

Review

Molecular Detection of Helminths in Stool Samples: Methods, Challenges, and Applications

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Abstract

Helminth infections caused by soil-transmitted species, like *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms, affect over one billion people worldwide, yet accurate diagnosis remains challenging due to low sensitivity of microscopy in detecting eggs in stool samples, especially in low-intensity infections. Molecular diagnostics, particularly PCR-based detection of helminth DNA in stool samples, have emerged as more sensitive and specific alternatives. Here we review advances in DNA extraction methods that overcome inhibitors in stool, multiplex PCR assays, and next-generation sequencing technologies enabling species differentiation and detection of drug resistance markers. These molecular tools enhance epidemiological surveillance and inform control strategies. Despite challenges such as sample complexity and cost, ongoing improvements in molecular diagnostics hold promise for more effective helminth detection and management in clinical and field settings.

Keywords: polymerase chain reaction (PCR); helminth DNA detection; parasite infection; diagnosis; stool sample analysis; fecal samples; DNA extraction methods; amplification; bead-beating; molecular diagnosis; next-generation sequencing (NGS); soil-transmitted helminths; PCR-based detection

1. Introduction

Helminthiasis are parasitic worm infections that affect both humans and animals worldwide, exerting a significant impact on public health and the economy. Accurate diagnosis is essential for effective treatment and for obtaining a precise epidemiological understanding of these diseases. Among helminthiasis, soil-transmitted infections caused by *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms (*Necator americanus* and *Ancylostoma duodenale*) are the most prevalent, affecting over one billion people globally. Traditional diagnostic methods, such as coproparasitological analyses (microscopic examination of stool samples for parasite eggs), support direct diagnosis but are limited in their ability to address deeper taxonomic or systematic questions. While stool analysis is highly specific for detecting parasite eggs, its sensitivity is low, resulting in many infected individuals being incorrectly reported as negative [1]. Serological tests can identify more individuals who have been exposed to parasites, but they cannot distinguish between current and past infections. Since the late 1990s, advances in molecular genetics have led to the development of strategies based on polymerase chain reaction (PCR) and, more recently, new



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sequencing technologies for helminth detection. Currently, PCR is effectively used to detect helminth DNA in environmental samples [2] as well as in various animal and human biological materials including blood [3], serum [4], urine [5,6], cerebrospinal fluid [7] and stool samples.

At the end of their complex lifecycle, most soil-transmitted helminths reside as adult worms in the human intestine, where female worms produce eggs that are expelled along with stool samples. However, these eggs are not always visible under microscopy. In particular, when the number of eggs per gram of feces (EPGs) is low, they can be difficult to detect using standard fecal sedimentation or flotation methods. In contrast, even a small number of eggs can often be detected by PCR, which is more sensitive. The first report on helminth DNA detection by PCR was by Gasser et al. in 1993, who developed a sensitive PCR cycle sequencing technique for amplifying and sequencing ribosomal DNA from single helminth eggs [8]. Subsequently, Romanova et al. used PCR and the random amplified polymorphic DNA (RAPD) method to identify helminth DNA from species such as *Trichinella*, *Fasciola*, *Echinococcus*, *Nematodirus*, and *Taenia* [9]. Another study described a highly sensitive PCR assay targeting a highly repeated DNA sequence of *Schistosoma mansoni*, capable of detecting DNA in fecal samples with as few as 2.4 eggs per gram, significantly more sensitive than the traditional Kato-Katz method [10]. PCR can also be standardized in multiplex formats to detect multiple soil-transmitted helminths simultaneously, as described by Phuphisut et al., achieving 87% sensitivity and 83% specificity compared to microscopic examination [11]. Additional protocols for helminth DNA detection have been described by Tun et al. [12] and using next-generation sequencing (NGS) by Pilotte et al. [13].

