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Molecular Detection and Epidemiological Assessment of *Giardia lamblia* in Symptomatic Patients from Wasit Province, Iraq: A Comparative Study Using Real-Time PCR (qPCR)

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Abstract
Giardia lamblia is the most prevalent intestinal protozoan responsible for waterborne gastrointestinal illness globally, especially in areas of the world with poor sanitation and water quality conditions, in developing countries. Although conventional microscopy is the primary technique used in routine diagnosis in Iraq, it is not very sensitive in the detection of low-density infections.
The present study was designed to investigate the molecular prevalence of G. lamblia in symptomatic cases of Wasit province (Iraq) and also to compare the results of microscopic examination with Real-Time PCR (qPCR) method.
The study was conducted from January to October 2025 with a cross-sectional study design on 200 patients who presented with symptoms in Al-Karama Teaching Hospital in Wasit Province. Formal-ether concentration microscopy and TaqMan-based Real-Time PCR of the small subunit ribosomal RNA (ssu rRNA) gene of the stool were examined. IBM SPSS version 26 was used for statistical analysis.
The prevalence of G. lamblia detected by qPCR was 19.0% (38/200), which was higher than the prevalence detected by microscopy (11.0% (22/200)) (P = 0.021). The ages 0-9 years had the highest prevalence rate (32.0%) and showed significant association with infection (P = 0.002). The sensitivity of the microscopy was 57.9% and the specificity was 100% compared to qPCR. The qPCR positive samples had significantly higher cycle threshold (Ct) values, representing low density infections that were not seen under conventional microscopy.
Giardiasis is still endemic in Wasit Province, especially children. Real-Time PCR was found to be more sensitive and reliable than microscopy, and is recommended to be an indispensable method for accurate epidemiological surveillance and clinical diagnosis.

Keywords: Molecular, Epidemiology of Giardia lamblia, RT-PCR, Iraq, Wasit, ssu rRNA.

1. Introduction

Giardiasis is a very prevalent protozoal infection of the intestine and is caused by a flagellate, Giardia lamblia (syn. Giardia intestinalis). Giardia duodenalis or Giardia intestinalis [1,2]. The disease poses a significant public health concern in developing nations, especially in areas where sanitation facilities and access to clean drinking water is limited, and personal hygiene habits are poor, and where there is overcrowding [3]. The World Health Organization estimates that giardiasis is one of the major causes of diarrheal diseases worldwide and that millions of cases are reported each year particularly in children and people with compromised immune systems [4].

It has a simple but very adaptable life cycle, consisting of two main stages: trophozoite and environmentally resistant cyst. Human infection takes place after the ingestion of full-grown cysts via contaminated water, food or

by direct person-to-person contact. Excystation takes place in the upper small intestine after being exposed to gastric acid and intestinal enzymes in the duodenum, and trophozoites are formed. These trophozoites adhere to the intestinal mucosa by means of an adhesive disc and can do damage to the intestinal epithelium, undergo villous atrophy, malabsorption and inflammatory changes [6]. The clinical manifestations of giardiasis include acute or chronic diarrhea, abdominal cramps, bloating, nausea, weight loss and nutritional deficiency [7].

Children are the most susceptible group to behavioral exposure, undeveloped immune system, and less awareness in hygiene and are the most affected group by giardiasis [8]. Chronic giardiasis in children has been linked to malnutrition, such as protein-energy malnutrition and deficiencies in micronutrients, growth retardation and cognitive impairment [9]. Multiple infections in early childhood have been shown in several epidemiological studies to have an important impact on physical and mental development [10].

In the developing countries, giardiasis is strongly related to environmental contamination and the lack of clean water. The parasite is very tolerant of the common chlorination methods used and will remain viable for extended periods in cold water systems [11]. This means that outbreaks are typically associated with municipal water contamination, sewage leakage, flooding and poor wastewater management [12].

In Iraq, intestinal parasitic infections are still endemic in many governorates because of the deterioration of infrastructure, environmental disruption related to war in Iraq and the lack of sanitation and water treatment systems [13]. The prevalence of giardiasis has been reported to be different between previous studies in Baghdad (11%), Basra (12%), Babylon (22%), Najaf (20%) and Diyala (13%), depending on the population studied and the diagnostic technique used [14,15]. Most of these studies, however, used only traditional microscopic assessment; and this has several diagnostic drawbacks.

