Prevalence, risk factors, challenges, and the currently available diagnostic tools for the determination of helminths infections in human

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Abstract
Soil transmitted helminthes (STH) are among the major public health issues in tropical and sub-tropical countries infecting more than 2 billion humans worldwide. STH causes considerable morbidity in children, affecting their cognitive development and physical growth. Endemic areas with poor sanitation and limited access to good quality water supply have the highest burden of STH infection. Various approaches to reduce and control the worm infections include the intermittent deworming of children with anti-helminthic drugs. Individual patient management and population based studies can only be successful upon using the diagnostic techniques with high accuracy. The lack of reliable tools for the provision of correct diagnostic results about the status and intensity of infection is a major challenge, as these factors varies considerably in areas of low infection intensities. The techniques currently available for the diagnosis of parasitic infections show limited specificity and sensitivity and as such several techniques in combination are normally used in diagnosing the variety of parasite species infection. This review article was planned to study the prevalence, risk factors, and the available diagnostic techniques for soil and water transmitted helminthes infections in humans. This article also discussed the challenges in diagnosis, treatment, and management of worm infections particularly helminthes. The articles available online on important portals like google scholar, PubMed, Digital Libraries, PakMediNet, Science direct, and the Directory of Open Access Journals (DOAJ) were searched for inclusion of the data in this review study. Duplicate studies and irrelevant reports describing the general aspects of parasite infections were excluded. This review study provides a comprehensive report on the prevalence, accurate diagnostic, and chemotherapeutic protocols to reduce the burden of worms infections. WHO suggests the chemotherapy for worm infections as feasible and cost effective strategy in schools due to the lack of proper policy for the prevention of intestinal worms.

Keywords
intestinal worms, parasite, soil transmitted helminthes, worm infestation

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Introduction

Intestinal worm infection is one among the major health problems throughout the world particularly in developing countries.\(^1\) Intestinal worms live and feed off another organism.\(^2\) Most of the infected children may carry different types of parasites that can live throughout the body and may infect the gastrointestinal tract.\(^3\) Adult worms lay eggs, the larvae hatched, grow, and developed into adult worm inside human body.\(^4\) *Taenia saginata* (Tap worm), *Enterobius vermicularis* (Pin worm) and *Trichuris trichiura* (Whipworm), *Ascaris lumbricoides* (roundworms), *Necator americanus* (Hookworms), and *Ancyclostoma duodenale* are among the most common helminths, each parasitizing hundreds of millions of people.\(^5\) Transmission of intestinal parasites is usually through contact with infected feces or ingestion of undercooked meat, drinking infected water, contaminated soil, and skin absorption.\(^6\) When unwashed, uncooked, contaminated food is ingested by mouth then parasite travels into intestine and reproduces itself causing symptoms by their toxicity like abdominal pain, diarrhea, vomiting, fatigue, dysentery, weight loss, muscle pain, skin irritation, sleep problem, and passing of worms in stool.\(^7\) In children, its rate is high due to soil transmission and nail biting.\(^8\) According to the World Health Organization (WHO) estimates, 870 million children live in the area of high prevalence. The most affected regions of the world include Africa, South Asia, and South America.\(^9,10\) Children with long/untrimmed nail, failure to wash hands before meals, bare footed, nail biting habit, and sucking of thumb have significant association with parasitic infection.\(^11\) School going children are more prone to develop intestinal worm’s infestation while playing in school lawns. Their hands become contaminated with infected soil and get infected when they put contaminated fingers in their mouth.\(^12,13\) The adult people are also at risk due to drinking contaminated water with parasites that colonize the gastrointestinal tract.\(^14\) Children using tube well water for drinking has significantly higher prevalence of intestinal worms than using tap water.\(^11,15\) Prevalence of intestinal worm infection is significantly lower in children using soap and water before meal and after defecation than children who just wash their hands by water.\(^16\) The prevalence of intestinal worms is 59.2% due to nail biting.\(^11\) Malnutrition in school-age children is common which leads to nutritional stress, adverse health, worse school performance, and cognitive impairment.\(^17\) Diagnostics play important role in the guidance for both deploying the existing resources for STH program, and the strategies for the implementation and evaluation of STH intervention. Currently used detection and quantification methods for STH-specific eggs have practical advantages of using inexpensive test kits and can easily performed in low-resourced field settings. The most common example is the Kato Katz method. However, these methods also have significant disadvantages poor reproducibility, lower than optimal sensitivity and moderate labor costs.\(^18\) There is a wide variety of intestinal worms but two most commonly used tests for diagnostic purpose are performed from stool sample and adhesive tape may be applied in order to find eggs of worms.\(^19\) The main aim of this review is to evaluate the prevalence of water and soil transmitted helminths (STH) and to provide an insight regarding risk factors, challenges and the available diagnostic techniques for parasitic infestations.

