Evaluation of Hookworm Diagnosis Techniques from Patients in Amhara Region, Ethiopia: Cross sectional study

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Research article

Keywords: Hookworm; Diagnosis Techniques; Evaluation; Amhara Region; Ethiopia

Posted Date: August 19th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-54259/v1

Abstract

Background: Inappropriate diagnosis could intimidate the prevention and control of Hookworm infection. This study was aimed at evaluating the performance of Hookworm diagnosis methods.

Methods*:* An institution based cross sectional study was conducted in Amhara region, Ethiopia. The study subjects were selected conveniently. Sensitivity, specificity, predictive values, test accuracy and agreements of the different hookworm diagnosis methods, namely; Test Tube Flotation (TFT), MacMaster (MM), Formol Ether Concentration (FEC), Kato katz (KK), and Direct wet mount microscopy (DWMM) were calculated. Composite reference standard (CRS) was used as a gold standard method. Kappa (κ) test was used to measure the level of agreement between diagnosis tests. Moreover, t-test was used to compare the diagnostic performance of the diagnosis methods.

Result*:* a total of 389 stool samples were collected from patients in the study. The overall prevalence of hookworm was 63.24%. Test Tube Flotation (TFT) was found to be the highest both in terms of sensitivity and diagnosis accuracy (100%). MacMaster (MM) was the second most sensitive test (68.7%), followed by FEC (44.3%) and KK (38.2%). On the other hand, DWMM had the lowest sensitivity (37.4%) and its diagnosis accuracy was also the least (60%). Only TFT had a perfect (Agreement=100%, kappa=1) with the CRS. The sensitivity of DWMM, KK, and FEC showed an increase in sensitivity as a function of increasing intensity of infection, but TFT and MM methods were not affected by the infection intensity.

Conclusion: Hookworm is still a public health problem in the study area. TFT is by far more sensitive than MM, FEC, KK and DWMM techniques.

Background

Hookworm is one of among the big three Soil-transmitted helminthes (*Ascaris lumbericoides*, *Trichuris trichuira*, and Hookworm). These three helminths infect more than two billion people worldwide and the disease burden might approach that of malaria(1). Soil-transmitted helminthic infections cause a great and frequently silent burden of morbidity and mortality on poor populations in developing countries that accounts approximately 85% of the NTD burden(2, 3). Despite the existence of control programs, hookworm's disease burden remains high. Currently, hookworm affects approximately 500 million people, with 5.1 billion at risk for acquiring infection worldwide(4).

Hookworm is the most common possible cause of iron deficiency anemia. It is a blood feeding intestinal worm and the mature larvae ingest the blood, rupture the erythrocytes, and degrade the hemoglobin by attaching to the gut wall. Hookworm changes feeding sites and during feeding secretes an anticoagulant, resulting in additional blood loss from the damaged gut wall. The number of adult hookworms and the fecal egg count, which is an indirect estimate of the number of worms, are strongly associated with poor iron status and has an adverse health effects. The most damaging effects of hookworm infections include impaired physical, intellectual and cognitive development of children, increased mortality in pregnant women and their infants and reduced work capacity of adolescents and adults (5, 6). Thus, prevention and control of hookworm infection and its associated health consequences is essential.

The prevention and control of hookworm infection involves many approaches like sanitary disposal of feces, early diagnosis, and chemotherapy and Health education (7–10). However, improper diagnosis and emergence of drug resistances could threaten the prevention and control of the parasite(11, 12). Misdiagnosis of Hookworm is unfortunate because a misdiagnosed patient may be given a treatment that is ineffective against the parasite, and therefore it would not alleviate the patient's suffering or stop the progressive wasting of health(13).

There are different methods of hookworm diagnosis. Conventional direct wet mount microscopy, concentration, FLOTAC, McMaster, Kato-katz, culture, and molecular analysis(14, 15). Direct stool microscopy is solely used in all health care service providers in Ethiopia for intestinal parasite detection including hookworm infection. It is generally believed that it has low sensitivity which might be affected by multiple factors.

