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Research Article

Evolution of Soil-transmitted Helminth Diagnosis: Transition from Traditional Microscopy to Advanced Molecular Techniques in the 21st Century

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Abstract

Soil-transmitted helminths (STHs), or geohelminths, are parasitic nematodes that spread through eggs and larvae found in contaminated soil. These infections are most common in tropical and subtropical regions, where poor sanitation exacerbates the risk. In the Americas, around 46 million preschool and school-age children are vulnerable to STH infections, which can lead to symptoms like intestinal distress, general malaise, and anemia. Diagnosing STHs is complicated due to intermittent egg shedding, limited trained personnel, and inadequate diagnostic tools. Traditionally, diagnosis relied on microscopy, a method with low sensitivity. However, the trend has shifted towards more accurate molecular diagnostics, particularly quantitative polymerase chain reaction (qPCR). This shift from conventional microscopy to molecular techniques, including serology, represents a significant advancement. The World Health Organization now advocates for the integration of molecular diagnostics into STH elimination programs, emphasizing the need for precise and timely diagnosis to reduce the morbidity and mortality associated with these infections.

Keywords: Soil-Transmitted Helminths (STHs); World Health Organization

Introduction

The soil-transmitted helminths (STH's; also called geohelminths) are a group of parasitic worm which belong to the phylum Nematoda and they are transmitted by eggs and larvae primarily through contaminated soil [1,2]. The STH's are mainly found in the tropics and subtropics especially in areas with poor sanitation. In the Region of the Americans, it is estimated about 46 million pre-school and school-age children are at risk of geohelminth infections [3,4]. STH's causes a wide range of symptoms such as intestinal manifestations, general malaise and weakness, Undiagnosed STH's can eventually lead to anemia. STHs infections thrive more in developing regions were there is a large shortfall of skilled professionals and the appropriate facilities to diagnose and combat the infection. Therefore accurate tests for the diagnosis of STHs are of uttermost necessity [5]. Historically; the diagnosis of soil-transmitted helminths (STHs) (e.g., *Ascaris*

lumbricoides, Hookworm (Ancylostoma duodenale, Necator americanus Trichuris trichiura and *Strongyloides stercoralis*) has relied on often-insensitive microscopy techniques [5]. Diagnosis of intestinal pathogens has recently in the past few years taken a different dimension with use of diagnostic tools like quantitative polymerase chain reaction (qPCR), a molecular diagnostic method. This then led to the progression of diagnostic techniques through the conventional techniques (microscopy, culture, and egg counting), the serology technique, and the molecular technique [5]. The World Health Organization has recommended that molecular tests be adopted to help for accurare and precise diagnosis of STHs infections in STH elimination programs. Thus, reducing the mortality and morbidity of soil transmitted Helminths significantly.

Soil transmitted helminths

Soil-transmitted helminths are a group of intestinal parasitic worm which belong to the phylum Nematoda and they are transmitted by eggs and larvae primarily through contaminated soil ("helminth" means parasitic worms) [1,2]. The soil-transmitted helminths of major concern to humans are: The roundworm *(Ascaris lumbricoides*)), the whipworm (*Trichuris trichiura*), *Necator americanus* and *Ancylostoma duodenale* (the hookworms) [3].

Transmission occurs through ingestion of contaminated food or water (Ascaris and Trichuris) or through skin penetration by larvae in the soil (hookworms). Once inside the human host, STHs mature and reproduce, with adult worms residing in the gastrointestinal tract (Ascaris and Trichuris) or attaching to the intestinal wall and feeding on blood (hookworms) [4,5].

Epidemiology of soil transmitted helminths

Globally, 1.7 billion people are infected with one or more STH. According to findings from a survey carried, the global prevalence of *A. lumbricoides* is reported to have a global prevalence of more than 1.2 billion, of which >50% of cases are seen in China. while 795 million was estimated for *T. trichiura* and another, 740 million for hookworm. About 50% of hookworm global prevalence is found in Sub-Saharan Africa and China [4], whereas the prevalence rate of *A. lumbricoides* was 819 million and *T. trichiura* was 464 million and hookworm was 439 million in which >50% of cases were seen in South Asia and Sub-Saharan Africa [5]. The difference in prevalence rate can be majorly due to the source of data and the scope of the review from where the data was obtained. Especially if the study it assessed only the population at risk of acquiring the infection and not the entire general population [6].