Literature search and inclusion criteria

For this general review, the articles available online on important portals like google scholar, PubMed, Digital Libraries, PakMediNet, Science direct, and Directory of Open Access Journals (DOAJ) were searched using the key words: diagnostics, intestinal helminths, water and soil-transmitted helminths, risk factors, challenges, and the diagnostic techniques used for determining the parasitic infection were included. The examples of diagnostic methods for parasitic infections are Kato-Katz method, formol ether floatation method, McMaster method; merthiolate-iodine-formaldehyde, sodium nitrate flotation, and the polymerase chain reaction (PCR). Textbooks information about parasitic infections are also included in this review.

Prevalence of STHs infections

World Health Organization (WHO) estimates that the major soil transmitted helminths contribute 5.18 million disability adjusted life years all around the world in 2010 and these main STHs include the hookworms (*Necator americanus* and *Ancyclostoma duodenale*), the whipworm (*Trichuris trichiura*), and the roundworm (*Ascaris lumbricoides*). Globally, an estimated 440 million individuals are...
infected with hookworms, 460 million with whipworms and 820 million with round worms.20

Intestinal parasitic infections remain a major public health problem with vast socioeconomic devastation among vulnerable rural communities and occurring predominantly in developing countries.21,22 According to 2014 WHO report, over 3.5 billion people were infected with intestinal helminths mainly by hookworms, T. trichiura, A. lumbricoides, H. nana, S. stercoralis, and T. saginata.23 Helminth infections grouped under geohelminthic; hook worm, T. trichiura and A. lumbricoides are extensively distributed in East Asia, China, the Americas, and sub-Saharan Africa.24,25 Geohelminthic infections affect over 2 billion people worldwide and 2/3rd of African countries having high risk areas has the prevalence of over 50%.26 A. lumbricoides is still one of the most prevalent IPIs with worldwide cases of 807 to 1221 million particularly in East Asia and sub-Saharan Africa. Additionally, the whipworm (T. trichiura) and hookworm are liable for 604 to 795 million and 576 to 740 million infections, respectively.27 WHO stated that worldwide 2.4 billion people do not have access to improved sanitation facility and 1.1 billion people do not have access to improved water supply sources, whereas approx. 2 billion people are infected with STHs. Consequently, about 2 million people, mostly children less than 5 years age die annually due to diarrheal diseases, and the populations of developing countries are the most affected ones, living under poverty, in poor health conditions and rural populations or periurban dwellers.28

Hookworm infections, in addition to Malaysia (21.0%), Bangladesh (22.3%), Nepal (30.7%), and Papua New Guinea (60.6%), remain common throughout sub-Saharan Africa. Contrary to that, hookworm infections are not found in North Africa and most of the Central Asia, Except Egypt with 6.0% prevalence. The prevalence of T. trichiura infections is 49.9% in Malaysia and 45.5% in Philippines as well as in Central America (28.4% in Venezuela to 5.1% in El Salvador) and Central Africa (38.8% in Equatorial Guinea to 11.8% in Central African republic). Figure 1 below shows the global distribution of the prevalence of each STH infection.29

It was found that of the three STH infections, A. lumbricoides have the widest distribution with increased transmission rates seen in Nigeria (25.4%), Cameroon (30.8%), and northwestern countries of Central sub-Saharan Africa (38.8% in Equatorial Guinea to 32.2% in Congo). This infection is found dispersed geographically among the southern countries of Central Latin America (Ecuador 35.8%, Colombia 26.0%, and Venezuela 28.4%) and Asian countries (the Philippines 33.6%, Afghanistan 36.0%, Malaysia 41.7%, and Bangladesh 38.4%).29 Global estimates suggest that over 568 million school aged children and 270 million pre-school children are included among the nearly 1.5 billion people globally infected with STHs. All these infected people are in need of treatment and prevention interventions. People carrying light intensity infections are usually asymptomatic whereas high morbidity are observed in people harboring heavy infections. Thus, variety of non specific and specific adverse effects such as cognitive impairment in children and reduced physical growth are the debilitating outcomes from the heavily infected people, as well as intestinal occlusion and anemia. Recent estimates suggest that four STHs namely the hookworms (Necator americanus and Ancylostoma duodenale), Trichuris trichiura and Ascaris lumbricoides infect over 480, 508, and 700 million people worldwide, respectively.29 In tropical countries, the highest prevalence is recorded with a total number of annually estimated deaths higher than 135,000. Crowded households are the more common source of clusters of infections.31 The transmission of STHs depend on the three principal conditions such as fecal contamination of soil, favorable soil conditions for the survival of eggs/ larvae, direct oral infestation or skin contact with contaminated soil and through the consumption of contaminated water and/or food.32 School going children (5–15 years) and the pregnant women are the most vulnerable groups for STHs infections. Endemic countries with limited healthcare facilities, absence of portable water and inadequate sanitary conditions are reported to have higher STHs infection.33 Prevalence data of intestinal worm infection in different countries is given in Table 1.

