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Prevalence of *Giardia duodenalis* in Stray Dogs in Istanbul and Diagnostic Comparison of Microscopy versus qPCR

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ABSTRACT

Giardia duodenalis is a common intestinal protozoan parasite affecting dogs and posing One Health concerns. We conducted a cross-sectional study of 2,485 fecal samples collected immediately after observed defecation from individually identified stray dogs presented to participating veterinary clinics in Ümraniye, Istanbul, to determine Giardia prevalence and compare the performance of conventional microscopy (direct smear and zinc sulfate flotation) with a real-time polymerase chain reaction (RT-qPCR) assay targeting the *Giardia* 5.8S rRNA gene. DNA was extracted using magnetic beads and amplified in a closed-tube qPCR assay. Using qPCR as the reference standard, we calculated sensitivity, specificity, and predictive values for each microscopy method and measured inter-test agreement (Cohen's κ). Real-time PCR detected *Giardia* DNA in 1,097 of 2,485 samples (44.1%). In contrast, microscopy identified only 298 positives (12.0%) by direct smear and 401 (16.1%) by $ZnSO_4$ flotation. Direct smear showed sensitivity 25.5%, specificity 98.7%, positive predictive value (PPV) 94.0%, and negative predictive value (NPV) 62.6%. $ZnSO_4$ flotation had sensitivity 34.6%, specificity 98.5%, PPV 94.8%, and NPV 65.6%. Combining both microscopy methods (positive by either) increased sensitivity slightly to 35.6%. Cohen's κ indicated moderate agreement between each microscopy method and PCR. These results confirm that PCR greatly increases detection of *Giardia* in canine feces, whereas conventional microscopy though highly specific misses most infections when only a single sample is examined. Our findings highlight the utility of molecular diagnostics for surveillance and control of canine giardiasis in urban settings.

Keywords: canine giardiasis, qPCR, molecular epidemiology, stray dogs, zinc sulfate flotation

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Introduction

Giardia duodenalis (also known as *Giardia lamblia* or *G. intestinalis*) is a flagellated protozoan parasite and a major cause of intestinal disease (giardiasis) in a wide range of hosts including humans, dogs, and cats (Uiterwijk et al., 2018). Giardiasis remains globally prevalent and continues to contribute substantially to diarrheal illness and morbidity in animals, particularly dogs (Emisiko et al., 2020). *G. duodenalis* is a globally distributed intestinal protozoan and a major cause of giardiasis, with an estimated 280 million symptomatic episodes annually. Closely linked with conditions of poverty, it has been recognized together with cryptosporidiosis within global neglected disease initiatives (Einarsson et al., 2016).

G. duodenalis is a species complex comprising eight genetic assemblages (A - H) with differing host ranges and epidemiology. In humans, infections are dominated by Assemblages A and B. A recent nationwide study from Norway (2022 - 2024) found B to be most prevalent in human cases (59%) followed by A (41%) within sub-assemblages, AII predominated in domestically acquired infections, whereas BIV was enriched among imported cases (Tipu et al., 2025). Multi locus sequence typing of domestic A isolates showed high diversity (20 MLST types in 21 isolates), and among the concurrently tested animal samples Assemblage E (livestock associated) was most common (62%) (Tipu et al., 2025).