In many Iraqi Hospitals, microscopy is still the routine diagnostic technique due to its simplicity and low price. However, sensitivity is poor and the technique still requires very high degree of operator experience, particularly when there is intermittent shedding of cysts or low numbers of parasites [16]. Therefore, it is not unusual to obtain a false negative result if only one stool specimen is tested [17]. Molecular diagnostic methods, however, including Real-Time Polymerase Chain Reaction (qPCR), offer extremely sensitive and specific detection through amplification of parasite specific- DNA sequences [18].

The high performance, short turnaround time and the high reproducibility of the Real-Time PCR methodology has made it one of the most advanced diagnostic tools to be developed for enteric protozoa [19]. The method can detect low density infections which cannot be seen in a microscope and it can help to monitor infections in endemic areas [20]. Additionally, qPCR assays for the small subunit ribosomal RNA (ssu rRNA) gene have proved to be very sensitive and efficient in the detection of Giardia lamblia [21].

Wasit Province is a unique epidemiological area in eastern Iraq due to its reliance on river water, high agricultural participation, livestock-human contact and old municipal water systems. The Tigris River continues to be the main source of water for domestic and agricultural purposes in the province and is a regular risk of transmission of the parasites by water in the province [22]. Also, water contamination or deterioration of the water supply network may play a major role in the transmission of intestinal protozoal infections.
Despite the environmental risk factors, there are only few molecular epidemiological data available about giardiasis in Wasit Province. Previous studies conducted at the local level were only based on the observation of the conventionally stained specimen by microscopy, without considering the molecular load of the infection [23].
Aim of the Study

The current study was aimed to evaluate the molecular prevalence of Giardia lamblia in symptomatic patients in Wasit Province, Iraq by Real-Time PCR (qPCR) and to compare it with conventional microscopic examination for diagnosis. Moreover, the study aimed to assess the epidemiological distribution of infection by age and gender and emphasize the significance of molecular diagnostic techniques for a better clinical diagnosis and epidemiological surveillance in endemic Iraqi context. This study aim to investigate the molecular prevalence of G. lamblia in symptomatic cases of Wasit province (Iraq) and also to compare the results of microscopic examination with Real-Time PCR (qPCR) method.

2. Materials and Methods

2.1 Study Design and Population

The study was a cross-sectional study conducted in Wasit Province, Iraq from January to October 2025. The study population was 200 number of patients of gastrointestinal complaints who visited Al-Karama Teaching Hospital in Al-Kut City with symptoms.

2.2 Ethical Approval

The study protocol was approved by the IRB of Wasit Health Directorate. All adult participants and/or guardians of pediatric participants gave written informed consent.

2.3 Sample Collection

Fresh samples of stool were collected in sterile leak-proof containers. Two portions of each sample were split into:
One part was examined with light microscopy.
The second part was frozen at −20°C for molecular studies.

2.4 Microscopic Examination

Formal-ether concentration technique was used to examine the specimens with conventional microscopy. Cysts and trophozoites of Giardia were looked for in prepared slides using light microscopy.

2.5 DNA Extraction

Stool samples were obtained from about 200mg and genomic DNA was extracted from the stool samples using the Presto™ Stool DNA Extraction Kit (Geneaid, Taiwan) as per the manufacturer's procedure with some modification.
To obtain effective disruption of Giardia cyst walls, mechanical lysis was done using zirconia/silica beads. The purity and concentration of DNA was determined with a NanoDrop™ spectrophotometer.

2.6 Real-Time PCR Assay

The TaqMan based Real-Time PCR assay targeting the small subunit ribosomal RNA (ssu rRNA) gene was used for the detection of Giardia lamblia.
Primer Sequences
• Forward Primer: 5’-GACGGCTCACACAACGGAAA-3’
• Reverse Primer: 5’-TGTTGTTCAGCCATAACGG-3’
• TaqMan Probe: 5’-FAM-CCCGGCGGTCCCTGCTAG-BHQ1-3’

PCR Reaction Mixture
The reaction mixture consisted of 20 µL per reaction of the following:
• 10 µL of 2× qPCR Master Mix
• 0.5 µM of each primer
• 0.2 µM of probe
• 4 µL template DNA
• Nuclease-free water (to volume)

Thermal Cycling Conditions
The following conditions were applied for the amplification:
• Denaturation at 95°C for 30 sec
• 40 cycles of:
• 95°C for 15 sec
• 60°C for 60 sec

Positive and negative controls were included in each run.