Improved diagnostic methods for helminths, particularly molecular tests, play a crucial role in identifying resistance mechanisms and monitoring the spread of resistance. These advances can inform the development of new drugs with novel modes of action or those targeting resistant strains. By elucidating the genetic basis of resistance, researchers can focus drug development on candidates effective against resistant parasites. Accurate diagnostics also enable clinicians and farmers to make informed decisions about drug selection and timing, based on the specific parasite species and their resistance profiles, thereby prolonging the effectiveness of existing drugs and helping to prevent further resistance development.

2. Methodology

This is a narrative review aiming to synthesize current understanding and existing literature on “molecular methods of helminth DNA detection” to explore historical development, methods, key trends and conceptual debates. Due to the broad nature of the topic, a systematic protocol was deemed unsuitable; instead, an expert-driven, flexible approach was adopted, guided by the authors’ knowledge and ongoing engagement with the field. Literature was gathered from MEDLINE database via PubMed search engine using broad keywords (see keyword section), like ‘helminth DNA detection’ and ‘PCR’ connected by AND/OR terms. No date restrictions were applied. References found through manual search (including forward and backward citations of articles found in the database search) were also taken in consideration. After a screening based on title, abstract and/or full text information, publications in line with the topic were identified. Selection criteria for cited literature included conceptual relevance, theoretical contribution, and methodological diversity to ensure a rich, representative sample. The collected literature was then thematically analyzed and organized to build a coherent narrative, highlighting key agreements, contradictions, and emerging gaps on our research topic.

3. Results & Discussion

3.1. Helminth Detection by PCR and the Identification of Resistance

Genes analyzed in PCR-based techniques for helminth detection are typically chosen for their species specificity and degree of polymorphisms, which allow for distinguishing between different helminth species. Some commonly targeted genes and genetic regions are presented in Table 1.

Table 1. Helminth genes used for DNA amplification by PCR.

Name	Locus	Description
Internal Transcribed Spacer (ITS) regions	ITS1 and ITS2	ITS regions are found in ribosomal DNA and provide high species specificity. These regions are commonly used for differentiating species like <i>Ascaris lumbricoides</i> , <i>Trichuris trichiura</i> , <i>Necator americanus</i> and <i>Ancylostoma duodenale</i> [14]. ITS has been typed in <i>Trichinella</i> , <i>Fasciola hepatica</i> , <i>Cysticercus ovis</i> , <i>Echinococcus granulosus</i> , <i>Nematodirus spathiger</i> [15] and <i>Clonorchis sinensis</i> [16].
18S rRNA gene	18S rRNA	This gene is highly conserved across eukaryotes but contains enough variation to distinguish between species. It is used in assays detecting <i>Strongyloides stercoralis</i> and other helminths [17,18].
Cytochrome c Oxidase Subunit I	COI	The COI gene is part of the mitochondrial DNA and frequently used for species identification due to its high level of sequence variability among different species. Largely used in first assays for cestode identification and <i>Schistosoma mansoni</i> [19,20].
NADH dehydrogenase subunit 1	ND1	The ND1 gene is part of the mitochondrial genome and is particularly useful for distinguishing closely related species. It is used in targeted assays for detecting <i>Ascaris</i> , <i>Onchocerca</i> , <i>Schistosoma</i> and others [21,22].
Beta tubulin gene	β -tubulin genes	The beta tubulin gene is used to detect helminths such as <i>Trichuris trichiura</i> and <i>Ascaris lumbricoides</i> [23]. Mutations in this gene are also associated with resistance to benzimidazole drugs [24,25].
High copy Number Non-Coding Repetitive DNA Elements	Various	High copy non-coding repetitive DNA sequences enhance the sensitivity of detection since they are present in multiple copies in the genome. There are highly sensitive PCR and qPCR assays for detecting soil-transmitted helminths as <i>Ascaris lumbricoides</i> and other helminths [13,26].

