment of the ssu rRNA gene was amplified by PCR analysis targeted through a specific primer set. The amplified PCR products were sent to a sequencing facility in South Korea to determine the sequence of the ssu rRNA gene in *Giardia lamblia*.

Results: The study did not find any correlations between infection rates and socioeconomic status or demographic variables. Microscopic examination identified (50) positive cases, of which (21) were also confirmed as positive through PCR. The ssu rRNA gene reported variation, and (21) different sequences of the gene show changing in one nucleotide which changes one amino acid. Following aligning of ssu rRNA gene sequences using NCBI-BLAST homology analysis, the samples showed the following percentages of identity with *Giardia lamblia* isolates from various countries: 99.59% and 100% with isolates from Australia, 100% with those from USA, 99.38% from Iraq, 99.59% from Sweden, and 99.59% from Spain.

Conclusion: Polymerase Chain Reaction (PCR) is a very dependable tool for identifying *Giardia lamblia.* The pathogenicity of *Giardia lamblia* may be impacted by the variations in sequencing alignment of ssu rRNA documented through bioinformatics and single nucleotide mutations.

Keywords: *Giardia lamblia*, Molecular analysis (PCR), ssu rRNA gene.

[1] **Department of Medical Microbiology, College of Health Science, Hawler Medical University, Kurdistan Region, Iraq.**

[2] **Department of Physiology and Microbiology, College of Medicine, Hawler Medical University, Kurdistan Region, Iraq.**

Correspondence: nurebiology@gmail.com

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A gastrointestinal parasite known as *Giardia duodenalis*, *Giardia intestinalis*, or *Giardia lamblia* infects the small intestines of humans and other animals. Globally, 0.4–7.5% of the population is infected with the protozoan parasite *Giardia duodenalis,* 8–30% live in less developed countries (1). *Giardia duodenalis* infections are caused by ingesting cysts in contaminated food or water. Asymptomatic *Giardiaduodenalis* infections are common in humans and usually clear within weeks without treatment (2). Asymptomatic illnesses can lead to malabsorption syndrome, a condition characterized by delayed growth in children, in nations with high poverty rates (3).

Gastrointestinal symptoms include diarrhea, pain, flatulence, nausea, and bloating are brought on by symptomatic disease (4). The eight genotypes that make up the *Giardia duodenalis* complex are called assemblages. A-H, these genotypes have the same morphology but have different molecular lineages. Humans and other mammals are infected by *Giardia duodenalis* assemblages A and B (5). Dogs and other canids (C, D), hoofed livestock (E), cats (F), rodents (G), and marine animals (H) are the usual hosts of assemblages C–H. However, new data indicates that circulating assemblages (like E) that are restricted to cattle may also infect humans, indicating that some assemblages may not be as host-specific and might therefore spread from non-human species to people (6). Four sub-assemblages (AI–AIV and BI–BIV) were identified by an allozyme analysis within assemblages A and B; of these, AI, AII, BIII, and BIV have been specifically identified in humans. Sub-assemblages AI–AIII within assemblages A have been validated by nucleotide sequence and phylogenetic analysis, with AI primarily isolated from animals, but humans are the primary source of AII. Furthermore, only two human cases of AIII have been reported recently; most cases have been in wild species, such as deer (7). Several recent reviews have discussed different assemblages of *Giardia duodenalis*, which may represent the existence of new Giardia species, but further biological and genetic studies are needed before confirming new species names (8). Several studies have suggested that the assemblages are genetically distant from each other, and several genomic analysis results also showed that the isolates of assemblages A, B, and E represent three different genetic populations. *Giardia duodenalis* is commonly identified in a variety of hosts using genetic markers like small subunit ribosomal RNA (ssu-rRNA), glutamate dehydrogenase (gdh), triosephosphate isomerase (tpi), and b-giardin (bg) genes. This allows for the identification of genetic diversity and population dynamics among *Giardia duodenalis* assemblages (9). Since the ssu-rRNA gene is a highly conserved and multicopied locus, it may be used in place of other methods to recognize and

