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Accuracy of Kato-Katz versus direct examination methods for diagnosing helminthiasis using preserved stool

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Abstract

Purposes: More than 1.5 billion people, or a quarter of the world's population, are infected by soil-transmitted helminth (STH). Children aged 2-14 years are the most susceptible to helminthiasis. In Indonesia, there are 60.4 million children infected with STH, with an average prevalence rate of 31.8%. Helminthiasis can cause growth and development disorders in children. Diagnosing helminthiasis could use qualitative (direct examination) and quantitative (Kato-Katz) methods. This study aimed to determine the accuracy of both methods in diagnosing helminthiasis using preserved stool specimens. **Methods:** This research design was an observational study with a cross-sectional approach. A total of 140 stool samples preserved using 10% formalin for 13 months were examined using a light microscope with the Kato-Katz and direct examination methods. **Results:** Among the 140 stool samples examined, 64 samples (45.71%) were found positive for STH using the Kato-Katz method, while with the direct examination method, 50 samples (35.71%) were positive for STH. The accuracy test showed that the sensitivity and specificity values of the Kato-Katz method were 86.79% and 79.31%, while the direct examination method was 64.15% and 81.60%, respectively. **Conclusions:** The Kato-Katz method was more sensitive than the direct examination method in diagnosing helminthiasis in the preserved stool. However, the direct examination method had a higher specificity value than the Kato-Katz method. In addition, the examination accuracy of fresh and preserved stool specimens in diagnosing helminthiasis was not significantly different.

Keywords: Kato-Katz; direct examination; soil-transmitted helminth; diagnostic test; accuracy test

INTRODUCTION

Soil-transmitted helminth (STH) infection is the most common helminth infection and is one of the neglected tropical diseases. More than 1.5 billion people, or a quarter of the world's population, are infected by STH. Cases of STH infection are generally caused by three types of helminths, namely A. lumbricoides with the highest number, followed by *T. trichiura* and hookworm (1.2). This occurrence is

closely related to the poor population with low sanitation and hygiene levels in many developing countries. The people most susceptible to STH infection are the children's age group, namely preschool and school-age or ages 2-14 years (2). In this age group, cases of STH infection in the Southeast Asian region are 117 million children (3). The prevalence of STH infection in Indonesia is also high, with 2.5-62% (4). In Indonesia, 60.4 million preschool and school-age children are infected with STH, with an average prevalence rate of 31.8% (3.5).

Several studies have been conducted to determine the prevalence of STH infection in Bengkulu province. A survey conducted on elementary school children in the Seluma Timur sub-district showed that the prevalence of STH infection was 16.7% (6). Furthermore, for elementary school children in Kungkai Baru village, Seluma district, the prevalence rate of STH was 59.7% (7). In Bengkulu, a study of the prevalence of STH infection conducted in elementary school children in the Fisherman village of Bengkulu city showed 9.19% (8).

STH infection is often chronic, so the impact it causes is only visible after a long time. Patients with mild STH disease usually do not show clinical symptoms. In contrast, cases of severe infection can cause various health problems, including abdominal pain, rectal prolapse, and delayed physical and cognitive growth in children. STH infection can also increase susceptibility to malaria, tuberculosis, diarrhea, and anemia (1). The death rate from this worm case also reaches 135 thousand people per year (9).

STH infection can be diagnosed by conducting a qualitative and quantitative stool examination. The most commonly used method for qualitative analysis is the direct examination method, while the Kato-Katz method is used for a quantitative study. Both of these methods are pretty practical and cost-effective (10). The direct examination method cannot determine the degree of worm infection and is less sensitive to mild conditions. In contrast, the quantitative Kato-Katz method can determine the degree of helminth infection. However, compared to the direct examination method, the Kato-Katz method requires more time to prepare the equipment (11,12).