STH prevalence is more common in rural areas when compared to urban areas. A recent study done at Vellore, India showed the prevalence of STH 9% in rural and 4.8% in urban areas [6,7]. However, the rates of *T. trichiura* (2.2%) and *A. lumbricoides* (3.3%) were higher in urban areas, while hookworm was more prevalent in rural areas (8.4%). This discrepancy may be due to differences in socioeconomic status, sanitation, and water supply [7]. In India, meta-analyses have shown that STH prevalence is higher in rural areas due to poor sanitation, inadequate water supply, and overcrowding [8]. Despite its high prevalence, soil-transmitted helminthiasis is often considered a neglected tropical disease, especially in underdeveloped rural areas, where it tends to be chronic rather than acute, making its socioeconomic burden difficult to quantify [6-8]. STHs are common in tropical and subtropical regions, where poor sanitation is widespread [9]. In the Americas, STH infections are prevalent, with an estimated one in three people infected. Around 46 million children between the ages of 1 and 14 are at risk, largely due to inadequate sanitation and clean water access [10].

In Nigeria, STHs are a significant public health concern, affecting both rural and urban areas, with higher rates in rural communities. Contributing factors include poor sanitation, lack of clean water, inadequate hygiene education, and poverty [6]. Children are particularly vulnerable due to their frequent contact with contaminated soil. In southwestern Nigeria, a pooled prevalence estimate (PPE) showed a 54.8% overall prevalence of STH infections among children aged 0-17 years. *Ascaris lumbricoides* was the most prevalent species at 44.6%, with high rates of *Strongyloides stercoralis*, *Trichuris trichiura*, and hookworms also observed [11].

Diagnosis of soil transmitted helminths in 21st century

Soil-transmitted helminths (STHs) significantly impair mental and physical development and contribute to anemia, especially in children. Although often asymptomatic, STH infections can sometimes lead to severe gastrointestinal symptoms. The World Health Organization (WHO) aimed to eliminate STHs as a public health issue in children by 2020 [10]. However, diagnosing parasitic diseases remains challenging due to the absence of physical

symptoms, limited trained personnel, and inadequate technology. The sporadic release of eggs or larvae further complicates diagnosis, highlighting the need for rapid and accurate diagnostic tests [12].

Various diagnostic methods for STHs are available, including traditional techniques like microscopy, culture, and egg counting, as well as more advanced serology and molecular methods. Conventional diagnostic techniques include Kato-Katz (KK), formol-ether (FE), sodium nitrate flotation (SNF), direct examination (DE), Kogar agar plate (KAP), merthiolate-iodine-formaldehyde (MIF), Baermann, McMaster, Harada-Mori, and the newer FLOTAC methods. Molecular diagnostics, such as polymerase chain reaction (PCR) and Loop-mediated isothermal amplification (LAMP), offer promising results but can be less sensitive, particularly in cases of light or multiple infections [9].

The progression of diagnosis of soil transmitted helminths in 21st century

Assessing the worm burden is crucial for understanding infection intensity and prognosis. The effectiveness of interventions and control strategies in endemic areas depends on accurate diagnostic tools, which are evaluated based on sensitivity and specificity [15]. Although microscopy has been the traditional method for detecting parasites, it often fails to detect infections due to issues like uneven egg distribution in stool, low egg count, and improper sample handling [15,16]. In highly endemic regions, less sensitive techniques may suffice to prevent morbidity [9,16]. Recent efforts have focused on advancing molecular diagnostics, particularly qPCR, to improve detection of intestinal pathogens, leading to a progression in diagnostic techniques from traditional to molecular approaches [9]. This then led to the progression of diagnostic techniques through the conventional techniques (microscopy, culture, and egg counting), the serology technique, and the molecular technique

Microscopy

There are several Microscopy-based techniques used to diagnose Soil transmitted helminths.