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differentiate *G. duodenalis* assemblages. However, because it is conserved and most PCR experiments only produce short amplified fragments, it is less helpful in identifying genetic variation within assemblages. However, when it comes to identifying genetic variation and classifying *Giardia duodenalis* populations at the sub-assemblage and genotype levels, the single-copy tpi, bg, and gdh loci are more sensitive. Nevertheless, these loci are not thought to be viable options for clinically detecting *Giardia duodenalis* (8, 10). There is consensus that these loci may genetically categorize various *Giardia duodenalis* assemblages. Although there is agreement on the ability of these loci to genetically classify different *G. duodenalis* assemblages, conflicting results have been reported regarding the efficacy of a single locus in distinguishing *G. duodenalis* populations into assemblages and sub assemblages (11). Thus, in order to enhance accuracy, a numeric multilocus genotyping (MLG) procedure was introduced, which involves the simultaneous analysis of at least two genes (tpi, bg, and gdh) Food handlers are among the most significant people who can spread intestinal parasite illnesses, such as *Giardia lamblia,* to other people (12).

The aim of the present study was to detect *Giardia lamblia* isolate from food handlers reside in Erbil city. The current investigation was undertaken due to the absence of prior work on the characterization of *Giardia lamblia* sequencing and phylogenetic analysis of ssu rRNA gene in this specific populations"

Materials and methods

Study Design and sample Collection

The present cross-sectional study was carried out from September 2024 to February 2025 on food handlers in Erbil city. Overall, 540 fecal samples were collected from food-handlers who annually visit to health centers to obtain a health certificate. Fresh fecal specimens were collected in a clean container. Each sample was labeled and immediately transferred to the parasitology and molecular units at Erbil International University for microscopic examination and molecular analysis.

Each participant was filled out a questionnaire that included basic personal information and demographics details-including their age, gender, duration of parasite illnesses, and history of other clinical problems.

A- Macroscopic examination

Stool samples were inspected for consistency (e.g. solid, semi-solid or liquid), color (e.g. brown, yellow), content (e.g. undigested food, worms), odor and any abnormalities, including the presence or absence of blood and mucus.

B- Microscopic examination

Microscopic analysis was performed using the saline and iodine wet mount

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technique. A clean glass slide was prepared for each sample with one drop of normal saline placed on one half slide and a drop of Lugals iodine on the other half, the size of a pinhead was taken two times from different areas of the same stool sample using a wooden stick (13). One of them was put on normal saline and mixed well, while the other swab mixed with Lugals iodine. Each preparation was covered with a cover slip and examined under light microscope using the magnification power 400 (40 x 10) to detect the presence of trophozoites and/or cysts of Giardia lamblia. About 1 gram of each fresh stool positive sample was stored at -20 ˚C without any preservative's solution for subsequent molecular analysis.

C- Molecular Approach

DNA extraction

Frozen stool samples were the primary source of genomic DNA, which was extracted using FavorPrep stool DNA isolation Mini Kit (50 preps), following the manufacturer's protocol with slight modifications. As a pre-treatment step, the frozen samples were weighed under cool temperature until the lysis buffer (Inhibit EX Buffer) was added to prevent DNA degradation. The isolated genomic DNA extracted from all positive stool samples were stored at -20 c for no longer than six months prior until used for running PCR and subsequent amplification. The purity of the extracted DNA was assessed using a Nanodrop spectrophotometer (Thermo, USA), which measures absorbance at (260/280 nm) (14).

Amplification of *Giardia lamblia* DNA by PCR

To confirm the presence of *Giardia lamblia*, PCR was performed using primers specific for 18S rRNA genes (15), using the isolated genomic DNA from the stool samples as a template. A 550 bp amplicon was amplified using a single PCR targeting the ssu rRNA region as shown in Table 1.

Thermocycle conditions for the 18S rRNA gene

The temperature profile consists of the following steps: a 5-minute initial denaturation at 95 °C, 35 cycles of denaturation at 95 °C for 40 seconds, primer annealing at 58 °C for 40 seconds, an extension at 72 °C for 1 minute, and a final 5-minute extension at 72 °C, (Table 2).