The overall prevalence rate of intestinal worms in different cities of Pakistan is increasing. Prevalence and intensity of intestinal worm infections are very high in rainy season while low in winter whereas, the infection rate is high in males compared to females that happen due to the suppression of immune system and the stimulatory effect of estrogen on immune response against GIT. Khan et al. investigated the prevalence of intestinal
Figure 1. The global distribution of STH infection prevalence by STH species. (a) Hookworm, (b) *Ascaris lumbricoides*, and (c) *Trichuris trichiura*; based on geostatistical models for sub-Saharan Africa and available empirical information for all other regions.
### Table 1. Prevalence data of intestinal parasites.

| Country         | Study location | Type of infection               | Percentage | Reference                        |
|-----------------|----------------|---------------------------------|------------|----------------------------------|
| Africa          | Tanzania       | Hookworms                        | 25.2%      | Mazigo et al.34                  |
|                 |                | Ascaris lumbricoides             | 1.6%       |                                  |
|                 |                | Enterobius vermicularis          | 0.67%      |                                  |
|                 |                | Trichuris trichiura              | 0.79%      |                                  |
|                 |                | Schistosoma mansoni              | 5.6%       |                                  |
|                 |                | Taenia species                   | 0.57%      |                                  |
|                 | Sub Saharan Africa | Hymenolepis nana                     | 13.8%      | Gelaw et al.35               |
|                 |                | Entamoeba histolytica            | 9.2%       |                                  |
|                 |                | Ascaris lumbricoides             | 5.9%       |                                  |
| Cuba            | Matanzas       | Ascaris lumbricoides             | 7%         | Cañete et al.36                  |
|                 |                | Trichuris trichiura              | 3%         |                                  |
|                 |                | Enterobius vermicularis          | 2%         |                                  |
|                 |                | Taenia species                   | 1%         |                                  |
| Egypt           | Damanhur       | Ascaris lumbricoides             | 14%        | Hegazy et al.37                  |
|                 |                | Enterobius vermicularis          | 3.4%       |                                  |
|                 |                | Ancyclostoma duodenale           | 5%         |                                  |
|                 |                | Hymenolepis nana                 | 0.2%       |                                  |
| Ethiopia        | Gambo area     | Ascaris lumbricoides             | 7.8%       | Wegayehu et al.38               |
|                 |                | Hymenolepis nana                 | 0.6%       |                                  |
|                 |                | Hookworms                        | 4.9%       |                                  |
|                 |                | Schistosoma mansoni              | 0.12%      |                                  |
|                 |                | Taenia species                   | 2.9%       |                                  |
|                 |                | Trichuris trichiura              | 2.8%       |                                  |
|                 | North Gondar   | Ascaris lumbricoides             | 48%        | Asrat et al.39                   |
|                 |                | Giardia lamblia                  | 41.9%      |                                  |
|                 |                | Entamoeba histolytica            | 27.3%      |                                  |
|                 |                | Schistosoma mansoni              | 15.9%      |                                  |
|                 |                | Hookworm                        | 11.5%      |                                  |
|                 |                | Trichuris trichiura              | 7%         |                                  |
|                 |                | Hymenolepis nana                 | 6.8%       |                                  |
|                 |                | Taenia species                   | 5.3%       |                                  |
|                 |                | Enterobius vermicularis          | 2.6%       |                                  |
|                 | Jawe Woreda    | Hookworm                        | 21.1%      | Hailu41                          |
|                 |                | Schistosoma mansoni              | 3.5%       |                                  |
|                 |                | Ascaris lumbricoides             | 3.9%       |                                  |
| India           | Pulwama        | Ascaris Lumbricoides             | 69.84%     | Wani and Ahmad42                 |
|                 | Chennaei, Tamil Nadu | Hymenolepis nana                         | 1.1%       | Rayan et al.43                |
|                 |                | Ascaris lumbricoides             | 21%        |                                  |
|                 |                | Trichuris trichiura              | 8%         |                                  |
|                 | Srinagar, Indian Occupied Kashmir | Ascaris lumbricoides                   | 28.4%      | Wani et al.44                   |
|                 |                | Giardia lamblia                  | 7.2%       |                                  |
|                 |                | Trichuris trichiura              | 4.9%       |                                  |
|                 |                | Taenia saginata                  | 3.7%       |                                  |
|                 | Mangalore      | Taenia saginata                  | 4.37%      | Hart and Hart4                   |
|                 |                | Hymenolepis nana                 | 0.38%      |                                  |
|                 |                | Trichuris trichiura              | 0.08%      |                                  |
| Iraq            | Baghdad        | Hymenolepis nana                 | 1.17%      | Al-Taie45                        |
|                 |                | Enterobius vermicularis          | 0.38%      |                                  |
|                 |                | Ascaris lumbricoides             | 8.21%      |                                  |
| Kenya           | Nairobi        | Trichuris trichiura              | 4.6%       | Mwanthi et al.46                 |
|                 |                | Hookworm                        | 0.1%       |                                  |
|                 |                | Schistosoma mansoni              | 0.4%       |                                  |
| Nepal           | Kaski          | Ascaris lumbricoides             | 2.1%       | Chandrashekhar et al.37         |
|                 |                | Trichuris trichiura              | 1.3%       |                                  |

