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Original Article

Prevalence and Molecular Detection of *Giardia intestinalis* in Erbil Province, Iraq: A Cross-Sectional Study

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ABSTRACT

Background: Protozoal infections are a major cause of diarrheal disease throughout the world. *Giardia intestinalis* is one of the most commonly identified intestinal protozoan parasites worldwide**.**

Objectives: This study was carried out to investigate the prevalence rate of Giardia intestinalis among laboratory visitors in Erbil Province and to identify the best and most accurate method for identification.

Methods: In a cross-sectional study conducted from June 2024 to March 2025, stool samples were examined from individuals who were referred to Erbil Medical Laboratory Center and some private laboratories for parasitic testing. Real-time Polymerase Chain Reaction (Rt-PCR) was performed for 96 samples, 20 of which had negative microscopy results. The study aimed to investigate epidemiological factors influencing giardiasis and to evaluate the performance of RT-PCR to detect parasites missed by microscopy.

Results: Giardia intestinalis was found in (1.48%) 74/5000 of total samples and 74/470 (15.74%) of protozoan infections. The highest infection 32 (6.8%) was detected in the 26–35 year old age group. Gender analysis revealed that 62 (13.19%) were male higher than female. Occupational analysis showed elevated infection rates among food-industry workers 14 (2.98%) and supermarket workers 11 (2.34%) workers. Among infected individuals, the common water sources included mixed tap water, along with intake of raw vegetables. Clinically,45 (9.57%) of infected persons were asymptomatic; symptomatic patients reported mild or intermittent gastrointestinal symptoms. PCR detected 87/96 (90.63%) compared to microscopy 74/96 (77.08%) in the same subset. Real-time PCR demonstrated higher sensitivity (98.65%) compared to microscopy by amplification of the gdh gene, while the sensitivity of microscopy was relatively low (83.91%) with a P value as highly significant ($p < 0.00001$).

Conclusion: Giardia intestinalis prevalence in Erbil is relatively low compared to other Iraqi regions. Surveillance strategies are needed and should be contextualized with molecular diagnostic procedures, taking possible limits on molecular detection into account.

Keywords: Prevalence, Rt-PCR, Giardia intestinalis, Erbil.

1 Introduction

Giardia intestinalis (syn. *Giardia lamblia*, *Giardia duodenalis*) is an intestinal protozoan that inhabits the small intestine of humans and animals, where it multiplies and leads to giardiasis, a diarrheal illness of global importance. In some developed countries *Giardia* is present in 2–5% of the population and 20–30% in developing countries [1] .Transmission primarily occurs through accidental ingestion of Giardia cysts in fecally contaminated food or water [2].The parasite is more prevalent in developing regions, largely due to inadequate sanitation and poor water quality. Epidemiological data indicate that infection rates are particularly high among children under 12 years of age[3].

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However, most of the protozoan infection studies are done on children, a study targets middle -age group from 15 to > 45 years old aligning with Chalabi's findings that nearly half of the intestinal parasite infestations in Erbil occur in individuals in this age group [4].

Conventional diagnostic tests, such as microscopy, are limited in their sensitivity and specificity, which can sometimes underdiagnose or misclassify protozoan infections. Molecular methods, especially polymerase chain reaction (PCR) based on genes such as the tpi gene, the gdh gene, and β-giardin (bg), have increased the diagnostic abilities of *Giardia* [5] .Several studies have in particular used phylogenetic analyses based on these genetic markers to identify the evolutionary relationships and transmission dynamics of individual assemblages.

To date, a total of eight genotypes (A to H) have been detected upon molecular analysis of *Giardia intestinalis*, but genotypes A

and B seem to be prevalent in humans [6] . These relationships seem to warrant additional investigation in terms of genetic distance and cospeciation. Assemblage A is more frequent in human and animal hosts, giving it a higher zoonotic potential, whereas assemblage B, which is primarily derived from human sources, exhibits significant genetic diversity [7]

Even in Iraq, molecular studies are only just beginning to unravel *Giardia intestinalis* epidemiology. Hassan in Erbil also suggests that dogs are reservoirs for it and could transmit it to humans [8] . In Kirkuk [9] confirmed that PCR techniques are sensitive diagnostic methods for this parasite. In Baghdad [10] a study on humans revealed that the total rate of *Giardia intestinalis* infection was 68/375 (18.13%) using three diagnostic methods (Microscopy, ELISA and RT PCR).

