The Sensitivity and Specificity Comparison Between Stool Wet Mount and Kato-katz Techniques for the Detection of Intestinal Helminthes

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Article

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Abstract

Background

In this prevalence study, the efficiency and sensitivity of stool wet mount and Kato-Katz smear techniques for the diagnosis of intestinal helminthes were compared to detect human intestinal helminths.

Objective

To assess the sensitivity and specificity between stool wet mount and Kato-Katz Techniques

Methodology

A total of 200 stool specimens were collected from school children aged 6 to 16 from four primary schools randomly selected in Badessa woreda, west Hararghe zone, eastern Ethiopia. Each specimen was smeared on one slide for every technique with normal saline for wet mount and a cellophane cover for Kato-Katz technique. The overall prevalence of the parasites in each school of the woreda was determined.

Result

The overall sensitivity of both techniques was calculated. Finally, the evaluation of sensitivity and specificity of both techniques was interpreted as Kato-Katz technique is more effective and sensitive than the stool wet mount technique.

Conclusion

To detect intestinal helminths especially from carriers and non-symptomatic patients Kato-Katz technique is a selective and sensitive method.

1. Introduction

Helminthes are known as parasitic worms (1). Helminthes of medically important are belonging to two phyla: the platy helminthes as flat worms and Nematy helminthes or round worms. Platy helminthes are further subdivided into Cestodes and Trematodes (2). According to some literatures, parasitic worms are categorized into three groups: Cestodes, nematodes, and Trematodes (1). Intestinal protozoa include amoebae, flagellates, and some coccidia. There are two diagnostic stages: vegetative or trophozoite stage and dormant or cyst stage. Both stages may be found in the same specimen (2). They are distributed all over the world. In developing countries such as Africa, their distribution is higher. In Ethiopia, almost all areas of the country helminthes are highly distributed. Majority of them are diagnosed microscopically at egg and larva stage according to their species. Microscopic examination of helminthes is the easiest and simplest technique in stool examination. There are different laboratory techniques to detect helminthes and intestinal protozoa; these include: Buffered Methylene Blue (BMB)

wet mount, Eosin wet mount, Concentration methods, permanent staining techniques, and Cellophane (Adhesive Tape) preparation. There are also different microscopic examinations (wet mounts) which include saline mount and iodine mount (2), or wet mount or thick smear technique or sometimes direct smear (3) and Kato-Katz technique called Kakuru technique (also called the Kakuru-Katz technique) are laboratory methods for preparing human stool samples prior to searching for parasite eggs (1).

In 1954, Kato and Miura were the first to introduce a new method, the “cellophane thick smear technique” which involved the principle of direct fecal sampling. It is different from the standard direct smear procedure in that a larger amount of fecal sample is employed and cellophane strips are used as cover slips instead of glass (3). According to some authors, direct smear is qualitative and Kato-Katz is a quantitative examination method (4).

Effective and efficient technique for the diagnosis of helminthes is essential. Therefore, comparison and evaluation of the diagnosing techniques is a crucial procedure prior to helminthes’ egg findings.

1.1 STATEMENT OF THE PROBLEM

Helminthe and intestinal protozoa infection is one of the health burdens in the world. One of the causes of prevalence and distribution of helminthes and intestinal protozoa are inappropriate use and inefficient application of the testing techniques. In developing countries the problem is aggravated. In Ethiopia, the application of an appropriate and evaluated testing techniques is not understood. Therefore, the prevention and control of helminthes and intestinal protozoa in the country is less effective due to the shortage, quality problem, and lack of evaluated techniques.

The accuracy of one technique in identifying individuals with soil-transmitted helminthes (STH) and intestinal protozoa infections is limited by day-to-day variation in helminthe egg excretion, confusion with other parasites and laboratory technicians’ experience (5).

The accuracy of other techniques is limited by immeasurable sample volume, time-consuming procedures, and unaffordable reagents, unequal sensitivity of the techniques to different parasites, and specificity of the technique to only certain parasite. As a result, the quality of diagnosing and treating of helminthes infection is low.

Intestinal protozoa and soil-transmitted helminthiasis (STH) are still to be major problems in health worldwide, especially in the tropical and subtropical regions. Although frequent, these infections are not always trivial. They can be the cause of a wide clinical spectrum ranging from apparently symptomless infections to life-threatening conditions such as intestinal obstruction in *Ascaris* infection. Therefore, this study is a help to promote and protect the highest attainable standard of health and to facilities for the diagnosis and treatment of helminthes and intestinal protozoa.