Clinically, mucosal alterations present as steatorrheic diarrhea following colonization of the proximal small intestine by ingested trophozoites that attach to enterocytes via the ventral adhesive disc with coordinated flagellar activity. This intimate adherence, along with parasite - host interactions, leads to villus blunting and epithelial injury, impaired nutrient absorption, and mucosal inflammation (Bartelt and Sartor, 2015; Klimczak et al., 2024). The clinical spectrum ranges from asymptomatic carriage to overt disease. When symptomatic, patients typically present with malodorous, fatty diarrhea (steatorrhea) accompanied by abdominal cramping, bloating, anorexia, and weight loss; nausea, belching, diffuse abdominal pain, malaise, and anaemia are also described (Hajare et al., 2022; Klimczak et al., 2024; Tipu et al., 2025). In early childhood, giardiasis has been associated with persistent diarrhea, malnutrition, linear growth faltering (stunting), and measurable deficits in cognitive development (Hatam-Nahavandi et al., 2024). These pathophysiological and clinical features together explain the substantial individual and public health burden posed by *Giardia* infections. On the other hand, many infected animals remain asymptomatic carriers; when clinical disease occurs, giardiasis most often presents with acute or chronic diarrhea, weight loss, and malabsorption. Young animals and those maintained in close quarters (e.g., kennels, shelters) are at elevated risk. Consistent with this pattern, Zhao et al. (2022) synthesized 33 studies from China (2001 - 2021) and estimated a pooled canine G. duodenalis prevalence of 11.2%, with markedly higher rates in Northwestern China (35.7%), higher prevalence in studies from 2010 onward (11.8% vs. 6.9% pre-2010), greater infection in puppies (<1 year: 12.2%) than adults (>1 year: 5.4%), assemblage A detected most frequently (5.2%), and no association with sex. In parallel, Mircean et al. (2012) demonstrated substantially higher risk in close housed dogs by ELISA, prevalence reached 50% in kennels and 47.7% in shelters compared with 34.1% in urban household dogs and 16.8% in rural household dogs; microscopy likewise yielded higher rates in shelters (16.5%) than in household settings (approx. 4 - 5%). Taken together, these findings underscore that crowding and communal housing constitute significant risk factors for canine giardiasis however, the heightened risk observed in kennels and shelters does not imply that individually housed, home living dogs are risk free. Sporadic exposure to contaminated environments and asymptomatic carriage can sustain transmission even among solitary household pets, warranting vigilance across all housing conditions.

For decades, conventional microscopy of stool samples (direct smear or fecal flotation) was considered the diagnostic as “reference standard”. However, microscopy requires considerable expertise and has its limited sensitivity. Previous studies have found that routine stool microscopy often detects only about half of *Giardia* infections relative to molecular assays (Uiterwijk et al., 2018). For instance, Emisiko et al. (2020) reported microscopy sensitivities of only 48–64% for G. duodenalis, indicating many cases are missed without PCR. In particular, quantitative real time PCR (qPCR) can amplify *Giardia* DNA to detectable levels even when only few organisms are present, dramatically improving sensitivity. Uiterwijk et al. (2018) reported a qPCR assay (targeting the small subunit rRNA gene) with 97% sensitivity for G. duodenalis in dogs, far outperforming microscopy (48% sensitivity) and even outdoing ELISA and DFA methods. Although some studies note that molecular tests can occasionally produce false positives (due to detection of non viable DNA or minor cross contamination etc.), overall specificity of well designed PCR assays is higher than other assays. For these reasons, PCR is increasingly used as a confirmatory diagnostic for giardiasis, especially in reference laboratories. Nonetheless, contemporary immunodiagnostic assays (e.g., coproantigen ELISA) and molecular methods (e.g., qPCR) provide substantially higher diagnostic yield and more definitive detection than microscopy alone. Nevertheless, molecular assays are not infallible. Rare false positives/negatives can arise from inhibitors, low template burden, or carryover contamination. So results should not be interpreted in isolation. In practice, no single test is perfectly reliable; best practice is to combine complementary techniques or perform serial stool examinations, and to interpret laboratory findings alongside the patient's clinical presentation and exposure history.

Here, we conducted a survey of stray dogs in Ümraniye (Istanbul) to establish the prevalence of G. duodenalis using two complementary approaches; light microscopy (direct smear and centrifugal flotation) and real time PCR. Our primary objective was to generate a robust, locality specific estimate of infection in free roaming dogs; secondary objectives were to compare the diagnostic yield of microscopy versus PCR, quantify their agreement, and describe basic correlates of infection in a One Health context. By pairing field sampling with broadly accessible diagnostics, this study aims to provide an objective prevalence estimate and a practical appraisal of method performance that can inform surveillance and control strategies for canine giardiasis in urban settings.

