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Comparative Study of Saline Wet Mount and Modified Kato-Katz Methods for Detection of Intestinal Helminths from Under 5-Year Children of Day Care Centres, Mingaladon Township

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Saline wet mount technique is commonly used for detection of helminthic infection in most laboratories of Myanmar. But this technique is less sensitive and could not determine the intensity of helminthic infection. The modified Kato-Katz technique is the best quantitative method to detect intensity. This study was to compare sensitivity, specificity and practicability of saline wet mount and modified Kato-Katz methods. The cross-sectional, comparative study was conducted from June 2013 to July 2014. A total of 100 stool samples were taken from under 5-year children who were attending the day care centres of Mingaladon. The detection rate of intestinal helminths by formol-ether concentration technique was 30% followed by modified Kato-Katz method and direct saline wet mount method (28% and 23%, respectively). Among these methods, the formol-ether concentration technique had better detection rate for helminthic eggs. The modified Kato-Katz had 93% sensitivity, 100% specificity and 98% accuracy for detection of intestinal helminths. For saline wet mount method, the sensitivity was 77%, specificity was 100% and accuracy was 93%. The results revealed that modified Kato-Katz method was shown to be more sensitive than saline wet mount.

Key words: Kato-Katz, Saline wet mount, Intestinal helminths, *Ascaris lumbricoides, Trichuris trichiura, Enterobius vermicularis*

INTRODUCTION

There is overwhelming evidence to show that intestinal worm infestation due to *Ascaris lumbricoides*, *Trichuris trichiura* and two hookworms *Ancylostoma duodenale* and *Necator americanus* and *Enterobius vermicularis* represents the leading cause of morbidity in pre-school and school-age children. In tropical regions, virtually all underprivileged children are carriers of intestinal parasites.[1]

Infestations by geohelminths are largely diseases of chronic morbidity and debilitation and, as such, the suffering is difficult to quantify at the population level. It is also a major cause of morbidity in school-age children who has the highest burden of worm infestation.[2]

Commonly used diagnostic methods for intestinal parasites rely on the detection of helminth eggs or larvae in human stool. These copromicroscopic approaches have drawbacks, such as low sensitivity for the detection of light-intensity infections. At present, the Kato-Katz technique is the most widely used method in epidemiological surveys pertaining to human intestinal helminth infections because of its simplicity, low cost, and the established

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system to stratify infection intensity into different classes based on cut-offs of egg-counts. The ether-concentration method is often used for the diagnosis of helminth infections. Importantly, it allows the concurrent diagnosis of intestinal protozoa, and is sometimes used in combination with the Kato-Katz method to enhance diagnostic sensitivity for helminth. An important feature of the ether-concentration method is that it uses preserved stool samples, fixed in either sodium acetate-acetic acid-formalin (SAF), or diluted formalin, thus allowing sample storage and analysis at later time points.[3]

Most hospital laboratories in Myanmar perform routine stool examinations by the direct wet mount technique only. Direct wet mount technique is simple, rapid and inexpensive, but it can miss low intensity infections if too much debris or fat is present in the preparation. The consequences of misdiagnosis can be grave. Therefore, there is the need for more accurate diagnosis of intestinal parasitic infections at the laboratory which functions as a teaching hospital laboratory. This study gave more diagnostic information for detection of various parasite densities from stool of patients.

MATERIALS AND METHODS

Laboratory-based, cross-sectional, comparative study was employed to identify intestinal helminths from stool specimens collected at day care centres. This study was carried out at the Department of Microbiology, Military Institute of Nursing and Paramedical Sciences (MINP), Yangon and day care centres in Mingaladon. This study was conducted from June 2013 to July 2014. A total of 100 stool samples were obtained from under 5-year children attending the day care centres as subjects. Children who had history of taking antihelmenthic drug in the two weeks prior to screening and contaminated samples (with urine, water and other materials) were excluded from the study.

Children were given plastic containers (125 ml) and instruction sheet for stool collection. Upon submission, each container was labeled with a unique identification number and sent within 1-2 hours to the Department of Microbiology, MINP. Stool samples were processed within 2-3 hours after reaching the laboratory.