Sensitivity and specificity are two indicators that show the validity of a diagnostic test. The higher the sensitivity and specificity of an examination method, the more accurate the examination method will be. Simplicity and ease in preparing tools and materials for an examination method can also be considered in diagnosing. The Kato-Katz method is the most sensitive to other methods in diagnosing helminth infection. In one study, the Kato-Katz method showed more sensitive results, although not much different than the direct examination method in diagnosing STH disease in fresh stool. The Kato-Katz method had 63.8%, 82.2%, and 59.5%, respectively, in detecting *A. lumbricoides*, *T. trichiura*, and hook helminths, while direct examination had a direct examination sensitivity of 52.1%, 62, 8%, and 42.8% (13).

Ideally, fresh stool specimens were used to establish the diagnosis of helminthiasis so that the detection and identification of worm eggs in the specimen were optimal. In addition to using fresh stool, both methods could also use the preserved stool as the specimen. Usually, stools were preserved in a 10% formalin solution. Stool preservation is required when sending to a remote laboratory or when it is necessary to identify difficult or rare parasites and cases that require re-examination or confirmation (14). Under favorable conditions, STH eggs could hatch within hours to days in their life cycle. However, with the addition of formalin solution, which could bind to the structure of the egg wall, it could be inhibited to prevent bias and errors during examination for diagnosis (1.14). A study conducted using the Kato-Katz method, mini-FLOTAC, and direct analysis of stool preserved with formalin for more than 12 months showed that STH eggs in the stool were still identifiable (15).

The limited laboratory facilities to diagnose cause unequal data distribution on helminthiasis prevalence in Bengkulu Province. This study had implications for the difficulty of using fresh feces as examination specimens. Most specimens must be transferred to a relatively far away laboratory which takes a long time. Hence, the use of preserved stool specimens was one of the solutions. Through this research, an analysis of the accuracy of the Kato-Katz method would be carried out, and a direct examination to establish the diagnosis of helminths using preserved stool specimens so that doubts about using these specimens could be removed.

METHODS

This research design was an observational study with a cross-sectional approach. Stool specimens used in this study were obtained from students in grades 2 to 6 in several elementary schools in the Kampung Melayu District, Bengkulu City. The stool specimen was collected 13 months earlier. The Kato-Katz method in the fresh stool was used as a sample in a previous study to diagnose helminthiasis. After being used in the study, the stool specimens were then preserved with 10% formalin solution then stored in the storage room of the Bengkulu University Microbiology Laboratory.

The sample was selected using a simple random sampling technique. Examination of 140 stool samples in this study was carried out at the same laboratory. The independent variable was the Kato-Katz method using a preserved stool and direct examination. In contrast, the dependent variable was the test accuracy.

Data collection began with checking formalin-preserved fecal specimens, selecting samples, recording sample initials, and examining samples with the Kato-Katz and direct examination methods and continued with calculating the sensitivity and specificity values of the two methods. This study used a diagnostic test analysis technique followed by testing the accuracy of the Kato-Katz and direct examination method of preserved stool using previous research data as the test standard. The data obtained from the study were then analyzed using the statistical software program SPSS version 16.0.

RESULTS

Table 1 found positive for STH using the Kato-Katz method, while 50 samples (36%) were positive for STH with the direct examination method.

Table 1. STH infection by type of examination method

Method	STH infection	n	%
Kato-Katz	Positive	64	45,71
	Negative	76	54,29
Direct Examination	Positive	50	35,71
	Negative	90	64,29
Total		140	100

Table 2 shows Kato-Katz method, samples with a single infection by *A. lumbricoides* and *T. trichiura* were 41 samples (29.29) and 16 samples (11.42%), respectively. A mixed infection of *A. lumbricoides* and *T. trichiura* was found in 6 samples (4.29%), while a mixed infection of *A. lumbricoides* and hookworm was found in 1 sample (0.71%). The total samples containing mixed infections examined by the Kato-Katz method was 7 (5%). On the direct examination method, 33 samples had a single infection with *A. lumbricoides* (23.57%) and *T. trichiura* 12 (8.57%). Mixed infections of *A. lumbricoides* and *T. trichiura* were also found on direct examination, 5 samples (3.57%). Based on the sample examination results using both methods, it was found that *A. lumbricoides* had the highest prevalence rate, followed by *T. trichiura*. In contrast, hookworm had a shallow prevalence rate (0.71%) and was only found in the form of larvae in the Kato-Katz method.