Nevertheless, Erbil Province is void of detailed information regarding the molecular epidemiology of *Giardia intestinalis*. In light of the aforementioned issues, the present study aimed to determine the prevalence of *Giardia intestinalis* among lab visitors during 2024 in Erbil Province by microscope and Rt-PCR, thereby contributing to a better diagnostic method, understanding of giardiasis transmission dynamics and informing public health interventions.

2 Material and Methods

A cross-sectional study through a parasitological examination of stool samples (N = 5000) in both sexes aged between 15 and 82 years was carried out from June 2024 to March 2025 to investigate the prevalence rate of enteric protozoa among individuals referred to Erbil Medical Laboratory Centre and some private laboratories in Erbil province, Kurdistan Region, Iraq. Clinical stool samples were collected in disposable, sterilized plastic containers. All patients' records were gathered, and a pre-structured questionnaire was created. The information obtained included the patient's age, gender, occupation and some clinical information related to clinical signs, and daily habits like drinking water and consuming fresh vegetables.

2. 1 Stool examination

Each sample was examined microscopically in normal saline and Lugol's iodine by direct wet-mount preparation. Depending on shape and size, the Giardia intestinalis (trophozoite or cyst) were observed at 40x. Furthermore, positive Giardia stool samples and any suspected samples from patient suffered from clinical signs of digestive disorder or samples with abnormal stool appearance were fixed and preserved in absolute ethanol by adding ethanol to Eppendorf tubes containing around 5 gm of stool to be in a 1:1 ratio, homogenized by a vortex mixer. Samples were stored at 4–7°C until DNA extraction.

2. 2 DNA preparation and PCR

Around 96 samples; 74 samples with positive giardiasis in addition to 22 samples from individuals with clinical digestive disorder and abnormal stool appearance who were negative by microscopy (no parasites identified after reading all the slide fields), were selected to be tested by real-time PCR. The genomic DNA of *Giardia intestinalis* was extracted from stool samples using the Stool Genome DNA Extraction kit (ELK Biotechnology Inc., USA) as per the manufacturer's protocol. The eluted DNA was brought up to 100 µl with nuclease-free water and stored at –20°C. The RT-PCR was performed after preparing the reagent reaction and reaction mixture according to the instructions of the *Giardia intestinalis* A-F Glutamate Dehydrogenase (gdh) kit (Genesig-UK). Add 15 µl from each reaction mix to labeled wells, then add 5 µl from the DNA template of 96 samples, negative control (5 µl of RNase/DNase-free water), positive control, standard control and endogenous control to qPCR experimental plate wells to make the final volume 20 µl in each well. The qPCR amplification protocol consists of 50 cycles in a thermocycler (Bio Rad, USA), with enzyme activation at 95°C for 2 minutes, denaturation at 95°C for 10 seconds, and data collection at 60°C for 60 seconds.

Interpretation of real-time PCR results was considered Positive results obtained when amplified between Cq 16-23 ,while negative when the Ct value was more than 30 or no amplification curve was obtained and regarding compromised samples, results were interpreted according to the Ct value and standard control.

2. 2 Ethical consideration: Approved by The Medical Ethics Committee of Erbil Polytechnic University EPU, No.26/0089 HRE in 2025/11/01.

2. 3 Statistical: Comparisons were made using the χ^2 test, and the test was considered significant if the P value was less than 0.05.

3. Results

A total of 5000 stool samples were examined during the period of the study and *Giardia intestinalis* was detected in 74 samples, microscopically revealed that infection rate was 15.7% (74/470) from positive intestinal protozoan infections (66.1% (311/470) samples were positive for *Entamoeba histolytica* complex and 18% (85/470 samples were positive for *Blastocystis* sp.), which represent 1.48% of the total 5000 examined samples, Table (1).

