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Confirmation of Soil-Transmitted-Helminths Infection Prevalence by Kato-Katz Kit Diagnostic in Elementary School Children in West Martapura River Banks, South Kalimantan Indonesia

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Abstract: Soil-Transmitted Helminths (STH) infections are the most widespread neglected tropical diseases, primarily affecting morbidity in School Age Children (SAC). STH infection in SAC remains a public health problem in Martapura River Banks. Early and accurate STH detection is essential to determine an effective treatment for reducing morbidity. The Health Department recommends detecting STH infection by Kato Katz (KK) but in public health centers only by the direct method. This research objective is to confirm STH infection prevalence by Kato-Katz (KK) in elementary school children in West Martapura River Banks, South Kalimantan, Indonesia, against the direct method. The modified KK templates were developed from acrylic material. A total number of 253 children 7-12 years old in four West Martapura elementary schools were examined for STHs eggs using both microscopies modified KK against direct method by two trained laboratory technicians. STHs infection prevalence was 50 (19.76%), interpreted as low prevalence by direct and 78 (30.83%), moderate by KK. The concordance between both methods was significantly based on Cohen's kappa (ķ=0.682). Even though the concordance of both methods was strong, the prevalence of STHs by modified KK was 30.08% higher than by Direct, which was only 20.33% positive. This study recommended using the KK for STHs diagnostic for prevalence measurement in low STHs infection.
Keywords: Kato-Katz; school-aged children; Soil-Transmitted Helminths diagnostic.

INTRODUCTION

Neglected Tropical diseases (NTDs) remain a public health problem in the world, especially among people who live in poverty. The World Health Organization recognizes one in 17 NTDs is Soil-Transmitted Helminths (STHs) infection. There are four species of STHs that common infected human: *Ascaris lumbricoides, Trichuris trichiura, Ancylostoma duodenale* and *Necator americanus.* The previous study reported that Indonesia has several hookworm cases (60 million) and the highest number of ascariasis and trichuriasis (more than 90 million cases). STHs infection causes loss of carbohydrates and proteins and blood loss. Therefore, it impacts a decrease in sufferers' health conditions, nutrition, intelligence, and productivity, reducing the quality of human resources. The prevalence of STHs infection in Indonesia generally remains high, especially in the underprivileged population, with poor sanitation. In 2019, the prevalence of STHs infection in several areas in Indonesia was between 2.5% - 62%. There was no

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data on the STHs infection surveillance in the Martapura river banks[1]. Current World Health Organization (WHO) guidelines for STH control include mass drug Administration (MDA) Programs based on prevalence measurements, aiming at reducing morbidity in pre-school-aged children (pre-SAC) and school-aged children (SAC) by lowering the prevalence of moderate-to heavy-intensity infections to <1%. Deworming Program in Indonesia since 2019 for SAC was implemented MDA for deworming based on surveillance by Kato Katz quantitative microscopy and provides albendazole treatment to school children biannually. Besides MDA, public health facilities provide for patients who come for treatment and positive fecal microscopic test results. Early and accurate diagnoses are essential to determine an effective treatment for reducing morbidity, especially in elementary school children[2].

The Kato Katz thick smear of stool specimens microscopy result was better than other quantitative concentration methods: stool centrifugation or floatation based. Kato Katz method is the gold standard of quantitative worm egg counts in the stool. WHO recommends the Kato Katz technique to diagnose STHs infection intensity at the community level because its simple, low cost, reproducible, and could use in a field-based epidemiological survey[3].

Although Kato Katz, as WHO's recommended method for STHs diagnostic at the community level, has been listed in Indonesia's health ministry regulation, the laboratory in the public health center has not equipped with the proper tools. The study by Arimaswati et al (2020) shows that the Kato Katz method is better than the direct slide method for detecting worm eggs in fresh stools (p-value was 0.002)[4]. Gunasari et al (2022) Using preserved stool specimens showed that the Kato-Katz method was more sensitive than direct examination (sensitivity was 86.79%)[5].

The Martapura Barat Health Center laboratory only detects STH infection with the direct wet stool method. To add information about the superiority of the Kato-Katz method compared to direct examination, more research is needed, so this study aims to confirm the prevalence of STH infection by Kato-Katz (KK) in school children. base on the banks of the West Martapura River, South Kalimantan, Indonesia, to the direct method.