2.7 Statistical Analysis

IBM SPSS Statistics version 26 was used to analyze data. Chi-square test was used to determine associations between infection prevalence and demographic variables. A p-value <0.05 was considered statistically significant.

3. Results

3.1 Diagnostic Performance

RT-PCR detected G. lamblia in 38 of 200 samples (19.0%) while only 22 cases were detected by microscopy (11.0%). Both diagnostic techniques were statistically different (P = 0.021). As illustrated in Figure 1, qPCR clearly outperformed conventional microscopy, detecting a markedly higher number of positive cases and achieving superior sensitivity against the molecular reference standard.

Table 1: Diagnostic Performance of Microscopy Compared with qPCR

Parameter	Microscopy	qPCR
Positive cases	22	38
Negative cases	178	162
Sensitivity	57.9%	100%
specificity	100%	100%

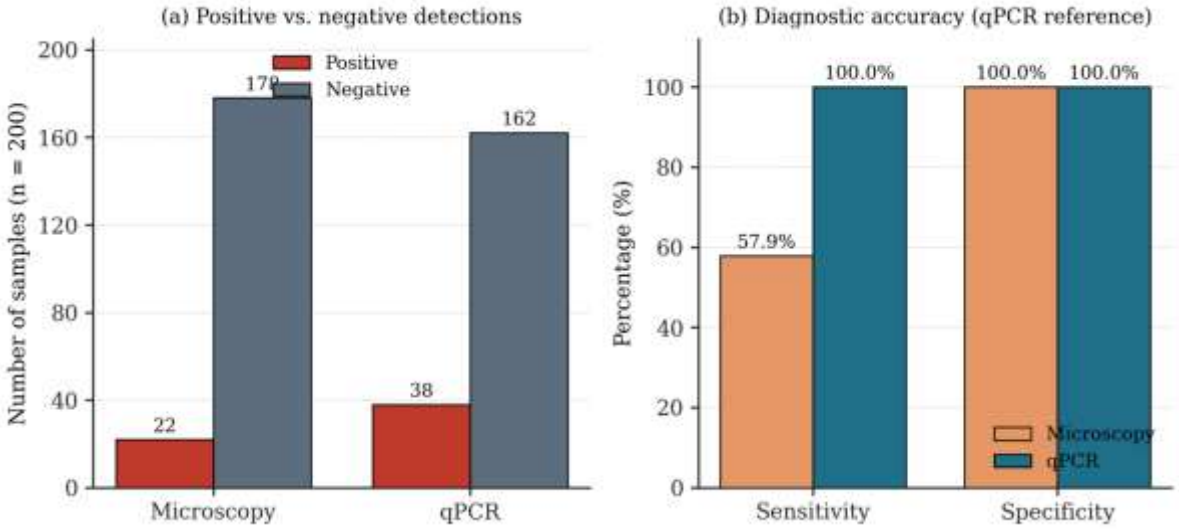


Figure 1. Comparative diagnostic performance of conventional microscopy and TaqMan-based Real-Time PCR (qPCR) for the detection of Giardia lamblia in 200 symptomatic patients. (a) Absolute numbers of positive and negative samples obtained by each method; (b) sensitivity and specificity of microscopy relative to qPCR as the reference standard.

3.2 Distribution According to Gender

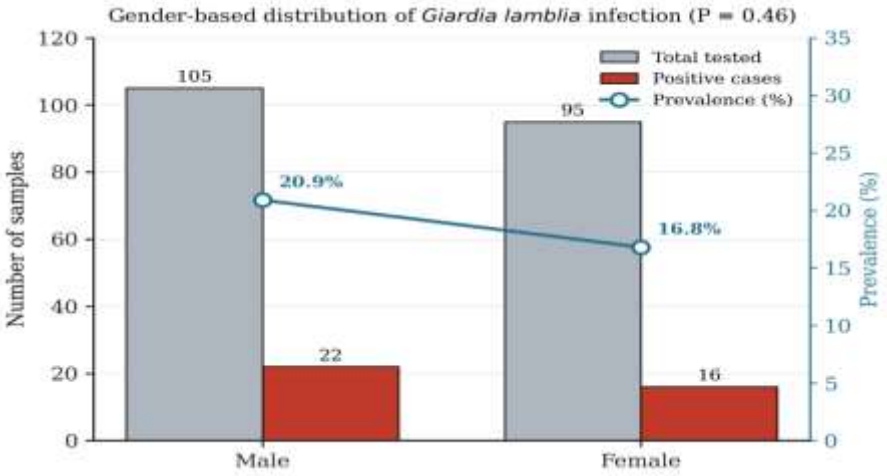
The male and female participants were 22 of 105 samples (20.9%) and 16 of 95 samples (16.8%) respectively, that were positive. There was no significant difference in the infection rate between males and females (P = 0.46). Figure 2 summarizes the gender-based distribution, showing a slightly higher prevalence in males (20.9%) than in females (16.8%), although this difference did not reach statistical significance.