Table 1: Overall prevalence of microscopically detected intestinal protozoa in the study population.

Protozoan	Positive cases (n)	% of total positive (n = 470)	% of total examined (n = 5 000)
***Entamoeba histolytica* complex**	311	66.17	6.22
Blastocystis sp.	85	18.09	1.7
Giardia intestinalis	74	15.74	1.48
Entamoeba histolytica* complex+*Blastocystis sp.	5	1.06	0.10

Entamoeba histolytica* complex+*Blastocystis sp.*+*Giardia	1	0.21	0.02
Total protozoan infections	470	100	9.40
Negative samples	4,530	—	90.60
Total examined	5,000	—	100

Note: Counts include both single and mixed infections.

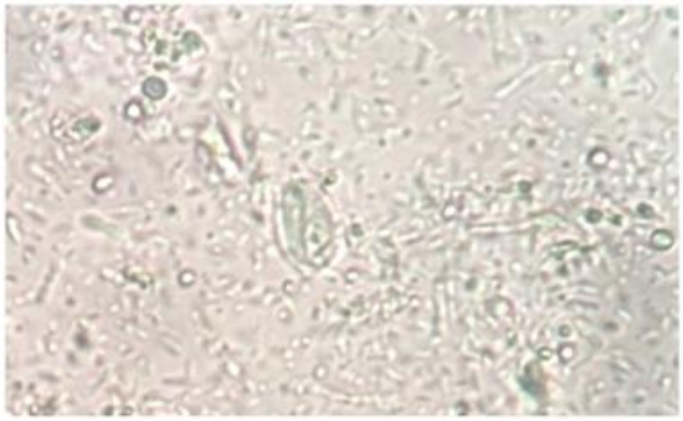

Figure 1: *Giardia intestinalis* cysts detected in human stool (magnification x100).

The higher percentage of infection was detected in male 62 (13.19 %), and the lower percentage was seen in female 12 (2.55%). Statistically, there was non-significant difference between male and female (*P*.value = 0.882), as shown in Table (2).

Table 2: Distribution of *Giardia intestinalis* concerning gender.

Gender	Total No. of protozoan infections	Total (%)	No. of *Giardia* cases	Giardiasis infection rate (%)	No. of other intestinal protozoan infection	Percentage of other intestinal protozoan infection (%)	P.value
Male	391	83.19	**62**	13.19	329	70.00%	0.882
Female	79	16.8	**12**	2.55	67	14.26%	
TOTAL	470	100	**74**	15.74	396	84.26%	

According to age groups, no significant differences (P.value = 0.64) were observed in the rates of infection with *G. intestinalis* among age groups, as the highest infection rate is 32 (6.81%) in the age group 26-35 years, followed by 16-25 years 28 (5.95%), and the lowest percentage of infection 3 (0.64%) in the age group > 45 years , as shown in Table (3).

Table 3: Distribution of Giardia infection according to age group.

Age Group	Total in Age Group	Total (%)	No. of Giardia cases	Infection rate (%)	No. of other intestinal protozoan infections	Percentage of other intestinal protozoan infection (%)	P value
16-25 years	149	31.70	28	5.96	121	25.74	0.6458
26-35 years	216	45.96	32	6.81	184	39.15	
36-45 years	81	17.23	11	2.34	70	14.89	
> 45 years	24	5.11	3	0.64	21	4.47	
Total	470	100	74	15.74	396	84.26	

Regarding the clinical manifestation 45 (9.57%) of cases were asymptomatic, followed by those who had nonspecific diarrhoea with P-values greater than 0.05, which refers to no clinical signs specific to giardiasis in this study (Table 4).

Table 4: Symptoms of lab visitors with positive cases of *Giardia*.

Symptoms	Total	Total percentage (%)	No. of Giardia cases	Giardiasis rate (%)	No. of other-protozoan infection	Percentage of other- protozoan infection (%)	P value
No symptoms	286	60.85	45	9.57	241	51.27	0.958
Sometimes diarrhea	140	29.79	21	4.47	119	25 .32	
Diarrhea + stomach pain	29	6.17	5	1.06	24	5.11	
Bloating	15	3.19	3	0.64	12	2.55	
TOTAL	470	100	74	15.74	396	84.26	

Comparison of stool appearance (Table 5) showed a greater infection rate in the passage of soft -consistency stool 28 (5.96%) and solid -consistency stool 26 (5.53%). P -values are highly significant (<0.0001).

