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A Microscopic and Molecular Investigation into the Prevalence of *Giardia duodenalis* in Stray Dogs in the Bitlis Province

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ABSTRACT: *Giardia duodenalis* is a globally distributed flagellated protozoan parasite infecting the gastrointestinal tract of humans, domestic animals, and wildlife. In dogs, infections are mainly associated with host-adapted assemblages C and D; however, the detection of zoonotic assemblages A and B in canine populations raises concerns regarding their possible role in the epidemiology of human giardiasis. Stray dogs may be particularly important in transmission dynamics because of their continuous exposure to contaminated environments and their potential to disseminate cysts in public areas. This study investigated the prevalence and molecular characteristics of *G. duodenalis* in 100 stray dogs from Bitlis Province, Türkiye, using microscopic examination and nested PCR targeting the β-giardin gene. *Giardia* cysts were detected by the native method in 3.0% (3/100) of samples, whereas nested PCR detected *G. duodenalis* DNA in 5.0% (5/100) of samples, indicating a higher detection rate with molecular analysis. No statistically significant association was found between infection status and sex ($p = 1.000$) or age group ($p = 0.386$), although positivity was numerically higher in dogs aged 0–1 month. Sequence analysis of the five PCR-positive samples revealed assemblage B in two isolates, assemblage C in two isolates, and assemblage D in one isolate, with 99.54–100% similarity to GenBank reference sequences. The detection of zoonotic assemblage B, together with dog-adapted assemblages C and D, suggests that stray dogs in Bitlis may have both veterinary and public health significance from a One Health perspective.

Keywords: Age, Assemblage, β-giardin, Nested PCR, Prevalence.

INTRODUCTION

Giardia duodenalis (syn. *G. intestinalis*, *G. lamblia*) is one of the most prevalent enteric protozoan parasites worldwide, infecting humans and a broad range of domestic and wild animals (Adam, 2021; Dixon, 2021). The parasite causes giardiasis, with clinical manifestations ranging from asymptomatic carriage to severe diarrhea, malabsorption, and weight loss (Einarsson et al., 2016; Adam, 2021). Transmission occurs primarily via the fecal–oral route through ingestion of environmentally resistant cysts from contaminated water, food, or direct contact with infected hosts (Dixon, 2021; Hublin et al., 2022).

Molecular studies have established that *G. duodenalis* comprises eight major genetic assemblages (A–H), which are morphologically indistinguishable but differ in host specificity and genetic structure (Ryan et al., 2021; Baroudi et al., 2026). Assemblages A and B possess broad zoonotic potential, whereas assemblages C through H display narrower host ranges (Ryan et al., 2021; Baroudi et al., 2026). Dogs are mainly infected with the host-adapted assemblages C and D, whereas zoonotic assemblages A and B have also been reported in canine populations, suggesting a possible but context-dependent role of dogs in the epidemiology of human giardiasis (Leonhard et al., 2007; Ryan et al., 2021; Sun et al., 2023). Stray dogs are at particular risk due to uncontrolled exposure to contaminated environments and may contribute to environmental contamination through cyst shedding in public areas (Symeonidou et al., 2017; Fazaeli et al., 2021). A recent meta-analysis estimated the global pooled prevalence of *Giardia* in domestic dogs at approximately 13%, with shelter and stray dogs exhibiting higher rates (Hatam-Nahavandi et al., 2025).

Microscopic examination has limited sensitivity due to intermittent cyst shedding and low parasite burdens (Symeonidou et al., 2017; Uiterwijk et al., 2018; Gabrielli et al., 2024), whereas PCR-based methods provide substantially greater sensitivity and specificity (Tan et al., 2016; Gabrielli et al., 2024). The β-giardin gene is among the most widely used molecular markers for *G. duodenalis* detection and assemblage determination (Cacciò et al., 2002; Lalle et al., 2005).

In Türkiye, published molecular data on canine giardiasis remain limited, and, to the best of our knowledge, no molecular data have been reported from Bitlis Province. Therefore, this study aimed to investigate the prevalence of *G. duodenalis* in stray dogs in Bitlis Province using microscopic examination and nested PCR, and to characterize the detected assemblages through β-giardin gene sequence analysis.

