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Application of 18S rRNA Gene-Based and β-Giardin Molecular Markers for Early Detection of *Giardia duodenalis* Infection

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ABSTRACT: Giardiasis and other intestinal parasite infections are still prevalent and a public health concern, especially in areas with inadequate sanitation and hygiene. *Giardia duodenalis* infections can cause mild to severe symptoms, such as chronic diarrhea, malabsorption, and growth impairment in children, so early detection is essential. The commonly used fecal microscopy test has a low sensitivity, particularly for infections with low parasite loads or in the absence of symptoms. The development of molecular diagnostic methods based on the polymerase chain reaction (PCR) provides a more accurate and sensitive alternative by using specific genetic markers. This review of the literature looks at the roles of the 18S rRNA and β-giardin genes as molecular markers for the early detection of *Giardia duodenalis* infection. The review's conclusions indicate that the 18S rRNA gene's high sensitivity and robust sequence stability make it suitable for initial screening and epidemiological surveillance. In contrast, the β-giardin gene is useful for genotype analysis and diagnostic confirmation because of its higher specificity. The combination of these two genes may be the most effective diagnostic strategy to improve the accuracy of early Giardiasis detection in both clinical and epidemiological settings.

KEYWORDS: *Giardia duodenalis*, 18S rRNA, β-giardin, PCR, early detection, molecular marker.

INTRODUCTION

The intestinal parasite *Giardia duodenalis*, sometimes referred to as *Giardia lamblia* or *Giardia intestinalis*, is the cause of giardiasis (Stephen et al., 2025). This illness is still a major global public health concern and is categorized as a neglected tropical disease (Savioli et al., 2006). An estimated 280 million people are thought to contract this parasite annually. Infections with *Giardia duodenalis* are common in developing nations with poor sanitation and low economic status (Tejan et al., 2023). Due to poor hygiene habits and exposure to tainted drinking water, children are the most susceptible to infection (Hajare et al., 2022). The *Giardia duodenalis* transmission cycle persists in the environment due in part to transmission from domestic animals and wildlife (Kuthyar et al., 2021). According to Prasertbun et al. (2012), some *Giardia duodenalis* infections are mild or asymptomatic, which permits the parasite to be continuously expelled by infected people without being discovered by standard microscopic examinations. Furthermore, the complexity of the parasite's epidemiological patterns is indicated by the variation in Giardia genotypes found in humans and animals; consequently, molecular approaches are necessary for disease surveillance and control (Ryan & Cacciò, 2013). Abdominal cramps, bloating, malabsorptive diarrhea (steatorrhea), and weight loss are clinical signs of Giardiasis or Giardia infection; however, in certain instances, *Giardia duodenalis* infection has been reported to be asymptomatic, making detection challenging (Stephen et al., 2025). Infections that go undiagnosed and untreated can cause health issues like malnutrition, growth disorders, and impaired cognitive function, all of which have a detrimental long-term impact on productivity and quality of life (Gutiérrez & Bartelt, 2024). Because chronic giardiasis can impede physical and mental development, it is especially concerning for children (Gutiérrez & Bartelt, 2024; Simsek et al., 2004). Giardiasis without typical symptoms can have long-term health effects, as evidenced by the fact that prolonged asymptomatic infection is also linked to stunted growth in children (Lehto et al., 2019). As a result, efforts to identify and manage this illness must be given careful consideration.

G. duodenalis infections can be found using a variety of diagnostic techniques. Fecal microscopic examination, which is still commonly used to diagnose giardiasis, has limitations because it is heavily reliant on sample quality and examiner skill, and detection failure frequently occurs when cyst or trophozoite excretion is low (Pouryousef et al., 2023). Although immunoassay techniques or antigen detection are more sensitive than traditional microscopy, they still have drawbacks, especially in cases where

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the parasite burden is low (Hooshyar et al., 2019). On the other hand, molecular techniques like PCR and qPCR target particular *Giardia duodenalis* genes, like the 18S rRNA or β-giardin genes, leading to higher sensitivity and specificity of detection; these techniques can identify parasite DNA in cases where the parasite burden is very low or when there are no symptoms (Verweij et al., 2003). The capacity to carry out molecular analysis or genotyping, which is utilized for epidemiological research and to comprehend genetic diversity, is another benefit of molecular methods (Hooshyar et al., 2019).