Molecular methods based on PCR include end-point PCR, real-time PCR (qPCR) and loop-mediated isothermal amplification (LAMP) [27,28]. LAMP, while less commonly used and less standardized than PCR, operates at a constant temperature, making it faster and simpler. This method is suitable for use in settings without sophisticated equipment and is well-suited for point of care applications. LAMP has demonstrated sensitivity and specificity comparable to PCR for detecting *Schistosoma* DNA [29]. Additionally, quantitative PCR has proven to be specific for detecting *Loa loa* microfilaremia, with cycle threshold values correlating with microfilarial density [3].

The choice of target gene should be guided by the specific goals of the study and the required level of taxonomic resolution. Mitochondrial genes (such as COI, ND1 and cytochrome b) are particularly effective for species-level identification and population genetics because of their high mutation rates and maternal inheritance. In contrast, ribosomal genes (16S, 18S and ITS) are better suited for broad phylogenetic and taxonomic

studies, especially when conserved universal primers are needed. Ribosomal genes are also advantageous when analyzing mixed-species samples, as their conserved regions facilitate universal amplification (Figure 1).

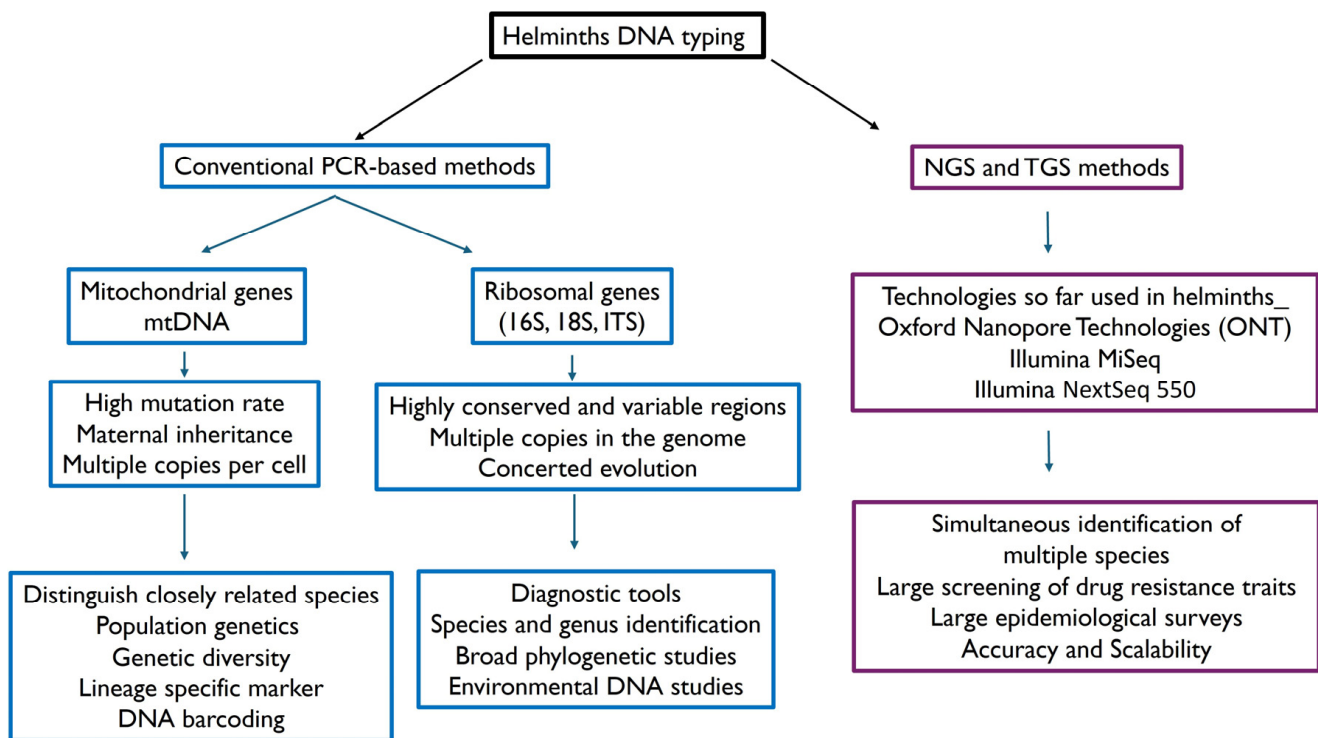


Figure 1. A schematic summary of molecular methods for helminth DNA typing and applications.