Although there exists different types of microscopic and a few advanced hookworm diagnosis methods(14), there are limited studies that show comparisons on their sensitivity. In this regard, an evaluation of these diagnosis techniques and identifying the most sensitive method of hookworm detection is crucial. Therefore, the data generated from this study could serve as an input for decision making among health care service providers, local health planners and policy makers.

Methods

Study Design, Period and Area

An institution based cross-sectional study was conducted from November 2019 to January 2020 in Debre Elias and Sanja districts of the Amhara region, Ethiopia. Debre Elias is 430 km far from Adis Ababa and 310 km from Gondar Town. Sanja is located 65 kms Northwest of Gondar Town and 792 km away from Addis Ababa. An estimated total population of Sanja Town is 26,000. Sanja has an altitude of 1800 m above sea level with annual rainfall ranging from 800 to 1800 mm and the average temperature of 25°C to 42°C.On the other hand, Debre Elias woreda has a total population of 82,150(16). There are two health institutions (one Health center and one Hospital) in each of the study areas. The study was conducted among adult patients who were visiting the health institutions.

Study population, sample size determination and sampling technique

All people who lived in Debre Elias and Sanja distict were our source population. Adult patients who were requested for stool microscopy in the health institutions were the study population. Patients who were not volunteer and those with watery stool samples were excluded.

The sample size was determined using a formula (Z^2*P (1-P)/d^2) for estimating single population proportion. The proportion of hookworm infection is assumed to be 50%. By considering a 95%

confidence level and a 5% expected margin of error the calculated sample size was 384. To minimize errors arising from the probable occurrence of noncompliance, 5% of the sample size was added to the calculated sample size and thereby increasing the sample size to about 400. The study subjects were conveniently selected during the study period.

Study variables

The outcome variable was positivity rate (prevalence) of hookworm infection, while gender and methods of hookworm diagnosis methods were the independent variables.

Laboratory works

Stool sample collection

Prior to the laboratory investigation, well-structured questionnaire was used to collect socio-demographic and other characteristics of the study participants. A clean, dry and leak proof container was used to collect about 20 g stool specimens. Then, the stool samples were processed following five types of microscopic stool examination methods at the health facilities.

Microscopic examinations of stool

All specimens were investigated using direct microscopy, Kato-Katz, Formol ether concentration, McMaster and Test tube-cover slide flotation methods following the protocols described before(10, 17) .

Direct Wet Mount Microscopy

a fresh stool sample (about 2 mg of stool) were placed on two slides with wooden applicator stick, emulsified with a drop of physiological saline (0.85%), covered with cover slides and examined under microscope using 10 × objectives.

Formol ether concentration

This test was performed by mixing around 1 g of faeces in 3–4 ml of 10% formaldehyde in a glass container and mix thoroughly. Two layers of gauze were placed in a funnel and strain the contents into a 15 ml centrifuge tube. Then, additional 3 ml of 10% formaldehyde and 3 ml of ether was added. The solution was mixed well and centrifuge at 1000 revolution for 3 min. The supernatant was removed and two slides were prepared from the sediment and finally examined with 10x objective of the microscope.

Kato-Katz technique

It was performed by transferring the sieved stool to the templates which delivers 41.7 mg of stool. The stool was covered with cellophane which was previously immersed with malachite green. Identification and quantification of the ova were done. Eggs counted per slide were multiplied by 24 to convert into number of eggs per gram (epg) of stool. The parasite load or intensity was defined as light, moderate, and heavy according to World Health Organization (WHO) guideline (18). Two Kato slides were prepared from each sample.

MacMaster

was performed by mixing 2 grams of stool with 30 ml of flotation solution (saturated sodium chloride solution at room temperature, density ~ 1.20). The fecal suspension was poured through a wire mesh to remove large debris. Then, 0.5 ml aliquot was 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 fecal egg count were expressed as eggs per gram of stool (EPG) and were obtained by multiplying the total number of eggs by 50.

