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ORIGINAL ARTICLE

Diagnostic performance of Mini Parasep® solvent-free faecal parasite concentrator relative to Kato-Katz and McMaster for the diagnosis of intestinal parasitic infections

Shimeles Adugna[a,1], Tadesse Kebede[a], Zeleke Mekonnen[b], Abraham Degarege[c,d,*], Song Liang[e] and Berhanu Erko[c]

[a]Addis Ababa University, College of Health Sciences, Department of Microbiology, Immunology and Parasitology, P.O. Box 1176, Addis Ababa; [b]Jimma University, School of Medical Laboratory Sciences, P.O. Box 378, Jimma; [c]Aklilu Lemma Institute of Pathobiology, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia; [d]Department of Epidemiology, Robert Stempel College of Public Health and Social Work, Florida International University, Miami, FL; [e]Department of Environmental and Global Health College of Public Health and Health Professions, University of Florida, Gainesville, FL 32610, USA; [1]Present address: Arsi University, College of Health Sciences, Department of Medical Laboratory Sciences, P.O. Box 396, Asella, Ethiopia

*Corresponding author: Department of Epidemiology, Robert Stempel College of Public Health and Social Work, Florida International University, Miami, FL, USA. Tel: +17865602008; Email: ameng002@fiu.edu

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Background: In this cross-sectional study, we compared the performance of Mini Parasep® solvent-free (SF) faecal parasite concentrator, Kato-Katz thick smear and McMaster techniques for the diagnosis of intestinal parasitic infections among children in Wosha Soyama Primary School, Ethiopia.

Methods: Stool samples were collected from 381 children and examined for intestinal parasitic infections using Mini Parasep® SF faecal parasite concentrator, Kato-Katz thick smear and McMaster techniques.

Results: About 86.1% of children were infected with at least one species of intestinal parasite based on combined results of the three techniques. The sensitivity and negative predictive values of Mini Parasep® SF, Kato-Katz and McMaster tests for detecting at least one species of intestinal parasite infections were 90.2% and 62.4%, 80.0% and 44.5%, and 55.2% and 26.5%, respectively. While Mini Parasep® SF was more sensitive in detecting *Ascaris lumbricoides, Schistosoma mansoni* and *Hymenolepis nana* infections, Kato-Katz was more sensitive in detecting *Trichuris trichiura* infection, and McMaster had higher sensitivity in diagnosing hookworm infection.

Conclusions: The Mini Parasep® SF faecal parasite concentrator technique showed better performance than the Kato-Katz and McMaster techniques for the detection of intestinal helminth infections in stool samples, particularly for *S. mansoni*, *A. lumbricoides* and *H. nana*. Hence, Mini Parasep® SF could be used as one of the suitable faecal examination methods for surveillance and monitoring of preventive chemotherapy of schistosomiasis.

Keywords: Diagnostic performance, Intestinal parasites, Ethiopia, Kato-Katz, McMaster, Mini Parasep® SF

Introduction

Reliable, sensitive and practical diagnostic tests are vital for patient screening, surveillance management, and for monitoring and evaluations of interventions.[1,2] Ideally, such tools must be accurate, simple, inexpensive and user friendly in field settings, particularly in developing countries, where resources are limited. More sensitive diagnostic tests are needed to establish an end-point of treatment towards the end of intervention programs, particularly when prevalence and intensity of infections become low.[1,2] Furthermore, diagnostic tests must also provide a result in time to initiate effective control measures involving treatment.[3]

More sensitive diagnostic tests help in the determination of endemicity of intestinal parasites with high confidence.[4] If a test sensitivity is inadequate, light infections might be missed and this runs the risk of stopping control programmes too early, before programme endpoints have been achieved. This is especially true as measurements of egg-based biomarkers, such as faecal egg counts, are inappropriate indicators of worm burden at the lighter intensities of infection. Furthermore, high-sensitivity tests are also required for surveillance once treatment has been stopped to detect the potential re-occurrence of infections.[1,2]