(Continued)
| Country       | Study location     | Type of infection          | Percentage | Reference                  |
|--------------|--------------------|----------------------------|------------|----------------------------|
| Nigeria      | Ilaje              | 
|              |                    | Hymenolepis nana           | 1.6%       |                            |
|              |                    | Necator americanus         | 0.4%       |                            |
|              |                    | Enterobius vermicularis    | 1.0%       |                            |
|              | Ilkoyl             | 
|              |                    | Ascaris lumbricoides       | 41.27%     | Awolaju and Morenikeji58   |
|              |                    | Trichuris trichiura        | 1.0%       |                            |
|              | Aragan (Araromi)   | 
|              |                    | Hymenolepis nana           | 1.7%       |                            |
|              |                    | Taenia saginata            | 1.0%       |                            |
|              | Oke –Omiru         | 
|              |                    | Ascaris lumbricoides       | 39.58%     |                            |
|              | Cappa              | 
|              |                    | Ascaris lumbricoides       | 38.46%     |                            |
| Pakistan     | Muzaffarabad       | 
|              |                    | Ascaris lumbricoides       | 3.8%       | Chaudhry et al.49          |
|              |                    | Hookworm                   | 2.4%       |                            |
|              |                    | Enterobius vermicularis    | 1.3%       |                            |
|              |                    | Trichuris trichiura        | 1.0%       |                            |
|              |                    | Hymenolepis nana           | 1.7%       |                            |
|              |                    | Taenia saginata            | 1.0%       |                            |
|              |                    | 
|              | Bannu              | 
|              |                    | Ascaris lumbricoides       | 15.0%      | Khan et al.50              |
|              |                    | Enterobius vermicularis    | 12.0%      |                            |
|              |                    | Hymenolepis nana           | 10.0%      |                            |
|              |                    | Taenia saginata            | 7.0%       |                            |
|              |                    | Ancyclostoma duodenale     | 1.0%       |                            |
|              | Rural Peshawar     | 
|              |                    | Round worm                 | 45.5%      | Ullah et al.51             |
|              |                    | Pin worm                   | 4.0%       |                            |
|              |                    | Hook worm                  | 3.5%       |                            |
|              |                    | Whip worm                  | 3.5%       |                            |
|              |                    | Hymenolepis nana           | 8.0%       |                            |
|              |                    | Taenia saginata            | 1.5%       |                            |
|              | Muzaffarabad       | 
|              |                    | Giardia lamblia            | 11.8%      | Chaudhry et al.49          |
|              |                    | Ascaris lumbricoides       | 3.8%       |                            |
|              |                    | Hookworm                   | 2.4%       |                            |
|              | Quetta             | 
|              |                    | Hymenolepis nana           | 34.0%      | Ahsan-ul-Wadood et al.52   |
|              |                    | Giardia lamblia            | 32.0%      |                            |
|              |                    | Entamoeba histolytica      | 29.0%      |                            |
|              |                    | Ascaris lumbricoides       | 4.0%       |                            |
|              |                    | Ancyclostoma duodenale     | 1.0%       |                            |
|              | Sukkar             | 
|              |                    | Giardia lamblia            | 36.19%     | Shaikh et al.53            |
|              |                    | Entamoeba histolytica      | 18.57%     |                            |
|              |                    | Hymenolepis nana           | 16.19%     |                            |
|              |                    | Ascaris lumbricoides       | 14.76%     |                            |
|              |                    | Ancyclostoma duodenale     | 7.24%      |                            |
|              |                    | Enterobius vermicularis    | 4.19%      |                            |
|              |                    | Trichuris trichiura        | 1.81%      |                            |
|              |                    | Taenia saginata            | 1.05%      |                            |
| Sudan        | Elengaz area, Khartoum | 
|              |                    | Hymenolepis nana           | 26.4%      | Gabbad and Elawad54        |
|              |                    | Taenia saginata            | 8.6%       |                            |
|              |                    | Enterobius vermicularis    | 6.2%       |                            |
|              |                    | Schistosoma mansoni        | 4.4%       |                            |
| United Arab | Sharjah            | 
| Emirates     |                    | Entamoeba histolytica      | 71.8%      | Dash et al.55              |
| Vietnam      | Southern Vietnam   | 
|              |                    | Giardia lamblia            | 17.5%      | Verle et al.56             |
|              |                    | Hookworm                   | 52.0%      |                            |
|              |                    | Trichuris trichiura        | 50.0%      |                            |
|              |                    | Ascaris lumbricoides       | 45.0%      |                            |
|              |                    | Giardia lamblia            | 3.0%       |                            |