Table 3 shows test standards from previous research data using the Kato-Katz method to examine

Table 2. STH infections by type of worm infection

STH type	Infection	Kato-Katz Method		Direct Examination Method	
		n	%	n	%
A. lumbricoides single	Positive	41	29,29	33	23,57
	Negative	99	70,71	107	76,43
Total		140	100	140	100
T. trichiura single	Positive	16	11,43	12	8,57
	Negative	124	88,57	128	91,43
Total		140	100	140	100
A. lumbricoides & T. trichiura	Positive	6	4,29	5	3,57
	Negative	134	95,71	135	96,43
Total		140	100	140	100
A. lumbricoides & hookworm	Positive	1	0,71	0	0
	Negative	139	99,29	140	100
Total		140	100	140	100

Table 3. STH infection based on previous study data

Method	STH Infection	Amount	Percentage
Kato-Katz	Positive	53	37,85
	Negative	87	62,15
Total		140	100

the same stool specimen in a new condition (not preserved). In this study, examination of the same 140 samples of stool showed that 37.85% were positive for STH, and 62.15% did not contain STH. This study used these data as a gold standard for testing the accuracy of the Kato-Katz and direct examination method in diagnosing helminthiasis in the preserved stool.

Table 4 shows the results of calculating the sensitivity, specificity, negative prediction, and positive predictive value of the Kato-Katz examination method on a preserved stool. As in Table 5, the sensitivity, specificity, negative prediction, and positive predictive values were calculated for the direct examination method on a preserved stool.

DISCUSSIONS

Soil-transmitted helminth infection

The examination results using the Kato-Katz method and direct examination of 140 samples of preserved stool showed that the prevalence of STH infection with each method was 45.71% and 35.71%. This study is in line with several theories which state that the average

Table 4. Accuracy of the Kato-Katz Method in Preserved Stool-1

Kato-Katz method in current study	Kato-Katz method in previous study		Total
	Positive	Negative	
Positive	46	18	64
Negative	7	69	76
Total	53	87	140

Sensitivity (%) $= \frac{46}{46+7} x\ 100\% = 86{,}79\%$

Specificity (%) $= \frac{69}{18+69} x\ 100\% = 79{,}31\%$

Negative predictive value (%)$= \frac{46}{46+18} x\ 100\% = 71{,}87\%$

Positive predictive value (%) $= \frac{69}{7+69} x\ 100\% = 90{,}78\%$

Table 5. Accuracy of Direct Examination Method in Preserved Stool

Direct Examination Method in Current Study	Kato-Katz Method in Previous Study		Total
	Positive	Negative	
Positive	34	16	50
Negative	19	71	90
Total	53	87	140

Sensitivity (%) $= \frac{34}{34+19} x\ 100\% = 64{,}15\%$

Specificity (%) $= \frac{71}{16+71} x\ 100\% = 81{,}60\%$

Negative predictive value (%) $= \frac{34}{34+16} x\ 100\% = 68\%$

Positive predictive value (%)$= \frac{71}{19+71} x\ 100\% = 78{,}88\%$

prevalence of STH in Indonesia ranges from 2.5-65% (4). According to WHO, the group most susceptible to infection by STH is the group of children with an age range of 2-14 years (2). Besides being supported by climatic factors, hygiene, and sanitation, children in this age group often have activities or play outside the home, so being infected with STH is also more significant (16). A study stated that STH infection in school-age children in Indonesia was around 31.8% (5).

In this study, the prevalence of STH infection was dominated by a single infection with *A. lumbricoides* and *T. trichiura*, while hookworm infection was the least. This finding is in line with CDC data that most cases of STH infection, respectively, are caused by *A. lumbricoides*, *T. trichiura*, and hookworm (1). In addition to single infections, mixed infections of *A. lumbricoides* and *T. trichiura*, and *A. lumbricoides* and hookworm were also found in lesser numbers. This occurrence was consistent with a study that found that the most common mixed infection was a mixture of *A. lumbricoides* and *T. trichiura* (13).