WHO recommends the use of the Kato-Katz technique in duplicate slides for the detection of soil-transmitted helminths (STH).[5] This method is simple, inexpensive and easy to use in field setting, and equipment can be reused.[6,7] However, the Kato-Katz thick smear needs to be prepared from fresh samples and sensitivity of the method decreases when intensity of helminth infection is low.[8] In addition, different STH species need different lengths of time to clear in order to be seen in a Kato-Katz thick smear under a microscope. Moreover, hookworm eggs disappear quickly after a Kato-Katz thick smear is prepared.[9] These characters limit applicability of the Kato-Katz method for large scale epidemiological surveys of STHs infections in areas where multiple infections with different helminth species is common—particularly when intensity of infections is low.[10] Furthermore, quantitative examination using the Kato-Katz method involves estimation of the number of eggs per gram of stool, based on the results of a fixed weight of stool (41.7 mg).[7] This may lead to a biased estimation of intensity of infection due to variation in density of the stool with the changes in the volume of the stool.[11]

Other techniques such as McMaster, FLOTAC and formol-ether concentration (FEC) are also available to diagnose STHs infections.[12] McMaster is a flotation method used for quantitative examinations of helminth infection.[13] Unlike the Kato-Katz method, the time difference between stool preparation and examination do not affect the diagnosis of hookworm infection in the case of the McMaster method.[10] In addition, the McMaster method is easy to use and does not need fully equipped laboratories, making it the method of choice for diagnosing helminth infection in field settings, particularly after treatment of individuals with anti-helminthic drugs.[14,15] However, the McMaster method was found to be less sensitive for detection of light intensity infection.[12] On the other hand, FLOTAC was shown to be more sensitive, but less feasible in field studies compared with the Kato-Katz and McMaster methods.[12,16–18] Similarly, the FEC method is available to detect parasite infection from both fresh and preserved samples,[19] but it is time-consuming, and the formalin and ether used during the sample preparation are also hazardous to health.

Recently, a closed, single use Parasep® SF faecal parasite concentrator method has been developed by DiaSys Europe to replace the traditional formalin-ether concentration method. Parasep® SF concentration method removes the debris and fat from faces through a two-stage filtration matrix (using fat dispersion chamber) without using chemical agents that could potentially harm laboratory personnel. In addition, the Parasep® SF needs less time to process, is user friendly and more cost effective for the diagnosis of helminth infection in large epidemiological surveys.[20] However, studies have reported varied levels of sensitivity of the Mini Parasep® SF faecal parasite concentration method for the diagnosis of parasite infections.[20–23] In addition, studies on sensitivity of the Mini Parasep® SF faecal parasite concentration method for diagnosis of STH infections are limited.

Altogether, the Kato-Katz, McMaster and Mini Parasep® faecal parasite concentration methods are less expensive, need less time to perform, and are simple and practical to use in field settings for the diagnosis of helminth infection. However, these microscope-based techniques have been shown to have different sensitivities and considerable variation in quantifying STH eggs, particularly in low transmission settings.[12] The influence of poor sensitivity and operator variability would be amplified, negatively impacting upon data reliability when using such methods, particularly where there is overlap of intestinal parasites. A sensitive, specific and simple-to-apply technique is necessary for mapping priority areas for control of helminth infection, for monitoring and evaluation of control programmes, or for surveillance purposes, as well as for the routine diagnosis of intestinal parasites. As the quantity of stool examined in Mini Parasep® SF faecal parasite concentration method is greater compared with the Kato-Katz and McMaster, it is here hypothesized that the Mini Parasep® SF faecal parasite concentration method will be more sensitive for the examination of different helminth species than the Kato-Katz and McMaster methods. The aim of this study was to compare the diagnostic performance of Mini Parasep® SF faecal parasite concentrator, Kato-Katz thick smear and McMaster technique for the diagnosis of intestinal parasitic infections among Wosha Soyama Primary School children, southern Ethiopia.

