

Genetic Characterization of *Giardia lamblia* in Infected Patients from Salah al-Din, Iraq, Using the *TPI* Gene

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ABSTRACT: This study evaluated *Giardia lamblia* infection in patients with diarrhea using the *tpi* gene and Nested PCR. One hundred stool samples were collected from patients attending Tikrit Teaching Hospital and health laboratories in Samarra and Ad-Dhuluiya between October 1, 2024, and July 1, 2025. Their ages ranged from month to 41 years. Microscopic examination revealed that 36 samples 36% were positive for the parasite, with a higher infection rate in males 51.2% than in females 24.7%, and the highest infection rate was found in the month to 10-year age group 62.2%. DNA was extracted from the samples, and 83 samples were suitable for molecular analysis. The first nested-PCR reaction for the *tpi* gene recorded bands of 605 bp in 42 samples, while the second reaction showed bands of 530 bp in 38 samples. All positive samples in the second reaction were classified as genotype A 90.4%.

Keywords: Diarrhea, *Giardia lamblia*, epidemiology, Nested PCR, *TPI* gene



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1. INTRODUCTION

Diarrhea is a major public health problem globally, ranking second among infectious disease-related deaths [1]. Giardiasis is a major cause of diarrhea, with an estimated 280 million cases annually [2]. The parasite is particularly prevalent in low-income countries with poor sanitation, while incidence rates are lower in industrialized countries [3]. The causative agent is *Giardia lamblia*, also known as *Giardia intestinalis* or *Giardia duodenalis*, one of the most common intestinal parasites in humans and many other mammals. Infections typically occur in children younger than five years of age, although this can be a problem in adults who are immunocompromised, and travelers to endemic regions [4, 5]. Diarrhea, bloating, and abdominal pain can result from either an asymptomatic infection or one with the symptoms. At times, it will cause chronic sequels, for instance, touchy gut disorder (IBS) [6]. Infective cysts remain resistant to chlorine making giardiasis one of the most common waterborne intestinal infections that require more effective disinfection methods such as ultraviolet radiation and ozone [7]. The *tpi* (triosephosphate isomerase) gene: One of the most widely used genes for the molecular diagnosis of giardiasis with the high sensitivity and specificity by nased PCR [8]. Furthermore, such marker considered as aleading candidate modifier for the separation of the parasite genetic populationsas this gene has high range of genetic sequences of the same gene andhigh accuracy for identifying among assemblages, suitable for used as abasic unit of information for improving genetic knowledge of the parasite infour populations of infected individuals [9].

2. MATERIALS AND METHODS

2.1 Sample collection

Stool samples were taken from 100 patients from people suffering from diarrhea and collected from Tikrit Teaching Hospital and some health laboratories in both Samarra and Al-Dhuluiya. Ages of the patients ranged from 1 month to 41 years, between October 1, 2024 to July 1, 2025. Immediately within the first 30 min since collection, a direct wet swab with physiological saline was performed on the samples paying particular attention to areas with a mucus- and lipid-rich environment where the parasite infection rates of *G. lamblia* parasite according to sex is likely to be found. Positive or questionable samples were aliquoted (100–200 mg) and placed in one 500 µl of (DNA Shield solution, Zymo Research, USA). These samples were subsequently kept at –20°C until the molecular analysis was conducted at the central laboratory at Tikrit University.

2.2 Molecular detection

Molecular testing was performed to detect *G. lamblia* using the *tpi* gene. DNA was extracted from stool samples using the Presto Mini gDNA Kit (Geneaid), followed by measurement of sample purity and concentration using a Nanodrop device at 260/280 nm wavelengths. To verify DNA quality and suitability for subsequent molecular steps, electrophoresis was performed on a 1% agarose gel. The results showed that 83 out of 100 samples contained DNA suitable for analysis.

2.3 Preparation of the primers

TPI gene-specific primers were obtained from Macrogen. The primers were prepared as working solutions at a final concentration of 10 pmol/µl after dissolving them in nuclease-free water, prior to use for gene amplification.

2.4 The primers

The amplification of *TPI* gene by nested PCR using two sets of primers as described in [10]. During the first round, the forward primer AL3543 was used (5'-AAAATATGCTGCTCGTCG-3'). As for the reverse primer AL3546 (5'-CAAACCTTITCCGCAAACC-3') to produce a 605 bp fragment.