MATERIALS AND METHODS

This research was a microscopy STHs egg detection by two trained laboratory technicians. There were 253 School -aged children (SAC) 7-12 years old in four West Martapura elementary schools who had no MDA deworming treatment for at least five months. These three elementary schools are located at the Martapura River banks in the West Martapura district. SAC's stool samples were examined for STHs eggs using both microscopies modified Kato-Katz and direct methods—the Kato-Katz modified by Rifqoh et al (2021), which was appropriate with WHO standards. The difference between the Kato Katz modification and the original is in the stool template. The template of the modified Kato Katz is made from acrylic resin material with a hole thickness of 2 mm, while the original is from PVC and has a hole thickness of only 1.5 mm[6]. Therefore the modified Kato Katz could take more stool specimens because of the more significant volume than the original (stool weight average is 43,22 mg while the original is 41,7 mg)

Duplication also did two hundred and fifty-three Kato-Katz thick smear stool preparations. The modified Kato-Katz template was placed on the microscope slide, filled the hole with the amount of sieved SAC's stool then removed the template. Place one

piece of adhesive strips over the stool sample 24 hours soaked in malachite green aqua-glycerol. Press gently the strips with another microscope slide to spread the stool. Place the slide with the strips upward, and keep the slide for 45 minutes at room temperature to clear the stool material. As the same KK method, two hundred and fifty-three direct wet mount stool preparation were done by duplication. One drop of saline solution on a microscopic slide, place one amount of SAC's stool, then emulsify thoroughly, and cover it with coverglass.

Both Kato-Katz and Direct method preparation and examination refer to the procedure in WHO bench aid of intestinal parasites were done in sequence, and the STHs Egg were identified microscopically using Olympus CX33 trinocular light microscope at 100x and 400x magnification. The positive result of this study was determined based on the presence of STHs Egg, while the negative was an absence. The species of STHs eggs were identified based on eggshells morphology and the distinctive features of STHs eggs, i.e., Ascaris lumbricoides, Trichuris trichiura, and Hookworm (Ancylostoma duodenale and Necator americanus)[7]

Data collected and edits to check completeness, continuity, and validation data. Data were entered into a computer program. Statistical analysis used Cohen's Kappa and computed the diagnostic parameter test: positive predictive value, negative predictive value, sensitivity, specificity, and accuracy. Approval for this study was obtained from the Ethics Commission of Rumah Sakit Daerah Idaman No. 63721K12124202209/EC/KEPK-1/10/2022.

RESULTS AND DISCUSSION

Table 1 shows characteristics (sex and age) based on identity information. 132 of 253 SAC (52.17 percent) were male, and 121 (47.83 percent) were female.

Table 1. Characteristics of School-Aged Children (SAC)

Characteristic		N (%)
Sex	Male	132 (52.17)
	Female	121 (47.83)
Age	7	28 (11.07)
	8	56 (22.14)
	9	53 (20.95)
	10	52 (20.54)
	11	57 (22.53)
	12	7 (2.77)
		253 (100)

Figure 1 shows *Ascaris lumbricoides* eggs (1A and 1B) and *Trichuris trichiura* eggs (2A and 2B) in SAC's stool by Kato Katz compared by Direct microscopic examination in 400x magnification. The microscopic identification in Kato Katz preparation shows the eggs absorb the malachite green despite the apparent morphology of *Trichuris trichiura* eggs which are specific features of its eggshell. However, the eggs were easily identified. The *Ascaris lumbricoides* decorticated eggs morphology microscopic identification in Kato Katz preparation contrasts with the background because the eggshell's thickness so that it couldn't absorb the malachite green.

