

The Ethiopian SORT IT Course

Diagnostic sensitivity of direct wet mount microscopy for soil-transmitted helminth infections in Jimma Town, Ethiopia

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Abstract

Introduction: Soil-transmitted helminthiasis (STH) remains a major public health problem in school children in Ethiopia. Although direct wet mount microscopy (DWMM) is the means to diagnose parasitic diseases in health care facilities in Ethiopia, it remains unclear what its diagnostic performance is for STH.

Methodology: A cross-sectional study was performed in Jimma Town (Ethiopia) and included 600 children from 10 primary schools. The diagnostic sensitivity of DWMM was compared to a composite reference standard (CRS) consisting of Kato-Katz, McMaster and Mini-FLOTAC. We also explored the impact of intensity of infection (the highest faecal egg counts (FECs; expressed as eggs per gram of stool (EPG)) across the CRS) on the diagnostic sensitivity of DWMM.

Results: Based on the CRS, there were 210 *Ascaris* (35.0%), 312 *Trichuris* (52.0%) and 102 hookworm cases (17.0%). The median intensity of infections equalled 2,057 EPG for *Ascaris*, 200 EPG for *Trichuris* and 110 EPG for hookworms. The sensitivity of DWMM was 73.8% for *Ascaris*, but was around 17% for both *Trichuris* and hookworms. The sensitivity significantly increased with intensity of STH. For *Ascaris*, the odds for detecting an infection intensity of 1,000 EPG was 6.2 times higher than detecting an infection of 100 EPG. For *Trichuris* and hookworms, these odds ratios were 7.1 and 14.

Conclusions: The diagnostic sensitivity of DWMM is low for STH, but it is able to detect those subjects that are in the highest need of treatment, and hence contributes to the global goal to eliminate STH as a public health problem.

Key words: Soil-transmitted helminths; direct wet mount microscopy; Kato-Katz; McMaster; Mini-FLOTAC; composite reference standard.

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Introduction

Soil-transmitted helminthiasis (STH) is caused by one of the four helminth species commonly known as the giant roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*) and hookworms (*Ancylostoma duodenale* and *Necator americanus*) [1]. These infections are common in the developing world and remain a major public health problem in the poorest settings [2]. Globally, STH contributes to a global burden of 1.9 million disability-adjusted life years [3]. In Ethiopia, it is estimated that 79 million people are at risk of STH, including 34.4 million children and 44.6 million adults [4].

To fight the morbidity caused by STH, the World Health Organization (WHO) recommends mass drug

administration (MDA) programs in which single oral doses of either albendazole or mebendazole are periodically administered to all children without any prior individual diagnosis [5,6]. The frequency of MDA is determined by the prevalence of STH. The drugs are administered twice a year when the prevalence exceeds 50% and once a year when the prevalence is at least 20% but less than 50%. In settings where the prevalence of STH is lower than 20%, individual diagnosis-treatment strategies are recommended. These large-scale deworming programs have recently received high political and scientific attention and with this, WHO aims to increase the coverage of MDA to children in need from the current 30% to at least 75% by 2020, and

this is envisaged to ultimately eliminate STH as a public health problem [7,8].

The standard method for STH diagnosis in these programs is the Kato-Katz thick smear. Beyond these programs, Kato-Katz is rarely used. Instead, the direct wet mount microscopy (DWMM) is the most frequently used routine diagnostic method for STH and other intestinal parasitic infections in health care facilities in Ethiopia and Africa at large [9]. Despite its cost effectiveness and the ease to perform, the diagnostic sensitivity of DWMM for the diagnosis of STH remains largely unknown. A poor diagnostic sensitivity, however, may result in missing those individuals that are most in need of treatment. This study aimed to determine the diagnostic performance of DWMM among school children in Jimma Town, South-West Ethiopia.

Methodology

Study design and population

This study was part of a cross-sectional study designed to compare the disease profiles of *Ascaris* infections using two serological assays with those obtained through copro-microscopy (Kato-katz, McMaster, and Mini-FLOTAC) in school children from Jimma Town (South-West Ethiopia; accepted for publication) [10]. This area was selected because previous studies indicated a high prevalence of STH (~50%) in school children [11]. The children were recruited from 10 primary schools, where 30 children age 5 to 10 year and 30 children age 14 to 18 year were randomly selected, resulting in a total of 600 children. After obtaining consent, children were asked to provide at least 5 g of stool in a clean, labelled and closed stool container. All samples were subsequently processed at the Neglected Tropical Disease Laboratory of Jimma University using DWMM, Kato-katz, McMaster and Mini-FLOTAC.