Detection of helminth DNA by PCR has also been used to evaluate parasite resistance to anthelmintic drugs. Furtado et al. used an amplification refractory mutation system (ARMS-PCR) to screen for single nucleotide polymorphisms (SNPs) in the beta-tubulin gene across multiple samples and identified a benzimidazole resistance-associated mutation at codon 200 in the beta-tubulin gene of *Ascaris lumbricoides* [24]. Oliveira et al. also confirmed SNPs in the beta-tubulin gene in *Trichuris trichiura* at codons 167, 198, and 200 providing evidence of the potential for drug resistance in these parasite populations in Brazil [25].

3.2. Methodological Challenges for Helminth DNA Extraction from Stool Samples

For protozoa, molecular typing based on DNA detection has proved effective [30]; however, challenges and controversies persist for helminths, primarily due to the difficulty of breaking open their hard and resistant eggs [31]. The ability to type helminth eggs is further limited by the complex nature of stool samples, the characteristics of helminth infections, and various technical limitations [32].

One important aspect to consider is the low DNA yield from stool samples. Helminth infections, particularly in regions with low transmission intensity or after treatment, often result in low parasite loads (low EPG), and hence, low amounts of DNA available for analysis [33]. Additionally, helminth DNA in stool can be degraded by environmental factors, digestive enzymes, and microbial activity, making it challenging to extract intact DNA. Storage and transport conditions also impact DNA integrity, as prolonged storage or exposure to high temperatures can cause DNA degradation [32]. Furthermore, the timing of stool sample collection relative to the helminth life cycle can influence the detection of eggs or larvae. Table 2 summarizes factors that can affect PCR detection of helminth DNA.

Table 2. Methodological challenges for helminth DNA detection in stool samples.

Step	Factor	Description
DNA extraction	Sample heterogeneity	Stool contains a complex mixture of substances including dietary residues, bacteria and other organic materials which can interfere with DNA extraction
DNA extraction/PCR amplification	Inhibitory substances	Bile salts, complex polysaccharides, humic acids and phenolic compounds can inhibit the PCR reaction leading to false-negative results
PCR amplification	Genetic variability	Helminths exhibit genetic variability which can affect the specificity of PCR primers and probes. Incorrect primer design can lead to cross-reactivity with non-target species.
PCR amplification	High amounts of non-target DNA	Feces contains a vast amount of microbial DNA and RNA derived from gut microbiota. The presence of non-target DNA can overwhelm the PCR reaction reducing sensitivity. Specificity in primer design and the use of qPCR can help to differentiate target DNA from background DNA.

Currently, various commercial kits are available for DNA isolation from fecal samples, and these can be used in both research and diagnostic settings (Table 3). These kits address different needs, such as the removal of inhibitors and compatibility with downstream applications. It is important to note that the type of sample matrix used for DNA extraction, as well as the specific steps taken to isolate or enrich helminth-derived products, are critical factors that affect the diagnostic sensitivity of PCR [34].

Table 3. DNA extraction kits optimized for stool samples.