Table 5: Relation between infection rate of *Giardia* and stool types.

Stool Type	Total samples	Total percentage (%)	No. of *Giardia* cases	Giardiasis rate (%)	No. of other protozoan infections	Percentage of other protozoan infections (%)	P value
Liquid	121	25.74	4	0.85	117	24.89	<0.0001
Semiliquid	173	36.81	16	3.40	157	33.40	
Soft	59	12.55	28	5.96	31	6.60	
Solid	117	24.89	26	5.53	91	19.36	
Total	470	100	74	15.74	396	84.26	

Among the 74 positive cases of *G. intestinalis* infection, the highest proportion was observed among unspecified jobs (working in hotels or barbershops, kindergartens or even unemployed), accounting for 26 cases (5.53%) of infections. The workers in food distribution companies represented 14 cases (2.98%) of the infections, followed by 11 cases (2.34%) who worked in supermarkets, which shows that more of the infected people are related to direct contact with food, even though they are not significant (Table 6).

Table 6: Risk factor of occupational categories deals with giardiasis.

Job Category	Total samples	Total (%)	No. of *Giardia* cases	infection rate of giardiasis (%)	No. of other - protozoan infection	Percentage of other protozoa (%)	P. value
Food companies	86	18.29	14	2.98	72	15.32	0.9985
Supermarket	66	14.04	11	2.34	55	11.70	
Restaurant	43	9.15	7	1.49	36	7.66	
Food Factory	33	7.02	6	1.28	27	5.74	
Bakery	27	5.74	5	1.06	22	4.68	
Sweet shop	21	4.47	3	0.64	18	3.83	
Coffee shop	15	3.19	2	0.43	13	2.77	
Other/Unemployed	179	38.09	26	5.53	153	32.55	
TOTAL	470	100	74	15.74	396	84.26	

The higher infection in people who don't care and drink any type of water, even tap water, is 54 (11.49%). P.value = 0.7502. Frequencies are similar to those that theoretically would be expected Table (7).

Table 7: Infection with *Giardia* is related to type of drinking water.

Drinking Water Source	Total	%	No. of *Giardia* cases	Giardiasis infection rate (%)	No. of other protozoan infection	(%)	P-value
Mixed (tap water and Filter/bottle water)	359	76.38	54	11.48	305	64.89	0.7502
Filter/bottle	88	18.70	16	3.40	72	15.31	
Bottle only	23	4.89	4	0.85	19	4.04	
TOTAL	470	100	74	15.74	396	84.46	

Among those 74 infected with giardiasis, infection rates were 52 (11.06%) of people who used to eat raw, uncooked vegetables, whereas P values were not significant (Table 8).

Table 8: Rate of infection related to consuming raw, uncooked vegetables.

Vegetable Intake	Total	%	No. of *Giardia* cases	infection rate%	No. of other- infection	%	P value
Yes	311	55.11	**52**	11.06	259	66.17	0.7179
Sometimes	144	30.64	**20**	4.26	124	26.38	
No	15	3.19	**2**	0.43	13	2.77	
TOTAL	470	100	**74**	15.74	396	84.26	

The use of RT-PCR techniques in the specific detection of *G. intestinalis* with a fluorescence of SYBER Green dye appeared very clear, as shown in figure 2 and 3, in the form of an amplification plot to the positive samples during 50 cycles.

Real-time PCR provides a highly sensitive molecular approach for detecting parasitic DNA; however, its results should be interpreted carefully. In some cases, both false-negative and false-positive outcomes may occur due to various technical and biological factors that can affect the consistency of the assay.A total of 96 stool samples (microscopically and clinically) were examined by the GeneSig *Giardia intestinalis* gdh real time PCR kit. The Cq values ranged between 13.69 and 30.13 for strong and moderate positives, while only four samples showed high Cq values (>31) which were identified as borderline/compromised (as negative in accordance with the kit interpretation criteria) as shown in Figure 2. Present results indicate that the qPCR method used in this study shows strong reproducibility. The qPCR developed in this study was microscopically tested on the *Giardia intestinalis* suspected clinical samples.