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MATERIALS AND METHODS

Sample Collection

In this study, fecal samples were collected from 100 stray dogs in the Bitlis region. Dog fecal samples were collected immediately after defecation, stored in sterile containers, and transported to the laboratory under cold chain conditions.

Microscopic Examination

All fecal samples were examined for the presence of *Giardia duodenalis* cysts under a light microscope using the native method.

Molecular Examination

DNA Extraction: Genomic DNA was extracted from all 100 fecal samples using the GeneMATRIX STOOL DNA Purification Kit according to the manufacturer's protocol. The extracted DNA isolates were stored at −20°C until subsequent molecular analyses.

Nested PCR: Amplification of the *G. duodenalis*-specific β-giardin gene region was performed using a nested PCR approach (Cacciò et al., 2002; Lalle et al., 2005). In the primary PCR, amplification was performed using the G7 (forward: 5'-AAGCCCGACGACCTCACCCGCAGTGC-3') and G759R (reverse: 5'-GAGGCCGCCCTGGATCTTCGAGACGAC-3') primers described by Cacciò et al. (2002). The primary PCR was carried out in a 20 µL total reaction volume containing 12.8 µL of DNase/RNase-free distilled water, 8 pmol of each forward and reverse primer, 4 µL of 5x Master Mix, and 1.6 µL of extracted genomic DNA. The thermal cycling conditions consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 60 s, annealing at 60°C for 60 s, and elongation at 72°C for 3 min, with a final extension at 72°C for 10 min.

In the nested PCR stage, the target gene region was amplified using the BG1F (forward: 5'-GAACGAGATCGAGGTCCG-3') and BG2R (reverse: 5'-CTCGACGAGTTCGTGTT-3') primers designed by Lalle et al. (2005). The secondary PCR was performed in a 20 µL total reaction volume containing 13.6 µL of DNase/RNase-free distilled water, 8 pmol of each forward and reverse primer, 4 µL of 5x Master Mix, and 0.8 µL of the primary PCR product. The thermal cycling conditions included an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 60 s, annealing at 55°C for 60 s, and elongation at 72°C for 3 min, with a final extension at 72°C for 10 min.

Agarose Gel Electrophoresis: Following PCR amplification, the products were stained with RedSafe™ Nucleic Acid Staining Solution and electrophoresed on a 1.5% agarose gel at 90 V for approximately 60 min. The resulting bands were visualized under UV light using a gel imaging system.

Sequence analysis

Five purified PCR products were sent to BM Labosis (Ankara) for Sanger sequencing analysis. The sequences were manually corrected and consensus sequences were generated using BioEdit software (http://www.mbio.ncsu.edu/bioedit/bioedit.html). The consensus sequences were compared with reference sequences in the NCBI GenBank database using the Basic Local Alignment Search Tool (Altschul et al., 1997).

Statistical analysis

Statistical analyses were performed using SPSS version 26.0. Sex and age group were considered categorical variables and expressed as numbers and percentages. Dogs were categorized into two age groups: 0–1 month and >1 month. Fisher's exact test was used to evaluate the associations between *G. duodenalis* positivity and sex or age group. A p value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Microscopic examination revealed *Giardia* cysts in three samples.

Nested PCR analysis yielded specific 511 bp bands for *G. duodenalis* in five samples (Figure 1)

G. duodenalis was detected in 6.0% (3/50) of female dogs and 4.0% (2/50) of male dogs. No statistically significant association was found between sex and *G. duodenalis* positivity (Fisher's exact test, $p = 1.000$) (Table 1).

