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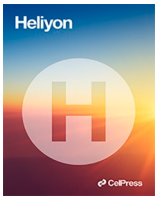

Research article

Evaluation of the diagnostic techniques in the detection of hookworm infestation among school children in Ethiopia: Cross-sectional study design

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ABSTRACT

Background: Hookworm infestation is an important public health concern especially in regions with poor sanitation and limited resources. In healthcare institutions in Ethiopia, wet mount microscopy with low performance has been used as the sole diagnostic technique. Sensitive diagnostic methods are essential for the proper identification of hookworm infection in national strategies for hookworm prevention and management. Thus, the objective of the article was to evaluate the performance of diagnostic techniques in the detection of hookworm infestation among school children in the Gozamin district, Northwest Ethiopia.

Methods: A cross-sectional study with 530 school children was conducted from February to April 2022 in Ethiopia. The study participants were selected by using systematic random sampling technique. Direct wet mount (DWM), Richie's, Kato-Katz (KK), and spontaneous tube sedimentation (STS) diagnostic techniques were utilized to process the stool samples. The data were entered into the Epi-data version 4.2 and the data was analyzed using SPSS version 25.0. The sensitivity, specificity, predictive values and test efficiency of the test were calculated with respect to the Composite Reference Standard (CRS) as the reference method. The degree of agreement with the methods of diagnosis was assessed by the Kappa value.

Results: The overall prevalence of hookworm was 34.9 %. The detection rate of STS, Richie's, KK and DWM techniques in hookworm detection was 30.2 %, 27.0 %, 22.3 % and 15.1 %, respectively. The sensitivity and test efficiency of STS to detect hookworm were 86.5 % and 95.3 %, for Richie's 77.3 % and 92.1 %, for KK 63.8 % and 87.4 %, for DWM method 43.2 % and 80.2 %, respectively. The agreement of STS, Richie's, KK and DWM techniques with CRS were perfect ($\kappa = 0.893$), perfect ($\kappa = 0.816$), substantial ($\kappa = 0.696$) and moderate ($\kappa = 0.498$), respectively in detecting hookworm parasites.

Conclusion: The prevalence of hookworm among school children was high. The STS method is superior to other methods for detecting hookworm infections. The Richie's technique was more successful in detecting the hookworm parasite than the KK and DWM techniques. It is important

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to implement the STS technique in routine diagnostic methods and in endemic areas of hookworm infection because it cheaper, easy to perform, and identifies a wide range of parasitic species.

1. Introduction

Hookworm infections are caused by the parasites *Necator americanus* and *Ancylostoma duodenal*, which are transmitted through contact with contaminated soil. An estimated 1.3 billion cases and 65,000 deaths occur each year from this parasite [1]. Hookworm infestation is an important public health concern especially in regions with poor sanitation and limited resources. Its control is a continuing priority in developing countries, as it directly impacts both individual well-being and economic productivity [2]. Hookworm is the most prevalent in resource-limited countries. In developing countries like Ethiopia, the burden of the disease and its importance to public health remain a priority [3].

Diagnostic techniques are crucial for testing for asymptomatic infections and evaluating the effectiveness of interventions. Likewise, diagnostic techniques function well enough to identify shifts in the infection intensity and prevalence in order to be used as a baseline for the evaluation and monitoring of program impact [4,5]. Finally, diagnostic techniques play an essential role in the management of patients and in the evaluation of the effectiveness of drug efficacy [6].

Diagnostic techniques with low sensitivity, such as the direct wet mount (DWM) technique, lead to a high chance of false negative results. Furthermore, the under identification of parasitic infestations may mislead the physicians [7–10]. The selection and application of the most sensitive diagnostic technique for clinical service and research is a significant challenge. The spontaneous tube sedimentation (STS) technique can be easily applied in developing countries with greater sensitivity, but it is not used for diagnostic purposes. The performance of STS compared to other diagnostic techniques is not well documented in Africa, particularly in Ethiopia [11].

The Richie technique is also a superior method for identifying intestinal nematode eggs than the DWM technique. This technique improves the diagnosis of hookworm because of the large number of stool samples examined compared to DWM [12–14]. Furthermore, the kato-katz (KK) technique for detecting hookworms can be enhanced by examining multiple smears from a single stool specimen [15]. As a result, the advancement and application of alternative diagnostic techniques with high sensitivity are limited in Ethiopia in general and in the study area in particular. Thus, the objective of this study was to evaluate the performance of STS, Richie's, KK and DWM techniques in the identification of hookworm infestation in the Gozamin district, Ethiopia. Therefore, health care service providers, local health planners and policymakers could benefit from the outcomes of this investigation.