There is no any study conducted on the efficiency and sensitivity of soil-transmitted helminthes and intestinal protozoa diagnosing techniques in Ethiopia. Thus, there is a need to generate further sensitivity

evaluation of the Wet mount and Kato-Katz techniques for the diagnosis of soil-transmitted helminthes and intestinal protozoa parasites in faecal samples. The objective of the present study was to fulfill the gap between soil-transmitted helminthes and intestinal protozoa diagnosis and application of a sensitive techniques.

2. Objective

To assess the Sensitivity and Specificity between stool wet mount and Kato-Katz Techniques

3. Materials And Methods

3.1 STUDY DESIGN

A Prospective, Cross-Sectional study was conducted to evaluate the sensitivity and specificity between wet mount and Kato-Katz techniques.

3.2 STUDY AREA

The study was conducted in Badessa woreda area of four schools. Badessa is a town and separate woreda in eastern Ethiopia. Located in the West Hararghe Zone of the Oromia Region, at the base of a spur of the Chercher Mountains 40 km south of the Addis Ababa - Djibouti Railway and 65 km east of Awash, this town has a latitude and longitude of 8°54'N 40°47'E Coordinates: 8°54'N 40°47'E with an elevation of 1761m above sea level. The climate is mostly warm with a dry and rainy season. Badessa, as the rest of the country, has experienced economic and social changes during the past one decade. As a result, there has been a marked improvement in the life style and in the health status of the inhabitants. Other crops in the area are vegetables, greens, cereals, and fruits.

Health care is delivered by government primary health care clinics, health centers, and private clinics of different levels started by physicians, public health officers, nurses, medical laboratory sciences personnel’s, and druggists.

About 200 students had given a stool specimens for both saline wet mount preparation and Kato-Katz preparation per each working day from Monday to Friday at selected schools.

3.3 STUDY PERIOD

The study was conducted from February 20, 2018 to July10, 2018. This includes the entire period of the study, including selection of the title and approval, preparation of the proposal, submission and approved training, pretesting, data collection, data analysis, report preparation, and dissemination of the results etc.

3.3.1 DATA COLLECTION METHOD

A Questionnaire was administered by the principal investigator to the children and it covered the following selected areas (Taking into account that these are the main characteristics commonly related to

intestinal parasitic infection): Age, Gender, Family Education, Finger nail status, Toilet use, Source of drinking water, Shoes wearing habits, residence with domestic animal(s) and anti-helminthic Treatment status before the study was conducted. All students who have no the habit of toilet use, no student was living with animal and no anti-helminthic treatment before 15 days prior to submit stool sample.

3.3.2 SPECIMEN COLLECTION METHOD

Prior to specimen collection Badessa woreda health office has reviewed and approved that a questionnaire and the study protocol has not any ethical and health effect on the students since there was no human tissue was used. Students were informed to submit faecal specimen in to a plastic cup with a tight fitting lid labeled with code. They were also informed to submit a faecal specimen of sized about thumb finger.

3.3.3 LABORATORY TESTING METHOD

Principle and procedure of both Kato-Katz and wet mount methods were followed the centers for disease control and prevention (CDC), world health organization (WHO) as well as international standards guidelines and protocols of stool sample collection and testing methods [13]. Badessa woreda health office has reviewed and approved that the principles and procedures were based on the WHO guidelines and protocols on human intestinal and soil transmitted parasites control and elimination.

3.4 POPULATION

3.4.1 SOURCE POPULATION

All primary schools in Badessa woreda, West Hararghe Zone, Eastern Ethiopia

3.4.2 STUDY POPULATION

All primary school students submitted faecal samples for intestinal helminths examination.

3.4.3 SAMPLING PROCEDURE AND SAMPLE SIZE

Random sampling techniques were applied to select four schools from 11 primary schools found in Badessa Woreda of West Hararghe Zone. Using a convenient sampling method, 50 students from four schools, and a total of 200 students were involved in the study.

3.5. QUALITY ASSURANCE

3.6. DATA AND SPECIMEN QUALITY ASSURANCE

Wet mount and Kato-Katz techniques were carried out by a trained one medical laboratory technologist and the principal investigator. Pilot study checking was done at Olinchity primary school before the actual laboratory testing to check the quality of materials, reagents, and all entire procedures. Diarrheic samples and anthelminthic medicated participants in the last 15 days before the stool examination were excluded.

3.7 STATISTICAL ANALYSIS

All data derived from microscopic examinations were compared for the two techniques. Statistical analysis was done using Statistical Package for Social Science (SPSS) statistical software version 15.