Saline wet mount method

A drop of saline was placed on the microscope slide. A small portion of the specimen was picked up with an applicator stick and mixed with saline drop. The cover slip was put separately and examined under the microscope.[4]

Modified Kato-Katz method

The cellophane strips were soaked in the glycerol-methylene blue solution for at least 24 hours before use. A small amount (approximately 0.5 g) of faeces was transferred onto a piece of newspaper. The screen was pressed on top of the faecal sample. Using the applicator stick, the upper surface of the screen was scraped across to sieve the faecal sample. The template was placed on a clean microscope slide. The sieved faecal material was transferred into the hole of the template and leveled with the applicator stick. The template was removed carefully so that all the faecal material was left on the slide and none was left sticking to the template. The faecal sample was covered on the slide with a glycerol-soaked cellophane strip. The upper surface of the cellophane was wiped off with a small piece of absorbent tissue if any glycerol was present on it. The microscope slide was inverted and pressed the faecal sample against the cellophane on a smooth surface to spread the sample evenly. The slide was not lifted straight up. The microscope slide was gently slided sideways while holding the cello-phane. Preparation of the slide was now complete. Any excess glycerol was wiped with a piece of absorbent tissue to ensure that the cellophane stays fixed. Stainless steel templates 1.5 mm thick, with a hole 6 mm in diameter, and thus designed to contain 41.7 mg of stool were used. Conversion of

egg counts/slide into eggs per gram (e.p.g.) was done by multiplication by 24.[5]

Concentrated method

Ten millilitre of 10% formalin was added to approximately 1 g of faeces and stirred using an applicator stick, until it became slightly cloudy suspension was obtained. A gauze filter was fitted into a funnel and placed the funnel on top of the centrifuge tube. The faecal suspension was passed through the filter into the centrifuge tube until the 7 ml mark was reached. The filter was removed and discarded the filter with the lumpy residue.

Three millilitre of ether or ethyl acetate was added and mixed well for one minute. This mixture was put into the centrifuge tube and centrifuged for one minute. The fatty plug (debris) was loosed with an applicator stick and poured away the supernatant by quickly inverting the tube. It was put in its rack and allowed the fluid on the sides of the tube to drain down to the sediment. It was mixed well and a drop was placed on a slide for examination under a cover slip. The area under the cover slip on the slide was examined for ova, cysts and larvae by using the x10 and x40 objectives.[6]

Data analysis

Sensitivity, specificity and accuracy were calculated by using the formula;

Sensitivity=TP/(TP+FN)×100;
Specificity=TN/(TN+FP)×100; and
Accuracy=(TP+TN)/all cases examined×100;
where TP=True positive, FN=False negative, TN=True negative, FP=False positive.[7]

Ethical consideration

Ethical approval was obtained from ethical committee of MINP and permission from the authorized person of study area.

RESULTS

Thirty out of 100 study units (30%) were detected to have intestinal helminthic infection while remaining (70%) were free from intestinal helminths based on the stool examination by formol-ether concentration technique (Fig. 1).

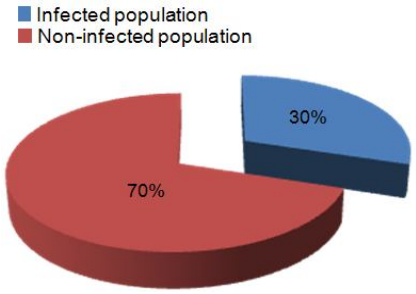
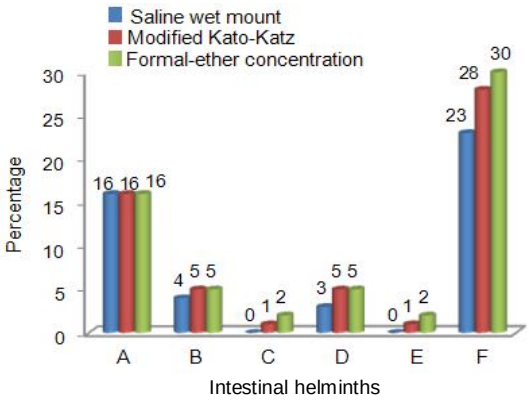


Fig. 1. Prevalence of intestinal helminthic infection among the study population

Of the total 30% infected cases, *Ascaris lumbricoides* was 16%, followed by *Trichuris trichiura* (5%), *Enterobius vermicularis* (2%), *Ascaris lumbricoides* and *Trichuris trichiura* (5%) and *Ascaris lumbricoides and Enterobius vermicularis* (2%).



A=*Ascaris lumbricoides*
B=*Trichuris trichiura*
C=*Enterobius vermicularis*
D=*Ascaris lumbricoides+Trichuris trichiura*
E=*Ascaris lumbricoides+Enterobius vermicularis*
F=Total

Fig. 2. Comparison of direct saline wet mount, formol-ether concentration method and modified Kato-Katz method for detection of intestinal helminths

The rate of infection for intestinal helminths detected by formol-ether concentration technique was 30% followed by modified Kato-Katz method (28%) and direct saline wet mount method (23%), respectively (Fig. 2).

Although 7 samples which were positive for helminths by formol-ether concentration turned out to be negative by saline method, all saline method positive results were concordance with formol-ether concentration result.