- **Direct Microcopy:** Historically, microscopy-based techniques, including the Kato-Katz method, direct smear examination, and formalin-ether concentration, have been widely used for STH detection [16]. While cost-effective and relatively simple, these methods have limitations, such as low sensitivity, especially in cases of low worm burden or early-stage infections. Moreover, the expertise required for accurate interpretation can be a barrier in resource-limited settings [17]. The basic procedure involves preparing a thin, smooth sample (using saline and iodine) and covering it with a cover glass. For dysenteric or unformed stool samples, a portion containing blood and mucus is selected for smear preparation, which is then examined under a microscope without adding saline or stain. The observer should carefully assess the shape, size, and bile staining of any larvae or helminth eggs present [18].
- **Egg counting Kato-katz technique**: The Kato-Katz technique remains the most widely used method for counting helminth eggs. The World Health Organization (WHO) advises analyzing two slides per sample using this technique. It's essential to examine the slides within 40–60 minutes after applying glycerol, as hookworm eggs may deteriorate and vanish. The observer should carefully assess the shape, size, and bile staining of any larvae or helminth eggs present. The intensity of infection, measured as eggs per gram of stool (EPG), is calculated by multiplying the number of eggs observed by 24 [17]. The Kato-Katz method is particularly effective for detecting *Ascaris lumbricoides* and *Trichuris trichiura*, though it is less sensitive for hookworm detection due to the eggs' fragility over time [12]. This method is widely used in field surveys for epidemiological studies due to its affordability and the minimal technical expertise required [9]. Despite its limitations, this method is still prevalent in field surveys due to its affordability and the minimal expertise required [19].
- **Formol-Ether Concentration Technique**: The Technique is a widely used method in specialized labs for diagnosing soil-transmitted helminths (STHs) [20]. Its key advantage is its speed and ability to concentrate various types of fecal parasites. This technique works with both fresh and preserved stool samples. Formol is used to inactivate pathogens, reducing the risk of infections in the laboratory [21]. Samples can be stored using sodium acetate-acetic acid-formalin (SAF) or diluted formalin for future analysis [22]. Over time, the method has been refined. In the Ridley

modification, feces are mixed with formol water, strained to remove larger particles, and then ether or ethyl acetate is added. The mixture is centrifuged, causing parasitic elements like cysts, oocysts, eggs, or larvae to settle as sediment, while debris remains suspended. The sediment is then examined under a microscope to detect and quantify the parasites [23].

- **Baermann technique:** The technique is used when the target diagnostic stage is a first-stage larva rather than an egg. It is considered one of the simplest morphology-based parasitological tests to perform and interpret [24].
- **Stoll's Dilution Egg-Counting Technique:** This technique is designed for counting eggs of nematodes and trematodes. This method is fast, cost-effective, and allows for egg quantification. It involves diluting 3 grams of feces in water or sodium hydroxide (for formed stool), shaking the mixture, and examining a small sample under a microscope. The egg count is then multiplied by 100 to determine the number of eggs per gram of feces [24].
- **Water Emergence Technique:** This involves making a hole in fresh stool, filling it with warm water, and incubating it at 35–37 °C for up to 3 hours. Larvae migrate into the water and are examined microscopically. This affordable method is suitable for resource-limited settings [24,25].
- **McMaster Method**: This method quantifies nematode infections by counting floating eggs in a chamber, offering higher sensitivity than the Kato-Katz technique, the McMaster was found to be more sensitive and provided accurate efficacy results [26,27].
- **Flotation techniques:** These use solutions with higher specific gravity than parasites, allowing the organisms to float while debris sinks to the bottom [24,27].