Materials and Methods

Study Area and Sample Collection

This cross-sectional study included 2,485 fecal samples collected in the Ümraniye district of Istanbul, Turkey. Samples were obtained from free-roaming stray dogs presented to participating veterinary clinics located in different neighborhoods of Ümraniye. Fecal material was collected non-invasively immediately after defecation was directly observed by the study personnel within the clinic premises (e.g., outdoor area/yard or designated holding/waiting areas). Only freshly deposited feces from an animal visually confirmed as a dog were sampled using a disposable spatula and placed into clean, labeled containers. To preserve parasite integrity, samples were kept at 4 °C and processed within 24 hours of collection. Each specimen was assigned a unique code. To minimize repeated sampling, only one fecal sample per dog was included; repeat visits (if any) were not resampled based on clinic visit records and/or contemporaneous identification of the dog.

Parasitological Examination

All stool samples were examined for G. duodenalis by direct wet mount microscopy (saline and Lugol's iodine) and by zinc sulfate ($ZnSO_4$) centrifugal flotation (33% w/v; specific gravity 1.18). Wet mounts were examined promptly under light microscopy (400×) for trophozoites and cysts. For $ZnSO_4$ flotation, approximately 2 g of stool was mixed with $ZnSO_4$ solution, strained, and centrifuged at 1500×g for 5 min; a coverslip was applied to the meniscus for 5 min and then examined microscopically (100× - 400×), with Lugol's iodine added as needed to enhance cyst visualization. A sample was considered microscopy positive if G. duodenalis cysts and/or trophozoites were observed by either method.

Nucleic Acid Extraction

Genomic DNA was extracted from the stool samples using a magnetic bead based nucleic acid purification system. We utilized the MagPro MGX-16 (Vitrosens Biyoteknoloji A.Ş.; Istanbul, Turkey) automated nucleic acid extraction device in combination with the corresponding MagFast 16 Total Nucleic Acid Purification Kit (Vitrosens Biyoteknoloji A.Ş.; Istanbul, Turkey) designed for this instrument. Each fecal sample (approx. 200 mg of stool, or an equivalent volume of stool suspension) was processed according to the kit's instructions for stool specimens. The sample was lysed in the kit provided lysis buffer to release nucleic acids, and the lysate was loaded into the MagPro MGX-16 along with prefilled reagent cartridges from the MagFast kit. The process yields purified nucleic acids eluted in a final volume of approximately 100 - 150 µL.

Upon completion of the automated extraction, the eluted genomic nucleic acids from each sample was collected in sterile tubes. All extracted nucleic acid samples were labeled and stored at -20 °C until PCR amplification.

PCR Amplification and Detection

Detection of *Giardia* DNA was performed using ChainproTM NGX16-4F (Vitrosens Biyoteknoloji A.Ş., Istanbul, Türkiye) real time PCR. We utilized the Vetfor® *Giardia* Detection qPCR Kit (Vitrosens Biyoteknoloji A.Ş., Istanbul, Türkiye) for this purpose. This lyophilized provides master mix containing Taq DNA polymerase, deoxynucleotides, $MgCl_2$, and target specific primers and TaqMan probes for G. duodenalis. The target locus for amplification is a conserved gene region in the *Giardia* genome, specifically the 5.8S ribosomal RNA gene, which allows specific identification of G. duodenalis and allows definitive identification of the parasite's DNA. An internal amplification control (IC) is included in the G. intestinalis TaqMan RT-PCR Detection Kits (Norgen Biotek Corp., Thorold, Canada) to verify that the PCR reaction has run correctly for each sample.

PCR reactions were set up according to the manufacturer's instructions. For each sample, 20 µL of extracted DNA was added directly to the lyophilized reaction pellet and gently resuspended; reactions were briefly centrifuged, sealed, and run on the ChainPro™ NGX16-4F according to the kit protocol. A kit-supplied positive control and a no-template control were included in every run, and each reaction contained an internal amplification control to monitor inhibition.

After the run, results were analyzed using the instrument's software. The amplification curves were examined for each sample. A sample was considered positive for G. duodenalis if it showed exponential amplification in the target (*Giardia*) channel with a cycle threshold (Ct) value within the manufacturer's defined cutoff. The positive control consistently yielded a proper amplification curve at the expected Ct, confirming that the PCR reagents were functioning. The no-template control remained no amplification, ensuring that no contamination was present in the reagents or environment.