2. Method

2.1. Study design, period and area

A cross-sectional study was carried out in children in primary education from February to April 2022 in the Gozamin district, Amhara region, Ethiopia. Gozamin district is one of the 151 districts in the Amhara National Regional State and one of the 18 districts in the East Gojjam Zone. Gozamin is situated 300 km from Addis Ababa, the nation's capital, and 270 km from Bahir Dar, the regional capital. The district is surrounded by the following: Machakle and Debre Elias Wereda to the west, Senan Wereda to the north, Baso Liben Wereda and Oromia National Regional State to the south, and Aneded and Debay Tilatgen Wereda to the east. The district has a total area of 1218 km2 with a total population of 132,883, of whom 66,348 are men and 66,535 women [16,17].

2.2. Inclusion and exclusion criteria

Students aged 6–14 years old, whose parents/carers gave consent and volunteered to participate, were included, whereas students who took antihelminthic drugs for the past two months prior to or during the data collection period were excluded.

2.3. Sample size determination and sampling technique

The sample size was calculated using a single population proportion formula $[(z_{/1\text{-}\alpha/2)})^2 \text{ X p (1-p)}/d^2]$. The 28.5 % prevalence was used from the previous study [18]. By considering, a 5 % of P-value, a 95 % confidence level and a 4 % expected margin of error, the calculated sample size were 489. To reduce the likelihood that noncompliance will occur, 10 % of the sample size was added to the determined sample size then the final sample size was 538. The data were collected from 3 randomly selected primary schools in the Gozamin district. The study participants were selected using systematic random selection procedure, with class roster acting as a sampling frame. Based on the total number of students in each school, the sample size was distributed in a proportionate manner.

2.4. Data collection and processing

Five grams of stool from each child was collected with a clean cup of stool and transported to the nearby health facility in less than an hour. The fresh stool samples were processed using DWM, KK, Richie's and STS techniques.

2.5. Direct wet mount technique

A fresh stool sample of about 2 mg was placed onto a slide using a wooden applicator stick, emulsified with a drop of physiological saline (0.85 %), covered with cover slides, and examined under the microscope [19].

2.6. Kato-katz technique

It was done by transferring the sieved stool to the templates, which deliver 41.7 mg of the stool. Cellophane, previously soaked in malachite green, covered the stool. The ova were identified and quantified. The number of eggs per gram of the stool (EPG) was determined by multiplying the counted eggs per slide by 24. The parasite load was categorized as light, moderate, or heavy according to World Health Organization guideline [20].

2.7. Richie's technique

A fresh stool sample of approximately 0.5 g was added to the sampling tube with 2.5 mL of formalin and 1 mL of ethyl acetate. During 3 min, it was thoroughly mixed and centrifuged for 1500 rotations. The sediment was mixed and examined under the microscope after removing the supernatant [21].

2.8. Spontaneous tube sedimentation technique

Three grams of the faeces specimen were weighed and homogenized in 10 ml of physiological saline. After that, the mixture was filtered via the surgical gauze into a 50 ml plastic tube that was filled with a saline solution up to 50 mL, clogged, and shaken vigorously. The supernatant was discarded after 45 min after the tube was left upright. The bottom sediment sample was taken, placed on a microscopic slide, and examined under a microscope [11].

2.9. Performance evaluation

The performance evaluation of DWM, KK, Richie's and STS hookworm detection techniques was calculated using the composite reference standard (CRS) as the reference standard. The CRC is crucial for the calculation of the sensitivity and specificity of a diagnostic technique in the absence of gold standard. Therefore, the performance of each diagnostic methods was determined by considering the combined results from each methods (any positive from diagnostic methods) as the CRS [22]. The Kappa value was used to evaluate the agreement between each diagnostic technique. For the Kappa value result, the following interpretations were made: 0 (no agreement), 0.01–0.20 (slight), 0.21–0.40 (fair), 0.41–0.60 (moderate), 0.61–0.80 (substantial), and 0.81–1.00 (perfect agreement) [23].

2.10. Data quality control

Representing 15 % (80 study participants) of the total sample size, the preliminary test examined issues related to sample collection, processing, and examination in children at Enidemata primary school children. Throughout the data collection period, the Principal Investigator verified the comprehensiveness, clarity and consistency of the data collected. Through the implementation of quality control measures during the pre-analytical, analytical and post-analytical phases, the reliability of laboratory results was assured. Two laboratory technologists were trained to collect data and detect parasites. The principal investigator investigated the erratic outcomes and determined the final outcome.