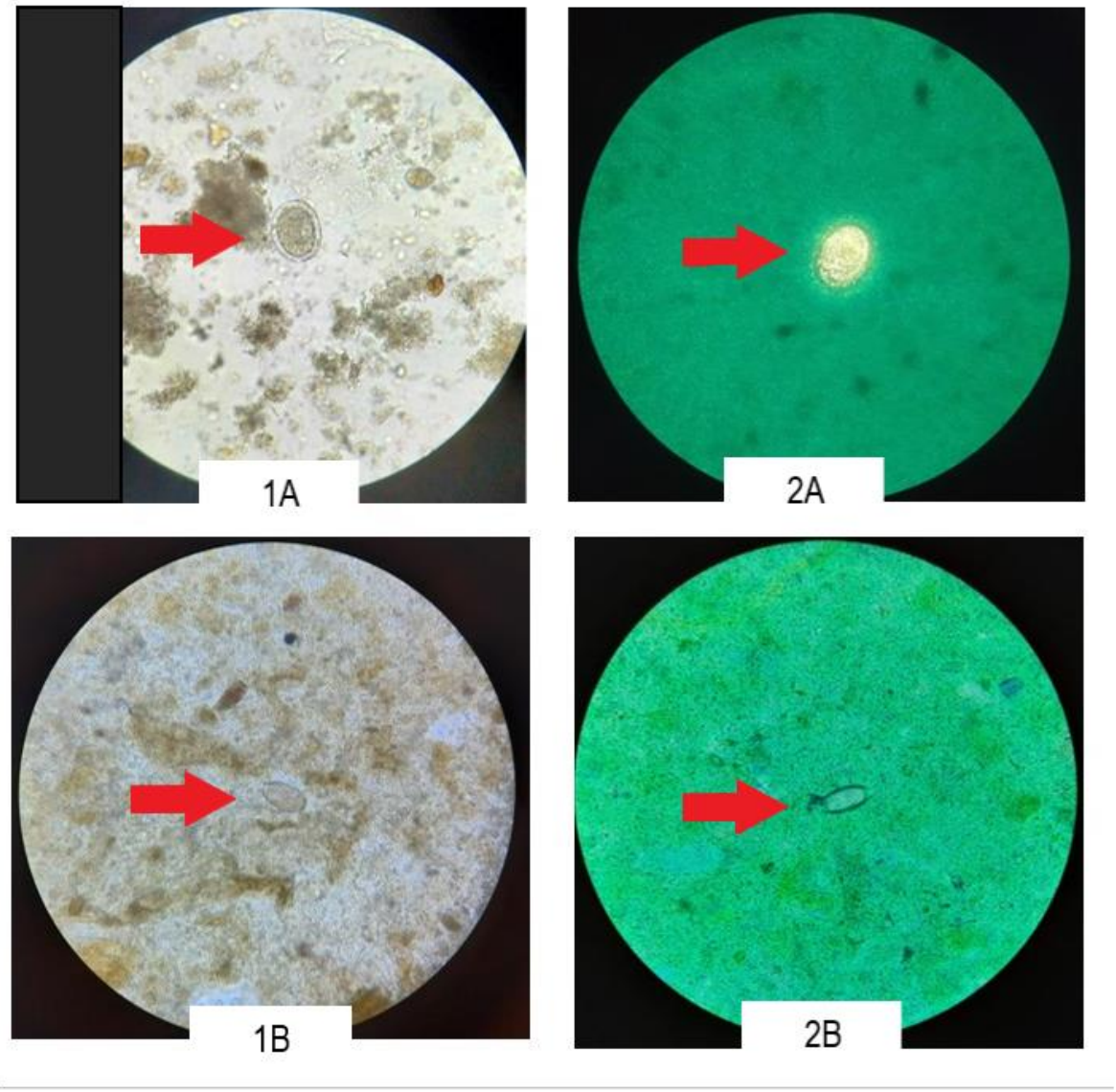

Figure 1. STHs eggs in SAC's stool by Direct (1A and 1B) and by Kato Katz (2A and 2B) microscopic examination 400x magnification; 1A.Decorticated *Ascaris lumbricoides* egg in Direct; 2A.Decorticated *Ascaris lumbricoides* egg in Kato Katz; 1B.Fertil *Trichuris trichiura* egg in Direct; 2B.Fertil *Trichuris trichiura* egg in Kato Katz

In the Direct wet mount, though *Trichuris trichiura* and *Ascaris lumbricoides* eggs still could be identified because of specific features of eggshells, the eggs are similar to the background, and some debris could cause the eggs to be unidentified. The Direct method only uses standard saline solution as the reagent, while Kato Katz uses the malachite green in aqua-glycerol. Glycerol in the Kato Katz reagent functions as a clearing agent. Therefore, the Kato Katz preparation has more clear background than the direct preparation with no clearing agent, so it's full of dirt, debris, etc. Before microscopic examination, Kato Katz preparation was incubated for at least 30 minutes at room temperature; according to Wisetmora et al (2023), the clearing process was increased by glycerol. In this study, the Kato Katz reagent is 1 ml malachite green 3% solved in 100 ml

distilled water and 100 ml glycerol. Hence it has almost 50% glycerol[8]. The study by Novita et al, (2022) shows that the preparation with glycerol 35% has the best clarity effect[9].