Second round, the forward primer AL3544 (5'-CCCTTCATCGGIGGTGAACTT-3') was used with the reverse primer AL3545 (5'-GTGGCCACACACICCCGTGCC-3') to produce a 530 bp fragment, with the same binding intensity of 50 °C.

2.5 Diagnosis of *G. lamblia* for the *tpi* gene using Nested PCR

The gene *tpi* was used for detecting the *G. lamblia* by using a two-stage nested PCR technique.

During the first round of PCR reaction, both primers AL3543 and AL3546 were used to target the gene, producing a 605 bp fragment. The components of reaction were prepared by adding 12 µL of master mix, 2 µL of each forward primer and reverse primer, and 3 µL of template DNA. After the reaction was complete, the PCR products were electrophoresed on a 2% agarose gel to confirm the presence of the presenting bands.

In the second round, the product of the first round PCR was used as the template DNA with primers AL3544 and AL3545. This reaction yielded a 530-bp band. The component of the reaction was prepared by adding 3 µL of the first reaction product, 2 µL of each forward internal primer and reverse internal primer, and 12.5 µL of master mix. After completion of the reaction, the nested PCR products were electrophoresed on a 2% agarose gel to confirm the formation of the presenting bands.

Both reactions were performed according to the same thermal program, which included an initial denaturation at 94°C for 10 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 45 seconds, and elongation at 72°C for 60 seconds, followed by an elongation phase. Final at 72°C for 10 minutes.

3. RESULTS AND DISCUSSION

Direct microscopic examination of 100 stool samples using the direct wet smear technique with 0.85% physiological saline showed that 36 samples (36%) were positive for one of the parasite stages (encysted or trophic), while 64 samples (64%) were negative. As shown in Table (1), and Figure (1).

Table 1. - Infection rates of *G. lamblia* parasite

Examined sample	Positive result	percentage	Negative result	percentage
100	36	36%	64	64%



FIGURE 1. The cyst stage of *G. lamblia* from a stained stool sample

The microscopic examination results in the current study, which showed an infection rate of 36%, were consistent with the study by Mostafa *et al.* [11] in Tikrit, which recorded 36.2%. The results of the study by [12] were lower by 14.3%, while the study by Haji & Bamarni [13] in Duhok recorded a rate of 15.55%. In contrast, the study by Mohammed *et al.* [14] in Kirkuk showed a higher rate of 58%. This discrepancy reflects the influence of environmental, behavioral, and health factors on the spread of the parasite. The results of the current study showed a higher infection rate among males compared to females, as the infection rate among males reached 51.2% compared to 24.7% among females, as shown in Table(2) .

Table 2. Infection rates of *G. lamblia* parasite according to sex

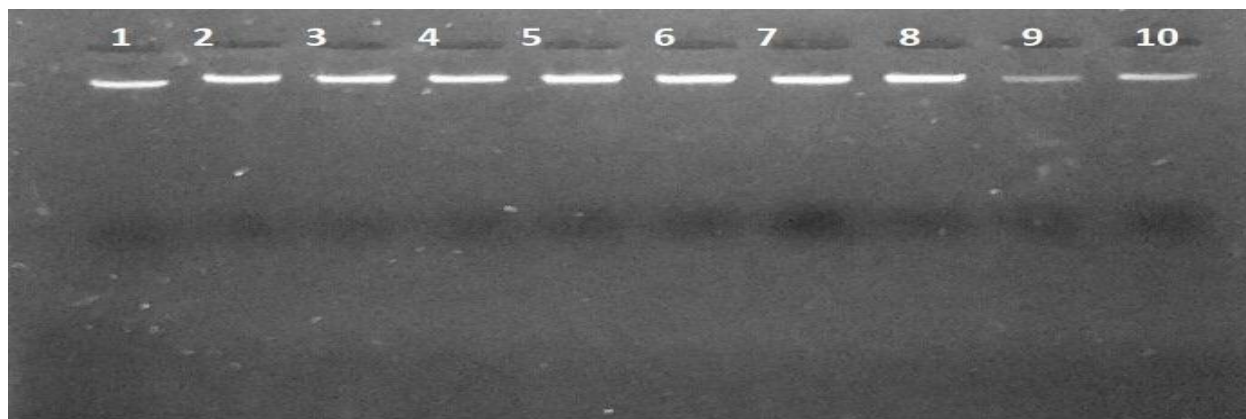
Percentage	Negative result	Percentage	Positive result	Examined sample	Sex
%48.8	21	%51.2	22	43	Male
%75.4	43	%24.7	14	57	Female
	64		36	100	Total number

The results of the current study are consistent with those reported in [15], where higher rates were observed among males (56.58%) compared to females (43.42%). However, these results differ from those of [16], which recorded higher rates among females (57.3%) compared to males (22.6%). The higher incidence rate among males can be explained by their increased exposure to environmental pollutants and poor adherence to health practices. The results showed that the highest infection rate was in the age group of months - 10 years (62.2%), then gradually decreased with age to reach the lowest rate in the age group of 32 - 41 years (11.8%), as shown in Table(3) .