Stool culture

The Koga agar plate culture is primarily utilized for cultivating *Strongyloides stercoralis*. In this method, a stool sample is innoculated on to the agar plate and incubated in a humid chamber at room temperature. After incubation, the plates are examined under a light microscope to identify the presence of *S. stercoralis* larvae. As the larvae move across the agar, they transport bacteria, which form visible tracks on the surface. The plates are then washed with a 10% acetyl-formalin solution, and the resulting liquid is centrifuged. The sediment is subsequently examined under a microscope [28]. This technique effectively distinguishes *S. stercoralis* larvae from those of other hookworms and is more sensitive than direct smear and fecal concentration methods [25,28].

The Harada-Mori technique

This was introduced in 1955. It utilizes filter paper onto which fecal material is applied and then placed in a test tube. The filter paper is kept moist by continuously adding water, creating ideal conditions for the hatching of ova and the development of larvae. Over time, several modifications to this method have been developed. This technique is straightforward and most effective when fresh fecal samples are used [29].

Centrifugation floatation

The merthiolate-iodine-formaldehyde concentration (MIFC) technique is a centrifuge-based method primarily used to detect protozoan parasites, although it is less sensitive for detecting other helminthes [30,31]. The process uses MIF solution, prepared by mixing 50 mL of 37% formaldehyde, 10 mL of 87% glycerin, and distilled water to make 1 liter. This solution acts as both a preservative and a stain, with potassium iodide (2 g in 10 mL distilled water) added for staining. Ether is used to dissolve fats in the stool sample. The MIF-preserved specimen is shaken vigorously, strained through gauze into a 15 mL centrifuge tube, and mixed with ether. After a brief rest period, the sample is centrifuged for 5 minutes at 1500 rpm, resulting in four distinct layers: ether, fecal debris, formalin, and sediment. The sediment, containing protozoa and helminth eggs, is carefully separated and a drop is placed on a glass slide for microscopic examination. Preparing a specimen with the MIFC technique takes about four minutes [25,31,32].

FLOTAC Techniques for Detecting Helminths Eggs : FLOTAC Techniques are advanced methods for detecting helminth eggs that combine flotation, centrifugation, and translation in a single apparatus [31,32]. FLOTAC is highly sensitive due to the larger stool sample size (about 24 times more than the Kato-Katz technique) and can analyze preserved samples for up to 83 days [33]. It is less time-consuming compared to Kato-Katz.

Sero-diagnosis

Traditionally, diagnostic methods for soil-transmitted helminths (STHs) have focused on identifying parasitic elements like eggs or larvae in stool samples. Recently, some studies have explored the use of enzyme-linked immunosorbent assays (ELISA) to detect copro antigens. These assays capture excretory/secretory (E/S) proteins from parasites using rabbit anti-E/S polyclonal antibodies, proving effective for diagnosing infections like *Strongyloides stercoralis* and hookworms [34,35]. However, antigen detection methods are less common for STHs compared to their application for other parasites, such as Plasmodium and protozoa [34,35].

Molecular (Nucleic acid–based method)

Advances in molecular diagnostics, including polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP), and quantitative real-time PCR (qPCR), have revolutionized STH diagnosis. These techniques offer rapid and precise quantification of STH eggs, with superior sensitivity that aids in monitoring treatment and control strategies [36].

The molecular targets used mainly are ITS-1, ITS-2, 18S, etc., Several different polymerase-chain reaction (PCR) based methods are available, i.e., conventional PCR, quantitative PCR (qPCR) and multiplex PCR reaction [36,37]. Droplet digital PCR, a third-generation technology, was developed for the absolute quantification of specific genes, including those of pathogens. Various PCR assay protocols, such as single, nested, real-time qPCR, and multiplex PCR, have been designed, allowing the selection of a method based on the target and resources. PCR techniques offer high sensitivity and specificity, requiring minimal starting DNA material.

Loop-mediated isothermal amplification (LAMP) is a novel technique that amplifies DNA with high specificity and efficiency under isothermal conditions of 60–65°C, using two to four sets of primers and a strand-displacement DNA polymerase. This method results in the accumulation of large amounts of target DNA, surpassing the specificity and sensitivity of PCR [9,42]. LAMP is a cost-effective alternative, as it uses a water bath instead of an expensive thermocycler, making it ideal for low- and middle-income countries (LMIC) focused on reducing STH-related morbidity [42].