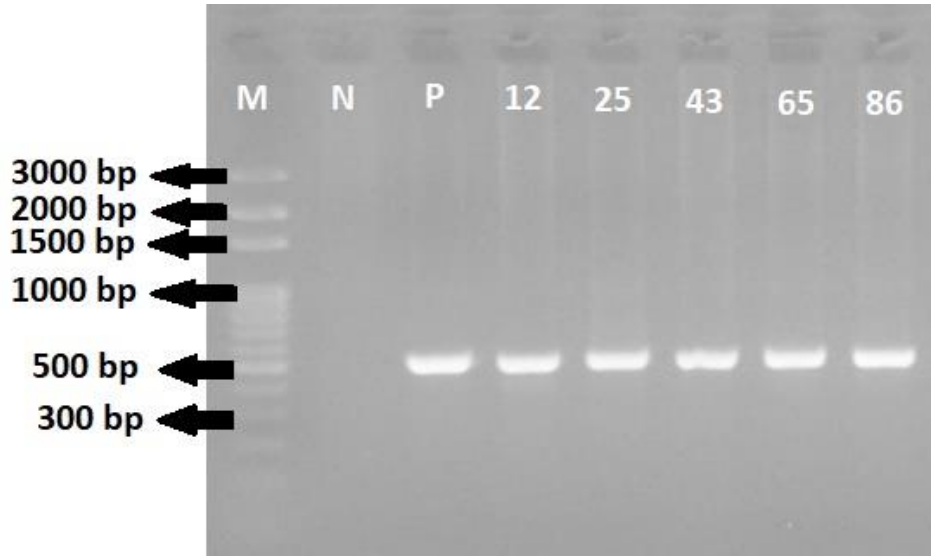

Figure 1. *Agarose gel image of samples that tested positive in the nested PCR (M: Marker, N: Negative control, P: Positive control, 12, 25, 43, 65, 86: positive samples).*

Table 1. *Distribution of Giardia duodenalis-positive samples according to sex*

Sex	Positive	Negative	Positivity (%)	P value
Male (n=50)	2	48	4.0	1.000
Female (n=50)	3	47	6.0	

G. duodenalis positivity was detected in 7.5% (3/40) of dogs aged 0–1 month and 3.3% (2/60) of dogs aged >1 month. No statistically significant association was found between age group and *G. duodenalis* positivity (Fisher's exact test, p = 0.386) (Table 2).

Table 2. *Age distribution of stray dogs testing positive for Giardia duodenalis.*

Age group	Positive	Negative	Positivity (%)	P value
0-1 month (n=40)	3	37	7.5	0.386
>1 month (n=60)	2	58	3.3	

Based on the sequence analysis, among the five *G. duodenalis* positive samples, two were assigned to assemblage B, two to assemblage C, and one to assemblage D, as shown in Table 3.

Table 3. *Comparison of results of the study samples generated using the NCBI Basic Local Alignment Search Tool*

Samples	Access codes of the most similar sample	Assemblage	Similarity ratio
12	GQ345010.1, OR921175.1, LC184018.1	**B**	**99.77%**
25	OR791779.1, PP918967.1, PX673974.1	**C**	**99.77%**
43	HM061151.1, PV368347.1, PQ154964.1	**D**	**100.00%**
65	LC184018.1, LC183972.1, PX673977.1	**B**	**99.56%**
86	LC437430.1, OR791778.1, PX557015.1	**C**	**99.54%**

To the best of our knowledge, this study provides the first molecular epidemiological data on *G. duodenalis* in stray dogs in Bitlis Province, Türkiye. The overall molecular prevalence of 5% falls within the lower range of globally reported rates. This rate is also comparable to recent molecular data from southeastern Türkiye, where *G. duodenalis* prevalence was reported as 4.0% in shelter dogs from Diyarbakır and 2.0% in shelter dogs from Batman (Aslan Çelik et al., 2023; Aslan Çelik et al., 2024).

A recent meta-analysis estimated the global pooled prevalence of *Giardia* infection in nonhuman mammalian hosts at 13.6% (Hatam-Nahavandi et al., 2025). The prevalence observed here is comparable to rates reported in Iran (1.6%; Fazaeli et al., 2021), Fujian Province, China (0.4%; Li et al., 2025), and Poland (6.0%; Piekara-Stępińska et al., 2021), but substantially lower than those documented in shelter dogs in Italy (41%; Agresti et al., 2022) and eastern Spain (36.5%; Adell-Aledón et al., 2018). These differences likely reflect variations in environmental conditions, population density, management practices, and diagnostic methodology.