Methods

Study area

A cross-sectional study was conducted in Wosha Soyama Primary School in Wondo Genet, southern Ethiopia, from December 2014 to July 2015.Wondo Genet is a resort town located in the southern Ethiopia (7°1′N 38°35′E) with an elevation of 1723 meters. Detailed descriptions of the study area are given in previous studies.[24]

Stool sample collection and examination

Simple random sampling method was employed to select children from each section in grades 1–8, using a table of random numbers, and when the selected child was absent, the student before or after the one indicated was considered for replacement. The selected volunteer children were supplied with piece of plastic sheet and applicator stick, and requested to bring a sizable stool sample. Stool specimens were then processed for microscopy using Kato-Katz thick smear, Mini Parasep® SF faecal parasite concentrator and McMaster technique. For Kato-Katz, a single 41.7 mg Kato-Katz thick smear was prepared and quantitatively examined for hookworm eggs within 30–60 minutes of preparation.[25] The Kato-Katz slides were also examined for other helminths within 24 hours of preparation. The results were recorded as eggs count per slide and multiplied by 24 to convert them into eggs per gram (epg) of stool.

The rest of the samples were transferred to a 20 ml plastic tube and preserved in 10 ml of 10% formalin for subsequent examination by Mini Parasep® SF and McMaster techniques. Part of the stool sample was examined by Mini Parasep® SF concentration method at Arsi University Collage of Health Sciences within one week of stool collection according to manufacturer instruction.[26] Eggs count per slide were recorded and multiplied by two to estimate the epg.[17]

Portion of stool specimens from same samples preserved in 10 ml of 10% formalin were examined by the McMaster egg counting technique at Aklilu Lemma Institute of Pathobiology (ALIPB) within 2 weeks of sample collection.[10] Briefly, 2 g of

stool was suspended in 30 ml of saturated salt (NaCl) solution at room temperature (density: 1.2). Then, faecal suspension that was sieved three times and mixed 10 times with 0.5 ml aliquots and added to each side of a McMaster slide chamber. Both chambers were examined under a light microscope (100× magnification). Faecal egg count (FEC) for each helminth species was calculated by multiplying FEC by 50. Microscopic examination of stool samples prepared using the Kato-Katz, Mini Parasep® SF and McMaster technique were done by the first author (SA) who is an experienced laboratory technologist.

Data analysis

Statistical analysis was performed using Stata version 13. Since there is no reference test for intestinal parasites, the sensitivity, specificity, negative predictive value and positive predictive values were calculated for each of the three methods considering the combined results from the individual methods (e.g. any positive from the three tests was regarded as positive) as the diagnostic 'gold standard'. Kappa values were estimated to determine the strength of agreement of the diagnostic methods and were interpreted as slight (0.01–0.20), fair (0.21–0.40), moderate (0.41–0.60), substantial (0.61–0.80) and perfect (0.81–0.99) agreement.[27] 95% CI values were estimated with p values <0.05 considered as statistically significant.

Results

Prevalence of intestinal parasitic infections

A total of 381 children were examined for intestinal parasites using Mini Parasep® SF faecal parasite concentrator, Kato-Katz thick smear and McMaster techniques. The mean age of the study participants was 9.67 years (ranging from 5 to 18) and 50.7% (193/381) of them were females. Out of 381 children, 86.1% (328/381) were found infected by at least one intestinal parasite species, while 53.8% (204/381), 50.4% (192/381), 34.1% (129/381), 14.7% (56/381) and 12.1% (46/381) were infected by *T. trichiura, A. lumbricoides, S. mansoni*, hookworms and *H. nana*, respectively, as determined by the combined results of the three techniques. *Taenia* spies, *G. lamblia*, *E. vermicularis*, and *E. histolytica/dispar* infections were less frequent among the study participants (Table 1).