Multiplex qPCR allows the simultaneous quantification and detection of multiple DNA sequences using various primer sets. Over the past decade, several multiplex qPCR assays have been developed for detecting STH infections, with some capable of identifying up to eight gastrointestinal parasites using species-specific primers/probes. Given the global focus on STH control and elimination in LMIC, there is a critical need for diagnostic assays that offer high sensitivity, specificity, reproducibility, and are suitable for limited-resource settings [45].

Advantages and limitations of microscopic and molecular techniques for diagnosis of soil-transmitted helminths

Advantages and disadvantages of various microscopic-based diagnostic techniques to detect STH's.

Microscopy-based techniques are simple and low-cost, but their sensitivity is affected by several factors, including intermittent excretion of parasite ova, low infection intensity that limits the detection, inappropriate transportation or sample storage [46,47].

Microscopy-Based Techniques	Advantages	Disadvantages	References
Direct Wet Mount Microscopy	Inexpensive, simple, and capable of detecting motile trophozoites.	Low sensitivity	[48,49]
Formol-Ether Concentration (Fec)	Inexpensive and crucial for detecting helminth ova.	Centrifugation is required, and this method does not detect unembryonated Ascaris spp. eggs.	[48,50]
Kato-Katz	WHO-recommended, gold standard, cost-effective, and capable of assessing the burden of infection.	Low sensitivity, unable to detect infections with low intensity.	[46,51]

Mcmaster	Affordable and simple to use, it has been widely utilized in human studies to assess anthelmintic cure rates. It is also highly sensitive for detecting low-intensity soil-transmitted helminth infections.	Requirement for a specialized counting chamber	[46,52]
Flotac	Simultaneous detection of various STHs, particularly in cases of low-intensity infections.	The process is intricate, requiring a specialized device and centrifugation with two distinct types of rotors..	[52,53]
Mini-Flotac	Simultaneous detection of various STHs.	The method is intricate, requiring specialized equipment, and it has reduced sensitivity for diagnosing Ascaris lumbricoides.	[46,52]
Baermann Technique	The method shows the highest sensitivity for detecting S. stercoralis larvae, making identification easier. It is nearly four times more sensitive than the Faecal Egg Count technique.	The process is time-consuming, necessitates a large volume of stool, and requires a fresh sample.	[46,54]
Fecpak^{g2}	Simultaneous detection of different STHs is a straightforward procedure that can yield results within an hour.	An internet connection is necessary	[52,53]

Table 1: Summarizes the advantages and disadvantages of several microscopy-based diagnostic methods for STH's diagnosis [55].

Advantages and disadvantages of molecular-based diagnostic techniques to detect STH's

Molecular approaches are increasingly utilised in monitoring and surveillance as they show higher sensitivity. Their capability to differentiate hookworm species is an advantage over the microscopic based technique, but despite their advantages, molecular techniques face challenges, such as the need for sophisticated laboratory infrastructure, trained personnel, and higher costs compared to microscopy. Standardization of protocols and quality control measures are essential to ensure reliable results across different settings. Additionally, the interpretation of molecular data requires bioinformatics expertise, highlighting the importance of capacity building and collaboration in implementing these methods effectively. Table 2 shows the advantages and limitations of various molecular tools used for STH detection.

Molecular Based Techniques	Advantages	Disadvantages	References
Conventional Pcr	The method is fast, easy to implement, and highly sensitive. It can detect mixed infections and is effective with both fresh and preserved fecal samples, all at a low cost.	Results need to be visualized using gel electrophoresis.	[56,57]
Real-Time Pcr	It allows simultaneous screening of multiple samples with enhanced sensitivity for all soil-transmitted helminths (STH). The process is fully automated, eliminating the need for gel electrophoresis, and provides quantified results.	It requires costly consumables, making it impractical for resource-limited settings. A high-tech laboratory is necessary, and optimizing qPCR efficiency can be difficult.	[58,69]

Dpcr	The method is fast, accurate, and highly sensitive. It effectively detects drug-resistant or high-pathogenicity sub-populations and can identify even low quantities of A. lumbricoides eggs.	The machines and reagents are costly	[60]
Lamp	It offers excellent sensitivity and specificity, is cost-effective, and features a straightforward procedure.	A precise and simple DNA extraction method is needed to avoid polyphenols in fecal samples that can inhibit DNA polymerase.	[46,61]

Table 2: [55].