Copro-microscopic methods

Direct wet mount microscopy

The DWMM was prepared by mixing a small amount of stool (about 2 mg) with a drop of 0.85 % NaCl on microscopic slides. A cover slip was added and the sample was subsequently examined using a 100× magnification. In case of doubt, the morphology of the eggs was confirmed using a 400x magnification [12].

Kato-Katz

A single Kato-Katz thick smear was prepared as described by the WHO. A square template with a hole diameter of 6 mm and depth of 1.5 mm was used. This

template allows the collection of approximately 0.0417 g of faeces. To avoid clearance of hookworm eggs, all smears were examined within 30 to 60 min after preparation for the presence of soil-transmitted helminth eggs. The number of eggs was counted separately for each helminth species and was multiplied by 24 to obtain the faecal egg counts (FECs; expressed in eggs per gram of stool (EPG)) [13].

McMaster

The McMaster egg counting method was performed as described by Levecke [14]. In short, 2 g of stool was suspended in 30 ml of flotation solution (the saturated salt solution at room temperature, specific density ~1.20). The faecal suspension was poured three times through a wire mesh to remove large debris. Then, 0.5 ml aliquots were added to each of the two chambers of a McMaster slide. Both chambers were examined under a light microscope using a 100x magnification and the FECs were obtained by multiplying the total number of eggs by 50 for each of the helminth species [15].

Mini-FLOTAC

The Mini-FLOTAC was performed by taking about 2 g of fresh stool, weighed into the Fill-FLOTAC container, followed by adding 38 ml of flotation solution (the same as for McMaster). Then, the stool was thoroughly homogenized using the homogenizer stick of the Fill-FLOTAC. The faecal suspension was filtered through the Fill-FLOTAC and subsequently added to both chambers of the Mini-FLOTAC device. After 10 minutes, the top part of the flotation chambers was translated and the Mini-FLOTAC was read under a light microscope using a 100x magnification for the presence of helminth eggs. The number of helminth eggs was counted as per species basis and multiplied by 10 to obtain the FECs [16,17].

Statistical analysis

The diagnostic sensitivity of DWMM equalled the proportion of individuals that was correctly identified as infected. In absence of a gold standard, we used a composite reference standard (CRS; combining Kato-katz, McMaster, and Mini-FLOTAC) as a substitute for a gold standard to determine the true infection status of the individuals. For this we assumed a 100% specificity for Kato-Katz, McMaster and Mini-FLOTAC. In addition, we explored the variation in sensitivity across different levels of infection intensity. To this end, the intensity of infection was stratified in 5 levels based on the quintiles of the highest FECs reported across the three different copro-microscopic methods of the CRS

Table 1. The number and prevalence of soil-transmitted helminth infections in 600 children from Jimma town, South-West Ethiopia, 2015.

	Kato-Katz	McMaster	Mini-FLOTAC	CRS	Prevalence (%)
Any STH	346	322	375	408	68.0
<i>Ascaris</i>	188	141	158	210	35.0
<i>Trichuris</i>	237	241	292	312	52.0
Hookworm	62	57	91	102	17.0

The number of cases was based a composite reference standard (CRS; combining Kato-Katz, McMaster, and Mini-FLOTAC) as a substitute for a gold standard to determine the true infection status of the individuals. For this we assumed a 100% specificity for Kato-Katz, McMaster and Mini-FLOTAC.

(level 1: FEC < 20th percentile (P20); level 2: P20 ≤ FEC < P40; level 3: P40 ≤ FEC < P60; P60 ≤ FEC < P80; level 5: FEC ≥ P80). Given the distinct difference in fecundity across the species, these levels of infection intensity were determined for the different soil-transmitted helminth species separately. Finally, a logistic regression model was built to test for any differences in diagnostic sensitivity of DWMM due to differences in intensity of infection. These models were fitted for each soil-transmitted helminth species separately with the test result of the DWMM as outcome and highest FEC across the CRS as covariate. The FECs were log transformed with base 10. The predictive power of these models was evaluated by the proportion of the observed outcome that was correctly predicted by the model. To this end, an individual probability > 0.5 was set as a positive test result, and negative if different. All results were transcribed and double-entered. The final analysis was completed in the statistical software R (R Foundation for Statistical computing, version 3.2.3). The level of significance was set at *p* < 0.05.