Although 2 samples which were positive for parasites by formol-ether concentration turned out to be negative by modified Kato-Katz method, all modified Kato-Katz method positive results were concordance with formol-ether concentration result.

In this study, all positive results with wet mounts and Kato-Katz techniques were concordance with formol-ether concentration technique result (Table 1).

Table 1. Results of Wet mounts and Kato-Katz techniques compared with the gold standard (FEC)

Method	Result	FEC		Total
		Positive	Negative	
Wet mount	Positive	23(TP)	0(FP)	23
	Negative	7(FN)	70(TN)	77
	Total	30	70	100
Kato-Katz	Positive	28(TP)	0(FP)	28
	Negative	2(FN)	70(TN)	72
	Total	30	70	100

FEC=Formal-ether concentration, TP=True positive, TN=True negative, FP=False positive, FN=False negative

Modified Kato-Katz method has 100% specificity, 93% sensitivity and 98% accuracy. Whereas saline method has 100% specificity, 77% sensitivity and 93% accuracy.

Intensity of intestinal helminths was classified by modified Kato-Katz method according to WHO cut-offs (1-4999 epg, 1-999 epg, 1-9999 epg for *A. lumbricoides*, *T. trichiura* and Hookworms, respectively). Among the infected children, light intensity for *Ascaris lumbricoides* was 23 and *Trichuris trichiura* was 10. There were no moderate and heavy infections in infected children.

The procedural steps for saline wet mount method were very few but procedural steps for modified Kato-Katz were few and formol-ether concentration were several. The time taken to identify the helminths by saline wet mount was less than 60 minutes but Kato-Katz was 60-90 minutes and formol-ether concentration was 90-120 minutes. In Myanmar, saline wet mount and Kato-Katz can be available in microscope equipped laboratory and formol-ether concentration can be available in reference laboratory. These data are presented in (Table 2).

Table 2. Operational characteristics of the methods

Chara-cteristics	Saline wet mount	Modified Kato-Katz	Formol-ether concentration
Procedural steps	Very few	Few	Several
Ease of per-formance	Very easy	Easy	Less easy
Time required	Results in less than 60 minutes	Results ready in 60-90 minutes	Results ready in 90-120 minutes
Materials required for testing	Physiological saline	Screen (stainless steel, nylon or plastic), template (stainless steel, plastic or card-board), cellophane, flat-bottomed jar, forceps, scrap paper, glycerol-methylene blue solution	Formalin, diethyl-ether, centrifuge, centrifuge tubes, gauze pads, test tube racks, fume chamber
Sensitivity	77%	93%	100%
Specificity	100%	100%	100%
Cost	Cheap	? Less expensive	? Expensive

DISCUSSION

In this study, overall positivity of stool helminth was 30%. The results showed that intestinal helminthiasis was one of the major health problems in children (under 5-year children) of the day care centres, Mingaladon Township.

The present study was comparable to the study of the prevalence of soil-transmitted helminthiasis among primary school children in Lanmadaw Township where the overall prevalence rate was 36.7%.[8] It was lower than the findings from the other studies where there were 69.5% and 61.67% among primary school children attending in North Okkalapa Township.[9, 10]

In the present study, prevalence of intestinal heliminthic infections detected by the

formol-ether concentration, modified Kato-Katz and the direct wet mount methods were 30%, 28% and 23%, respectively. The direct wet mount exhibited the lowest performance and less sensitive than the formol-ether concentration and modified Kato-Katz methods.

In a case study at the Komfo Anokye Teaching Hospital in Ghana, the formol-ether concentration method gave the highest prevalence (11.1%) of helminthic parasites. The direct wet mount and Kato-Katz methods detected total prevalence of 3.2% and 5.1%, respectively. Kato- Katz method showed good agreement with the formol-ether concentration in the detection of hookworms, *Trichuris trichiura*, and *schistosoma mansoni*.[11]

Findings from the present study confirmed earlier reports that the traditional direct wet mount screening test is less sensitive hence the employment of modified Kato-Katz and formol-ether concentration as the confirmatory tests in routine laboratory examination of stool samples will significantly reduce misdiagnosis of intestinal helminthic infections and its attendant public health consequences.

The use of direct wet mount alone as an indicator of intestinal parasitic infections is also suggested to be insufficient. However, in most laboratories in Myanmar, the direct wet mount is the preferred stool parasitological detection technique. This shows that, since the use of direct wet mount as a confirmatory test will significantly increase misdiagnosis of intestinal helminthic infections, the use of another diagnosing method is mandatory to decrease the consequences caused in the community due to intestinal helminthic infections.