Table 2: Distribution of Infection According to Gender

Gender	Total Tested	Positive Cases	Prevalence (%)
Male	105	22	20.9
Female	95	16	16.8

Total	200	38	19.0

Figure 2.



Distribution of Giardia lamblia infection by gender among the study population (n = 200). Grey and red bars denote the number of individuals tested and the number of qPCR-positive cases, respectively, while the blue line indicates the corresponding prevalence (%).

3.3 Distribution According to Age

Children under 10 years had the highest prevalence rate (32.0%), adolescents aged 11 to 25 (13.8%) and adults over 25 (8.3%). There was a significant relationship between age and the prevalence of infection (P = 0.002). As depicted in Figure 3, the prevalence of infection declined progressively with increasing age, peaking among children under 10 years (32.0%) and confirming a statistically significant age-related trend.

Table 3. Distribution of Infection According to Age Groups

Age Group	Total Tested	Positive Cases	Prevalence (%)
<10 years	75	24	32.0
11–25 years	65	9	13.8
>25 years	60	5	8.3

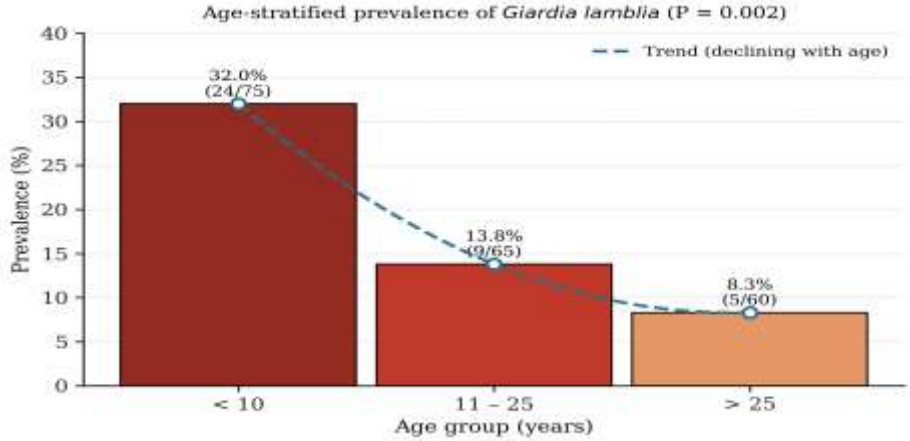


Figure 3. Age-stratified prevalence of Giardia lamblia infection among symptomatic patients. Bars represent the observed prevalence (%) within each age group, with positive/total counts shown above each column, and the dashed blue curve depicts the declining infection trend with increasing age.

3.4 Ct Value Analysis

The mean Ct value of all positive samples was 26.4 ± 4.2. Lower Ct values were observed for samples that were positive by microscopy and qPCR than for those that were only positive by qPCR, suggesting that microscopy-negative samples had lower parasite loads.

4. Discussion

The present study revealed that, Real-Time PCR significantly added to the detection of Giardia lamblia infection in the symptomatic patients of Wasit Province in comparison to conventional microscopic examination. Microscopy reported 11.0% of cases while the molecular prevalence was 19.0% in this study. It can be suggested that the true incidence of giardiasis in endemic populations in Iraq may be significantly higher than indicated with traditional microscopy.

In line with previous international studies which have shown that molecular assays are more sensitive than the traditional microscopic assays [18,19], the detection rate by qPCR is higher than the rate of detection obtained by the traditional microscopic assay. It is broadly accepted that microscopy is sensitive but has a low sensitivity because of variable parasite density, low numbers and intermittent shedding of cysts, and the level of experience of the observer [16,17]. In the current study, microscopy was not able to detect 16 positive samples and molecular diagnostics using qPCR was the only method to detect occult infections.