The need for progression of Diagnosis of Soil Transmitted Helminths in 21st century

Soil-transmitted helminths (STHs) pose significant health risks, particularly in resource-limited settings where sanitation and hygiene standards are suboptimal [55]. There is no doubt on how conventional diagnostic methods, such as microscopy, have been the cornerstone of STH detection for decades. But however, advancements in molecular techniques of STH diagnosis have revolutionized the field, offering greater sensitivity, specificity, and the potential for multiplexing [52,55].

In the 21st century, the progression of diagnosing Soil Transmitted Helminths (STH is very important for several reasons):

- **Accuracy**: Traditional diagnostic methods like stool microscopy have limitations in terms of sensitivity and specificity. The rapid, highly sensitive properties of qPCR make it suitable for diagnosing STH over an insensitive and labor-intensive age old conventional methods. Many studies have compared the sensitivities of both methods. According to these studies, for *Ascaris lumbricoides*, microscopy had a sensitivity of 70–88% while molecular tests had a sensitivity of 85–100%; for hookworms, sensitivity of microscopy was reported 30–88% versus molecular techniques with sensitivity of 75–100%; for *Strongyloides stercoralis*, sensitivity of microscopy was 16–50% versus molecular techniques 76–93%; for *Trichuris trichiura*, microscopy had the sensitivity of 88% while molecular techniques had the reported sensitivity of 100% [58]. Advancements in diagnostic techniques can offer more accurate identification and quantification of STH infections, reducing false negatives and positives [55].

- **Early Detection**: Improved diagnostic methods can enable early detection of STH infections, allowing for prompt treatment and prevention of complications. Early intervention is particularly crucial in children, as STH infections can impair growth, cognitive development, and school performance. In early-stage infections, parasite eggs or larvae may not appear in stool, but however antibody assays and polymerase chain reaction (PCR) can aid in the diagnosis [9,55].

- **Monitoring and Surveillance**: Advanced diagnostic tools facilitate better monitoring of STH prevalence and transmission dynamics. This data is essential for designing targeted intervention strategies and assessing the impact of control programs, for example DNA extraction platforms and nucleic acid detection methods are two important molecular approaches to diagnosis and surveillance of STH [52].

- **Drug Resistance Monitoring**: With the widespread use of anthelmintic drugs in mass deworming programs, there's a growing concern about the development of drug-resistant STH strains. Advanced diagnostic methods can help in monitoring drug efficacy and detecting emerging resistance patterns. For example the microscopic-based Kato-Katz has low sensitivity in the detection, and also serology-based assays can be more sensitive but are parasite species-specific. But however, the highly sensitive PCR methods have been developed to be multiplexed to allow multi-species detection [55].

Conclusion

The transition from microscopy to molecular techniques for diagnosing soil-transmitted helminths (STH) in the 21st century marks a significant advancement in the field of parasitology and public health. While microscopy has been the gold standard for many years, molecular methods offer enhanced sensitivity, specificity, and efficiency in detecting STH infections. This progression has revolutionized our understanding of the prevalence, distribution, and transmission dynamics of STH, leading to more targeted and effective control and elimination strategies. As we continue to refine and implement these molecular tools, we move closer to achieving the goal of global STH control and eventual eradication, ultimately improving the health and well-being of millions worldwide.

Recommendations

One key recommendation for the progression of diagnosis of soil-transmitted helminths (STH) from microscopy to molecular techniques in the 21st century is to ensure widespread adoption and integration of molecular methods into existing diagnostic protocols. In a way to achieve this adoption, the following can be done through capacity building, infrastructure development, integration into surveillance and control programs and research and innovation.

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