Table 1. Primer sequences were used in the PCR

Gene	Primer	Sequence (5' to 3')	Product size	
			bp	Tm
18S rRNA	Forward	5-GGA GGT AGT GAC AAT AAATC-3	550bp	59C
	Reverse	5-TAA GAC TAC GAG GGT ATC TA-3		

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Gel electrophoresis

Agarose (1.2%) was prepared using 1× TAE buffer solution and safe red dye. The amplified PCR products were loaded after being stained with loading dye. The gel was initially run at 5 Volt/cm, after which the voltage was increased to 75-100 volts and maintained for 45 minutes.

Sequencing of DNA

The PCR product of 21 samples (using only forward primer) was sequenced by ABI Prism Terminator Sequencing Kit (Applied Biosystem) at Microgene Center in Korea. Chromatograms of the 18S rRNA gene were edited and base calls were checked using the Finch TV program software.

Data analysis: Finch TV chromatogram viewer software was used to convert the chromatograms to FASTA format. Manual editing of the DNA sequences in the ABI file was done using BioEdit v.7.0.5. To determine, the degree of homology with the nearest species, the results of the sequence editing process were examined using the BLAST (Basic local alignment search tool) NCBI.

Ethical considerations: The study was approved by the Research Ethical Committee of the College of Health Sciences, Hawler Medical University. Written andoral consents were obtained from all participants prior to sample collection. With assurance of confidentiality. All personal information was kept private.

Statistical analysis: Data was analyzed using the statistical package for social science (SPSS statistical for windows, version 23.0). Test of significance included the Pearson's Chi-Squar test (or Fishers Exact test for sparse data). Statistical significance was set as a P-value <0.05.

Result

The study included a total of 540 participants, comprising 80 females and 460 males. The examined demographic and socioeconomic characteristics, including gender, age group, education level, living conditions, and residency, shown in Table 3 did not indicate any statistically significant association with infection rates (P >0.05). The infection rate was higher among males (82.6%) than females (15%); however, the difference was not statistically significant (P = 0.055). Rates of infection were also quite constant among demographic subgroups defined by age, level of education, housing situation, and geographic location. The age group of (>19) exhibited the higher infection rate (9.88%), with no significant difference (P = 0.979). Participants in primary school had the (highest infection rate) (9.52%), however, the disparities among educational levels were not statistically significant (P = 0.970).

The infection rate was highest among individuals with poor living conditions (10.17%), although there was no significant link (P = 0.949). Infection rate

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among rural residents (9.74%) were higher compared to urban residents (9.07%), however the difference was statistically not significant (P = 0.821).

Table 2: PCR Amplification Reagents

No.	PCR components	Concentration	Volume (µl)
1	Master Mix	2x	25
2	Forward Primer	10 Pmol	2
3	Reverse Primer	10 Pmol	2
4	DNase free Water	-	17
5	Template DNA	50ng/µl	4
Total			50

Table 3. Infection rates across demographic and socioeconomic groups characteristics of *Giardia lamblia* infection rates detected by direct microscopic examination

Variant	No. of participants	Positive (%)	Negative (%)	P-value
Gender				
Female	80 (14.81%)	12 (15.00%)	68 (85.00%)	0.055
Male	460 (85.19%)	38 (82.6%)	422 (91.74%)	
Age-group				
>19	81 (15%)	8 (9.88%)	73 (90.12%)	0.979
20–30	295 (54.62%)	27 (9.15%)	268 (90.85%)	
>30	164 (30.37%)	15 (9.15%)	149 (90.85%)	
Educational level				
Primary school	147 (27.22%)	14 (9.52%)	133 (90.48%)	0.970
Secondary school	289 (53.52%)	27 (9.34%)	262 (90.66%)	
Graduated	104 (19.26%)	9 (8.65%)	95 (91.35%)	
The living conditions the individual				
Poor	59 (10.93%)	6 (10.17%)	53 (89.83%)	
Medium	431 (79.81%)	39 (9.05%)	390 (90.95%)	0.949
Good	50 (9.26%)	5 (10.00%)	45 (90.00%)	
Residency				
Urban	386 (71.48%)	35 (9.07%)	349 (90.43%)	0.821
Rural	154 (28.52%)	15 (9.74%)	139 (90.26%)	
Total	540 (100%)			