Other studies conducted in low resource environments have also shown that qPCR is superior to other methods used for enteric protozoa detection [20]. In endemic communities, Worku et al. found that diagnostic sensitivity was over 30% higher with molecular assays than microscopy [6]. Similarly, Fletcher et al. highlighted the higher degree of reproducibility and analytical precision that can be achieved using qPCR compared to morphological examination [3].
The prevalence of giardiasis in the present study is similar to the molecular prevalence in neighboring countries in the Middle East. Similar prevalence rates were shown in studies done in western Iran, ranging from 18% to 21% and with a significant number of the cases being located in provinces geographically and environmentally close to eastern Iraq [24]. However, slightly lower rates have been reported in Jordan and Turkey, which may reflect the improvements in centralized water treatment and sanitation systems in the country [25].

The strong relation between age and prevalence of infection in this study is an important epidemiological trend. The highest infection rate (32.0%) was found in children under 10 years old, a finding that has been confirmed by previous Iraqi and international reports [8,9]. Many factors can contribute to the susceptibility of children to giardiasis, such as their immature immune system, low levels of understanding about hygiene, interpersonal relationships and greater contact with contaminated environments.

Giardiasis affects children more than the acute gastrointestinal signs and symptoms. A chronic infection may be responsible for the impairment of nutrient absorption due to the villous atrophy and dysfunction of the epithelium of the small intestine [7]. Protein-energy malnutrition, growth retardation, anemia and cognitive deficits have been linked to persistent malabsorption in early childhood [9,10]. Pediatric giardiasis therefore is an infectious disease as well as a developmental and nutritional problem.

The environmental and infrastructural conditions in Wasit Province are likely to play a significant role in the transmission of giardiasis. The Tigris is an important source of water for drinking and agriculture to the province. Contamination can occur, however, with enteric pathogens due to aging municipal water systems and sewage leakage [22]. The cyst stage of the parasite is also highly resistant to chlorination and can persist in water for extended periods of time [11].

The agricultural livelihood activities of Wasit Province could also add to the risk of transmission via contamination of irrigation water and the increased interaction between livestock and humans. Based on the results of previous regional investigations, zoonotic transmission was proposed as a possible important mechanism of Giardia circulation within rural communities [14]. Genotyping was not done in the current study but zoonotic transmission pathways should be taken in to consideration in future epidemiological investigations.

In this study, analysing the cycle threshold (Ct) values gave important insights into parasite burden. Samples that were positive using only qPCR had significantly higher Ct values than samples that were positive using both methods, meaning that they contained lower concentrations of parasites. In previous molecular studies, high Ct values were correlated with subclinical or low-density infection [21], a finding similar to the one observed here. It is unclear why traditional microscopy is not able to identify many positive cases as these were the findings.

The results obtained in the present study support the use of molecular diagnostic methods in clinical routine in Iraqi health care institutions. Microscopy is still cheap and readily available, but is not always sensitive enough to lead to underdiagnosis and ongoing transmission. Some of the benefits of Real-Time PCR are significant in diagnostic accuracy, public health surveillance, and public health management [19,20].

In terms of public health, the undiagnosed giardiasis cases could remain as potential reservoirs for further community infections, particularly in overcrowded, unsanitary conditions. Therefore, early and accurate molecular diagnosis can play an important role in reducing transmission rates and better patient outcome.

There are a number of limitations in the present study. Seasonal evaluation of infection patterns was not possible with the cross-sectional design. Additionally, molecular genotyping of Giardia assemblages was not conducted, allowing neither differentiation of zoonotic versus anthroponotic transmission nor the determination of the zoonotic assemblage. For future studies, the use of multilocus genotyping, environmental water analysis and a larger multicenter sampling should be considered.

Despite these findings, the current study is one of the first molecular epidemiological studies conducted for giardiasis in Wasit Province and the necessity of using advanced molecular diagnostics in endemic Iraqi environments. The study also has its own limitations.The study is also limited in some aspects.

The current study has its limitations due to the cross-sectional design and the lack of a seasonal analysis. Additionally, molecular genotyping of Giardia assemblages was not carried out. Future studies should include multilocus genotyping and environmental sampling to gain insight into transmission.

5. Conclusion

The present study revealed that Giardia lamblia infection was found to be prevalent in Wasit Province, Iraq among the symptomatic patients. Real-Time PCR proved to be much more sensitive than conventional microscopy and was able to detect low intensity infections that would not have been detected otherwise.

Implementation of molecular diagnostic techniques in routine clinical care is highly recommended for the enhancement of diagnostic accuracy, epidemiological monitoring and public health control strategies.

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