In the present study, modified Kato-Katz has 100% specificity, 93% sensitivity and 98% accuracy respectively. For saline wet mount method, the sensitivity was 77%, specificity was 100% and accuracy was 93%. The results revealed that modified Kato-Katz was shown to be more sensitive than saline wet mount. However, specificity of both modified Kato-Katz and saline wet mount method were 100%. The accuracy of modified Kato-Katz and saline wet mount methods were 98% and 93%, respectively.

In this study, saline wet mount method missed *Trichuris trichiura* (1%), *Enterobius vermicularis* (1%), *Ascaris lumbricoides* with *Trichuris trichiura* (2%) and *Ascaris lumbricoides* with *Enterobius vermicularis* (1%) of infected individuals, respectively, as compared to modified Kato-Katz method. Though saline wet mount method is quick to prepare and inexpensive, it can miss egg of helminths. Thus in this study, a significant number of the infected population was missed by saline wet mount method as compared to the modified Kato-Katz method.

In the study of the accuracy of diagnosis of intestinal helminthes at the Komfo Anokye Teaching Hospital, Kumasi, sensitivity and specificity of the wet mount method were found to be 29.2% and 100%, respectively. By using Kato-Katz method, the sensitivity and specificity were 92.8% and 100%, respectively. Formol-ether concentration method has 100% specificity, 93.33% sensitivity for broad range of parasite ova, larvae and cysts and 98% accuracy, respectively.[12]

The present study reconfirmed that the direct wet mount was less sensitive than the modified Kato-Katz method. The pattern of sensitivity and specificity also suggested that modified Kato-Katz method could suffice for a confirmatory test in routine examination of stool specimens for intestinal helminths. This approach will improve the detection of helminths from stool specimens for accurate diagnosis of intestinal helminth infections, for effective management of patients and the communities.

In the present study, the intensity of intestinal helminthiasis was light in all of the subjects. Predominantly light intensity

observed in this study could be attributed to the frequency of de-worming exercise practiced within the study population. Most of the children receive antihelminthic drugs every 3-6 months.The light intensity of infection detected in this study was similar to that of children from a day care centre in Matanzas city where the light infections of *Ascaris lumbricoides* and *Trichuris trichiura* were found.[13]

In another study, intensities of *Ascaris lumbricoides*, *Trichuris trichiura* and hook-worm were determined by stoll's dilution egg counting technique and revealed that light infection was seen in 32.22%, 15% and 1.67%, respectively. Moderate infection was 3.33% for *Ascaris lumbricoides* and 0.56% for *Trichuris trichiura*. None of the children have heavy infection.[10]

Of the three parasitological methods used, the direct wet mount was found to be the simplest, most affordable and required minimum labour and skill to perform. It is rapid to perform, involving very few steps to complete. It does not require any special equipment or materials other than physio-logical saline. Other authors have described similar characteristics.[14-16]

Among 100 stool specimens examined for helminth eggs, the wet mount method gave a sensitivity of only 77% relative to the recovery rate of helminth parasites with the formol-ether concentration. The findings of the present study as well as others have shown that the wet mount method was the least sensitive method among the three parasitological methods evaluated.[12, 17]

The modified Kato-Katz method was found to be relatively rapid and easy to perform, required minimal training and involved few steps to complete. The Kato-Katz technique is a useful tool for the quantification of egg counts to determine infection intensities. These qualities make the modified Kato-Katz the most frequently employed method in research works.[18, 19]

Based on the findings of the present study, the modified Kato-Katz method is more effective and efficient than saline wet mount method for diagnosis of intestinal helminthic infections in assessment of anthelminthic drug sensitivity, and epide-miological and clinical studies.

Conclusion

Saline wet mount technique is easy to use, but it is less sensitive than the modified Kato-Katz method and unable to count the helminth eggs. Findings from the present study showed that using direct wet mount only significantly increased the mis-diagnosis of intestinal helminth infections.

If modified Kato-Katz technique is adopted as a routine method in diagnosing intestinal helminths or is incorporated with direct smear method, stool microscopy will continue as the most important diagnostic method and at the same time reducing the traces of prevalence of intestinal helminths ensuing from misdiagnosis.

The arguments used for recommending the modified Kato-Katz technique in the field are that it would be the best quantitative method available; that it is cheap, easy to learn and to perform with little special material needed; that no hazardous products have to be used.

In conclusion, the present study revealed that modified Kato-Katz technique showed a better sensitivity than the traditional direct wet mount method. Therefore, the employment of modified Kato-Katz technique as a confirmatory test in routine laboratory examination of stool and in epidemiological studies will significantly aid in accurate determination and manage-ment of intestinal helminth infections in the community.

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