worm infection in primary school children (5–10 years age) living in district Bannu, Pakistan. They found that 54% of the children in the study population have infection with seven types of intestinal worms including Ancyclostoma duodenale, Giardia lamblia, Entamoeba histolytica, Taenia
The prevalence data of each worm infection is given in Table 1. Most common infection was *Ascaris lumbricoides* (15%) and least infection was *Ancyclostoma duodenale* (1%). Abdominal discomfort and pain was the most common clinical presentation in children suffering from intestinal worm infection. Other complaints were cough, anemia, itching, urticaria, vomiting, bloody diarrhea, and diarrhea in percentage of 7.41%, 18.52%, 9.26%, 3.7%, 7.4%, 3.7%, and 12.9%, respectively. Mehraj et al. reported that helminthiasis is endemic worldwide. Humid and hot tropical climate, lack of access to potable water, poor hygiene, illiteracy, and poverty are the major risk factors for intestinal worm infections.

**Risk factors**

The risk of acquiring STH infection cannot be attributed by only one factor but because of coexistence and amalgamation of multiple factors including environmental, behavioral, social, and biological factors both at individual and community level. These factors include the lack of personal hygiene, substandard living conditions and poverty. Studies in other tropical countries postulated that the rate of infection is influenced by the environment and the behavior of local residents. Potential risk factors explored were markers of social advantage (home ownership, occupation, education, and TV/radio ownership), demographic factors (ethnicity, place of residence either urban/rural, mother’s age, and gender), and the factors associated with lifestyle and housing (use of soap, place of cooking, domestic animals, method of waste disposal, water source, type of toilet, and roof type). Poor personal hygiene, inadequate availability of clean water, poor sanitation, low socioeconomic status, and the rural areas are the major risk factors for STH infection. Densely populated home environment, dirty playground, and the poor hygiene of child and the mother or caregiver are also the risk factors for STH infection in preschool children.

**Challenges**

The control of STHs involves several challenges, among which few are highlighted. First challenge for the control of STH is the proper mobilization of funds required for successful action. In contrast to some of the neglected tropical diseases (NTDs) with visible morbidity like lymphatic filariasis (LF) and onchocerciasis which have raised funding successfully for implementing the control activities and globally little funds are available for implementing the control of STH alone. Therefore, it is advantageous for the endemic countries to avail the opportunities generated by the impetus and new partnership for NTD control resulting from the growing financial resources all over the world.