Data Analysis

Diagnostic results from microscopy and PCR were entered into a spreadsheet for analysis. The prevalence of *Giardia* infection was calculated for each method as the proportion of samples testing positive out of the 2485 total samples. Using the PCR assay as the reference standard, we evaluated the performance of the conventional methods. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were computed for the direct smear and $ZnSO_4$ flotation methods, with 95% confidence intervals for each estimate. In these calculations, a positive was defined as a sample positive by PCR, and a negative was a sample negative by PCR; microscopy results were compared against these criteria. To assess the benefit of using both microscopy methods in parallel, we also calculated the combined microscopy sensitivity (any microscopy method positive vs. PCR as reference). Agreement between each pair of diagnostic tests (direct vs. PCR, flotation vs. PCR) was measured by Cohen's kappa coefficient. The strength of agreement was interpreted using standard benchmarks (values >0.75 indicating excellent agreement). For statistical comparison of detection rates, McNemar's chi-square test was employed to compare paired proportions (e.g. the difference in positivity between direct smear and PCR on the same specimens). A two sided p-value < 0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS (version 26.0, IBM Corp., Armonk, NY, USA).

Ethics

In accordance with Turkish legislation, activities limited to collecting fecal material from the environment without handling or intervening on live animals fall outside the scope of animal experimentation, therefore, this study did not require approval from a Local Animal Experiments Ethics Committee (HADYEK) (Official Gazette No. 28914, 15 Feb 2014). This interpretation is consistent with university HADYEK bylaws that explicitly list "collection of feces or bedding" among procedures not subject to ethics approval.

Results

Out of 2,485 stool specimens examined, real-time PCR detected 1,097 positive cases (44.1%), far exceeding the positives found by direct microscopy (298 cases, 12.0%) and $ZnSO_4$ flotation (401 cases, 16.1%). Table 1 summarizes the agreement between the three diagnostic methods. Notably, PCR identified a large number of infections that were missed by conventional microscopy. Direct microscopy alone detected only 25.5% of the PCR-confirmed positives, and $ZnSO_4$ flotation alone detected 34.6%. Even combining both microscopic methods (direct smear plus flotation) only increased the detection to about 35.6% of the PCR-positive cases implying that nearly two-thirds of infections would have been missed without the PCR assay. On the other hand, the specificity of the microscopy-based methods was very high; only a small number of PCR-negative samples were false-positive by microscopy or flotation (for example, 18 samples were microscopy positive/PCR negative, and 21 were $ZnSO_4$ positive/PCR negative, as shown in Table 1). This corresponds to an estimated specificity above 98% for both microscopy and $ZnSO_4$ flotation in our study, reflecting that these methods rarely yield positive results in truly uninfected samples.

Table 1. Comparison of results obtained by direct microscopy, $ZnSO_4$ flotation, and PCR (reference standard) on 2,485 stool specimens. Each combination of test outcomes is shown with the number of specimens observed, along with the interpretation assuming PCR as the reference-standard indicator of infection.

Direct Microscopy	$ZnSO_4$ lotation	PCR (Reference Standard)	No. of Specimens
Positive	Positive	Positive	270
Positive	Negative	Positive	10
Negative	Positive	Positive	110
Negative	Negative	Positive	707
Positive	Positive	Negative	2
Positive	Negative	Negative	16
Negative	Positive	Negative	19
Negative	Negative	Negative	1,351
Total			**2,485**

Interpretation based on PCR results; any PCR positive sample is considered a infection, while PCR-negative samples are considered uninfected. “False” results by other methods can be inferred where a non-PCR method is positive but PCR is negative (false positives by that method), or where PCR is positive but a non-PCR method failed to detect the parasite (false negatives by that method).