Several genes are targeted by molecular techniques, and choosing genetic targets is crucial to maximizing the effectiveness of molecular detection. Because of its high copy number and conservation, the 18S rRNA gene is widely used to detect parasites even in situations where DNA levels are low, including when cyst numbers are extremely low (Narayanan et al., 2021). Conversely, genotyping and molecular epidemiological analyses are made possible by the β-giardin gene, which offers genetic variation that can be used to distinguish between Giardia assemblages or strains. This allows for the detection of the parasite as well as the understanding of transmission patterns and sources of infection (Volotão et al., 2007).

According to studies comparing PCR targets, the β-giardin gene is crucial for the parasite's genetic differentiation, while the 18S rRNA gene has better diagnostic sensitivity (Weinreich et al., 2022). Based on scientific evidence, this review attempts to assess and contrast the use of β-giardin and 18S rRNA as molecular targets for the early detection of *Giardia duodenalis* infection. A deeper understanding of the benefits and drawbacks of the target genes (18S rRNA and β-giardin) and their implications for the diagnosis of giardiasis is anticipated through analysis of different journals.

CHARACTERISTICS OF GIARDIA DUODENALIS

The two main stages of Giardia duodenalis, a pathogenic parasite of the gastrointestinal tract, are an infectious cyst form that is excreted in feces and an active trophozoite form that resides and reproduces in the host's small intestine (Stephen et al., 2025). The trophozoite, which is motile and pear-shaped, can be found in the feces of people who have severe diarrhea. However, because it cannot survive in the outside world for very long, routine examinations are unable to identify it (CDC, 2024). Cysts, on the other hand, are able to survive in the external environment and spread giardiasis through the fecal-oral route because of their robust walls (CDC, 2024). Both types can be seen in feces, but because cysts are more stable and can be found in formed stool samples, they are more commonly targeted for detection (CDC, 2024). Because the excretion of parasites in feces is sporadic, trophozoites and cysts may not always be found in fecal samples even though the person is infected. Thus, to maximize the detection of *Giardia duodenalis* in diagnostic tests, repeated sample collection and the application of sedimentation techniques are needed (Uchôa et al., 2017).

Giardia duodenalis also exhibits a high degree of genetic variability, with multiple genetic assemblages or groups. Humans are most commonly infected by assemblages A and B (Ahmad et al., 2020). *Giardia duodenalis* identification can be impacted by genetic differences between assemblages because these differences affect primer compatibility and amplification efficiency (S. M. Cacciò & Ryan, 2008a; Rafiei et al., 2020; Ryan & Cacciò, 2013). The accuracy of molecular examination results can be improved by selecting gene targets more appropriately through an understanding of assemblage variation and its genetic characteristics ((S. M. Cacciò & Ryan, 2008a; Rafiei et al., 2020; Ryan & Cacciò, 2013).

The selection of gene targets that remain constant throughout the parasite life cycle is crucial to ensure consistent molecular detection results because different stages of the *Giardia duodenalis* life cycle, such as trophozoites and cysts expelled in feces, cause variations in gene activity at each stage (Faghiri & Widmer, 2011). Because the 18S rRNA gene has a high copy number and can be found in various parasite stages, it is frequently used in molecular studies and offers high PCR and qPCR sensitivity (Jothikumar et al., 2021; Narayanan et al., 2021). Furthermore, the β-giardin gene is expressed in both the trophozoite and cyst stages because the functional proteins it encodes form the parasite's adhesive structures and cyst wall ((Jenkins et al., 2009; Molina-Gonzalez et al., 2020). Despite differences in parasite stage and genetic diversity within fecal samples, molecular analyses can produce reliable, accurate results by using stable, representative gene targets.

PRINCIPLES OF MOLECULAR DETECTION IN *GIARDIA*

Due to their superior sensitivity, specificity, and stability, molecular diagnostic techniques can be selected as diagnostic instruments (Xiao & Feng, 2017). The identification of parasite DNA found in host fecal samples is the basis for the molecular detection of giardiasis (Morris et al., 2025). In order to separate parasite DNA from debris that could impede the amplification process, it is first extracted from fecal samples. Only Giardia DNA is identified and amplified after the extraction process because particular primers