Test Tube Flotation

was performed by mixing 2 grams of stool with 30 ml of flotation solution 2 (saturated sodium chloride solution at room temperature, density ~ 1.20). The fecal suspension was poured into 10 ml test tube through a wire mesh to remove large debris. Then, a cover slide was placed to the top of the test tube. This allowed ova of the parasite were adhering to the cover slide. Finally, the cover slides were placed under light microscope and examined using a 10x objectives.

Quality Assurance Mechanisms

To avoid observer bias, two experienced laboratory personnel performed the microscopic examination of the slide smears blindly and independently. Independent readings of slides by the laboratory personnel were checked by another expert. The results of their observation were recorded for later comparison on separate sheets. A quality control was done by repeating all discordant results.

Data Analysis

Data was analyzed using SPSS software. To estimate the sensitivity, specificity, PPV, and NPV, the standard formula was used i) sensitivity = TP/ (TP + FN) × 100%; ii) specificity = TN/ (TN + FP) × 100%; iii) PPV = TP/ (TP + FP) × 100%; and iv) NPV = TN/ TN + FN) × 100%. T-test was used to compare their sensitivities. The kappa (κ) test was used to measure the level of agreement among the tests. A κ value of 0.2–0.60 represents a fair to moderate agreement, a κ-value of 0.60–0.80 represents a substantial agreement beyond chance, whereas a κ-value of > 0.80 represents almost perfect agreement beyond chance(19).

Result

Hookworm prevalence rate by diagnosis techniques

A total of 389 study participants were enrolled in the study. Out of these, 221(56.8%) were males and 168 (43.2%) were females. The mean age of the study participants was 32.9 ± 13.78 years and most (88.4%) of them were from 18 to 45 years of age. Out of 389 patients, who were subjected for intestinal parasitological investigation; 246, 169, 109, 94, and 92 were positive using TFT, MM, FEC, KK, and DWMM techniques, respectively. Overall, the prevalence of hookworm among patients in the study area was 63.23% (Table 1).

Table 1
Hookworm positivity rate by diagnosis techniques among the study participants

Diagnosis tools	Hookworm infection	
	Number of positives, n (%)	Number of negatives, n (%)
TFT	246(63.23)	143(36.76)
MM	169(43.44)	220(56.55)
FEC	109(28.02)	280(71.97)
KK	94(24.16)	295(75.83)
DWMM	92 (23.65)	297(76.34)
CRS	246(63.23)	143(36.76)
CRS-composite reference standard		

Performance of Hookworm diagnosis techniques using CRS as gold standard

Computation of the sensitivity of the laboratory diagnosis methods showed that the TFT had the highest sensitivity (100%). Moreover, its diagnosis accuracy was 100%. MacMaster was the second both in terms of sensitivity (68.7%) and diagnostic accuracy (80%), followed by FEC, and KK. The prevalence of hookworm using TFT and MM showed statistically significant discrepancy with a difference rate of 20% ($p < 0.001$). The present finding also showed that DWMM had the lowest sensitivity (37.4%) and diagnostic accuracy (60%). All tests had 100% specificity and positive predictive values. TFT and MM had 100% and 65% NPVs, respectively, while the others had 48 to 51%. The sensitivity, specificity, and diagnostic accuracy, positive and negative predictive values are summarized in Table 2.