Brand/Kit Name	Manufacturer	Sample Type	Key Features
QIAamp Fast DNA Stool Mini Kit	QIAGEN	Stool	Fast processing time, high DNA yield, inhibitor removal. Enrich host DNA
QIAamp PowerFecal Pro DNA Kits	QIAGEN	Stool	Fast processing time, high DNA yield, inhibitor removal. Enrich microbial DNA
ZR Fecal DNA MiniPrep	Zymo Research	Stool, gut material	Inhibitor removal, bead-beating step, easy to use spin column format
NucleoSpin Soil	Macherey-Nagel	Soil, stool	Effective inhibitor removal, broad sample compatibility
Stool DNA Isolation Kit	Norgen Biotek	Stool	High sensitivity for low-DNA samples
GeneJET Genomic DNA purification kit	Thermo Fisher Scientific	Various including stool	Simple protocol, broad compatibility with various sample type
PowerSoil DNA isolation kit	QIAGEN	Soil, stool	Ideal for samples with high inhibitor content. Bead-beating step designed to lyse tough cells.
MAGNA Pure LC DNA isolation kit	Roche	Stool	Employs bead beating for the mechanical disruption of cells in faecal samples. High throughput, automation compatible

One of the most important steps in extracting DNA from helminth parasites is the use of bead-beating techniques, which mechanically disrupt the external structures of helminths (such as the tegument, cuticle, or eggs), thereby releasing DNA for subsequent purification and helping to separate fecal material that may trap DNA. These beads, typically made of glass, zirconia, or stainless steel, are placed in a tube with the fecal sample and a lysis buffer. Comparative analyses of DNA extraction methods have demonstrated that bead-beating improves the detection of helminth DNA in stool samples [35]. The study by Devyatov et al. also emphasized the importance of bead-beating for effective DNA extraction from helminth eggs [36]. Other studies have confirmed that bead-beating prior to DNA extraction is a highly efficient approach for detecting *T. trichiura* DNA in human stool samples [37,38]. A summary of the DNA extraction workflow is presented in Figure 2. Alternatively, a study by Monteiro et al. (2018) successfully detected helminth DNA from Kato-Katz slides, supporting the use of Kato-Katz thick smears as a source of helminth DNA for large-scale genetic, epidemiological, and drug-resistance studies [39].

Detailed Workflow for DNA extraction from stool samples (Helminths DNA)

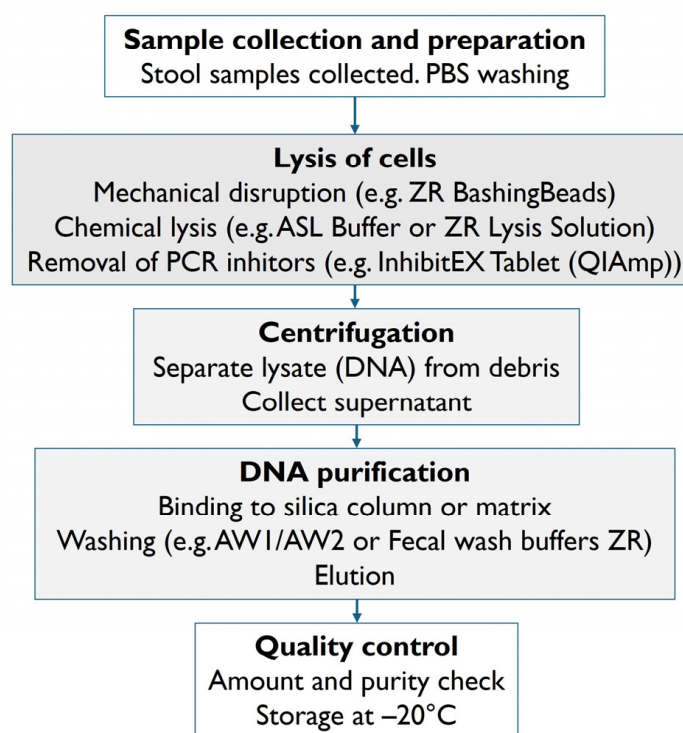


Figure 2. A workflow of key steps for helminth DNA extraction from stool samples.