Table 3.- Infection rates of *G. lamblia* parasite according to age

Percentage%	Positive result	Examined sample	Ages
%62.2	23	37	Month -10 years
%36.4	8	22	11-21 years
%12.5	3	24	22-31 years
%11.8	2	17	32-41 years
36%	36	100	Total number

The results of the current study are consistent with those reported in [17], which recorded the highest infection rate in young age groups (≤ 10 years) at 81.25%. However, they differ from those reported by Zuhair & Al-Maki (2007)[18], which found the highest infection rate in the 21–30 age group. The higher infection rate among children can be explained by weak immune systems, lack of health awareness, and frequent exposure to polluted environments. DNA was extracted from a total of 100 fecal samples. The results showed successful extraction in 83 samples, while 17 samples did not achieve sufficient quality in terms of DNA quantity or purity. The results were verified using electrophoresis, as shown in Figure (2), which shows the resulting bands of DNA extracted from the samples.

**FIGURE 2.** DNA bands extracted from stool samples after electrophoresis on 1% agarose gel.

The results of the current study are consistent with those of [19], where the success rate of DNA extraction from samples reached 44.3%. In contrast, the study by (Faria *et al.*, 2023)[20] showed a marked decrease in extraction efficiency, reaching 18%. This discrepancy reflects the significant importance of the quality of the extracted DNA in achieving efficient molecular diagnosis of *G. lamblia*. A nested-PCR was performed for the *tpi* gene. In the first reaction, 83 samples were examined, and the results revealed the appearance of distinct bands approximately 605 base pairs in size when compared to the standard size guide (DNA ladder) in 42 samples, as shown in Figure (3)

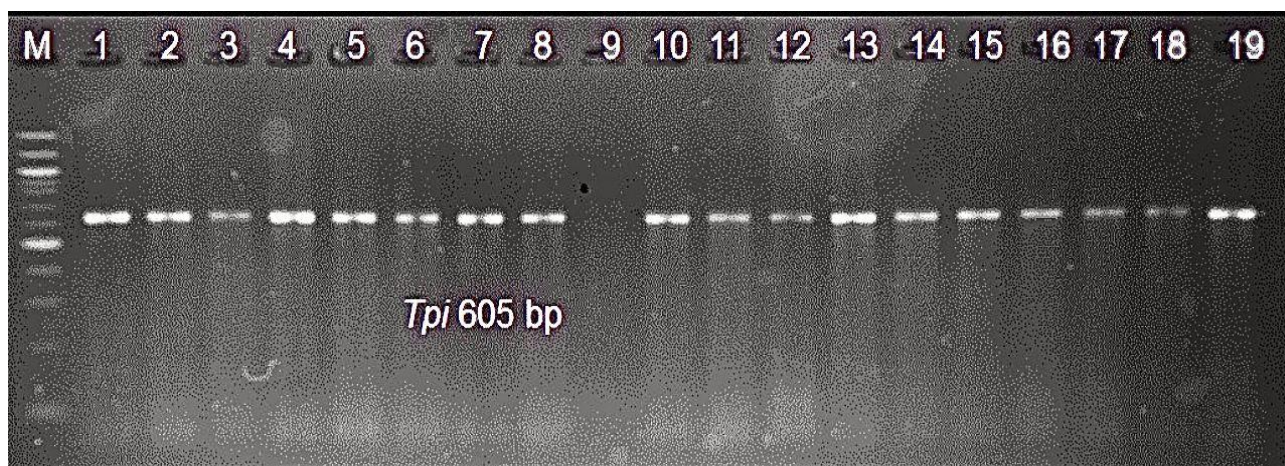


FIGURE 3. The result of the first Nested PCR reaction for the *tpi* gene for samples (1-2-3-4-5-6-7-8-10-11-12-13-14-15-16-17-18-19) represents the positive samples with a size of approximately 605 base pairs. Electrophoresis was carried out in a 2% agarose gel with a standard size indicator of 100 base pairs. Samples (9) represent the negative samples.