Table 2
Performance of Hookworm diagnosis techniques compared to CRS

Diagnosis Tools	Sensitivity %(95% CI)	Specificity %(95% CI)	PPV %(95% CI)	NPV %(95% CI)	Diagnosis accuracy
TFT	100(98, 100)	100(97, 100)	100	100	100 (99,100)
MM	68.7 (62,74)	100(97,100)	100	65(60,69)	80(75,84)
FEC	44.3 (48,50)	100(97,100)	100	51(48,54)	65(60,70)
KK	38.2(32,45)	100(97,100)	100	48(46,51)	61(56,66)
DWMM	37.4 (31,44)	100(97,100)	100	48(47, 51)	60(55, 65)

Performance of Hookworm diagnosis techniques based on infection intensity

The mean intensities of infections were expressed as eggs per gram of stool (EPG). Out of the 246 hookworm positive study participants, 77 (31.3%), and 92 (37.4%) were grouped under light and heavy infection category, respectively; while the remaining 77 (31.3%) were not categorized to any of the infection intensity since they were only detected by the TFT. The sensitivity of DWMM, KK, and FEC techniques showed an increase in sensitivity as a function of increasing intensity of infection. On the other hand, the sensitivity of TFT and MM methods were not affected by the infection intensity. Amazingly, TFT detected a significant number of hookworm infections (a total of 77) which were not identified by the internationally accepted STHs diagnosis tools (MM and KK techniques) (Table 3).

Table 3
Performance of Hookworm diagnosis techniques based on infection intensity category

Diagnosis tools	Number of positives, n (%)		
	Uncategorized infection intensity, n = 77	Light infection intensity, n = 77	Moderate infection intensity, n = 92
TFT	77	77(100)	92 (100)
MM	0	77(100)	92 (100)
FEC	0	17(22)	92 (100)
KK	0	2(2.6)	92 (100)
DWMM	0	3(3.9)	89 (96.7)
CRS	77	77(100)	92 (100)

Degree of agreement of Hookworm diagnosis techniques with CRS (gold standard)

Test tube flotation technique had perfect agreement (Agreement = 100%, kappa = 1) with CRS, followed by MM (moderate agreement = 80%, kappa = 0.67), and FEC (substantial agreement = 64%, kappa = 0.36). On the other hand, DWMM had low degree of agreement (fair agreement = 60%, kappa = 0.30). The degree of agreements of hookworm diagnosis techniques with composite reference standard (CRS) are summarized in Table 4.

Table 4
Degree of agreement of Hookworm diagnosis techniques with gold standard (CRS)

Diagnosis tools		CRS		Total	Agreement	Kappa value
		+	-			
TFT	+	246	0	246	100	1
	-	0	143	146		
MM	+	169	0	169	80.2	0.67
	-	77	143	220		
FEC	+	109	0	109	64.7	0.36
	-	137	143	280		
KK	+	94	0	94	61	0.31
	-	152	143	295		
DWMM	+	92	0	92	60	0.30
	-	154	143	297		

Kappa < 0: No agreement, 0.00 -0.20: Slight agreement, 0.21–0.40: Fair agreement, 0.41–0.60: Moderate agreement, 0.61–0.80: Substantial agreement, 0.81- 1.00: Almost perfect agreement, CRS-composite reference standard

Discussion

Ethiopia is one of the hotspot areas for hookworm and other STHs in the world (20–22). Thus, an integrated hookworm prevention and control measure is needed. Appropriate diagnosis is one of the most important tools in fighting of the disease. It is recommended to use Kato-Katz, FEC and MacMaster methods for the detection of human soil transmitted helminthes (STHs) including hookworms. All of these and other techniques rely on visual examination of a small sample of stool to determine the presence and number of the parasite's ova with different sensitivities especially in low transmission areas (23, 24). It is clear that they are helpful in the disease diagnosis; however, they may not be equally sensitive and could also have their own limitations. For instance, DWMM is solely used in almost all health care facilities in Ethiopia and other developing countries due to its low cost, and easy procedure. Nevertheless, it is undoubtedly known that the sensitivity of DWMM is poor (25, 26). In spite of this, there are little studies that have evaluated the clinical sensitivity of DWMM compared to other microscopical techniques for the diagnosis of intestinal parasitosis including Hookworm infection.