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attach to parasite target genes like β-giardin or 18S rRNA (Nantavisai et al., 2007; Weinreich et al., 2022). Numerous molecular techniques, including conventional PCR, nested PCR, and real-time PCR (qPCR), can be used to amplify DNA; these techniques all operate on the same fundamental principle (Hussain et al., 2025; Kralik & Ricchi, 2017; Quan et al., 2018). By increasing the quantity of target DNA fragments, this amplification procedure makes it possible to detect them (Kumagai & Furusawa, 2024).
The sensitivity and specificity parameters can be used to assess the efficacy of diagnostic tests (Vicente et al., 2024). The capacity to reject signals from non-target sources or other organisms is measured by specificity. It shows how well a diagnostic technique can identify people who don't have the illness as negative cases (Trevethan, 2017). The goal of specificity is to precisely identify negative cases and avoid the potential for false negative results or misdiagnosis (Trevethan, 2017). Sensitivity, on the other hand, describes a test's capacity to find positive cases among people who are thought to have the illness, allowing for precise disease detection within a population. The goal of specificity is to precisely identify negative cases and avoid the potential for false negative results or misdiagnosis (Trevethan, 2017).
Molecular detection performance is influenced by a number of factors, such as the quality of DNA extracted from fecal samples, which must be free of contamination and degradation in order to increase DNA amplification efficiency (Vicente et al., 2024). Furthermore, if they are not eliminated through appropriate extraction techniques, fecal inhibitors like bile and complex polysaccharides can inhibit polymerase enzymes, decreasing PCR sensitivity (Elmahallawy et al., 2024; Monteiro et al., 1997). To avoid non-specific amplification and guarantee precise Giardia detection, factors pertaining to the design of efficient and specific primers and probes are essential (Elmahallawy et al., 2024). In the end, improving molecular procedures for a particular sample is a crucial step to guarantee diagnostic accuracy and reduce the likelihood of false-positive or false-negative errors (Vicente et al., 2024).

18S rRNA GENE AS A MOLECULAR MARKER

The 18S rRNA gene encodes the small subunit ribosomal RNA in eukaryotic organisms. It has an important plays a significant structural role in the ribosome and plays a role in protein translation, reflecting species variation in eukaryotic samples (Pisarev et al., 2008). Due to its comparatively high copy number within the genome, which facilitates the detection of parasite DNA during PCR analysis, this gene is frequently employed in Giardia detection (Mala & Hamza, 2016). The sequence of the 18S rRNA gene is comparatively stable because it is conserved. Giardia isolates and strains do not significantly alter it, which makes it appropriate for high-sensitivity PCR and qPCR techniques for early detection (Narayanan et al., 2021). Research has demonstrated that extremely low levels of Giardia DNA can be found using qPCR that targets the 18S rRNA gene (Weinreich et al., 2022). As a result, the 18S rRNA gene is commonly employed as a primary molecular marker in giardiasis molecular diagnosis (Weinreich et al., 2022).
Due to its low sequence variation, the 18S rRNA gene has limitations for differentiating Giardia assemblages despite being extremely sensitive (Wu et al., 2022). Research has demonstrated that the 18S rRNA gene is less effective than the β-giardin or tpi genes in identifying genotypic variations (Pouryousef et al., 2023; Rafiei et al., 2020; Wu et al., 2022). For assemblage-based molecular epidemiological analysis, the 18S rRNA gene alone is therefore less ideal (Gatei et al., 2003). Furthermore, in complex fecal matrices, less specific primer design may raise the risk of non-target amplification (Kounosu et al., 2019). PCR targeting the 18S rRNA gene is very sensitive and specific for identifying Giardia infection, according to several studies using fecal samples (Lee & Kwak, 2023; Ng et al., 2005; Verweij et al., 2003). Consistent detection performance in clinical populations and low-prevalence community settings has been reported in recent diagnostic evaluations (Veeken et al., 2025). The 18S rRNA gene is a dependable target for preliminary molecular screening before additional genetic analysis because of these benefits (Weinreich et al., 2022). Thus, in PCR-based giardiasis diagnosis, the 18S rRNA gene is still advised as a primary molecular marker (Pallant et al., 2015; Weinreich et al., 2022).