The prevalences of any intestinal parasitic infection, as determined by Mini Parasep® SF faecal parasite concentrator, Kato-Katz thick smear and McMaster egg counting techniques, were 77.7% (296/381), 68.8% (262/381), and 47.5% (181/381), respectively. The Mini Parasep® SF faecal parasite concentrator technique detected more cases of *S. mansoni* and *H. nana* infections compared with the other two techniques. On the other hand, the Kato-Katz technique detected higher prevalence of *T. trichiura* infection than the Mini Parasep® SF and McMaster egg counting techniques. None of the children diagnosed positive for *S. mansoni* by either the Kato-Katz or the Mini Parasep® SF faecal parasite technique were found positive by the McMaster egg counting technique.

Average egg counts

The average egg counts per gram of stool (epg) for different helminths by Mini Parasep® SF faecal parasite concentrator, Kato-Katz thick smear and McMaster techniques are presented in Table 2. Generally, Kato-Katz test yielded the highest count for most helminth parasites, followed by the McMaster technique and Mini Parasep® SF faecal parasite concentrator.

Sensitivity, specificity, negative and positive predictive values

Pooled stool examination results obtained by the combined Mini Parasep® SF, McMaster and Kato-Katz thick smear techniques were used as the gold standard to estimate sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of each technique in detecting different species.

Table 1. Prevalence of intestinal parasitic infections as determined by three techniques among children (n=381) in Wosha Soyama Primary School, Wondo Genet, southern Ethiopia

Parasite species	Mini Parasep® SF faecal parasite concentrator	Kato-Katz	McMaster	Combined*	p-value
A. lumbricoides	46.7	31.2	29.4	50.4	<0.001
Hookworms	6.3	1.8	8.7	14.7	<0.001
S. mansoni	31.8	15.2	NPF	34.1	NA
T. trichiura	37.5	44.4	16.3	53.8	<0.001
H. nana	11.3	2.4	8.9	12.6	<0.001
Taenia species	5.5	1.8	NPF	6.3	NA
G. lamblia	1.1	NPF	NPF	1.1	NA
E. vermicularis	0.8	0.5	NPF	0.8	NA
E. histolytica/dispar	2.1	NPF	NPF	2.1	NA
Any intestinal parasite	77.7	68.8	47.5	86.1	<0.001

NA, not applicable; NPF, no parasite found.
Combined*, positive by at least one method (Mini Parasep® SF faecal parasite concentrator, Kato-Katz or McMaster).

The Mini Parasep® SF technique was found to have higher sensitivity and NPV in the diagnosis of infection with *A. lumbricoides* and *H. nana* than the Kato-Katz thick smear and the McMaster techniques. Kato-Katz thick smear technique had higher sensitivity and NPV for the identification of *T. trichiura* than the Mini Parasep® SF and McMaster techniques. Notably, for the infection of hookworms, the McMaster technique had higher sensitivity and NPV compared with the Mini Parasep® SF and the Kato-Katz thick smear techniques (Table 3).

The Mini Parasep® SF parasite concentrator had a higher sensitivity than the Kato-Katz technique in detecting *S. mansoni* infection. All children were negative for *S. mansoni* infection as determined by McMaster technique. However, specificity and PPV of detecting the different intestinal parasites were similar (100%) in all the three tests (Table 3).

Table 2. Average number of eggs counted per gram of stool by the three techniques among children in Wosha Soyama Primary School, Wondo Genet, southern Ethiopia

Helminth species	Mini Parasep® SF faecal parasite concentrator	Kato-Katz	McMaster
A. lumbricoides	210.1	3013.1	1902.6
T. trichiura	16.9	242.7	254.8
S. mansoni	17.1	177.0	NPF
H. nana	28.5	36.0	1057.3
Hookworms	11.8	33.6	91.2
E. vermicularis	4.7	24.0	NPF
Taenia species	29.6	4683.4	NPF

NPF, no parasite found.