The present study evaluated the performance of five types of stool examination methods (TFT, MM, FEC, KK, and DWMM) for hookworm diagnosis using their composite as a gold standard method. It has confirmed that DWMM has poor sensitivity. On the other hand, Test Tube Flotation technique was found to be a more sensitive, cheaper, and easier to apply in the routine practices of hookworm identification. It was reported that both of the sensitivity and diagnostic accuracy of this method was 100%. This is almost three times more sensitive than the commonly used DWMM (37%). Low sensitivity of DWMM might be related with the use of only small amount of the stool sample (only 2 mg of stool is used in DWMM compared to about 4 gm for Test Tube Flotation technique). Moreover, the presence of large stool debris materials in the DWMM may conceal the parasitic ova. The sensitivity of DWMM in this study (37%) is similar with other studies done in Ethiopia (25, 27). This suggests that DWMM has resulted in around 63% false negative reports during hookworm diagnosis. This substantially underestimates the prevalence of hookworm infection. Moreover, it has a great impact in the control and elimination programs of hookworm and other soil transmitted helminthes infections. Thus, this study may encourage using TFT as a confirmatory test for hookworm infection in order to break the transmission cycle and ultimately reduce its morbidity and mortality.

The finding of the present study also demonstrated that MM is the second most sensitive test with a sensitivity and diagnostic accuracy of 68.7%, 80%, respectively. Even though it is one of the most recommended diagnosis methods for soil transmitted helminthes by World Health Organization, its sensitivity is lower by about 33% compared to TFT in the current study. Formol ether concentration technique and KK were also ranked as the third (44.3%) and fourth (38.2%) sensitive tests, respectively in this study. However, this is lower than the previous study carried out in Gondar in which their sensitivities were reported from 69-72.4% (27). The observed differences in the sensitivities among different studies could be due to the infection intensity variation. Moreover, it might be related with the difference in the skill of the laboratory personnel. The observation of lower sensitivity of MM, KK and FEC than TFT from the current study indicates that another better diagnostic tool is necessary during patient diagnosis, monitoring and evaluation of hookworm infection following intervention.

According to the current study, the sensitivity of most of the stool examination methods increases when more eggs are excreted in stool. FEC, KK, and DWMM identified hookworm from only 22%, 2.6%, and 3.9% of the light infections, respectively. However, DWMM, KK, and FEC have detected the parasite from 97, 100, 100 percents of moderately infected study participants, respectively. This may suggest that they will most likely not to miss the moderate-to-heavy intensity of hookworm infections, which is mostly associated with morbidity (28, 29). Hence, these three techniques will be able to diagnose those individuals who are in the highest need of treatment. Nonetheless, their inability to detect light infections properly may make them to have insignificant role in the evaluation of hookworm infection following MDA. Thus, TFT might be taken as the best method over the other available diagnosis methods.

The specificity of all of the stool examination methods described in this study was 100%, and this is in line with findings of a study done in Gondar which revealed that DWMM, FEC and KK had greater than 97% of sensitivity (27). The current study also analyzed the level of agreement of the various types of hookworm diagnosis methods with composite reference standard. The reproducibility of the TFT compared to CRS had a perfect agreement (Agreement = 100%, kappa = 1).This indicates that the TFT is

100% as sensitive as the gold standard technique for the disease diagnosis. Next to TFT, MacMaster showed moderate agreement (agreement = 80%, kappa = 0.67), followed by Formol Ether concentration, and Kato-Katz technique. Importantly, DWMM showed the lowest (Agreement = 60%, kappa = 0.30) and this may suggest its little role in the diseases diagnosis. Generally, this study found an encouraging outcome and implies that TFT could be the most preferred technique for hookworm infection detection.

Limitation

There is no any similar study conducted so far. As a result, this has made difficulties in making rigorous discussions on this finding.