β-GIARDIN GENE AS A MOLECULAR MARKER

A significant structural protein found in the ventral disc structure of Giardia duodenalis' cytoskeleton is encoded by the β-giardin gene, which is crucial for the parasite's attachment to the host intestine (Lourenço et al., 2012). This gene is dispersed throughout the cytoplasm and is found on the exterior of the cyst wall (Wang et al., 2025). According to (Fu et al., 2022), the β-giardin protein is unique to Giardia and is hardly present in other organisms. These features make β-giardin an appropriate molecular target for the precise identification of the parasite (Fu et al., 2022). The β-giardin gene is used in studies to characterize Giardia in addition to detecting infections (S. Cacciò et al., 2002).

The β-giardin gene's high specificity for Giardia, which makes it an appropriate confirmatory target, is one of its key benefits (S. Cacciò et al., 2002; Guy et al., 2004). This gene can be used for genotyping and assemblage determination, especially for assemblages A and B, in addition to detecting Giardia. This is due to strain variation in the β-giardin gene's DNA sequences (S. Cacciò et al., 2002; Guy et al., 2004). According to comparative studies, the more conservative rRNA genes are not as informative for epidemiological analysis as the β-giardin marker (Weinreich et al., 2022). In order to increase the precision of genetic analysis of Giardia isolates, the β-giardin gene is often employed in multilocus genotyping techniques (Shamsi et al., 2025).

The β-giardin gene has fewer copies than the 18S rRNA gene, despite its high specificity. This condition may make it more difficult to detect parasites, especially in samples with low parasite burdens (Esmailikia et al., 2017; Weinreich et al., 2022). Although their specificity values are still high, recent meta-analyses have also revealed that PCR assays that target the β-giardin gene typically have lower sensitivity than 18S rRNA-based PCR (Shamsi et al., 2025). Because of this condition, using the β-giardin gene as the only marker for initial screening is less effective (Pallant et al., 2015). The β-giardin gene is suggested by a number of studies as an extra marker, especially during the confirmation and genotyping phases after initial detection using more sensitive gene targets (S. Cacciò et al., 2002; Fu et al., 2022; Shamsi et al., 2025).

COMPARISON OF THE 18S rRNA AND β-GIARDIN GENES

The choice of target genes is crucial for the early molecular detection of *Giardia duodenalis* infection. Due to its high copy number and excellent detection sensitivity, the 18S rRNA gene is frequently employed as a primary target (Lee & Kwak, 2023; Verweij et al., 2003; Weinreich et al., 2022). According to comparative research, real-time PCR molecular techniques that target the 18S rRNA gene can screen clinical samples with 100% sensitivity and 100% specificity, which makes them very effective for identifying infection even at low parasite burdens (Weinreich et al., 2022). On the other hand, due to its lower copy number in the genome, the β-giardin gene typically exhibits lower sensitivity as a screening target, even though it can still yield high specificity results (Guy et al., 2004; Hijjawi et al., 2018; Shamsi et al., 2025; Weinreich et al., 2022). Both genes function well in terms of specificity, but β-giardin has an extra benefit. The β-giardin gene is frequently used to confirm positive results because it is highly specific to Giardia and rarely shows cross-reactivity with other organisms (Cacciò, De Giacomo and Pozio, 2002; Hijjawi et al., 2018; Weinreich et al., 2022; Shamsi et al., 2025).. Furthermore, β-giardin sequence variation can be used to identify assemblages, especially assemblages A and B, which are sometimes difficult to distinguish using the more conservative 18S rRNA target, even though 18S rRNA is very sensitive in identifying the organism's overall presence (Weinreich et al., 2022).

Because the 18S rRNA gene is a part of ribosomal RNA and has comparatively consistent sequences across different Giardia isolates, it tends to be more stable in terms of target stability (Shumanskaya et al., 2025). Because of this feature, the 18S rRNA gene can be used for epidemiological surveillance and screening large populations (Moreno et al., 2018; Narayanan et al., 2021; Weinreich et al., 2022). Furthermore, this gene typically produces reliable detection results in samples with low parasite loads and under various clinical conditions (Narayanan et al., 2021; Shumanskaya et al., 2025). On the other hand, there is more genetic variation in the β-giardin gene between Giardia isolates. The sequence differences offer an advantage for identifying the genetic traits of the parasite, even though this variation may impact detection sensitivity (Hijjawi et al., 2018; Shamsi et al., 2025). As a result, the β-giardin gene is crucial for molecular epidemiological research, source tracing of transmission, and genotyping analyses and assemblage determination (Shamsi et al., 2025; Weinreich et al., 2022).