Using PCR as the benchmark, we can derive the diagnostic performance of the other two methods. Direct microscopy achieved an approximate sensitivity of only 25.5% (detecting 280 of 1,097 positives) and a specificity of 98.7%. Its positive predictive value (PPV) was 94% (most microscopy positive samples were positives), but the negative predictive value (NPV) was only 62.6%, due to many infections being missed. $ZnSO_4$ flotation showed slightly better sensitivity (34.6%) but similarly high specificity (98.5%), with a PPV of 95% and NPV 65.6%. These findings illustrate that while microscopy based methods have excellent specificity, they lack sensitivity when only a single stool specimen is examined. In practical terms, the PCR assay detected about three times as many infections as $ZnSO_4$ flotation and nearly four times as many as direct microscopy, a highly significant difference ($p<0.001$) in diagnostic yield. This trend is consistent with other reports where molecular tests have vastly outperformed conventional ova-and-parasite examination; for example, a recent study found a broad qPCR panel identified 55% more positive samples than zinc sulfate centrifugal flotation (679 vs. 437 positives) in a large sample set (Leutenegger et al., 2023).

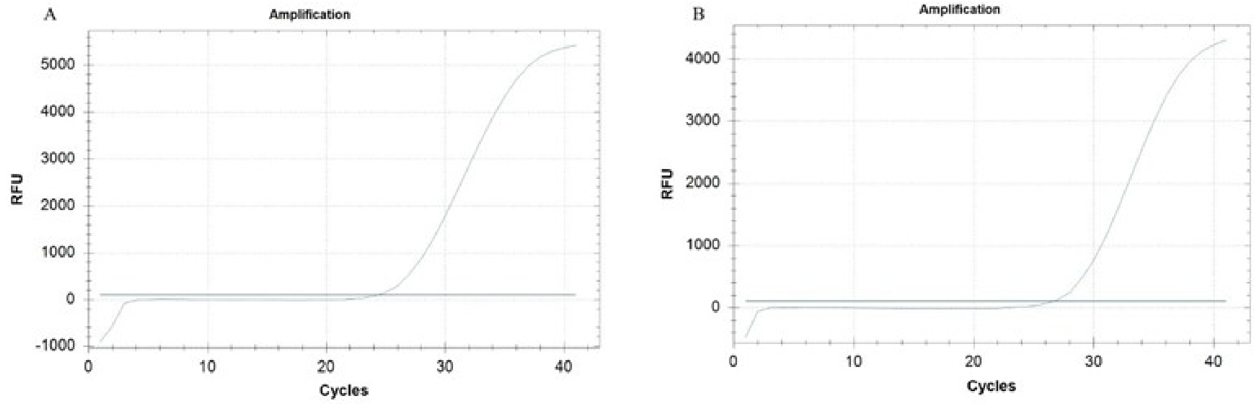

Figure 1. Representative real-time PCR amplification curves for G. duodenalis detection. Ct values were 24.34 for sample (A) and 26.60 for sample (B). Reactions were run for 40 cycles. The no-template control (NTC) showed no amplification.

Discussion

Our data show a marked increase in parasite detection when using PCR instead of conventional microscopy. The PCR method, which targets parasitic DNA, was able to identify infections even in cases where cysts or trophozoites were present in very low numbers or not observed microscopically. This enhanced sensitivity of PCR is well documented in the literature. In fact, PCR has largely replaced microscopy in some settings due to its generally higher sensitivity and broad detection capabilities. By amplifying parasite genetic material, PCR can reliably detect infections that would be missed by visual examination, especially in light or asymptomatic infections (Knopp et al., 2008). For instance, Elsafi et al. (2013) reported that real-time PCR (and a parallel immunoassay) reached 85.7% sensitivity for *Giardia lamblia* and *Cryptosporidium parvum* detection in single stools, notably higher than conventional microscopy. Similarly, in a veterinary context, Leutenegger et al. (2023) showed that a multiplex fecal qPCR could recover significantly more positives (overall parasite frequency 679 vs. 437 by flotation) and co-infections than routine $ZnSO_4$ concentration, underscoring the superior pickup rate of molecular diagnostics (Leutenegger et al., 2023). These findings align with our observation that PCR-positive cases vastly outnumbered microscopy positives, highlighting the inadequacy of relying on microscopy alone for sensitive parasite detection.