After using the products of the first reaction as a template, the second reaction was performed using primers to amplify a smaller part of the gene, 530 base pairs in size, which represents the A genotype. This stage showed positive results in 38 samples, as shown in Figure.(4)

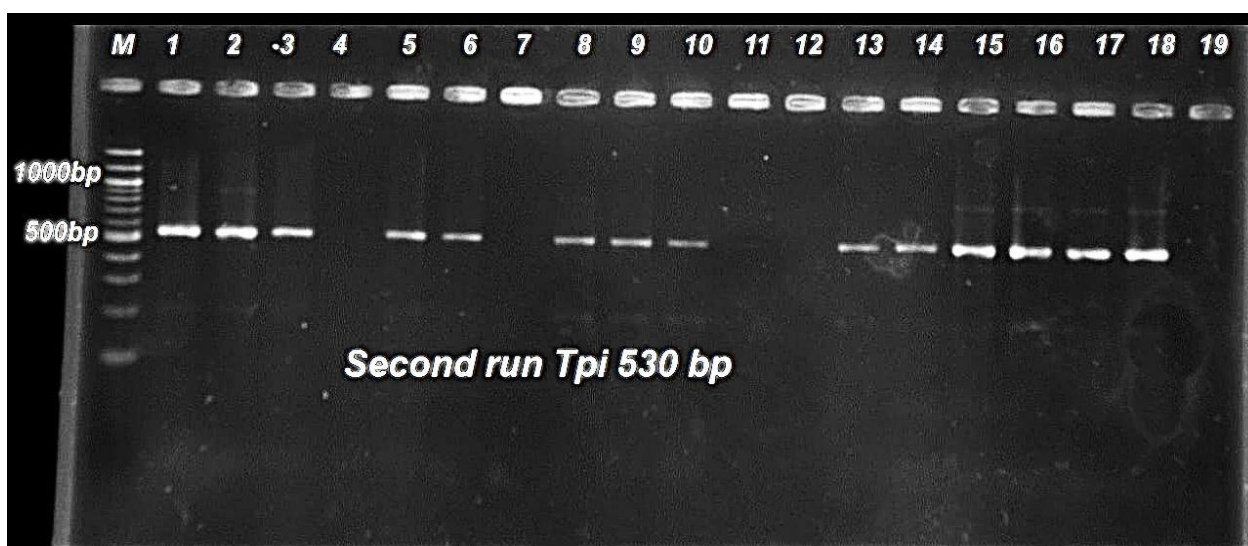


FIGURE 4.- The result of the second reaction, Nested PCR, for the *tpi* gene for samples (1-3-2-5-6-8-9-10-13-14-15-16-17-18), represents the positive samples with a size of approximately 530 base pairs, electrophoresis was carried out in a 2% agarose gel with a standard size indicator of 100 base pairs, while samples (4-7-11-12-19) represent the negative samples.

Molecular analysis showed that all positive samples in the second reaction (38 samples) directly belonged to genotype A, representing 90.4% of positive samples. This reflects the clear prevalence of this type in the studied area and confirms the effectiveness and accuracy of the Nested PCR technique in identifying *G.lamblia* genotypes. These results support the findings of [5] in Egypt, where type A recorded the highest prevalence at 45.7%, and [21] in Iran, where type A recorded the highest prevalence at 58.3%. The *TPI* gene is a pivotal gene in *Giardia lamblia*, encoding triosephosphate isomerase, a key enzyme in the glycolysis pathway. This enzyme catalyzes the reversible reaction between dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (G3P), a central step in the conversion of glucose to energy (ATP). This is critical for the parasite, given its near-total reliance on glycolysis as its primary energy source in a low-oxygen intestinal environment [22]. The *TPI* gene is expressed at a relatively high level during the feeding phase

and is considered an evolutionarily conserved gene among eukaryotes, indicating its biochemical importance. Its genome sequence also possesses a degree of genetic diversity sufficient to distinguish between different parasite assemblages, making it a popular target for studies. Molecular epidemiology [23]. The *TPI* gene is used as an effective molecular marker in PCR techniques, particularly in nested PCR protocols, to identify and characterize different genetic isolates of *G. lamblia* and to differentiate between human and animal isolates. Molecular analysis of this gene has also revealed the presence of homogeneity and diversity within genotypes A and B, which are the most common in human infections [23].

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