Ethical Considerations

The study was approved by the Institutional Review Boards of Jimma University (Ethiopia; reference number: RPGC/181), Ghent University (Belgium; reference number: 2015/0801 and study registration number: B670201526293), and the International Union against Tuberculosis and Lung Disease (Paris, France;

reference number: 107/18). Written informed consent and assent was obtained using informed consent forms in two local languages (Afaan Oromo and Amharic).

Results

Prevalence and intensity of soil transmitted helminths

A total of 600 school children participated in this study, including 318 (53.0 %) boys and 282 (47.0 %) girls. Based on the CRS, 408 (68.0 %) children were infected with at least one soil-transmitted helminth. There were 210 *Ascaris* (35.0 %), 312 *Trichuris* (52.0%) and 102 hookworm cases (17.0%) (Table 1). The median (quintiles intensity of infections (expressed as eggs per gram of stool (EPG)) equalled 2,057 EPG for *Ascaris* (P20: 120; P40: 775.2; P60: 3,960.0 and P80: 8,025), 200 EPG for *Trichuris* (P20: 72.0; P40: 150.0; P60: 258.4; P80: 632) and 110 EPG for hookworms (P20: 48.0; P40: 100.0, P60: 182.0 and P80: 366.0).

Diagnostic sensitivity of direct wet mount microscopy

The sensitivity of DWMM was 73.8% for *Ascaris*, but was only around 17% for both *Trichuris* and hookworms (Table 2). For each of the three STHs, the sensitivity increased with the intensity of infection. For *Ascaris*, the sensitivity ranged from 30.2% for the lowest infection intensities (level 1; FEC < 120.0 EPG) to 94.3% for the highest levels of infection intensity (level 5; FEC ≥ 8025.0 EPG). For both *Trichuris* and hookworms, the sensitivity ranged from nearly 0% to

Table 2. The sensitivity of direct wet-mount microscopy for the diagnosis of soil-transmitted helminths among 600 school children in Jimma Town, South-West Ethiopia, 2015.

	Test result of direct wet-mount microscopy				Diagnostic sensitivity (95% confidence interval)
	True positive	False negative	False positive	True negative	
Any STH	208	200	8	184	51.0 (46.2-55.9)
<i>Ascaris</i>	155	55	14	376	73.8 (67.2-79.5)
<i>Trichuris</i>	53	259	2	286	17.0 (13.1-21.7)
Hookworm	18	84	2	496	17.6 (11.1-26.7)

In absence of a gold standard, we used a composite reference standard (CRS; combining Kato-Katz, McMaster, and Mini-FLOTAC) as a substitute for a gold standard to determine the true infection status of the individuals. For this we assumed a 100% specificity for Kato-Katz, McMaster and Mini-FLOTAC. The CRS was positive if at least one egg was found by either Kato-Katz, McMaster or Mini-FLOTAC, and negative if no eggs were found in Kato-Katz, McMaster and Mini-FLOTAC.

42.9% between the lowest (*Trichuris*: < 72.0 EPG; hookworms: < 48.0 EPG) and the highest level (*Trichuris*: ≥ 632.0 EPG; hookworms: ≥ 366.0 EPG) of infection intensity (Figure 1). The logistic regression confirmed these explorative analyses for each of the three soil-transmitted helminths. For *Ascaris*, the odds for detecting an infection intensity of 1,000 EPG (= 10³) is 6.2 times (95% confidence interval: 3.7 – 10.4) higher than detecting an infection of 100 EPG (= 10²). For both *Trichuris* and hookworms, odds for detecting an infection intensity of 1,000 EPG is 7.1 (95% confidence interval: 3.6 – 14.0) and 14 (95% confidence interval: 3.6 – 57.1) higher than detecting an infection of 100 EPG. Each of the models correctly predicted test outcomes in at least 80% of the cases.