These results show the advantages of the qPCR detection method established in this study, which are more sensitive than traditional microscopy. Table 9 revealed that, real-time PCR was positive for 87 out of the 96 samples (90.63%), while microscopically it was positive for 74 (77.08%) showing a sensitivity (83.9%) of microscopic techniques. Among 74 positive samples by microscope which tested by PCR, 73 positive samples by both techniques, and only one microscopy-positive sample did not amplify (invalid) giving PCR a sensitivity of 98.65% compared to microscope. Out of the 22 negative samples by microscope, which were clinically suspected of intestinal protozoan infection, 14 tested positive for giardiasis by PCR. Given the moderate sensitivity of microscopy, some of these 'false negatives' are likely true infections that were not detected.

Table 9: Diagnostic comparison between microscopy and Real-time PCR for detection of *Giardia lamblia*.

Real time PCR results	Stool samples (N=96)						
	Positive Microscopically	(%)	Negative Microscopically (Abnormal Clinically)	(%)	Total	Total (%)	P.value
Positive	73	98.65	14	63.64	87	90.63	**(p< 0.00001)**
Negative	1	1.35	8	36.36	9	9.38	
Total	74	100	22	100	96	100	
Sensitivity of PCR			**98.65%**				
Sensitivity of microscope			83.91%				

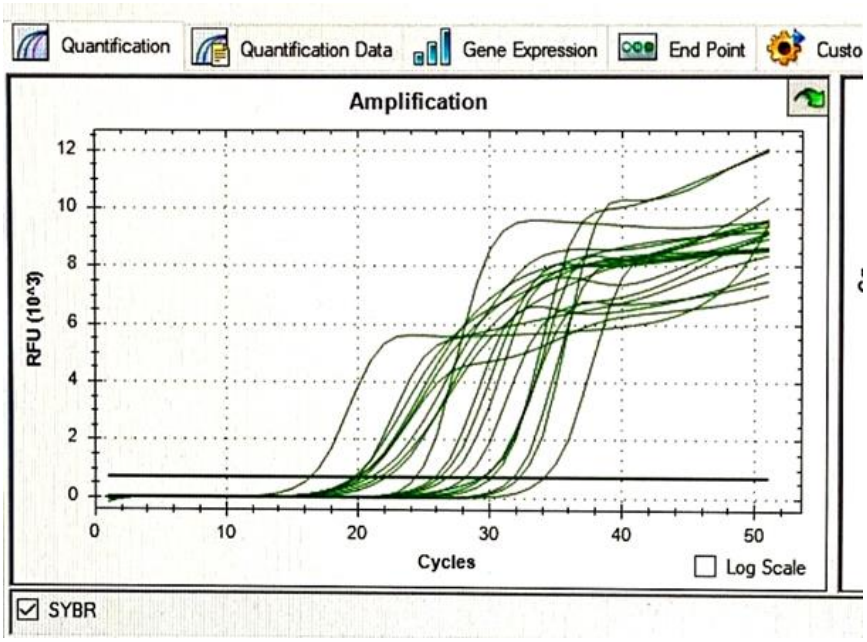

Figure 2: Amplification Plot of the region of the gdh gene within 50 cycles which represented by using of the SYBER Green fluorescence dye.

4 Discussion

Diarrheal diseases remain a major contributor to global morbidity and mortality burden of childhood worldwide, as per the data available from the World Health Organization (WHO) 2024 [11;12]. In present results from the cross-sectional study conducted in Erbil city, *Giardia lamblia* was identified in 74/5000 cases, and the rate of infestation was 1.48% (15.74 % from all protozoan infections).