Second challenge is the expansion of the coverage of regular deworming as a public health intervention to approach the individuals at risk of morbidity caused by STH infections. In context of multidisease integrated control, the integration of helminth control within community directed interventions in sub-Saharan Africa is taking advantage of the long experience, LF control programs and gains of onchocerciasis through the establishment of community directed treatment with ivermectin (CDTI) in all countries supported by the African Programme for Onchocerciasis Control—APOC. Yet, STHs are more widely distributed in most of the countries than both the LF and onchocerciasis. Hence, the challenge will be to expand the MDA coverage in areas with no CDTI.

The third challenge is the failure of the cost-effective anti-helminthic drugs in achieving the desired results because of inadequate waste drainage system, inaccessibility of safe water, poor sanitation, and favorable climate for intestinal parasitic infection transmission in developing countries. The fourth challenge is the proper monitoring and evaluation of diagnostic tools. Regular monitoring and evaluation is crucial for ensuring the efficient implementation of the programs providing maximum benefits to the beneficiaries. This is particularly challenging providing the commonly used diagnostic techniques with low sensitivity in low transmission zones resulting in significant increase in number as far as programs are successful. Therefore, it is required to develop and validate alternative diagnostic techniques to address this issue.

Some other important challenges for the control of STHs infections are the sustainability of STH control programs, implementation of operational research, strengthening the institutional capacity and partnership, and the development of new drugs. Therefore, the long term viability of control...
Program is based on strengthening the health system. Additionally, the reinforcement of the capacity of community ownership and the school system is required for the efficient participation of the communities and schools in disease control. Finally, the support for drug discovery and development along with operational research is essential to fulfill gaps and improving the implementations of the control. For example, to understand some key issues like epidemiology, assessment of morbidity, efficacy testing of drugs, and drug resistance monitoring is of vital importance. Surveillance of STH infections, improvement in water supply, preventive chemotherapy, and health education may be helpful in minimizing these challenges and further spread of infection.64

**Strategies for control**

The delegates of World Health Assembly crafted a resolution in 2001, which encouraged the member states to sustain and intensify their efforts for the elimination and control of STH and schistosomiasis infections. Furthermore, the Assembly also called the United Nations Organizations, Non Government Organizations, and bilateral agencies to strengthen their support to endemic countries for the control, prevention, and elimination of communicable diseases.

The control strategy of STH infection is geared towards controlling the morbidity throughout the series of deworming of people at risk in endemic areas. WHO considered the women of childbearing age (including breastfeeding women and women pregnant in the 2nd and 3rd trimesters), school and preschool age children, and adults involved in high risk occupations like teapickers, miners are vulnerable and at risk of STH infection.65

There are three main interventions or strategies for the control of STH infection, such as treatment with anthelmintic drugs, health education, and improved sanitation.66

Treatment with anthelmintic drug (also called deworming) aims in reducing the morbidity by decreasing the worm burden. Individuals residing in endemic areas and those without prior diagnosis are at risk and the recommended control strategy is the periodic medicinal treatment. WHO suggested once a year treatment administration in areas of more than 20% STH infection prevalence. However, twice a year treatment should be planned when the infection prevalence is more than 50%.67

The control strategy for preschool and school age children can be integrated with school health programs or child health days for efficient treatment administration. Deworming activities allow easy provision in the propagation of hygiene and health education of public health programs like improving sanitation and promotion of hand washing practices in schools, thus proven to be advantageous and a good entry point. With such strategy, in 2013 more than 368 million school age children in endemic countries have been treated with anthelmintic medicines. Mebendazole (500 mg) and Albendazole (400 mg) are the WHO’s drug of choice used as medicines for STH infections and are affordable, effective, easily accessible, and safe. These medicines are made available to all endemic countries through their health ministries being donated by WHO.65

In contrast to these, health education and improved sanitation aim to control the infection transmission and reinfection through reducing the water and soil contamination using the methods that usually cut across several sectors (e.g. infrastructure, health education, and veterinary services). These interventions have shown to be effective and sustainable.68

**Diagnostic tools for parasitic infections**

Several diagnostic tools are available and being used for the investigation of parasitic infections particularly STHs. Since a single “gold standard” test (with 100% accuracy) does not exist for detection of intestinal parasites. Some diagnostic techniques are briefly described below.