3.3. Helminth qPCR Reagents

After obtaining the lysate and isolating the DNA, PCR reactions can be set up as either single-plex or multiplex assays. Single-plex PCR amplifies a single target DNA sequence in one reaction, allowing for the specific detection of one helminth species. In contrast, multiplex PCR enables the simultaneous amplification of multiple target sequences in a single reaction, facilitating the detection of several helminth species at once. This approach is particularly useful for diagnosing mixed infections (Table 4). For example, Basuni et al. reported a pentaplex real-time PCR assay for the simultaneous detection of *Ancylostoma* spp., *Necator americanus*, *Ascaris lumbricoides*, and *Strongyloides stercoralis* in stool samples [40].

Table 4. Examples of multiplex and singleplex approaches for helminth DNA typing.

Parasite Name	Target Gene/Region	PCR Type	Reference
<i>Ascaris lumbricoides</i>	Internal Transcribed Spacer 1 (ITS1)	Multiplex	Phuphisut et al., 2014 [11]
<i>Trichuris trichiura</i>	Internal Transcribed Spacer 2 (ITS2)	Multiplex	Phuphisut et al., 2014 [11]
<i>Necator americanus</i>	18S rRNA	Multiplex	Phuphisut et al., 2014 [11]
<i>Strongyloides stercoralis</i>	18S rRNA	Singleplex	Verweij et al., 2009 [17]
<i>Schistosoma mansoni</i>	NADH dehydrogenase subunit 1 (ND1)	Singleplex	Pontes et al., 2002 [10]
<i>Echinococcus granulosus</i>	Cytochrome c oxidase subunit 1 (COI)	Singleplex	Bowles et al., 1992 [41]
<i>Taenia solium</i>	Cytochrome c oxidase subunit 1 (COI)	Singleplex	Nunes et al., 2005 [42]

Currently, several commercial kits are available for the detection of helminths by PCR, designed to simplify DNA extraction and amplification by providing all necessary reagents for target helminth DNA (Table 5). For example, Autier et al. validated the Allplex™ GI-Helminth(I) Assay, a commercial multiplex PCR kit capable of detecting multiple helminths—including *Ancylostoma* spp., *Ascaris* spp., and *Enterobius vermicularis*—and found high concordance with microscopy, especially when bead-beating pretreatment was used [43].

Table 5. Commercial PCR kits for helminth DNA detection.

Kit Name	Manufacturer	Target Helminths	Description	PCR Type
FastTrack Helminth Detection Kit	FastTrack Diagnostics	<i>Ascaris lumbricoides</i> , <i>Trichuris trichiura</i> , <i>Necator americanus</i> , <i>Strongyloides stercoralis</i>	Multiplex PCR kit designed for the detection of common soil-transmitted helminths in stool samples.	Multiplex PCR
RIDA® GENE Parasitic Stool Panel	R-Biopharm AG	<i>Ascaris lumbricoides</i> , <i>Trichuris trichiura</i> , <i>Strongyloides stercoralis</i>	Multiplex real-time PCR kit for the detection of multiple helminth species in stool samples.	Multiplex Real-Time PCR
GeneXpert® Strongyloides	Cepheid	<i>Strongyloides stercoralis</i>	Real-time PCR test for the detection of <i>Strongyloides stercoralis</i> in stool samples, using the GeneXpert system.	Singleplex Real-Time PCR
BioGene Helminth Panel	BioGene	<i>Ascaris lumbricoides</i> , <i>Trichuris trichiura</i> , <i>Ancylostoma duodenale</i> , <i>Necator americanus</i>	A comprehensive multiplex PCR panel for the detection of common soil-transmitted helminths in stool samples.	Multiplex PCR
RealStar® Helminth PCR Kit	Altona Diagnostics	<i>Schistosoma</i> spp., <i>Fasciola</i> spp., <i>Opisthorchis</i> spp.	Real-time PCR kit for the detection of various helminths in stool and tissue samples.	Multiplex Real-Time PCR
Amplidiag® Bacterial and Parasite Panel	Mobidiag	<i>Schistosoma</i> spp., <i>Strongyloides stercoralis</i> , <i>Ascaris lumbricoides</i>	Multiplex PCR kit for simultaneous detection of bacteria and helminths in stool samples.	Multiplex PCR

3.4. Some Examples of Molecular Typing of Helminth DNA: Opportunities and Novel Approaches

Molecular typing of helminth eggs recovered from stool samples provides valuable insights, including the identification of zoonotic infections and the estimation of drug resistance. The following examples illustrate the utility of these molecular approaches.