This results is slightly less than the meta-analysis conducted by Chalabi in Erbil from 2011 to 2021, which reported 4.24% total intestinal parasites and a *Giardia* prevalence of 29.54% among them [4] and a meta-analysis conducted by Naqid in Zakho from 2018 to 2022 with a total protozoan infection rate of 18.65% out of these *Giardia intestinalis* was 25.31% with a significant decrease after 2020 [13]. Comparing the current study with the data records mentioned above, the observed decline in infection rates may be associated with improved living conditions and increased hygiene awareness especially after the COVID-19 pandemic .

This is supported by the findings of Chalabi who showed a decrease in the total infection rate in 2020 [4] and the significant decrease of giardiasis in Mexico after the impact of COVID-19 [14] . Higher infection rates have been associated with socioeconomic conditions such as poor sanitation [15, 16] .

The prevalence of *Giardia intestinalis* in the current study was lower than infection rate of 10.31% that was reported in Kirkuk by Salman et.al. and less data revealed in Erbil(Soran) and Sulimani (Chamchmal) by Khudhair 2020 , where the prevalence rate was 4.2% and 8.5% respectively , indicating that the occurrence of giardiasis has been decreasing compared to previous years [17 , 18] .The observed differences may be linked to the diversity of citizens across various factors, including environmental conditions, nutrition, health-related factors, the specificity of the diagnostic techniques and the sizes of samples in the survey [19].

Internationally, prevalence also varies. In Turkey, a meta-analysis reported a mean prevalence of 17.26% *for years 2012-2018* [20], while in Iran, rates ranged from 1.4% to 39.5%, with an overall prevalence of 4.6% [21] and 1.6% in other studies [22].The observed differences may be linked to the diversity of citizens across various factors, including environmental conditions, nutrition, health-related factors, the specificity of the diagnostic techniques used, and the sizes of study samples in the survey [19] .The highest percentage of *Giardia* infestation was in the 26-35-year-old group, followed by the 15-25 age group 32(6.8%) and 28 (5.95%) respectively. Suggesting age-specific exposure linked to adult occupational or social behaviors. A strong correlate to Chalabi's findings is that nearly half of the intestinal parasite infestations in Erbil occur with those individuals aged between 15 and 44 years, a demographic that is largely active outdoors, eating outside or occupationally associated with food [4]. Especially in our study most of males` work was primarily associated with food and the food industry. Although males comprised 62 (13.19%) of *Giardia* cases, there was no significant gender disparity same as found by previous studies [4, 17, 23].This contrasts with Naqid`s study, where he found a higher rate of *Giardia* seen in females in Zakho (55%) with a significant gender difference [13].

Differences in infection patterns may reflect environmental exposure as well as biological factors, including hormonal influences on immunity [24].The disparities in infection rates between the two genders can also be attributed to differences in endocrine-immune interactions. Moreover, sex steroids, particularly androgen in males and estrogen in females, influence

various aspects of host immunity, with androgen lowering immune competence. The increased intensity of infection in males compared to females may be due to their low immunity [25]. The influence of steroid hormones on disease resistance genes and the behaviors that increase male susceptibility to infection is noteworthy [24] .One could also attribute the low prevalence in females to a greater emphasis on hygiene care [26]. The observed inconsistencies probably indicate variations among different age groups and the contexts in which they have been exposed. Males may have an increased infection rate due to their greater engagement in activities and more frequent exposure to environmental conditions compared to females, especially since most participants were food handlers, and males are more engaged in this job than females in Iraq.

The highest infection proportions appeared in the food industry (food distribution companies) 14 (2.98%) and supermarket workers 11(2.34%). Concerning the risk of environmental contamination with *Giardia intestinalis*, Saber and Bakr found that (3.6 %) rate of giardiasis among food handler less than present results even though there was no significant differences among occupations [23]. Also, it was higher among the food industry and supermarket workers who handle raw food, and their attitude in restaurants related to poor hygiene practices and cross contamination [27].

Although plausible, food-handling occupations in Iraq have not been conclusively linked to giardiasis risk in published adult studies, likely due to a focus on pediatric or general public samples rather than occupational categories.