*Chi-square, P ≤0.05 considered statistically significant

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As presented in Table 4: (78.7%) of the total participants were asymptomatic, whereas (21.3%) experiencing symptoms. The rate of positive results was higher among symptomatic individuals compared to asymptomatic individuals (13.03% vs. 8.24% respectively). However, this difference was not statistically significant P=0.145.

Table 5, revealed that direct microscopic inspection identified 50 positive cases (9.26 %) out of 540 samples. In contrast PCR analysis detected 21 positive cases (42%) among the 50 samples tested.

Table 4. Comparison of microscopy results of *Giardia lamblia* between symptomatic and asymptomatic groups

Symptomatic	**No.of participants**	**Positive (%)**	**Negative (%)**	**P-value**
Symptomatic	115 (21.3)	15 (13.04)	100 (86.96)	
Asymptomatic	425 (78.7)	35 (8.24)	390 (91.76)	0.145
Total	540 (100)	50 (9.26)	490 (90.74)	

***** Fisher's Exact Test, P≤0.05 considered statistically significant

Table 5. Comparison of diagnostic methods by direct Microscopy vs. PCR of *Giardia lamblia*

Method	**Positive (%)**	**Negative (%)**	**Total**	**P-value**
Direct microscopic	50 (9.26 %)	15 (13.04)	540	
Polymerase Chain Reaction (PCR)	21 (42%)	29 (58%)	50	< 0.001

*Chi-square, P ≤0.05 considered statistically significant

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As presented in Figure 1, agarose gel electrophoresis a distinct PCR product of approximately 550 base pairs in multiple samples, indicating successful amplification of the target gene. Lane M, contained a DNA ladder ranging from 100 bp to 3000 bp, serving as a molecular size reference. Variations in band intensity may reflect differences in DNA concentration or amplification efficiency.