4. Results

4.1. STUDY SUBJECTS

This study was based on two hundred stool samples collected from school children aged 6–16 years. The study was conducted in four primary public schools; fifty students were participated from each school. At a mean age of 11 years, 67% (134 Females) and 33% (66) Males were tested for the study. The Female-to-Male ratio of the study was about 2:1.

The Socio-demographic characteristics of the study subjects were shown according to Table 1 below.

Table 1
Socio-demographic characteristics of the study subjects of Badessa Woreda, West Haraghe, in February, 2018

Variable	Group	Investigated Students	Percentage (%)
Gender	Female	134	67
	Male	66	33
Total		200	100
Age Group	6–9 Years	76	38
	10–13 Years	74	37
	≥14 Years	50	25
School Name	School 1	50	25
	School 2	50	25
	School 3	50	25
	School 4	50	25
Total		200	100
Family Education level	Grade 1–4	199	99.5
	Grade 5–8	1	0.5
Total		200	100
Finger nail Status	Trimmed	129	64.5
	Not trimmed	71	35.5
Total		200	100
Drinking water Source	River	139	69.5
	Well	61	30.5
Total		200	100
Shoes Wearing Habits	Occasional	90	45
	Not at all	110	55
Total		200	100

The study showed that 76 (38%) of the study subjects were from the age group of 6–9 years, 74 (37%) from the age group of 10–13 years, and 50 of the study subjects (25%) were from ≥ 14 years of age group. About 99.5% (199) of the study subjects were from the family educational level of grades 1–4. All

study subjects had no habits of toilet use (100%) and none of them treated with anthelminthic drug (100%) before the study was conducted (Table 1).

Other variables such as; drinking water source, finger trimming status, and bare footing habits were directly related to the prevalence of intestinal helminths in the study subjects.

The following table (Table 2) showed the result concordance with different variables and p- value of the significant variations.

Table 2
Socio-Demographic Characteristics and Personal hygienic habits in Relation to intestinal Helminth infection in the study population

Variables	Group	Positive (%)	Negative (%)	Total (%)	P value
Gender	Female	90 (67.2)	44 (32.8)	134 (67.0)	P = 0.015(0.021) OR 2.04[1.12–3.73]
	Male	33 (50.0)	33 (50.0)	66 (33.0)	F = 0.781 (0.626-0.9)
Total		123 (61.8)	77 (38.5)	200 (100)	M = 1.19[1.08–2.35]
Age Group	6–9 Years	72 (94.7)	4 (5.3)	76 (38.0)	P = 0.000
	10–13 Years	40 (54.1)	34 (45.9)	74 (37.0)	P = 0.000 OR = 0.048Neg.= .753[.679-.851]Pos.=15.79[2.21–2.616]
	≥14 Years	11 (22.0)	39 (78.0	50 (25.0)	P = 0.000
Total		123 (61.5)	77 (38.5)	200(100)	OR = 0.110(.019-.249)
Drinking Water Source	River Water	36 (25.9)	103 (74.1)	139 (69.5)	P = 0.000 OR = 6.9[2.588–18.79] Neg.=1.33[1.17–1.516] Pos.=.191[0.078–0.47]
	Well Water	1 (1.6)	60 (98.4)	61 (30.5)	
Total		37 (18.5)	163 (81.5)	200 (100)	P = 0.385 OR = 1.4 = 0.987[.962-1]
Finger nail Status	Trimmed	60 (46.5)	69 (53.5)	129 (64.5)	
	Not Trimmed	63 (88.7)	8 (11.5)	71 (35.5)	
Total		123 (61.5)	77 (38.5)	200 (100)	
Shoes Wearing habits	Occasional	5 (5.6)	85 (94.0)	90 (45.0)	
	Not at all	32 (29.1)	78 (70.9)	110 (55.0)	
Total		37 (18.5)	163 (81.5)	200 (100)	
Family Education	Grade 1–4	123 (61.8)	76 (38.2)	199 (99.5)	

Variables	Group	Positive (%)	Negative (%)	Total (%)	P value
	Grade 5–8	0 (0.0)	1 (0.5)	1 (0.5)	
School Names	School 1	31(62.0)	19 (38.0)	50 (25.0)	
	School 2	9 (18.0)	41(82.0)	50 (25.0)	
	School 3**	62 (124)	5 (10.0)	67 (33.5)	
	School 4*	45 (90.0)	5 (10.0)	50 (25.0)	
Total		147 (73.5)	70 (35.0)	217 (108.5)	

*Multiple Infections of: Hook Worm + *Enterobius vermicularis* 7 (3.5%)

**Hook Worm + *Schistosoma mansoni* 17 (8.5%)

Total 24 (16.3%)

High parasitic prevalence was observed among the age group of 6–9 years (P = 0.000). Therefore, among all ages, this age group was the highest (accounted for about 38% followed by the age group of 10–13 Years, 37% and ≥ 14 Years was 25%) (Refer Table 2).