Discussion

Almost all health care facilities in developing countries use DWMM due to its low cost, easy procedure and ability to detect a wide range of intestinal parasites, including but not limited to STH (e.g. protozoa). Although it is generally accepted that the

sensitivity is poor, there are little studies that have assessed the diagnostic sensitivity of DWMM for STH. The present study confirmed the poor sensitivity (< 75%), but it also highlighted important differences both between and within soil-transmitted helminths. The diagnostic sensitivity of DWMM for *Ascaris* infection was significantly higher compared to that for both *Trichuris* and hookworm, and the sensitivity increased with intensity of infections. Both findings are not unexpected and are in line with previous reports in the literature [9,15,18]. In essence, the sensitivity increases when more eggs are excreted in stool (see Figure 1). As a consequence, it is also not surprising that the sensitivity for *Ascaris* is high, as the fecundity of this helminth species is notably higher than that of other helminth species. One female *Ascaris* worm lays about 200,000 eggs per day [19], whereas the daily egg output ranges from 9,000 – 30,000 for hookworms [20] and from 3,000 to 5,000 for *Trichuris* [21] This difference in fecundity was also observed in the present study, the median FECs being highest for *Ascaris* (2,057 EPG), followed by *Trichuris* (200 EPG) and hookworms (110 EPG). It is therefore of utmost importance that the sensitivity data is interpreted with the FECs in mind.

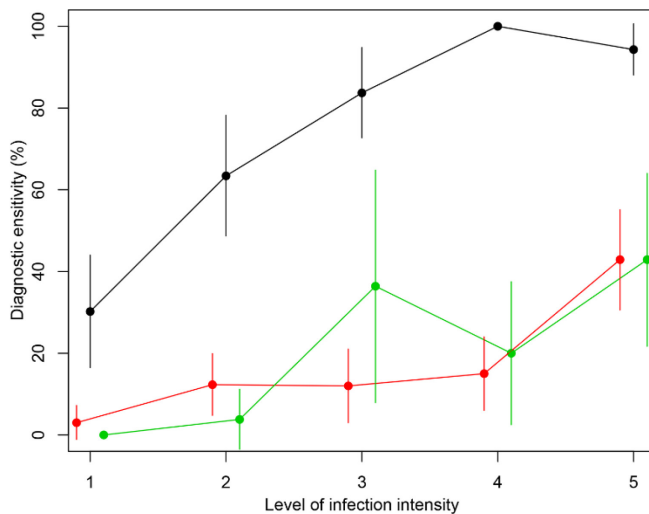
Although it is clear that DWMM fails to detect infections of low intensities, it will most likely not miss the moderate-to-heavy intensity infections, which cause most of the STH-associated morbidity [22]. For example, the WHO classifies *Ascaris* infections in the moderate-to-heavy intensity category when FECs are at least 5,000 EPG [7,8] but the DWMM already has a high sensitivity (> 94%) when FECs are 3,960 EPG. Hence, DWMM will be able to diagnose those individuals that are in the highest need of treatment. A similar pattern is to be expected for the other two soil-transmitted helminths, particularly when the levels of intensity observed in this study are far below those defined for moderate-to-heavy intensity infections (*Trichuris*: 1000 EPG and hookworm: 2,000 EPG).

It is also important to note that reducing the prevalence of moderate-to-heavy intensity infections below 1% is the ultimate goal of MDA programs [7,8]. As such, the DWMM has an important role to play to identify those subjects that present with moderate-to-heavy intensity infections at health facilities, but are not covered in MDA programs.

Conclusion

In conclusion, the diagnostic sensitivity of DWMM is low for STH, particularly for *Trichuris trichiura* and hookworm, and when intensity of infection is low. However, DWMM is able to detect those subjects that

Figure 1. Diagnostic sensitivity of direct wet mount microscopy across different levels of *Ascaris*, *Trichuris* and hookworm infection intensities in school children in Jimma Town, South-West Ethiopia, 2015.



This Figure illustrates the diagnostic sensitivity of direct wet mount microscopy across different levels of *Ascaris* (black lines), *Trichuris* (red lines) and hookworms (green lines). The dots represent the point estimate, whereas the vertical lines reflect the 95% confidence intervals. The intensity of infection was stratified in 5 levels based on the quintiles of the highest FECs reported across the composite reference standard (level 1: FEC < 20th percentile (P20); level 2: P20 ≤ FEC < P40; level 3: P40 ≤ FEC < P60; P60 ≤ FEC < P80; level 5: FEC ≥ P80). Given the distinct difference in fecundity across the species, these levels of infection intensity were determined for the different soil-transmitted helminth species separately. The quintiles were 120.0, 775.2, 3,960.0 and 8,025.0 EPG for *Ascaris*, 72.0, 150.0, 258.4 and 632.0 EPG for *Trichuris* and 48.0, 100.0, 182.0 and 366.0 EPG for hookworms.

are in the highest need of treatment, and hence contributes to the global goal to eliminate STH as a public health problem.

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