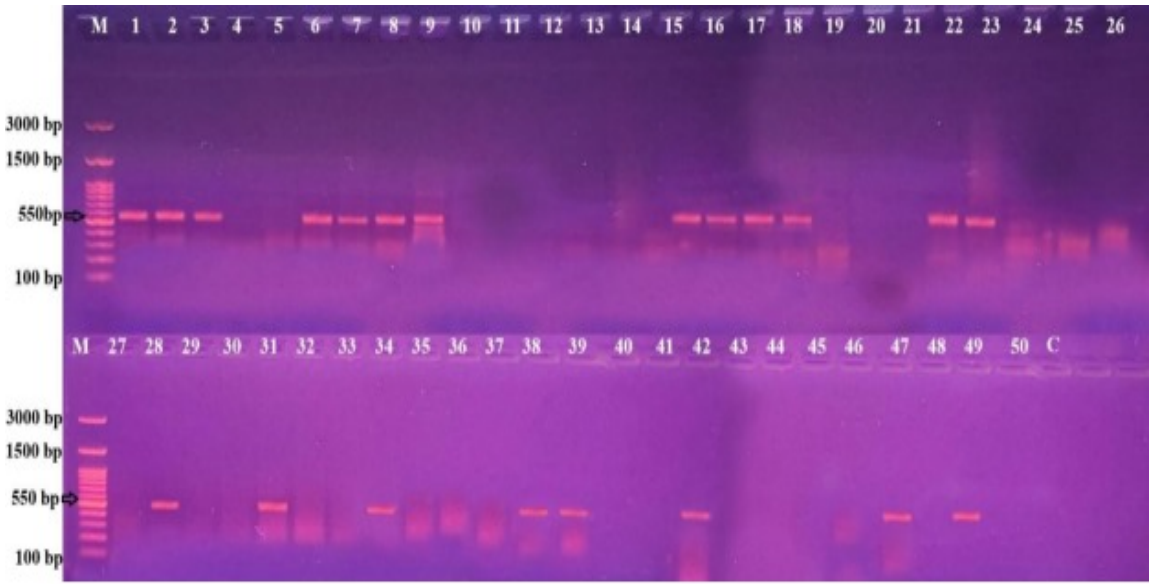

Figure 1. Demonstrates the PCR amplification of the 18S rRNA gene, which targets a 550bp fragment using specific primers. In the 50 stool samples that were microscopically positive, 21 samples exhibited the anticipated PCR band, which suggests the presence of the parasite. The remaining 29 samples did not exhibit any amplification

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Sequence alignment and submission of 18S rRNA gene to GenBank

The 18S rRNA gene sequences were verified for quality using BioEdit v7.0.5. By comparing the query sequences with other biological sequences, in the present study, we used the Basic Local Alignment Search Tool (BLAST), which is accessible at the National Center for Biotechnology Information (NCBI) website, to compare and align the 18S rRNA partial gene sequences with other biological sequences to find similarities with *Giardia* and investigate variant nucleotides between Iraqi and other country of *Giardia,* as shown in Table 6.

Table 6. The GenBank accession numbers for the18S rRNA gene partial sequences of *Giardia intestinalis*

Name	Accession No.	Gene name
Giardia intestinalis	PV587099	18S rRNA
Giardia intestinalis	PV587100	18S rRNA
Giardia intestinalis	PV587101	18S rRNA
Giardia intestinalis	PV587102	18S rRNA
Giardia intestinalis	PV587103	18S rRNA
Giardia intestinalis	PV587104	18S rRNA
Giardia intestinalis	PV587105	18S rRNA
Giardia intestinalis	PV587106	18S rRNA
Giardia intestinalis	PV587107	18S rRNA
Giardia intestinalis	PV587108	18S rRNA
Giardia intestinalis	PV587109	18S rRNA
Giardia intestinalis	PV587110	18S rRNA
Giardia intestinalis	PV587111	18S rRNA
Giardia intestinalis	PV587112	18S rRNA
Giardia intestinalis	PV587113	18S rRNA
Giardia intestinalis	PV587114	18S rRNA
Giardia intestinalis	PV587115	18S rRNA
Giardia intestinalis	PV587116	18S rRNA
Giardia intestinalis	PV587117	18S rRNA
Giardia intestinalis	PV587118	18S rRNA
Giardia intestinalis	PV587119	18S rRNA

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DNA sequencing analysis and Alignment of nucleotide of the 18S rRNA gene

Submitted sequences of the 18S rRNA gene from *Giardia lamblia* were dispatched to Macrogen Molecular Company in Korea. A mutation or variation of nucleotides appear among 21 submitted sequence of the 18S rRNA gene was identified according to alignment in the program of MEGA version11, which shown in Figure 2 that reveals the substitution of thymine (T) for guanin (G).

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PV587111-Giardia
CGGCCGGTGCGCGCGTCCCGCCGGCCGCCCATGGAAACCGGGAGGCTCCGGGCTCTGGGG