Because the 18S rRNA gene is a crucial part of ribosomal RNA, it is highly conserved, which makes it stable among Giardia isolates and appropriate for use as a molecular detection target for epidemiological surveillance and large-scale population screening (S. M. Cacciò & Ryan, 2008b; Moreno et al., 2018). According to Narayanan et al. (2021) and Weinreich et al. (2022), this stability allows for consistent detection of infections with low intensity or in asymptomatic individuals across various assemblages and geographical regions. The β-giardin gene, on the other hand, exhibits greater genetic variability due to its encoding of a structural protein involved in parasite adhesion, which may impact detection sensitivity but offers benefits for additional genetic analysis (S. Cacciò et al., 2002; Hijjawi et al., 2018; Shamsi et al., 2025). β-giardin is a more informative marker for genotyping, phylogenetic analysis, and differentiation of Giardia assemblages in detailed molecular studies because of this variability (Shamsi et al., 2025; Weinreich et al., 2022). The following table summarizes the key features of β-giardin and 18S rRNA as molecular markers.

No	Aspect	18S rRNA	β-Giardin
1.	Sensitivity	Higher	High
2.	Specificity	Not too specific assemblage	Very specific
3.	Clinical application	Initial screening, including cases with low parasite burden	Specific confirmation, genotyping, and epidemiological studies

β-giardin is utilized as a complementary marker for confirmation and genotyping, while the 18S rRNA gene is consistently suggested as an initial screening target based on usage patterns in the literature (S. Cacciò et al., 2002; Fu et al., 2022; Moreno et al., 2018; Shamsi et al., 2025; Weinreich et al., 2022). Numerous studies have also demonstrated that combining both genes in a single assay, like multiplex PCR or real-time qPCR, is an ideal strategy because it can combine the specificity and genotyping ability of β-giardin with the high sensitivity of 18S rRNA, improving overall diagnostic accuracy (Hijjawi et al., 2018; Ng et al., 2005; Shamsi et al., 2025; Weinreich et al., 2022).

CLINICAL IMPLICATIONS AND RESEARCH DIRECTIONS

Particularly in children, early detection of *Giardia duodenalis* infection is crucial for avoiding complications like growth disorders, malabsorption, and chronic diarrhea (Messa et al., 2021). Early detection of *Giardia duodenalis* infection allows for prompt and suitable treatment, which reduces the severity of the illness and its long-term effects on nutritional status (Leung et al., 2019; Shamsi et al., 2025). Furthermore, early detection reduces the fecal-oral route of parasite transmission, particularly in unsanitary areas (Adam, 2021; Weinreich et al., 2022). Additionally, asymptomatic infections that are frequently overlooked by standard microscopic examinations can be found using molecular techniques (Elmahallawy et al., 2024). As a result, both individual and community-level giardiasis prevention and control initiatives can benefit from the application of molecular techniques (Elmahallawy et al., 2024; Messa et al., 2021; Shamsi et al., 2025).

Because PCR techniques that target the 18S rRNA and β-giardin genes are more sensitive and specific than fecal microscopic examinations, they are highly relevant in reference laboratories (Leung et al., 2019). Particularly in samples with low cyst counts or subpar specimen quality, these techniques enable more precise diagnostic confirmation (Elmahallawy et al., 2024; Weinreich et al., 2022). Molecular markers facilitate more thorough mapping of Giardia prevalence and genotype distribution in epidemiological research (Hijjawi et al., 2018; Shamsi et al., 2025). Furthermore, this strategy has a great chance of being used in endemic regions with sufficient laboratory referral systems (Messa et al., 2021; Weinreich et al., 2022). Thus, incorporating molecular techniques into surveillance and diagnostic systems can enhance epidemiological data quality and facilitate more successful Giardia control measures (Shamsi et al., 2025).

CONCLUSION

It has been established that the β-giardin gene and 18S rRNA are reliable molecular markers for identifying *Giardia duodenalis* infection. Because of its greater sensitivity, the 18S rRNA gene is more appropriate for early screening and the detection of infections with low parasite burdens. The β-giardin gene, on the other hand, is more specific, which makes it helpful for Giardia genotype analysis and diagnostic confirmation. Therefore, using the 18S rRNA and β-giardin genes together may be the best way to detect giardiasis early for both clinical and epidemiological surveillance purposes.

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