A majority of infected individuals ,45 (9.57%) were asymptomatic and symptomatic ones mainly reported intermittent diarrhea or abdominal discomfort. Stool types were most commonly soft 28 (5.96%) or solid 26 (5.53%) with minimal liquid stool 4 (0.85%). The relation between *Giardia* infection and stool consistency was significant, whereas giardiasis relation with clinical signs were not significant statistically (P.value > 0.0001 and P.value = 0.958, respectively). Saber and Bakr findings supported our results in that (98.6%) of participants didn't show any symptoms [23]. This was consistent with clinical knowledge that *Giardia* infections are frequently mild or sub clinical with many carriers asymptomatic [28] . Most infected participants used mixed water (11.49%), mostly tap water (in Erbil, many cities depend on well water), and sometimes bottled water (treated with RO and ozone), with fewer using sterilized water. The water source was not significantly associated with infection (P.value = 0.7502). The standard chlorine levels employed to eliminate bacteria in municipal water supplies are ineffective in inactivating *Giardia* cysts [29] and *Giardia* cysts have been isolated from water supplies in different parts of the world [30] . Additional behavioral factors including increased water and beverage consumption in hot weather could potentially be a source of infection [30]. Water treatment infrastructure is not differentiated across Iraq leading to significant differences in water quality from one region to another[31] .Fecal contamination from poor sewage and water quality primarily contributes to the burden of these protozoan parasites in developing countries. In Iraq, the fresh and tap water could be polluted with pathogenic protozoan parasites such as *G. lamblia* in some provinces [32] . Another source of infection was raw vegetable intake, which was high among infected individuals (intake of green vegetables was always 52 (11.06%), sometimes eating green vegetables 20 (4.26%), but statistical testing showed no significant association). Nevertheless, market surveys from Iraq support vegetable contamination as a plausible exposure route. In Soran district (Erbil- Kurdistan Region), 48.4% of raw vegetable samples were contaminated with various intestinal parasites, although *Giardia* was not predominant [33] . In Duhok, among the 166 vegetable samples, 4.2% harboured *Giardia lamblia* cysts [34] . In Thi Qar, vegetables such as leek and basil had high parasite contamination—especially *Giardia* at 71.1% of protozoan concentration within contaminated samples [35].These findings underscore the potential but complex role of raw produce in transmission even if not statistically significant here.

Whereas low income and poverty are high-risk factors for increased infection with intestinal parasites, several studies have shown higher prevalence as a result of lower socioeconomic conditions [15, 16] . It appears that changes in the prevalence of *G. intestinalis* infection could be due to different factors including seasons of sampling, kind of consumed water, different laboratory tests, climate and environmental factors [21].*Giardia intestinalis* was detected in 1.48% of examined individuals, representing 15.7% of protozoan infections, this prevalence is considerably lower than historical Iraqi data, where rates ranged from 4% to over 40% depending on region and population. Results obtained by the qPCR method performed in this study showed strong reliability and sensitivity. It achieved a positive detection rate of 87 /96 (90.63 %) for *Giardia intestinalis* in suspected clinical samples, compared to only 74/470 (15.7%) with microscopy. It also showed high specificity, which indicates its superior benefits over standard PCR in addition to consistency with previous research findings. While the positivity in Hussein et.al `s study 25/42 (59.52%) [36] may be due to using different techniques and different genes because he uses multiplex nested RT PCR with tpi gene. Beyhan and Taş in Turkyie revealed positive as 38/94(40.4 %) and 75/94 (79.8%) using a microscope and real time PCR respectively[37] .Sow et al in Senegal record that 72 /98 samples (73.5%), whereas microscopic examination was positive in 37/98 (37.7%) samples, aligning with our results in superior sensitivity of real time PCR compared with microscopy [38] .

These results agree with Al-kayat (2013) who revealed (90.8%) of samples were positive for *G. intestinalis* by RT-PCR assay[39] ,and agreed with a study conducted in Canada by Xiao et al[40] that detected all specimens that were positive for *Giardia* in a microscope and gave positive results by RT-PCR 15/15(100%)without recorded any false negatives .Studies advise using RT-PCR rather than conventional PCR to eliminate the risk of contamination in the processing of samples that may give false negative ,which support the higher sensitivity of RT-PCR[41].