PV587119-Giardia
CGGCCGGCGCGCGCGTCCCGCCGGCCGCCCAGGGAAACCGGGAGGCTCCGGGCTCTGGGG

PV587114-Giardia
CGGCCGGCGCGCGCGTCCCGCCGGCCGCCCAGGGAAACCGGGAGGCTCCGGGCTCTGGGG

PV587115-Giardia
CGGCCGGCGCGCGCGTCCCGCCGGCCGCCCAGGGAAACCGGGAGGCTCCGGGCTCTGGGG

PV587117-Giardia
CGGCCGGCGCGCGCGTCCCGCCGGCCGCCCAGGGAAACCGGGAGGCTCCGGGCTCTGGGG


PV587099-Giardia
CGGCCGGCGCGCGCGTCCCGCCGGCCGCCCAGGGAAACCGGGAGGCTCCGGGCTCTGGGG

PV587118-Giardia
CGGCCGGCGCGCGCGTCCCGCCGGCCGCCCAGGGAAACCGGGAGGCTCCGGGCTCTGGGG

PV587108-Giardia
CGGCCGGTGCGCGCGTCCCGCCGGCCGCCCATGGAAACCGGGAGGCTCCGGGCTCTGGGG

PV587116-Giardia
CGGCCGGCGCGCGCGTCCCGCCGGCCGCCCAGGGAAACCGGGAGGCTCCGGGCTCTGGGG

PV587105-Giardia
CGGCCGGTGCGCGCGTCCCGCCGGCCGCCCATGGAAACCGGGAGGCTCCGGGCTCTGGGG
          ******* ********************** ****************************
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Figure 2. nucleotide sequence alignment of the 18S rRNA gene from different Giardia isolates.Conserved regionsare highlighted, while sequence variations are indicated in yellow and blue. The observed sequence diversity among the isolates suggests underlying genetic heterogeneity, which may be associated with functional or phenotypic differences among the strains

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NCBI-BLAST homology results

All of the samples compared to reference sequences in GenBank show high levels of similarity to *Giardia lamblia* (100% to 99.59%). There is widespread genetic similarity across regions, as the strains that have been identified are associated with various countries such as Australia, India, Sweden, and the United States. The match rate toU09491 (USA) is 100%., 99.55% match the Sweden -based XR_005248679, and 99.59% match the Australia -based AF199447 and AF199444, respectively. With a match rate of 99.59% to PQ512127 Australia and 99.38% to HQ179634 (Iraq), as shown in Table 7.

Phylogenetic tree analysis

Phylogenetic study of the 18S rRNA gene revealed that the majority of *Giardia lamblia* isolates belonged to a single clade, suggesting that this gene is highly conserved and shared among local strains. There was a cluster of these isolates with GenBank accession codes starting with "PV" next to generic reference sequences. Slight variation in genetic makeup was indicated by a small number of isolates, including KP159521 and MT951204.The presence of *Giardia muris* as an out group in the tree's root system proved that it had evolved separately and was a good candidate for rooting as a separate branch. In addition to demonstrating that the 18S rRNA gene is preserved within *Giardia lamblia*, the relatively small genetic distance (~1%) between *Giardia lamblia* and the *Giardia muris* group shows how this *Giardia* species differs from others, as shown in Figure 3.

Table 7. Global reference sequences for *Giardia lamblia* isolates analyzed by NCBI-BLAST

Parasite samples	Accession Number	Query Cover %	Identic Number %	Genbank Accession Number	Genbank Identification	Country
PV587101		100	100	U09491	*Giardia lamblia*	USA
PV587102		100	100	U09492	*Giardia lamblia*	USA
PV587103		100	100	AF199446	*Giardia lamblia*	Australia
PV587104 PV587105		100	100	M54878	*Giardia lamblia*	Portland
PV587099 PV587106		100	99.59	XR_005248679	*Giardia lamblia*	Sweden
PV587107		100	99.59	AF199447	*Giardia lamblia*	Australia
PV587106 PV58710		100	99.59	PQ512127	*Giardia lamblia*	Spain
		100	99.38	HQ179634	*Giardia lamblia*	Iraq