**Direct wet preparation**

The examination of feces by direct microscopy through wet preparation is essential for the detection of parasitic elements like the motile larvae of *Strongyloides stercoralis*. It is also used for the detection of high concentration of eggs of helminthes infection with *Ascaris lumbricoides*. It is rapid and inexpensive but not often used in control programs because it is only semi-quantitative. However, lower sensitivity of the direct wet mount technique has been reported for the detection of low intensity infection.69 It is routinely used for the detection of protozoan parasites like trophozoites of *Giardia lamblia*, *Entamoeba histolytica*, and
rarely *Balantidium coli*. A thin smear of fecal sample is examined under light microscope for the detection of eggs/larvae/trophozoites of parasite species. An iodine or eosin preparation is also required to identify the oocytes/cysts of intestinal protozoa. The wet mount method has been chosen for the routine diagnosis due to its easy use, cost effectiveness, and time saving compared to other techniques. The parasites detection rate using wet mount method in single stool examination is very limited because of its poor sensitivity.

### Kato-Katz technique

This is most widely used technique to determine the prevalence and intensity of STHs infection, and is recommended for the quantification of STH eggs in stool of human by the WHO. The advantages of this technique over the other copro-microscopic methods are high sensitivity, quantification of eggs and inexpensive with minimal infrastructure requirement.

### Formol-ether concentration method

This method is commonly used for the diagnosis of STHs infection and is performed in specialized laboratories. The formol used in this method inactivates the organism, thus minimizing the risk of laboratory acquired infection from fecal pathogen. It is a rapid method. This method is used for the diagnosis of STHs and intestinal protozoa. Its diagnostic sensitivity is greatly improved when used along with Kato-Katz method for helminthes infection. Formol-ether concentration method (FEC) method is highly sensitive than Kato-Katz method to diagnose the soil transmitted helminthes and *Taenia* species infections.

### Zinc sulfate flotation method

This method is used for concentrating the eggs of *Trichuris trichiura* but also cysts of *Entamoeba histolytica* and *Giardia lamblia*. The solution of zinc sulfate with specific gravity 1.180 to 1.200 is used in this technique. Fecal sample (1.0 gram) is emulsified in tap water followed by the removal of debris through straining. Then the sediment is suspended in ZnSO₄ solution (4 mL) following centrifugation. The cyst and eggs float on to the top after allowing the suspension for 30 to 45 min. The eggs/larvae are collected by placing the cover slip on the tube and then transferred to a glass slide for microscopic examination. A published study revealed that zinc sulfate flotation technique is advantageous over the centrifugal sedimentation method to significantly detect the eggs of light helminths like those of *E. vermicularis* and *T. trichiura* in faeces.

### Saturated sodium chloride flotation method

This is a very important and cost effective tool commonly practiced for concentrating the eggs of *A. lumbricoides* and *Hook worm*, particularly in field surveys. It works on the same principle as that of above described zinc sulfate technique but saturated solution of sodium chloride is used as flotation solution.

### FLOTAC techniques

The use of FLOTAC technique in diagnosing STHs in humans have been suggested in recent studies and these techniques have been extensively used in the fields of veterinary sciences. FLOTAC techniques are used for counting the helminthes egg in fecal sample both in veterinary and human. It has the possibility of testing up to 83 days old preserved stool samples. It has less time consuming and takes only about 12 to 15 min from preparation to microscopic analysis for detecting helminthes eggs. This technique is highly sensitive, precise, accurate, and cost effective to diagnose the helminth and protozoan infections in animals and humans. However, centrifugation is required in this technique, and hence might be out of reach in resource limited settings. The modified FLOTAC technique called Mini-FLOTAC technique also has high sensitivity for the diagnosis of protozoan and helminth infections in animals and human fecal and urine sample.

### Stoll’s dilution egg-counting method

Stoll’s technique is a rapid and inexpensive technique that offers the possibility of egg quantification. This technique uses 3 g of feces by making 15 times dilution with water in a screw cap container. In case of using formed stool sample, sodium hydroxide (0.1 mole/L) is recommended. The solution is homogenized in the screw capped container. With the help of Pasteur pipette, the suspension (0.15 mL) is transferred onto a glass slide and
examined microscopically after placing cover slip over it. The number of eggs per gram feces is quantified by multiplying the eggs counted by 100.84

**Quantitative fecal examination through McMaster method**

This technique is useful for the quantitative determination of nematode worm infection load which is expressed in eggs per gram feces. It is a fast method and can easily recover the debris free floating eggs that are loaded into a counting chamber.59 Several studies evaluated the sensitivity of this method. McMaster was found to be more sensitive for the detection of STHs when compared to Kato-Katz technique providing more efficacy and accurate results.85