Agreement of the test results

The Mini Parasep® SF faecal parasite concentrator had higher agreement with the 'standard' (combined results of the three techniques) in detecting infections of *A. lumbricoides*, *H. nana* and *S. mansoni*, followed by *T. trichiura* and hookworm infections (Table 4). The Kato-Katz offered higher agreement with the 'standard' in detecting *T. trichiura*, followed by *A. lumbricoides* and *S. mansoni* infections. However, the agreement of the Kato-Katz with the 'standard' was modest in the case of hookworm and *H. nana* infections. The McMaster egg counting had highest agreement with the 'standard' in detecting *H. nana* followed by hookworm infections and then *A. lumbricoides*, but the agreement in the case of *T. trichiura* was poor.

Discussion

The purpose of this study was to evaluate the performance of Mini Parasep® SF faecal parasite concentrator, Kato-Katz thick

Table 3. Sensitivity, specificity, NPV and PPV of the three techniques in diagnosing intestinal parasite infections among school children in Wosha Soyama Primary school children, Wondo Genet, southern Ethiopia

	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)
Mini Parasep® SF faecal parasite concentrator				
A. lumbricoides	92.7 (89.0, 96.4)	100 (98.0, 1.0)	93.1 (90.0, 96.5)	100 (97.0, 1.0)
Hookworms	42.7 (29.7, 55.7)	100 (99.0, 1.0)	93.4 (88.0, 94.0)	100 (83.0, 1.0)
S. mansoni	93.0 (88.6, 97.4)	100 (98.0, 1.0)	96.5 (94.2, 98.8)	100 (96.0, 1.0)
T. trichiura	69.8 (63.5, 76.0)	100 (97.0, 1.0)	74.0 (69.0, 80.0)	100 (97.0, 1.0)
H. nana	89.6 (81.0, 98.1)	100 (90.0, 1.0)	98.5 (97.7, 99.7)	100 (90.0, 1.0)
Any species	90.2 (87.0, 93.4)	100 (92.0, 1.0)	62.4 (52.0, 72.7)	100 (98.0, 1.0)
Kato-Katz				
A. lumbricoides	62.0 (55.1, 68.9)	100 (98.0, 1.0)	72.1 (66.7, 77.5)	100 (96.0, 1.0)
Hookworms	12.5 (4.2, 21.8)	100 (0.99, 1.0)	86.9 (83.6, 90.4)	100 (56.0, 1.0)
S. mansoni	44.6 (36.0, 53.1)	100 (98.0, 1.0)	77.7 (73.5, 82.5)	100 (92.0, 1.0)
T. trichiura	82.4 (77.2, 87.6)	100 (97.0, 1.0)	83.0 (77.9, 88.1)	100 (97.0, 1.0)
H. nana	18.8 (7.7, 29.7)	100 (99.0, 1.0)	89.5 (86.4, 92.3)	100 (63.0, 1.0)
Any species	80.0 (75.6, 84.2)	100 (92.0, 1.0)	44.5 (35.6, 53.4)	100 (98.0, 1.0)
McMaster				
A. lumbricoides	58.3 (51.0, 65.3)	100 (98.0, 1.0)	70.3 (64.8, 75.8)	100 (96.0, 1.0)
Hookworms	58.9 (46.0, 71.8)	100 (99.0, 1.0)	93.4 (90.8, 96.0)	100 (87.0, 1.0)
T. trichiura	30.2 (23.7, 36.3)	100 (97.0, 1.0)	55.2 (47.8, 62.5)	100 (93.0, 1.0)
H. nana	70.8 (57.9, 83.7)	100 (99.0, 1.0)	96.0 (93.9, 98.1)	100 (87.0, 1.0)
Any species	55.2 (50.0, 60.6)	100 (92.0, 1.0)	26.5 (20.4, 32.6)	100 (97.0, 1.0)

95% CI, 95% confidence interval; PPV, positive predictive value; NPV, negative predictive value.