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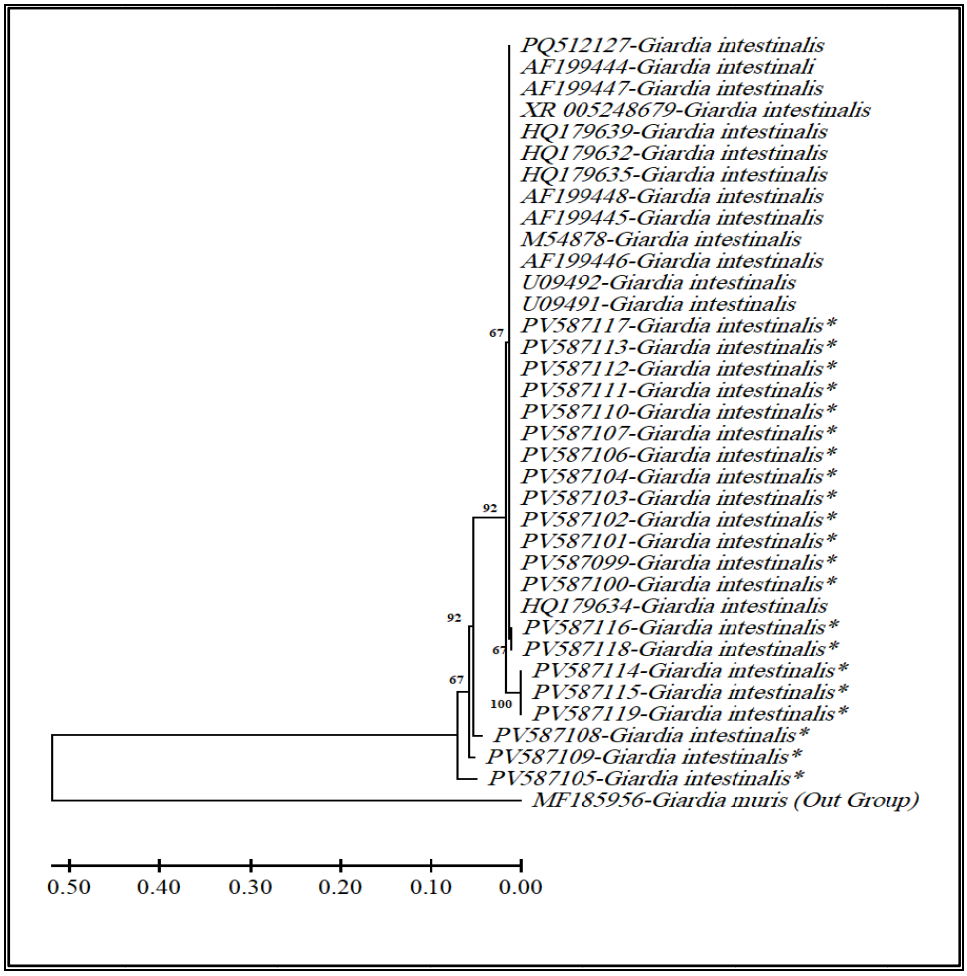

Figure 3. The phylogenetic tree showing the evolutionary relationships among *Giardia lamblia* isolates includes samples from Kurdistan, Iraq (marked with *). The Tamura-Nei Maximum Likelihood algorithm, which is part of MEGA11, was used to build it. Model, and the validation process involved the usage of 100 bootstrap repetitions. The research utilized *Giardia muris* as a control group or (out group) and depended on partial 18S rRNA gene sequences

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Discussion

Giardia lamblia is among predominant intestinal protozoa which cause diarrhea in human being. The parasite till now is considered a major enteric parasite worldwide, especially in food handlers who have an important role in spreading the parasite (16). In the present study out of 540 stool samples examined for intestinal parasites, 50individuals tested positive for *Giardia lamblia*cysts.