Conclusion

The present study highlighted that the prevalence of hookworm infection is being underreported in Ethiopia due to the use of poor sensitive test methods. TFT is almost three and 1.5 times more sensitive in the diagnosis of hookworm infection than DWMM and MacMaster technique, respectively. It is also by far more sensitive than KK and FEC techniques. A part from its better sensitivity, TFT is simple and does not require expensive materials. Thus, we recommend that laboratory professionals, who are found in hookworm endemic areas, should stick to TFT for its diagnosis. We also advocate that TFT has to be used as a major method of hookworm diagnosis during implementation and monitoring of mass drug administration.

Abbreviations

DWMM-Direct Wet Mount Microscopy, EPG-Eeggs per gram of stool, FN-False Negative, FP-False Positive, FEC-Formol Ether Concentration, KK-Kato Katz, MDA-mass drug administration, MM-MackMaster, NPV-Negative Predictive Value, NTD-Neglected Tropical Disease, PPV-Positive Predictive Value, STH-Soil Transmitted Helminthiasis, TFT-Test Tube Floation Technique, TN-True Negative, TP-True Positive, WHO - World Health Organization

Declarations

Ethics approval and consent to participate

The study protocol was reviewed and approved from Research and Ethical Review Committee of University of Gondar (Reference number of the letter is O/V/P/RCS/05/358/2018). Support letter was also obtained from East Gojam and Central Gondar Zone Health Administration Offices. The benefit and risk of this project was explained to the district health facilities and study participants. Both informed verbal and written consent was obtained from each study participant.

Consent for publication

Not applicable in this section

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Funding

The research was funded by University of Gondar. The University did not have any scientific contribution for the paper.

Authors’ contributions

AJZ conceived the study, involved in data collection, analysis and wrote the first draft of the manuscript. AA, YT, MB, TS, and MA critically reviewed the manuscript. All authors reviewed and approved the manuscript.

Acknowledgements

We would like to thank University of Gondar for allowing us to do this research. We are also grateful to all study participants and data collectors.

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References

1. Utzinger J, Keiser J. Schistosomiasis and soil-transmitted helminthiasis: common drugs for treatment and control. Expert opinion on pharmacotherapy. 2004;5(2):263-85.
2. Hotez PJ, Kamath A. Neglected tropical diseases in sub-Saharan Africa: review of their prevalence, distribution, and disease burden. PLoS neglected tropical diseases. 2009;3(8):e412.
3. Childers KAG. A Survey of the Prevalence of Gastrointestinal Parasites and Associated Risk Factors in Children in a Rural City of the Dominican Republic: Virginia Tech; 2014.
4. Bartsch SM, Hotez PJ, Asti L, Zapf KM, Bottazzi ME, Diemert DJ, et al. The global economic and health burden of human hookworm infection. PLoS neglected tropical diseases. 2016;10(9):e0004922.