The poor sensitivity of single-sample microscopy in our study (25-35%) is in line with other studies indicating that a single stool exam often detects only a fraction of infections. Because parasite shedding is often intermittent and organisms may be sparse in any given sample, examining multiple specimens or using concentration methods is necessary to improve microscopic detection. Traditional guidelines have recommended analyzing at least three stool specimens collected on different days to increase the yield of ova-and-parasite examination. Indeed, multiple studies have shown that employing concentration methods and repeat sampling significantly raises microscopy's sensitivity for example, zinc sulfate flotation can detect substantially more cases than direct smear alone. In our data, the direct smear and $ZnSO_4$ methods together identified roughly 36% of infections (when results were combined), illustrating a small gain from using both methods in parallel. This modest improvement reinforces the recommendation that multiple methods in combination be applied for parasite diagnosis whenever PCR or other highly sensitive tests are not available. Still, even two microscopic methods together failed to catch nearly two-thirds of the PCR-confirmed cases in a single sample, which underscores the limitations of microscopy for routine screening.

In terms of specificity, our findings confirm that microscopy has very high specificity in experienced hands, as false positives were rare. Direct identification of parasite ova/cysts under the microscope especially when concentration techniques and appropriate staining are used yields few spurious positives. We observed 18 instances where microscopy was positive but PCR was negative and 19 instances where $ZnSO_4$ flotation was positive but PCR was negative. Because each sample was tested by qPCR in duplicate with strict run controls (no-template controls, positive controls, and internal extraction/inhibition controls), we recorded no PCR false positives or false negatives; duplicate results were concordant and all control criteria were met. Accordingly, these discordant findings are most plausibly explained by non-PCR factors (e.g., reader/artifact errors on microscopy, concentration artifacts with flotation, or sampling heterogeneity with low organism burden) rather than errors in the PCR assay. Although we did not encounter PCR misclassification in this dataset, the performance of qPCR is bounded by its analytical characteristics and pre-analytical variables. For completeness, the overall PCR positivity proportion was 44.1% (1097/2485; 95% CI 42.2-46.1%). In general, qPCR assays operate within a finite analytical measurement range, are susceptible to inhibition and variable extraction efficiency, and require careful contamination control and verification of amplification efficiency. Nonetheless, despite being a powerful tool, qPCR requires meticulous quality control and that results should be correlated with clinical and epidemiological context.

A practical advantage of conventional microscopy is its breadth of detection. A trained microscopist can potentially detect any parasite present in the sample including unexpected species as well as other diagnostic clues (e.g. white blood cells, helminth eggs, protozoan cysts of different types) in the same examination. Our findings concur that a well designed molecular panel could replace the labor intensive process of multiple stool microscopy without loss of diagnostic yield. It is worth noting that microscopy is laborious and operator dependent results can vary with the examiner's expertise, and maintaining skilled staff is challenging (Doğan et al., 2012). PCR, on the other hand, can be automated, standardized, and is less subject to individual interpretation errors.

From a public health and clinical perspective, the substantially higher detection rate of PCR has important implications. Patients with low intensity infections (who might have been falsely negative by microscopy) can be correctly diagnosed and treated, reducing ongoing transmission in the community. PCR's ability to detect co-infections is also superior, as shown by its identification of mixed parasite infections that were missed by microscopy in other studies (Leutenegger et al., 2023; Elsafi et al., 2013). Furthermore, molecular tests can provide genotypic information (e.g. species subtype or drug resistance markers) that microscopy cannot. For instance, our PCR protocol could theoretically be extended to characterize *Giardia* assemblages offering epidemiological insights and guiding therapy. These additional benefits of PCR-based diagnostics higher throughput, potential for automation, and richer data align with the direction of modern clinical parasitology moving toward molecular methods.