Accuracy of Kato-Katz and direct examination of preserved stool

Of 140 samples examined with the Kato-Katz method, 64 (45.71%) were positive, while the direct examination was 50 (35.71%) positive for STH. This difference could be caused by the number of specimens prepared for the two methods. The Kato-Katz method used a double-slide, with each preparation requiring approximately 41.7 mg of stool. The direct examination method used a single slide and only needed 20 mg of stool per preparation. This difference might make the scope for finding eggs or larvae in the Kato-Katz method broader than the direct method (14).

The positivity rate between the two methods in this study was not much different. This study aligns with examining fresh stool specimens using the Kato-Katz and direct examination method. The study showed that the prevalence of *A. lumbricoides*, *T. trichiura*, and hookworm using the Kato-Katz method were 63.8%, 82.2%, and 59.5%, respectively, while using the direct examination method was 52.1%, 62.8%, and 42.8% (13).

This study's accuracy test used standards from previous studies that detected STH infection in fresh stool using the Kato-Katz method. After the accuracy test, the Kato-Katz method obtained sensitivity and specificity values of 86.79% and 79.31%, while the direct examination method obtained sensitivity and specificity values of 64.15% and 81.60%, respectively. From these results, it could be that the Kato-Katz method was more sensitive but less specific when compared to the direct examination method. The results of the accuracy test in this study were slightly different from the results of several previous studies, which obtained sensitivity and specificity values for the direct examination method of 89.09% and 100% (17), as well as 96.16% and 100% in other studies comparing direct examination method with Kato-Katz on the fresh stool (18). This study might be due to the different types of stool specimens used in the examination; previous studies used fresh and preserved stool. Of course, the morphological appearance of worm eggs in the fresh stool would be better than in preserved stool, so the number of eggs detected and identified would be more. However, the accuracy of the two examination methods for diagnosing helminths in stool preserved for 13 months compared to the fresh stool in the previous study did not significantly differ. Another study comparing fresh and preserved stool examination for more than 12 months using the

Kato-Katz method, mini-FLOTAC, and direct examination also stated that STH eggs in the preserved stool could still be detected. This is because formalin contains compounded formaldehyde that binds to protein structures to prevent the autolysis of worm eggs (19).

Research limitations

Some of the stool samples found in a liquid state or the form of a solution made the sample-making process more difficult because the extra effort was needed to collect, move, and keep the sample on the slide. Of course, this affected the mass of stool used as a specimen to make preparations.

The positivity rate for STH infection in preserved stool was higher than in previous studies using fresh stool specimens. This possibility could be caused by differences in the distribution of STH egg density in fresh and preserved stool specimens. Preserved stools were likely to spread the eggs more evenly than before preservation because when preserved, the stool specimen would be stirred in such a way until it dissolved in a 10% formalin solution. As a result, when a small portion of the specimen was taken for examination, the probability of finding STH eggs would be greater than in fresh unstirred stool specimens. Moreover, the number of STH eggs in the stool samples was not too much because most patients with STH infection only had mild conditions.

No hookworm eggs were found in this and previous studies, but only the larval stage was located. This condition could occur because the thin hookworm egg wall structure was straightforward to lyse when making preparations. Identification of hookworm eggs in fresh stool was recommended to be carried out less than 1 hour after collecting stool specimens because hookworm eggs were effortless to lyse. In contrast, examining the preserved stool for less than 15 days was better because hookworm eggs would begin to degrade after 15-30 days (20).

CONCLUSION

The conclusion that could be drawn from this study was that the Kato-Katz method was more sensitive than the direct examination method for diagnosing helminths in preserved stool. However, the direct examination method was more specific than the Kato-Katz method. In addition, the accuracy of examining fresh and preserved stool specimens for diagnosing helminths was not significantly different. It is necessary to conduct further research on the effect of the length of time for preserving stool with formalin on the sensitivity and specificity of a worm infection examination method. Storage of preserved fecal specimens needs to be carried out properly; avoid exposure to direct sunlight and ensure the storage container is tightly closed to prevent leakage and specimen damage.

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