Due to substantially high sensitivity and specificity in both qualitative and quantitative analysis, real-time PCR an advanced techniques used widely to detect nucleic acids of interest in any type of parasitological sample such as protozoa. Based on our findings and previous studies, real-time PCR can be considered a reliable confirmatory method. The challenge remains with the fact that false-negative and false-positive results are possible, which needs accuracy with respect to the interpretations of outcomes due to other biological properties affecting the assay's consistency [42]. Proper sampling protocols, excellent laboratory practices, high-quality extraction, and real-time RT-PCR kits are essential to reduce the risk of inaccurate results [43] .

The sensitivity of real-time PCR assays for identifying parasites in stool samples was higher than that of microscopy, with the former method being able to detect more *Giardia intestinali*s, as indicated by the rates given in (Tables 8). This method is beneficial for diagnostic laboratories and practical field applications, especially in epidemiology studies or assessing the efficacy of mass drug administration programs[38]. Earlier studies also suggest the superiority of real-time PCR over microscopy for detection of intestinal parasites [44-46] .

The benefit of real-time PCR assays in detecting low DNA copy numbers [47] ,emphasizes their usefulness in controlling neglected tropical diseases by identifying reservoirs and assessing transmission and treatment efficiency. However, it underscores the continued relevance of traditional parasitological diagnosis due to its low cost and ability to detect pathogens not targeted by the PCR assay, especially in low-resource endemic settings, and its ability to detect infections that are not specifically targeted by the real-time PCR assay.

Interestingly, our study revealed that the majority of *Giardia*-positive cases exhibited no clear symptoms with physically abnormal stool and had low cyst counts in their stool samples. While microscopy detected *Giardia* in some liquid and in solid and soft stool samples, real-time PCR identified more samples as *Giardia*-positive (90.6%). This is due to liquid stools are diluted and challenging to examine microscopically, while real-time PCR is a highly sensitive method, demonstrating its higher sensitivity. Studies showed that RT-PCR techniques are able to detect *G. intestinalis* at concentrations even 1 trophozoite (the lowest detectable concentration = 0.1 pg). Real-time PCR is capable of identifying even minimal DNA quantities, leading to positive results after additional amplification cycles when DNA concentration is low [48] .

Real-time PCR is capable of detecting low amounts of DNA, but in the case of low amounts of extracted DNA, additional cycles need to be developed. The reasons for false-negative or weak-positive results may be due to DNA degradation after extraction, inhibitors in stool, missing samples or technical problems and/or insufficient sampling. Schuurman et al noted a variance between microscopy and RT-PCR results, highlighting the need for a third reference standard for comparison[49] .Although microscopy provides a rapid diagnosis, real-time PCR is more sensitive and specific, particularly for low-intensity or mixed infections. Both experience and parasite load are required for microscopic diagnosis [44].

Unlike microscopy, which has its limitations in epidemiological studies, molecular techniques such as real-time PCR are a reliable method for the diagnosis of helminths and intestinal protozoa [46].The sensitivity of real-time PCR is capable of detecting low levels of infection, facilitating case detection, and assessing the impact of treatment [45].Compared to standard PCR, it minimizes contamination hazards and reagent costs [44, 50] .It is essential to monitor the occurrence of *Giardia intestinalis* with wide usage of molecular methods for the detection of *Giardia intestinalis* . Third-party comparisons are needed to assess sensitivity and specificity of laboratory techniques. Also, *Giardia intestinalis* assemblages A and B in relation to symptomatology and socio-demographic factors; additional research is warranted.

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7 Conflict of interests

The authors declare no conflicts of interest.

8 Conclusion

In conclusion, *Giardia* prevalence in Erbil was relatively low but showed higher occurrence among young adults (26–35 years). There were no significant associations with age, gender, occupation, water source, symptoms or vegetable intake, maybe due to sample and methodological limitations. Microscopy is convenient for routine diagnosis especially in detecting poly parasitism and real-time PCR is more sensitive and specific. The sensitivity of real-time PCR is capable of detecting low levels of infection, facilitating case detection, and assessing the impact of treatment.

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