Table 4. Agreement of the three techniques with the 'standard' (combined results of the three methods) in diagnosing intestinal parasite infections among school children in Wosha Soyama Primary School, Wondo Genet, southern Ethiopia

Methods	Intestinal parasites	Kappa-value
Mini Parasep® SF faecal parasite concentrator	*A. lumbricoides*	0.93
	Hookworms	0.56
	S. mansoni	0.87
	T. trichiura	0.68
	H. nana	0.94
Kato-Katz	*A. lumbricoides*	0.62
	Hookworms	0.20
	S. mansoni	0.51
	T. trichiura	0.81
	H. nana	0.29
McMaster	*A. lumbricoides*	0.58
	Hookworms	0.71
	S. mansoni	NA
	T. *trichiura*	0.29
	H. nana	0.81

NA, not available.

smear and McMaster techniques for the diagnosis of intestinal parasitic infections. Mini Parasep® SF method showed a greater sensitivity and NPV for diagnosing intestinal helminth infections, particularly for *A. lumbricoides*, *S. mansoni* and *H. nana* species than the Kato-Katz thick smear and the McMaster techniques. The Kato-Katz technique showed better sensitivity and NPV for detecting *T. trichiura* infection, while the McMaster techniques showed better sensitivity and NPV for detecting hookworm infection. However, the ability of Mini Parasep® SF faecal parasite concentrator technique in determining the intensity of helminth infection was lower than that of Kato-Katz thick smear and the McMaster for all helminth parasites. Kato-Katz was better in estimating intensity of *A. lumbricoides* and *S. mansoni* infection, but McMaster was better for estimating intensity of *H. nana*, hookworm and *T. trichiura* infections. A study that involved participants from Africa, Asia and South America also showed greater intensity of *A. lumbricoides* infection when diagnosed using the Kato-Katz method than the McMaster.[10] Another study in Argentina also estimated a higher intensity of *A. lumbricoides* infection, but lower intensity *H. nana* infection based on the Kato-Katz method as compared with the McMaster method.[28] This could perhaps be due to the fact that the Kato-Katz has higher multiplication factors than the Mini Parasep® SF faecal parasite concentrator and McMaster methods.[25,29] In addition, the Kato-Katz method involves examination of a relatively larger quantity of stool (41.7 mg) after screening the debris through screening. However, the McMaster method examines small stool size (~20 mg) without screening the artefacts/debris.

The varying degree of sensitivities among the three techniques for detecting different helminth species could be due the inherent difference in each technique and biological nature of helminth species. For example, hookworms excrete relatively few number of eggs. Moreover, the glycerin used in Kato-Katz method can affect hookworm eggs over time.[25,30,31] This may explain, the poor performance of the Kato-Katz in diagnosing hookworm infection observed in the present study. Similarly, the reason for the poor performance of the Kato-Katz thick smear in detecting *H. nana* could be due to the size (30–40 μm) and nature of the shell (yellowish/transparent and thin) of the eggs that make it to be easily destroyed between 30–120 minutes after the smear is prepared.[32] On the other hand, detection of parasites using the McMaster method involves flotation of eggs using the flotation solution (concentrated sodium chloride). As the buoyancy of eggs is not similar for the different intestinal parasites, performance of the McMaster method may not be identical for the diagnosis of different helminth species. For example, *S. mansoni* egg and unfertilized *A. lumbricoides* eggs will be less likely detected using the McMaster chambers due to the heavy size.[29]