Despite clear advantages, PCR is not without drawbacks in practice. Cost and accessibility have been limiting factors in many settings. Conventional microscopy is inexpensive and widely available, whereas multiplex PCR assays require specialized equipment, reagents, and expertise, often at a higher cost per test. Until recently, PCR-based parasite panels were not routinely available in many clinical labs, and turnaround times could be slower. There is also the consideration of target spectrum, a PCR panel must include the parasites of interest in a given region; otherwise, a pathogen not covered by the primers will go undetected. In contrast, an observant microscopist might catch an unusual parasite or a commensal organism incidentally. For these reasons, many experts have advocated a combined approach: using PCR as a front-line screening tool for common parasites, while retaining microscopy (and other methods like antigen tests) as complementary techniques. In resource-rich laboratories, an increasingly common practice is to perform a multiplex PCR for major protozoan parasites (such as *Giardia, Cryptosporidium, Entamoeba histolytica*, etc.) and simultaneously use ova-and-parasite concentration methods for helminths, ensuring no parasite goes unnoticed. In resource-limited settings, however, microscopy will continue to play a central role, and improvements in training and technique (e.g. use of concentration, multiple samples, quality control between observers) are critical to maximize its yield.

In practical terms, PCR detected about three times more positive samples than either microscopic method reflecting its ability to detect low-burden infections that visual examination misses.

Conclusion

In conclusion, this study clearly illustrates that real-time PCR offers a substantially higher diagnostic yield for intestinal parasites compared to traditional microscopic techniques. PCR was able to detect the majority of infections, including many that were missed by both direct smear and $ZnSO_4$ flotation, thereby demonstrating its value as a high sensitivity reference method. Direct microscopy (with or without concentration) remains highly specific and can identify a broad range of parasites, but its sensitivity is severely limited when only one stool sample is analyzed. These findings support the adoption of molecular methods as a routine diagnostic tool for intestinal parasitic infections, especially in clinical scenarios where maximum sensitivity is required (e.g. for symptomatic patients or outbreak investigations). By incorporating PCR into routine practice either as a replacement for or adjunct to microscopy laboratories can improve detection rates and ensure timely treatment for infected individuals, ultimately contributing to better patient outcomes and reduced transmission. Given the practical considerations, a balanced approach is recommended: leverage PCR-based panels for their superior sensitivity and speed, while using conventional methods to complement PCR and to cross-verify results in cases of ambiguity. Ongoing improvements in PCR accessibility, cost-effectiveness, and test panel breadth are likely to further cement PCR's role as the new reference standard in parasite diagnostics. Our results, in concordance with the growing body of literature, underline that molecular diagnostics can significantly enhance the accuracy of parasite detection a crucial step toward more effective management and control of parasitic diseases in both clinical and public health settings.

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Conflict of Interest

One author is an employee of Vitrosens Biyoteknoloji A.Ş. All other authors declare no competing interests.

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References

Bartelt, L. A., & Sartor, R. B. (2015). Advances in understanding Giardia: Determinants and mechanisms of chronic sequelae. F1000Prime Reports, 7, 62. https://doi.org/10.12703/p7-62

Doğan, N., Öz, Y., Koçman, N. Ü., & Nursal, A. F. (2012). Comparison of individual differences in the direct microscopic examination in the diagnosis of intestinal parasites. Turkish Journal of Parasitology, 36(4), 211–214. https://doi.org/10.5152/tpd.2012.51

Einarsson, E., Ma'ayeh, S., & Svärd, S. G. (2016). An up-date on Giardia and giardiasis. Current Opinion in Microbiology, 34, 47–52. https://doi.org/10.1016/j.mib.2016.07.019

Elsafi, S. H., Al-Maqati, T. N., Hussein, M. I., Adam, A. A., Hassan, M. M. A., & Al Zahrani, E. M. (2013). Comparison of microscopy, rapid immunoassay, and molecular techniques for the detection of Giardia lamblia and Cryptosporidium parvum. Parasitology Research, 112(4), 1641–1646. https://doi.org/10.1007/s00436-013-3319-1