There were significant Variations of the parasites between the above variables (Table 2): gender (P = 0.15 (0.021), OR = 2.04 [1.12–3.73], Female (0.781[0.626-0.9)) and Male (1.19 [1.1.08–2.35]); Drinking Water source (P = 0.000) OR = 0.048 (.006-.8); Finger nail status (P = 0.000) or = 0.110 [.049-.249]; Shoe wearing Habits (P = 0.000) OR = 6.9 ([2.588–18.791] (Refer Table 2).

Toilet use, treatment status with anthelminthic drugs before the study, and residence with domestic animal(s) were excluded from the relations due to the following data: All study subjects had no habits of toilet using (Open-air defecations were practiced (100%); none of them treated with anthelminthic drugs before the study was conducted (100%); all study subjects were not live with domestic animal (s).

Prevalence of intestinal helminths detected in faecal samples of all study subjects was summarized for the four schools participated in the study in Table 3.

Table 3 Prevalence of Intestinal Helminths detected from 200 faecal samples from four Public Primary Schools in Badessa Woreda, West Hararghe Zone.

Helminthic Parasites (Ova / Eggs)

Schools	No of Students Examined	*Ascaris lumbricoides*	*Enterobius vermicularis*	Hook Worm	*Schistosoma mansoni*	Total
School 1	50	10 (20%)	7 (14%)	12 (24%)	2 (4%)	31(62%)
School 2	50	4 (8%)	2 (4%)	3 (6%)	0 (0.0)	9 (18%)
School 3	50	0 (0.0)	0 (0.0)	17 (34%)	45 (90%)	62 (124%)*
School 4	50	10 (20%)	7 (14%) **	28 (56%)	0 (0.0)	45 (90%)
Total	200	24 (19.5%)	16 (13%)	60 (48.8%)	47 (38.2%)	147 (73.5%)
* Multiple Infections of: Hook Worm and *Schistosoma mansoni*						
** Multiple Infections of Hook Worm and *Enterobius vermicularis*						

Table 4 represents a further analysis of the detected cases by the two techniques applied for the study.

COMPARISON OF FAECAL SAMPLES DETECTED BY WET MOUNT AND KATO-KATZ TECHNIQUES

A Total of 147 intestinal Helminth Parasitic cases were detected by wet mount and Kato-Katz techniques. From these cases, the Kato-Katz technique detected 147 cases of intestinal Helminths from 123 positive students [100%], whereas; the Wet mount technique detected only 45 [30.6%] which were positive cases by the Kato-Katz technique. There was a significant difference between Wet mount and the Kato-Katz technique [P = 0.000]. There was a far apart between the two methods [Kappa value = 0.241] (Refer Table 4).

Table 4 Comparison of the numbers and percentages of cases detected by Wet mount, Kato-Katz, and Both Techniques

Number of detected cases (Detection Rate, %)

Helminths [Ova /Egg]	Number of Positive Cases [n = 147]	Positive by Wet mount Only	Positive by Kato-Katz Only	Positive detection by Both Methods	Total
Ascaris lumbricoides	24	0 [0.0]	19 [79.2%]	5 [20.8%]	24
Enterobius vermicularis	16	0[0.0]	16 [100%]	0 [0]	16
Hook Worm	60	0[0.0]	34 [56.7%]	26 [43.3%]	60
Schistosoma mansoni	47	0[0.0]	33 [70.2%]	14 [29.78%]	45
Total	147	0 [0.0]	102 [69.4%]	45 [30.6%]	147 [100%]

4.2 DISCUSSION

The current study demonstrated that the prevalence of intestinal helminths were very high in the area when we compared with the previous studies. There were significant variations of all species in all schools (refer Table 3).

This study demonstrated that the variations of intestinal helminth distribution in the woreda areas/ schools required further study. School 3 primary school is the largest in *Schistosoma mansoni* due to highly intensified irrigations are developing whereas; School 4 primary school is the largest in Hook Worm infection. All samples were examined by Wet mount and Kato-Katz techniques, according to the standardized procedure described elsewhere (2, 9). One slide was prepared per person for each technique (Wet mount and Kato-Katz techniques), and egg findings from Kato-Katz preparation were conducted 30 minutes after its preparation.