On the other hand, a meta-analysis of 17 studies showed lack of significant difference in the sensitivity of Kato-Katz and McMaster methods for the diagnosis of *A. lumbricoides*, hookworm and *T. trichiura* infection.[12] Although sensitivity of the Kato-Katz (~62.0%) and McMaster methods (58.3%) was comparable for the diagnosis of *A. lumbricoides* infection, sensitivity of the Kato-Katz method was better than the McMaster methods for the diagnosis of *T. trichiura* and sensitivity of the McMaster methods was better than the Kato-Katz method the for the diagnosis of hookworm infection. The current study used combined results of the Mini Parasep® SF faecal parasite concentrator, Kato-Katz thick smear and McMaster techniques as a gold standard for estimating the sensitivity of Kato-Katz thick smear and McMaster techniques. However, almost all the studies included in the meta-analysis by Nikolay et al.[12] did not employ Mini Parasep® SF faecal parasite concentrator techniques for detection of STH infection. In addition, all the studies included in the meta-analysis did not involve a similar 'gold standard' to evaluate the performance of the Kato-Katz thick smear and McMaster techniques for the diagnosis of hookworm and *T. trichiura*.

While Parasep® SF method detected nine parasite species and the Kato-Katz detected seven parasite species, McMaster technique detected only four parasite species. McMaster didn't detect *S. mansoni* egg, which was the third most prevalent helminths in the present study. This confirms previous finding that Mini Parasep® SF better detect STH and *Schistosoma* species than the McMaster method.[33]

Although the present study has generated important information on the comparative performance of Mini Parasep® SF faecal parasite concentrator, Kato-Katz thick smear and McMaster techniques for the diagnosis of intestinal parasitic infections, it is not without flaws. Fresh stool samples are not used for all the three methods and the formalin used for preservation might have an effect on the parasite stages in the stool sample. In addition, the cost and technical issues (field applicability, the time it takes, etc.) were not assessed for the three techniques. Thus, it was not possible to report the exact amount of time spent and resources used for processing individual samples. However, on rough estimation, the Kato-Katz was the most time-consuming method,

followed by the Parasep SF technique for preparing, cleansing and examination of the stool samples. McMaster was the least time-consuming to prepare, clean and read stool samples. This agrees with previous reports.[17,28] As there is no 'gold standard' for the examination of intestinal parasites, we have used the combined results from the three individual methods (e.g. any positive from the three tests was regarded as positive) as the diagnostic 'gold standard'. However, using more sensitive and specific tests such as the quantitative polymerase chain reaction (qPCR) method as a 'gold standard', would a more accurate estimate of the performance of the three methods for the diagnosis of intestinal parasitic infections.

Conclusions

The Mini Parasep® SF faecal parasite concentrator technique performs better than the Kato-Katz thick smear and the McMaster techniques for diagnosing intestinal helminth infections, particularly *S. mansoni*, *A. lumbricoides* and *H. nana* infection in stool samples. However, the Kato-Katz technique is better for the diagnosis of *T. trichiura*, while the McMaster technique would be a better choice for the diagnosis of hookworm infection. Kato-Katz would be a better choice still for estimating intensity of *A. lumbricoides* and *S. mansoni* infection, but McMaster would be a better choice for estimating intensity of *H. nana*. It is suggested here that decision on choice of these methods in epidemiological survey could be made based on the dominance of the specific parasite species in community and the objective of the study.

Authors' contributions: SA, TK, ZM, AD and BE designed the study. SA, with further support from TK and BE, collected and examined the stool specimens. SA and AD analysed and interpreted the data. SA prepared the first draft. TK, ZM, AD, SL and BE reviewed and improved the manuscript. All authors read and approved the final version of the manuscript.

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Competing interests: None declared.

Ethical approval: Ethical clearance was obtained from Ethics and Review Committee of the Department of Microbiology, Immunology and Parasitology, Collage of Health Sciences, Addis Ababa University [Ref No.= 4/T/para/2015]. Permission to conduct the study was obtained from local administration and school directors. Informed written consent was obtained from parents/guardians of the children, while the children gave their assent before participating in the study. All study participants found positive for STHs were treated with a single dose of 400 mg albendazole and those found positive for schistosomiasis, *H. nana* and *Taenia* species were treated with appropriate doses of praziquantel.

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