5. Olsen A, Magnussen P, Ouma J, Andreassen J, Friis H. The contribution of hookworm and other parasitic infections to haemoglobin and iron status among children and adults in western Kenya. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1998;92(6):643-9.
6. Koukounari A, Estambale BB, Njagi JK, Cundill B, Ajanga A, Crudder C, et al. Relationships between anaemia and parasitic infections in Kenyan schoolchildren: a Bayesian hierarchical modelling approach. International journal for parasitology. 2008;38(14):1663-71.
7. Zheng Q, Chen Y, Zhang H-B, Chen J-X, Zhou X-N. The control of hookworm infection in China. Parasites & vectors. 2009;2(1):44.
8. Ziegelbauer K, Speich B, Mäusezahl D, Bos R, Keiser J, Utzinger J. Effect of sanitation on soil-transmitted helminth infection: systematic review and meta-analysis. PLoS medicine. 2012;9(1):e1001162.
9. Knopp S, Mgeni AF, Khamis IS, Steinmann P, Stothard JR, Rollinson D, et al. Diagnosis of soil-transmitted helminths in the era of preventive chemotherapy: effect of multiple stool sampling and use of different diagnostic techniques. PLoS neglected tropical diseases. 2008;2(11):e331.
10. Steinmann P, Utzinger J, Du Z-W, Jiang J-Y, Chen J-X, Hattendorf J, et al. Efficacy of single-dose and triple-dose albendazole and mebendazole against soil-transmitted helminths and Taenia spp.: a randomized controlled trial. PloS one. 2011;6(9):e25003.
11. Ásbjörnsdóttir KH, Means AR, Werkman M, Walson JL. Prospects for elimination of soil-transmitted helminths. Current opinion in infectious diseases. 2017;30(5):482-8.
12. Tchuenté LT. Control of soil-transmitted helminths in sub-Saharan Africa: diagnosis, drug efficacy concerns and challenges. Acta tropica. 2011;120:S4-S11.
13. Wei K-Y, Yan Q, Tang B, Yang S-M, Zhang P-B, Deng M-M, et al. Hookworm Infection: A Neglected Cause of Overt Obscure Gastrointestinal Bleeding. The Korean journal of parasitology. 2017;55(4):391.
14. Cheesbrough M. District laboratory practice in tropical countries: Cambridge university press; 2006.
15. Glinz D, Silué KD, Knopp S, Lohourignon LK, Yao KP, Steinmann P, et al. Comparing diagnostic accuracy of Kato-Katz, Koga agar plate, ether-concentration, and FLOTAC for Schistosoma mansoni and soil-transmitted helminths. PLoS neglected tropical diseases. 2010;4(7):e754.
16. CAC. summery and statistical report of the 2007 population and housing census addis abeba. population and housing census commition. 2008:57-60.
17. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J mol biol. 1975;98(3):503-17.
18. Schistosomiasis WECotCo, Organization WH. Prevention and control of schistosomiasis and soil-transmitted helminthiasis: report of a WHO expert committee: Who; 2002.
19. DG A. Practical Statistics for Medical Research. London, U.K2001
20. Erosie L, Merid Y, Ashiko A, Ayine M, Balihu A, Muzeyin S, et al. Prevalence of Hookworm infection and haemoglobin status among rural elementary school children in Southern Ethiopia. Ethiopian

Journal of Health Development. 2002;16(1):113-5.

21. Demissie F, Petros B, Kebede A. Hookworm species distribution among school children in Asendabo town, Jimma Zone, South West Ethiopia. Ethiopian Journal of Health Sciences. 2008;18(2).
22. Worku L, Damte D, Endris M, Tesfa H, Aemero M. Schistosoma mansoni infection and associated determinant factors among school children in Sanja Town, Northwest Ethiopia. Journal of Parasitology Research. 2014;2014.
23. Organization WH. Bench aids for the diagnosis of intestinal parasites: World Health Organization; 2019.
24. Organization WH, UNICEF. Prevention and control of schistosomiasis and soil-transmitted helminthiasis: World Health Organization/Unicef joint statement. Geneva: World Health Organization, 2004.
25. Mengist HM, Demeke G, Zewdie O, Belew A. Diagnostic performance of direct wet mount microscopy in detecting intestinal helminths among pregnant women attending ante-natal care (ANC) in East Wollega, Oromia, Ethiopia. BMC research notes. 2018;11(1):276.
26. Yimer M, Hailu T, Mulu W, Abera B. Evaluation performance of diagnostic methods of intestinal parasitosis in school age children in Ethiopia. BMC research notes. 2015;8(1):820.
27. Endris M, Tekeste Z, Lemma W, Kassu A. Comparison of the Kato-Katz, wet mount, and formol-ether concentration diagnostic techniques for intestinal helminth infections in Ethiopia. International Scholarly Research Notices. 2013;2013.
28. van der Werf MJ, De Vlas S. Morbidity and infection with schistosomes or soil-transmitted helminths. Rotterdam: Erasmus University. 2001:1-103.
29. Anderson RM, Turner HC, Truscott JE, Hollingsworth TD, Brooker SJ. Should the goal for the treatment of soil transmitted helminth (STH) infections be changed from morbidity control in children to community-wide transmission elimination? PLoS neglected tropical diseases. 2015;9(8).