Molecular assays on stool samples can help trace infection routes and estimate zoonotic sources of infections. For example, Areekul et al. found evidence suggesting a potential role of dogs in pediatric trichuriasis in rural Thailand, as they detected coinfections with *T. trichiura* and *T. vulpis* through PCR and ITS2 cloning, and identified both *Trichuris* species eggs in local dogs [44]. Similarly, sequencing of *nad1* and *cob* genes recently confirmed the presence of *E. multilocularis* eggs in shepherd dogs and wolves in north-western Italy [45], enabling precise mapping of the parasite's geographical range and species-level discrimination of cestode eggs.

Pyrosequencing following PCR amplification of the beta-tubulin gene has recently been used on stool samples to optimize methodology and estimate resistance allele frequencies in *Trichuris trichiura* and *Necator americanus*, indicating potential for large-scale surveys [46]. While conventional PCR methods are sensitive and specific, they are relatively limited in detecting multiple infections or unknown pathogens because they rely on primers targeting known genomic regions. In contrast, next-generation sequencing (NGS) and long-read metabarcoding approaches offer clear advantages over conventional PCR by enabling broader detection capabilities.

NGS employs massive parallel sequencing, involving DNA fragmentation, library preparation, sequencing, and reassembly, which generates large amounts of data useful for various biological applications. These include improved simultaneous identification of species in stool samples and large-scale screening for drug resistance traits in helminths. Originally developed for biodiversity studies in different environments, these methods have been adapted to analyze complex mixtures in fecal samples, encompassing both microbiota and parasites.

NGS also offers the advantage of enabling whole-genome sequencing, which serves as a foundation for numerous additional studies. It allows comprehensive screening for specific SNPs or unknown variants relevant to taxonomic and biological trait identification, and facilitates the discovery of novel pathogens, as NGS does not require prior knowledge of the pathogen's genome.

Despite NGS and TGS (third generation sequencing) not yet being widely available in many countries, they are becoming increasingly accessible globally, thus offering higher accuracy and scalability and representing a promising development for diagnostics and large-scale epidemiological screening applications for helminthiasis and parasite risk forecasting. Recently such advanced methods were applied to blood samples and filarial nematodes of medical and veterinary importance [47], enabling the detection of known filarioid species, previously undescribed species, and coinfections.

Nevertheless, stool samples remain a challenging material for advanced sequencing methods. For example, standard PCR targeting ITS regions has been combined with NGS-based multiplex barcoding to screen pooled fecal samples for ascarids such as *Parascaris* and strongylids in Australian Thoroughbred horses [48]. In this context, the true potential of NGS-based methods lies in their ability to simultaneously provide diagnostic information and detect drug resistance, which is particularly important for infections in grazing ruminants [49]. When specific diagnostic molecular regions are unavailable, these technologies offer opportunities to develop novel, multi-parallel PCR-based diagnostic assays, as demonstrated by Pilotte et al., who identified repetitive DNA elements as alternative targets for precise diagnosis of soil-transmitted helminths [13]. This approach, combined with bioinformatics, enhances species specificity and detection limits. Avramenko et al.