James Emisiko, Nathan Shaviya, Clement Shiluli, Ronald Wamalwa, Bernard Jumba, Jeremiah Zablon, Fidelis Mambo, & Mustafa Barasa. (2020). Comparison of Microscopy and PCR for Detection of Giardia Lamblia and Entamoeba Histolytica in Human Stool Specimens in a Resource Limited Setting in Western Kenya. Ethiopian Journal of Health Sciences, 30(6). https://doi.org/10.4314/ejhs.v30i6.6

Hajare, S. T., Chekol, Y., & Chauhan, N. M. (2022). Assessment of prevalence of Giardia lamblia infection and its associated factors among government elementary school children from Sidama zone, SNNPR, Ethiopia. PLOS ONE, 17(3), e0264812. https://doi.org/10.1371/journal.pone.0264812

Hatam-Nahavandi, K., Ehsan Ahmadpour, Mostafa Rezaeian, Rahimi, H. M., Ahmadreza Meamar, Badri, M., Eslahi, A. V., Hossein-Ali Rahdar, Solmaz Sepahi, Hamed Mirjalali, & Carmena, D. (2024). Occurrence and assemblage distribution of Giardia duodenalis in symptomatic and asymptomatic patients in southeastern Iran (2019–2022). Gut Pathogens, 16(1). https://doi.org/10.1186/s13099-024-00666-0

Klimczak, S., Kacper Packi, Rudek, A., Sylwia Wenclewska, Kurowski, M., Kurczabińska, D., & Agnieszka Śliwińska. (2024). The Influence of the Protozoan Giardia lamblia on the Modulation of the Immune System and Alterations in Host Glucose and Lipid Metabolism. International Journal of Molecular Sciences, 25(16), 8627–8627. https://doi.org/10.3390/ijms25168627

Knopp, S., Mgeni, A. F., Khamis, I. S., Steinmann, P., Stothard, J. R., Rollinson, D., Marti, H., & Utzinger, J. (2008). Diagnosis of Soil-Transmitted Helminths in the Era of Preventive Chemotherapy: Effect of Multiple Stool Sampling and Use of Different Diagnostic Techniques. PLoS Neglected Tropical Diseases, 2(11), e331. https://doi.org/10.1371/journal.pntd.0000331

Leutenegger, C. M., Lozoya, C. E., Tereski, J., Andrews, J., Mitchell, K. D., Meeks, C., Willcox, J. L., Freeman, G. L., Richmond, H. L., Savard, C., & Evason, M. (2023). Comparative study of a broad qPCR panel and centrifugal flotation for detection of gastrointestinal parasites in fecal samples from dogs and cats in the United States. Parasites & Vectors, 16(1). https://doi.org/10.1186/s13071-023-05904-z

Mircean, V., Györke, A., & Cozma, V. (2012). Prevalence and risk factors of Giardia duodenalis in dogs from Romania. Veterinary Parasitology, 184(2–4), 325–329. https://doi.org/10.1016/j.vetpar.2011.08.022

Tipu, J. H., Tabassum, A., Klotz, C., Sivertsen, A., Afset, J.-E., Gaustad, P., Sandven, L., Brekke, H., Lund, H. M., Lier, T., Tverelv, L. R., Robertson, L. J., & Hanevik, K. (2025). Diversity of domestic and imported assemblages of Giardia in Norway and multi-locus sequence typing of domestic assemblage A isolates. International Journal for Parasitology. https://doi.org/10.1016/j.ijpara.2025.08.018

Uiterwijk, M., Nijsse, R., Kooyman, F. N. J., Wagenaar, J. A., Mughini-Gras, L., Koop, G., & Ploeger, H. W. (2018). Comparing four diagnostic tests for Giardia duodenalis in dogs using latent class analysis. Parasites & Vectors, 11(1). https://doi.org/10.1186/s13071-018-3014-2

Zhao, Z.-Y., Li, M.-H., Lyu, C., Meng, X.-Z., Qin, Y.-F., Yang, X.-B., Ma, N., Zhao, Q., Zhang, Y., & Jiang, J. (2022). Prevalence of Giardia duodenalis Among Dogs in China from 2001 to 2021: A Systematic Review and Meta-Analysis. Foodborne Pathogens and Disease, 19(3), 179–191. https://doi.org/10.1089/fpd.2021.0073