This study has an agreement in Kato-Katz technique Sensitivity with some of the previous studies (1, 3, 5, 8, 9, 12, 14, and 15); However, the study conducted in other areas (4, 6, 7 and 17) revealed that Kato-Katz technique was less sensitive for soil transmitted Helminths including Hook Worm infection. The present study found that Kato-Katz technique is more sensitive than Wet mount technique for all intestinal helminths infections. In contrast to the previous study (4), the present study examined a single slide per person and concluded that the Kato- Katz technique, when used with the wet mount, is sensitive, appropriate and efficient for the examination of intestinal helminthiasis.

As to the relationship between prevalence sexes, the study demonstrated that the infection rate was higher in females in comparison to males. The study also demonstrated that the prevalence of intestinal Helminths was related with drinking river water, bare footing, and not trimming the finger nails. However, within the limited scope of this study, the relationships between using toilets, family education, antihelminthic treatment, and residence with domestic animals and prevalence of intestinal helminths were given less attention according to the following data. All study participants used no toilet (100%), 199 (99.5%) families had grade 1–4 educational level, all participants were not treated with any

antihelminthic drug before the study was conducted (100%), and all participants were not living with any domestic animal(s).

The overall prevalence of Intestinal Helminths in the woreda was 73.5%. This may be due to poor personal and environmental hygiene like other areas of the country.

The most serious consequence of Hook Worm infection is chronic blood loss from the small intestine, which leads to iron deficiency anemia, particularly in children and women of childbearing age, whose physiological needs for iron are greater. In areas where Hook Worm anemia is present, 50% or more of the population may have hemoglobin values below the normal range and some of these may have severed degrees of anemia (10).

The overall prevalence of intestinal Helminths among the study subjects were led by Hook Worm infection with 48.8% followed by *Schistosoma mansoni* with 38.2%, *Ascaris lumbricoides* with 19.5% and 13% *with Enterobius vermicularis*. There were significant variations of the parasites among schools. School 3 public primary school was the highest prevalence with 214% including multiple infections of Hook Worm and *Schistosoma mansoni.* School 4 was 48.8% in Hook Worm infection and Hook Worm and *Enterobius vermicularis* double infection was observed.

This is the first report on the sensitivity of Kato-Katz technique, after the examination of Hook Worm infection on 30 minutes after preparation.

4.3. CONCLUSSION

The current study concluded that the Kato- Katz technique for *Schistosoma mansoni* and Soil-transmitted intestinal Helminths examination in faecal samples. It also a selective technique for field survey to investigate the prevalence and drug resistance of the parasites. It seems necessary to indicate that Kato-Katz technique is not appropriate for intestinal examination in areas where the species may exist.

4.4. RECOMMENDATIONS

High magnitude of Hook Worm in School 3 and *Schistosoma mansoni* in School 4 areas calls for immediate intervention. School 1 and School 2 areas also need nonspecific control measures.

The study wants to indicate that a comprehensive control strategy for helminths infection should include:

Ensuring the wide availability of anithelmintics for *schistosomiasis* and soil-transmitted helminth infections in all health services in endemic areas;

Ensuring good case management of symptomatic cases (example, IMCI);

Regular treatment of all children at risk, including adolescent girls; through school-and community-based initiatives;

Treating pregnant women at risk through antenatal care and other women's health programs;

Ensuring a saved water supply and adequate sanitation facilities in all schools;

Ensuring provision of potable water and sanitation facilities at house or community level;

Promoting good hygiene and sanitation practices among school children and caregivers (Hand washing, finger nail trimming, using of latrines, and use of footwear) through community development activities and in school curricula.

Declarations

Ethics approval and consent to participate

The study was reviewed and approved by Ambo University Woliso Campus ethics and research review committee. Written and verbal informed consent for participation was obtained from students, teachers, and schools principals as the study poses no risk to them. All those who harbored pathogenic intestinal Helminthic infections were given an anthelminthic drug (Praziquantel) to be treated appropriately. In this study there was no human tissue is collected and not used as one of the study material.

Consent for publication

Not applicable (N/A)

Availability of data and materials

The full data for this study is available from the corresponding author when there is a reasonable need.

Competing interests

The author declares that he has no competing interests.

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The author's contributions

The author was fully participated in gap finding, proposal writing, involved in the study design, data collection, data analysis, report writing, writing the manuscript, and edition of the revised manuscript.

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Limitations Of The Study

Unlike other studies conducted in many areas of the world, the current study did not consider the parasitic count per person. The smallness of the sample size (200 school Children) was also another drawback of the current study. Within the limited scope of the current study, there was incapability to determine the specific reason of variations of distribution of the intestinal helminths in the woreda except that in School 3 areas in which irrigations are highly extended.

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