described the first application of deep amplicon sequencing to study parasitic nematode communities in cattle, introducing the concept of the gastrointestinal “nemabiome” [50]. More recently, metabarcoding using high-throughput sequencing has enabled the simultaneous identification of multiple helminth species within mixed samples, providing a comprehensive understanding of species composition and diversity. Various target genes, such as mitochondrial 12S and 16S rRNA and nuclear genes like ITS-2 and 18S rDNA, have been explored for metabarcoding, revealing the diversity of parasitic communities and enabling detection of drug-resistant genetic variants, which is valuable for monitoring anthelmintic resistance [51]. However, genetic variation can significantly impact the detection of helminth parasites, as many PCR assays have been developed using a limited range of geographically restricted parasite isolates. Low-coverage genome sequencing of STH samples from 27 countries revealed substantial genetic variation within and between helminth populations, including copy number and sequence variants in diagnostic target regions [52]. Such variation, especially within primer and probe binding sites of qPCR assays, can affect diagnostic sensitivity and specificity, potentially leading to false-negative results, particularly in low-intensity infections. To minimize the impact of genetic variation, qPCR assays should be redesigned to target more conserved genomic regions, and it is essential to validate these assays using diverse samples from various geographic regions to ensure robust performance across different helminth populations.

In the context of environmental samples, a recent study on soil contamination by *Echinococcus granulosus* in a highly endemic region of Italy demonstrated the utility of molecular approaches for estimating human health risks associated with exposure in educational farms [49]. To improve DNA capture from cestode eggs, a chelex bead pre-treatment was used. The advent of NGS and TGS, with declining costs and reduced reagent requirements, now enables the processing of many samples in pools, facilitating high-throughput screening of stool samples [13].

In settings where NGS technologies are not available, LAMP and qPCR are reliable methods for identifying low-intensity infections of *Ascaris lumbricoides**. LAMP, in particular, is noted for its applicability in resource-limited settings due to its cost-effectiveness, simplicity, and rapid results [53]. A recent review provides a detailed comparison of various molecular methods, including their sensitivity, specificity, and cost-effectiveness relative to traditional approaches [54].

4. Conclusions

PCR offers significantly higher sensitivity and specificity than microscopy for detecting helminths, particularly in low-intensity or mixed infections. However, despite its greater sensitivity, PCR has limitations in detecting DNA when only a few eggs or larvae are present in the sample. Accurate quantification of helminth burden using PCR can be challenging due to variability in DNA extraction efficiency and the presence of inhibitors. Additional drawbacks include potential contamination with phylogenetically related taxa, sample complexity, the presence of inhibitors, higher costs, and the need for highly trained personnel.

Detecting helminth DNA requires optimized DNA extraction protocols to remove or neutralize inhibitors, careful primer design, the use of internal controls to monitor inhibition, and the development of standardized, cost-effective methodologies suitable for diverse settings, particularly in low-resource areas where helminth infections are most prevalent. Commercial PCR kits for helminth DNA detection are increasingly used in research, specialized clinical diagnosis, and public health monitoring but are not yet standard in all settings. Epidemiological studies aiming to evaluate the prevalence and distribution of helminths infections may benefit from including molecular methods, especially in large-

scale surveys in which comprehensive detection is crucial. PCR methods also facilitate a better understanding of helminth genetic diversity at both species and population levels, enable tracking of drug resistance, and provide insights into population structure.

Molecular detection of helminth DNA can also be applied to environmental samples such as soil and water to assess transmission risks in public health studies. As technology advances and costs decrease, and with the development of portable and miniaturized PCR devices, molecular diagnostics for helminth infections are likely to become increasingly common in both clinical and field settings in the near future.

Beyond parasite identification and diagnosis, next-generation sequencing enables comprehensive analysis of the helminth genome, including the identification of genes essential for parasite survival and reproduction and the discovery of potential drug targets such as enzymes, structural proteins, and proteins involved in key metabolic pathways that influence transmission and invasion. Additionally, NGS facilitates the identification of genetic loci under selection—such as mutations associated with anthelmintic drug resistance, pathogenicity, or immune evasion—and supports studies on the emergence and spread of drug resistance.

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Conflicts of Interest: The authors declare no conflicts of interest.

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