The higher PCR detection rate than microscopy (5% vs. 3%) is consistent with previous findings. Hassan (2021) reported 10.46% by flotation versus 23.53% by PCR in Iraqi stray dogs, while Inpankaew et al. (2014) documented 2.1% by microscopy versus 10.6% by PCR in Cambodian dogs. The two additional samples detected exclusively by PCR likely reflect low cyst burdens or intermittent shedding below the microscopic detection threshold.

No statistically significant associations were found between *Giardia duodenalis* positivity and sex or age group, consistent with findings from China (Zhang et al., 2017; Liao et al., 2020) and Brazil (Osmari et al., 2021). The numerically higher prevalence in dogs aged 0–1 month compared with those aged >1 month (7.5% vs. 3.3%), although not statistically significant, is biologically plausible given the immature immune systems of very young animals (Agresti et al., 2022; Murnik et al., 2023; Hatam-Nahavandi et al., 2025). Similar tendencies for higher positivity in younger dogs have also been reported in southeastern Türkiye, although age categories differed among studies (Aslan Çelik et al., 2023; Aslan Çelik et al., 2024).

The *Giardia duodenalis* assemblage distribution, including assemblage B (n = 2), assemblage C (n = 2), and assemblage D (n = 1), is noteworthy and partly overlaps with recent molecular data from southeastern Türkiye. Aslan Çelik et al. (2023) detected assemblages B, D, and E in shelter dogs from Diyarbakır, whereas Aslan Çelik et al. (2024) reported assemblages C and D in shelter dogs from Batman. The predominance of canine-specific assemblages C and D is consistent with the global pattern observed in dogs (Zhang et al., 2017; Piekara-Stępińska et al., 2021; Figueiredo et al., 2023; Hsu et al., 2023; Sun et al., 2023; Szydłowicz et al., 2024; Jańczak et al., 2025; Li et al., 2025;). The detection of zoonotic assemblage B in 40% of positive samples is of particular public health significance, as assemblage B can infect both humans and animals (Hatam-Nahavandi et al., 2025; Ryan et al., 2021). Notably, assemblage B was also detected in shelter dogs from Diyarbakır, suggesting the possible circulation of zoonotic *G. duodenalis* genotypes in dog populations of eastern and southeastern Türkiye. (Aslan Çelik et al., 2023). Assemblage B has been similarly reported in dogs from Spain (Adell-Aledón et al., 2018), Cuba (Puebla et al., 2017), Canada (Julien et al., 2019), Italy (Agresti et al., 2022), and Poland (Jańczak et al., 2025). The absence of assemblage A is consistent with studies from China (Zhang et al., 2017), Iran (Fazaeli et al., 2021), and Fujian Province (Li et al., 2025), though it contrasts with reports from Iraq (Hassan, 2021), Spain (Mateo et al., 2023), and Italy (Agresti et al., 2022).

Several limitations should be acknowledged. The use of a single genetic locus (β-giardin) may limit the robustness of assemblage assignment, as multilocus genotyping using β-giardin, glutamate dehydrogenase, and triosephosphate isomerase provides more comprehensive molecular characterization (Zhang et al., 2017; Adell-Aledón et al., 2018; Szydłowicz et al., 2024). The relatively small sample size, cross-sectional design, and use of the native microscopic method rather than concentration techniques represent additional limitations. Future studies should incorporate larger sample sizes, multiple sampling time points, and multilocus genotyping to provide a more comprehensive understanding of the epidemiology of *G. duodenalis* in eastern Türkiye.

CONCLUSION

This study reports a 5% molecular prevalence of *G. duodenalis* among stray dogs in the Bitlis province of Türkiye, with PCR showing higher sensitivity than microscopic examination (5% vs. 3%). Sequence analysis revealed the presence of both canine-specific assemblages (C and D) and the zoonotic assemblage B, indicating that stray dogs in this region may harbor genotypes with zoonotic potential. No significant associations were found between *G. duodenalis* positivity and sex or age group, although positivity tended to be higher in dogs aged 0–1 month. These findings provide the first molecular epidemiological data on canine giardiasis in the Bitlis region and underscore the importance of continued molecular surveillance, larger-scale studies employing multilocus genotyping, and integrated One Health approaches to better understand the transmission dynamics and public health significance of *G. duodenalis* in this area.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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