2.11. Operational definitions

False positive (FP): the individual does not have the condition but tests positive for the condition.
Sensitivity (SN): is the probability that a truly infected individual will test positive (sensitivity = TP/(TP + FN)).
Specificity (SP): is the ability of a method to identify non-infected individuals correctly (specificity = TN/(TN + FP)).
Positive predictive value (PPV): the probability that those testing positive by the method are truly infected (PPV = TP/(TP/FP)).
Negative predictive value (NPV): the probability that those testing negative by the test are truly uninfected (NPV = TN/(TN + FN)).
Test efficiency (TE): the overall ability of the test to correctly identify positives from negatives and implies the absence of false positives& false negatives (TE= (TP + TN)/(TN + TP + FN + FP)).

2.12. Data analysis

The data were entered into Epidata version 4.2 and analyzed with the statistical software SPSS version 25.0. The SN, SP, NPV and PPV of each diagnostic method were calculated relative to the CRS. The degree of agreement between the diagnostic techniques was ascertained by estimating the kappa value at 95 % CI. The P-value <0.05 was regarded as statistically significant.

2.13. Ethical consideration

This research was done in accordance with the Declaration of Helsinki. The ethical clearance was granted by the ethical review committee of the Department of Medical Laboratory Science, College of Medicine and Health Science, Debre Markos University, Ethiopia (DMLS/ser/105/2022). School permission was granted from the schools where the research was carried out. The parents/guardians of each student were given written informed consent. Each study participant was given assent. Finally, study participants who had intestinal parasites were sent to a local medical center for care.

3. Results

3.1. Socio-demographic characteristics of the study participants

Five hundred and thirty (n = 530) students participated in the present study with, 1.5 % non-response rate. There were 280 male participants (52.8 %) and 58.5 % of the school children were in the age group of 6–10 years. Four hundred and eighty-six (91.7 %) participants resided in rural areas, and 425 (80.2 %) adhered to the Orthodox faith (Table 1).

3.2. Prevalence of hookworm

The overall hookworm prevalnce was 34.9 % (95 % Confidence interval (CI): 30.9%–39.1 %) with a CRS. Using the STS, Richie's, KK and DWM techniques, hookworm infection was 30.2 % (95 % CI: 26.4%–34.2 %), 27.0 % (95 % CI: 23.4–30.9 %), 22.3 % (95 % CI: 18.9–26.0 %), and 15.1 % (95 % CI: 12.3–18.4 %), respectively (Table 2).

3.3. Performance of diagnostic methods in the identification of hookworm infection

The STS method had superior sensitivity (86.5 %) and NPV (93.2 %) in the diagnosis of hookworm as compared to the Richie's (SN) = 77.3 %, NPV = 89.1 %), KK (SN = 63.8 %, NPV = 83.7 %) and DWM (SN = 42.3 %, NPV = 76.7 %) methods (Table 3).

3.4. Test efficiency (TE) of diagnostic methods

The overall test proficiency of STS, Richie's, KK and DWM in diagnosing the hookworm parasite was 95.3 %, 92.1 %, 87.4 % and 80.2 %, respectively (Table 4).

3.5. Agreement of the diagnostic methods in the identification of hookworm infection

The κ agreement between the STS with the CRS for the diagnosis of hookworm was perfect ($\kappa = 0.893$). The agreement of Richie's method with the CRS was perfect in the identification of hookworm ($\kappa = 0.816$). On the other hand, in the identification of hookworms, the KK method ($\kappa = 0.696$) agreed substantially, while the DWM method ($\kappa = 0.498$) agreed moderately (Table 4).

4. Discussions

Hookworms and other soil-transmitted helminths are common in Ethiopia, but they have not been well reported until now. As a result, an appropriate clinical and public health diagnosis is important for battling helminth infection [24]. In this study, four kinds of diagnostic techniques (STS, Richie's, KK and DWM) were evaluated for hookworm diagnosis, using their CRS as the gold standard. The present article revealed that the prevalence of hookworm was 34.9 % (95 % CI: 30.9%–39.1 %). The findings are similar to a reported prevalence of Northwest Ethiopia (33.2 %) [25] and Southern Ethiopia (36.1 %) [26]. However, the present hookworm prevalence was higher than findings from Nigeria (16.8 %) [27], Brazil (12.6 %) [28], Cambodia (9.6 %) [29] and Thailand (6.6 %) [30]. These variations could be caused by differences in financial standing, sampling period, and environmental and geological characteristics of

Table 1
Socio-demographic characteristics of school children among the studied population.