**Antigen detection methods**

The so far described methods are based on detecting the parasitic elements (eggs/cysts/larvae) in stool sample. However, the use of enzyme linked immunosorbent (ELISA) assay capturing coproantigens has been reported in some published studies. The assay is based on the principle of assay is based on the principle of capturing the parasites excretory/secretory proteins by using specific polyclonal antibodies. Antigen detection methods are effective for the diagnosis of hookworm and *S. stercoralis* infections but have not been explored for the diagnosis of STH. However, immunological tests have been successfully used for the diagnosis of various protozoan and food borne helminth infections.86,87

**Polymerase chain reaction**

Currently the use of molecular methods with higher diagnostic precision, such as PCR, is recommended for morphologically indistinguishable species.58 PCR is a DNA based detection method with tremendous success in bacteriology and virology, and now efforts are made toward the use of PCR as the first line diagnostic tool for parasitic infection. PCR reactions involves the repetitive cycle of three steps including denaturation, annealing/primer binding, and amplification/extension by Taq DNA Polymerase.89 Molecular methods have high sensitivities and specificities and require very small amount of DNA as the starting material and with the major requirement is to know the target DNA sequence in order to design the primers of interest for amplification. DNA damage in stool samples, contaminants amplification, requiring well trained personnel, and the unavailability of infrastructure in low resource settings are some of the drawbacks of molecular methods.90 Studies have reported that both the qPCR and digital PCR have the ability to detect very small amount of *A. lumbricoides* with higher sensitivities. So, these techniques are very useful to control transmission in detecting STH infections particularly in low intensity infection settings. The multiplex qPCR method allows the simultaneous detection and quantification of several target DNA sequences. These methods use the combination of multiple primer sets for the amplification of DNA in real time. Several multiplex qPCR assays have been developed for STH infection detection in past decade.91 Global efforts toward the control and elimination of STH infection in LMIC urged the need of alternative diagnostic assays demonstrating the excellent reproducibility, consistency and high throughput92 or with high specificity and sensitivity but potentially inexpensive and can be used in limited resource settings.93

**Prevention of worms infection**

Many species of the intestinal helminths can be killed by treating with Albendazole or Mebendazole.94 This can only control the disease for a short time period in a target population and re infection occurs soon. For long term prevention, measures should be adopted to stop the transmission of intestinal worms.95 Proper washing of hands with soaps after defecation and before feeding the children should be done. Properly cooked and washed vegetables should be used. Proper water supply and improving the sanitation is not possible in short time span so patient should adopt those measures which can be practiced on personal behalf to protect themselves from intestinal worm infection.96 Soil-transmitted infections are among the most common infections causing abdominal pain, diarrhea, general malaise, weakness, and chronic intestinal blood loss. Literature review shows that the people with poor hygiene and the school going children are usually developing intestinal worms.97 Intestinal worm infestation is a matter of great concern especially for third world countries because these countries are overcrowded
with improper and poor sanitation system and water supply which are the root cause for this disease and favors the transmission of this infection.98 Almost 27% of entire school and preschool age children in the world need treatment with anti-helminthic drugs.99 Maintaining hygiene may decrease the rate of intestinal worms.100 According to World Health Organization, all those areas in which this disease is endemic, their people must adopt preventive therapy.94 If the risk of this infection is up to 20%, they should use preventive chemotherapy with anti-helminthic drug once a year and if the risk is more than 50%, they should adopt twice a year preventive chemotherapy.100 This review makes a case for standard school-based plan to administer chemotherapy to decrease the burden of intestinal worm infestation in school going children in Pakistan.

Conclusion and suggestions
Accurate diagnosis of parasitic infections is crucial for both the management of individual patient and population based studies like trials for drug efficacy and the surveillance programs for the control and elimination of parasitic diseases in human public health. The decision made for the diagnosis is dependent on clinical laboratory testing in clinical medicine. Several available techniques for the detection of STH infections differ significantly in their sensitivity, field applicability, simplicity, and the cost but still no gold standard technique with 100% accuracy exist. This is the need of the hour to make efforts in developing a gold standard test for the diagnosis of parasitic infections. The detection and identification of protozoans and helminthes can be challenging in developing countries; however, the occurrence of parasitic infection and higher positivity rate provide the basis for the enhancement of expertise in developing countries. Artifacts in stool samples show close resemblance with parasites, particularly to an inexperienced microscopist in reporting such structures as parasites. A successful control of STHs infections across high endemic areas call to strengthen health system and interventions, to ensure the access of drugs against helminthes in all health services, co-implementation, and the synchronized use of different anthelminthic drugs, health education, adequate sanitation, promotion of access to safe water, and the resource mobilization to sustain control activities.

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