Among the foodhandlers, include in this study, male constituted the vast majority (91.74%). This gender disparity may be attributed to greater employment opportunities for men in the restaurant industry, particularly in Erbil city northern Iraq, where males are more frequently engaged in food service role. Nearly majority of the individuals examined in a study of Sudanese food handlers were men (92.9%) (17). Similar patterns were observed in investigations conducted in (Iran), (Al Dewanya, Iraq) (16, 18). The microscopic inspection of stool samples in this study indicated no statistically significant differences in infection rates based on age, gender, education level, or housing situation (19). Consistent with earlier research from (Brazil), (Korea) (16, 17). This study disagreed with previous research that identified people in their age group (>19) as being at high risk for giardiasis because the infection rate was (9.1%) in this age range, though it conflicts with the findings of the studies in (Al-Diwanya, Iraq), (Brazil) (19, 20). given the strong association between gender and age and the prevalence of intestinal parasites. These findings are consistent with those of a global study that identified giardiasis as a major health concern among younger population, with substantial variation observed across demographic factors. Notably, place of residence appeared to be a significant determinant; with infection rates higher among rural resident (9.74%) compared to urban counter parts (9.07%). This discovery agrees with the study in (Danimark) (21). The study indicated that rural residents had higher infection rates, which were explained by factors such as drinking well water, poor sanitation, and limited access to health care, reporting that the gap in residency is not statistically significant. Additionally, there was no statistically significant difference between symptomatic and asymptomatic persons, based on microscopic examination the microscopy results (P = 0.145). Interestingly, (8.24%) of the positive instances were discovered among the asymptomatic participants. It is suggested that many infected individuals do not develop symptoms, making them potential parasite carriers, consistent with the study in (Iran) (22). Only 21 out of 50 samples successfully amplified the 550 bp 18S rRNA gene fragment using PCR. One major drawback of microscopy is that it is not a reliable method for distinguishing

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between different species of *Giardia*. Despite the great sensitivity and specificity of molecular detection techniques, their expensive cost prevents them from being routinely used as a laboratory test procedure in the majority of endemic locations (23). The amplified PCR producers (50) samples of patients' feces of the current study were send to laboratory in South Korea for DNA sequencing to characterize the genetic profiles of *Giardia lamblia* isolates. The nucleotide thymine (T) has replaced guanin (G) in the 18S rRNA gene sequence, according to sequence alignment results. Based on the research in Brazil, this alteration may affect the nucleotides' structure and function (24). In the study using NCBI-BLAST to determine homology sequence identity, (50) samples were compared to GenBank reference sequences. The reference sequences came from a variety of countries, including (USA, Spain, Australia, Portland, Sweden and Iraq). The highest percentage of identity was 100% with the USA sample (U09491) and (U09492) respectively, followed by 100% with the Australian sample (AF199446, AF199445), (99.59%) with the Sweden sample (XR_005248679), (99.59%) with the Spain sample (PQ512127) and (99.38%) with Iraq (HQ179634). These findings are in agreement with several studies-conducted in (Iran) and (China) (25, 26). The results showed that all of the PCR samples were identical to the NCBI-BLAST isolates with the accession number (AF199446), a study conducted in Diyala, Iraq (27), found that the 18S rRNA sequences of the local isolates were very similar to one that was found in the UK, specifically in the NCBI GenBank database (accession number X76903.1). The phylogenetic tree shows that the *Giardia lamblia* isolates are quite similar to each other and cluster together, proving that they are really the same species. On the other hand, due to their physical separation, *Giardiamuris* seems to constitute a different species. In contrast to the study in Babylon, Iraq (28, 29), that examined the phylogenetic tree based on the 18S rRNA gene, the *Giardia lamblia* isolate and the out-group *Giardia muris* have a (1%) total genetic variation. With respect to the *Giardia lamblia* isolates, which are indistinguishable from both the USA strain and the out-group strain, the overall genetic variation among the isolates was about (0.50%) (30).

Conclusion

The study found that polymerase chain reaction (PCR) is the most reliable method for detecting and identification of *Giardia lamblia,* achieved through the *amplification* of 550bp fragment of 18SrRNA gene. However, a noted limitation of PCR its relatively higher coast and longer processing time compared to other diagnostic assays. The study also found that asymptomatic carriers of *Giardia lamblia* among food handlers have a potential risk for

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transmission as they may contaminate the food they prepare, thereby infecting consumers.

The pathogenicity of *Giardia lamblia* may be impacted by the large variations in sequencing alignment of 18S rRNA caused by bioinformatics and single nucleotide mutations. These changes alter the structure and function of nucleic acids. According to the results of NCBI-BLAST homology analysis, the samples showed genetic similarity to *Giardia lamblia* strains reported from several countries.

Competing interest

The authors declare that they have no competing interests.

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