Variables	Category	Frequency (n)	Percentage (%)
Sex	Male	280	52.8
	Female	250	47.2
Age	6–10	310	58.5
	11–14	220	41.5
Resident	Rural	486	91.7
	Urban	44	8.3
Religion	Orthodox	425	80.2
	Muslim	97	18.3
	Protestant	8	1.5

n, number; %, percentage.

Table 2
Prevalence of hookworm using DWM, KK, Richie's and STS techniques among the studied population.

Diagnostic methods	Total examined	Pos (n (%))	95%CI
DWM	530	80 (15.1 %)	12.3–18.4
KK	530	118 (22.3)	18.9–26.0
Richie's	530	143 (27.0)	23.4–30.9
STS	530	160 (30.2)	26.4–34.2
Combined	530	185 (34.9)	30.9–39.1

Pos, Positive; Neg, Negative; n, number; %, percentage; CI, Confidence interval.

Table 3
The performance of diagnostic methods in the identification of hookworm infection against the gold standard among the studied population.

Tests	Result	CRS as "Gold" standard					
		Pos	Neg	Sensitivity	SP	PPV	NPV
				% (95 % CI)	% (95 % CI)	%	% (95 % CI)
DWM	Pos	80	0	43.2(36.3–50.4)	100(98.9–100)	100	76.7(72.5–80.3)
	Neg	105	345				
KK	Pos	118	0	63.8(56.6–70.4)	100(98.9–100)	100	83.7(79.9–87.0)
	Neg	67	345				
Richie's	Pos	143	0	77.3(70.7–82.7)	100(98.9–100)	100	89.1(85.7–91.9)
	Neg	42	345				
STS	Pos	160	0	86.5(80.8–90.7)	100(98.9–100)	100	93.2(90.2–95.4)
	Neg	25	345				

Pos, Positive; Neg, Negative; %, Percent; CI, Confidence interval; SP, specificity; PPV, positive predictive value; NPV, negative predictive value.

Table 4
The test efficiency and test agreement of diagnostic methods in the diagnosis of hookworm infection against the CRS among the studied population.

Tests	Result	CRS as "Gold" standard			
		Pos	Neg	TE	TA
				% (95 % CI)	κ value(95 % CI)
DWM	Pos	80	0	80.2(76.5–83.5)	0.498(0.424–0.572)
	Neg	105	345		
KK	Pos	118	0	87.4(84.2–89.9)	0.696(0.632–0.761)
	Neg	67	345		
Richie's	Pos	143	0	92.1(89.4–94.1)	0.816(0.763–0.868)
	Neg	42	345		
STS	Pos	160	0	95.3(93.1–96.8)	0.893(0.852–0.934)
	Neg	25	345		

Pos, Positive; Neg, Negative; CI, Confidence interval; TA, Test agreement; TE, Test efficiency, 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.

the study areas.

The STS detection rate for the diagnosis of hookworm was 30.2 % (95 % CI: 26.4%–34.2 %) which is comparable with previous study carried out in Ethiopia [18,31] and Peru [13]. The detection rate of Richie's method of identifying hookworm infection was 27.0 % (95 % CI: 23.4–30.9 %), which is comparable to the finding from rural Bahir Dar, Ethiopia (23.7 %) [32]. The KK method also identified 22.3 % (95 % CI: 18.9–26.0 %) hookworm parasites, which is in line with studies done in Ethiopia with a detection rate of (24.16 %) [33] and (25.1 %) [18].

The sensitivity of STS technique was 86.5 % (80.8%–90.7 %) in the detection of hookworm. The finding of this study is lower as compared to study done in Ethiopia (92.2 %) [18] and Peru (100 %) [13], but it is higher as compared to previous study done in Ethiopia (77.8 %) [31] for the detection of hookworm. The present finding also demonstrated that the sensitivity of Richie's and KK were 77.3 % and 63.8 %, respectively in hookworm identification, which is higher than that of an earlier report in Ethiopia [31,33]. The reasons for the variation could be difference in inter-personal competence, sample size, geographic area covered, and environmental sanitation. Another possible justification might be due to differences in endemicity of hookworm species. The sensitivity of DWM 43.2 % (CI: 36.3%–50.4 %) is consistent with previous studies done in Ethiopia (37.4 %) [33] and (37.9 %) [34].

The diagnostic techniques have varied in their sensitivity in the identification of soil transmitted helminths [35]. For instance, in the current study, the STS (86.5 %) was more sensitive than Richie's (77.3 %) and KK (63.8 %) for the diagnosis of hookworm infection, which is consistent with previous reports in Ethiopia [18,31]. The current study has shown that the DWM technique has poor test efficiency and sensitivity (43.2 %). The STS technique, on the other hand, was found to be more sensitive, less expensive, and easier to apply in the routine laboratory diagnosis of hookworm than the other ones. The sensitivity of the STS method is almost two times

higher than that of the most common DWM technique (43.2 %). The low sensitivity of DWM could be explained by the small amount of faeces sample used (DWM uses about 2 mg of the stool, whereas STS uses around 3 g of the stool). It is also possible that the parasite ova in the DWM are hidden by the huge stool detritus.

The Richie's technique (77.3 %) had a higher sensitivity than the KK technique (63.8 %) for the detection of hookworm infection, which is comparable with earlier reports in Ethiopia [31,34,36] and in India [37]. The better performance of Richie's than KK in hookworm identification might be due to the rapid degeneration of delicate hookworm eggs over time [10] and small amount of stool samples was processed via the KK approach [13]. Furthermore, the observed discrepancies can result from glycerin-induced hookworm eggs disappearance after delays between the production of the KK smear and microscopic analysis [38,39]. Likewise, a study conducted in Ethiopia [34,40,41], India [42] and Peru [13] stated that DWM has a lower sensitivity for the identification of hookworm than KK and Richie's method. Therefore, the magnitude of hookworm infestation is greatly underestimated in Ethiopia.

The agreement of the STS technique ($\kappa = 0.893$) and Richie's technique ($\kappa = 0.816$) with the CRS was perfect for hookworm identification, which is comparable with previous study conducted in Ethiopia [31]. Moreover, the KK method agreed substantially ($\kappa = 0.696$) in the detections of hookworm with the CRC. It is not similar with the studies done in the city of Gondar, Ethiopia ($\kappa = 0.57$) [12] and in Tanzania ($\kappa = 0.34$) [24]. The difference may occur as a result of CRS method and differences in hookworm endemicity. The lower sensitivity of DWM, KK and Richie's than STS from the present study indicates that another better diagnostic tool, like the STS technique, is essential for the identification of hookworm in routine diagnosis and research purposes. More so, it cheaper, easy to perform and diagnoses a variety of parasitic species [11].

5. Strength and limitation of the study

The study's strength was the random selection of schools and study participants. The pre-analytical, analytic, and post-analytical phases of quality control were utilized to guarantee the identification of parasites and the validity of laboratory findings. In this study, the prices and timeliness of diagnostic techniques were not compared. Furthermore, the information pertaining to the study area's economic and religious circumstances were not assessed.

6. Conclusion and recommendation

Hookworm was found to be in high prevalence among school children in the study area. The STS method is superior to other methods for detecting hookworm infections. The Richie's technique was more successful in detecting the hookworm parasite than the KK and DWM techniques. It is important to implement the STS technique in routine diagnostic methods and in endemic areas of hookworm infection because it requires less equipment, is simple to perform, and identifies a wide range of parasitic species.

CRediT authorship contribution statement

Abebe Fenta: Writing – original draft, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Destaw Kebede:** Visualization, Supervision, Resources, Investigation. **Adane Tilahun:** Validation, Software, Resources. **Bewket Mesganaw:** Supervision, Formal analysis, Data curation. **Adane Adugna:** Writing – review & editing, Visualization, Formal analysis, Data curation. **Wubetu Yihunie:** Supervision, Methodology, Data curation. **Habtamu Belew:** Software, Methodology, Investigation. **Desalegn Abebaw:** Writing – review & editing, Data curation. **Gashaw Azanaw:** Writing – review & editing, Validation, Supervision.

Ethics approval and consent to participate

This research was done in accordance with the Declaration of Helsinki. The ethical clearance was granted by the ethical review committee of the Department of Medical Laboratory Science, College of Medicine and Health Science, Debre Markos University, Ethiopia (DMLS/ser/105/2022). School permission was granted from the schools where the research was carried out. The parents/guardians of each student gave written informed consent. Each study participant gave assent. Finally, participants in the study with intestinal parasites were referred to a nearby health facility for treatment.

Consent for publication

Not applicable.

Availability of data and material

The data used in this investigation can be obtained from the author upon reasonable request.

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Not applicable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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LIST OF ABBREVIATIONS

CRS	Composite reference standard
DWM	Direct wet mount
EPG	Egg per gram
FN	False negative
FP	False positive
KK	Kato-katz
NPV	Negative predictive value
PPV	Positive predictive value
Sn	Sensitivity
Sp	Specificity
STS	Spontaneous tube sedimentation
TE	Test efficiency
TN	True negative
TP	True positive

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