Table 2. STHs Eggs Qualitative in Direct Microscopic Examination Result

Eggs of Species	Number of Specimens N (%)
Negative STHs	203 (80.24)
Ascaris lumbricoides	28 (11.07)
Trichuris trichiura	22 (8.70)
Ascaris lumbricoides & Trichuris trichiura	0 (0.00)
Total Number (%)	253 (100)

Table 2 shows STHs eggs qualitative in Direct wet mount microscopic result for STHs Infection with the number of negative STHs was 202 (79.84 percent), 28 (11.07 percent) *Ascaris lumbricoides,* 22 (8.70 percent) *Trichuris trichiura* and no specimen resulting both of *Ascaris lumbricoides* and *Trichuris trichiura.* According to WHO (2019), direct wet mount preparation only needed about 2 mg of stool specimen. Therefore, it was less representative of all specimen conditions than a large specimen in another preparation method[7].

Table 3. Egg Per Gram (EPG) Number in Kato Katz Microscopic Examination Result

Eggs of Species	Number of Specimens with EPG 0-69 N (%)				Total N (%)
	EPG 0	EPG 23	EPG 46	EPG 69	
Negative STHs	175 (69.17)	-	-	-	175 (69.17)
Ascaris lumbricoides	-	32 (12.65)	11 (4.35)	1 (0.39)	44 (17.39)
Trichuris trichiura	-	16 (6.32)	9 (3.56)	7 (2.77)	32 (12.65)
Ascaris lumbricoides & Trichuris trichiura	-	1 (0.39)	1 (0.39)	-	2 (0.79)
					253 (100)

Table 3 shows STHs eggs quantitative in Kato Katz microscopic result for STHs Infection with the number of negative STHs was 175 (69.17percent) and 44 (17.39 percent) *Ascaris lumbricoides,* 32 (12.65 percent) *Trichuris trichiura* and 2 (0.79 percent) both of *Ascaris lumbricoides* and *Trichuris trichiura*—intensity of STHs infection interpretation by total number of STHs eggs each species.

Kato Katz method as a quantitative method, the intensity of STHs infection was interpreted by the total number of STHs eggs per gram stool (EPG) of each species. EPG was calculated from the factor and the number of STHs eggs in Kato Katz's preparation. A factor is a number calculated from 1 gram or 1000 mg divided by the average stool weighing in all holes of Kato Katz templates calibration[7].

In this study, we use the Kato Katz template with 43,22 mg of stool average; thus, the factor was 23. EPG result was calculated and then used to classify the infection intensity as light, moderate, or heavy. According to WHO (2002), the severity of STHs infection is light, moderate, or heavy-intensity of infections as follows *Ascaris lumbricoides,* 1 to 4999 EPG, 5000 to 49999 EPG, and ≥50000 EPG, and *Trichuris trichiura*: 1 to 999 EPG, 1000 to 9999 EPG, and ≥10000 EPG[10].

Table 3 shows the intensity of *Ascaris lumbricoides* was only as light intensity based on all specimen numbers in EPG by modified Kato Katz calculation was between 1-4999 EPG as follows *Ascaris lumbricoides* eggs with 23 EPG was 32 (12.65 percent), 46 EPG was 11 (4.35 percent), 69 EPG was 1 (0.39 percent) and co-infection with *T*richuris *trichiura* 23 EPG was 1 (0.39 percent) and 46 EPG was 1 (0.39 percent). Its similarly to *Ascaris lumbricoides*, the intensity of *Trichuris trichiura* was only as light intensity based on all specimen numbers in EPG by modified Kato Katz calculation was between 1-999 EPG as follows *Trichuris trichiura* eggs with 23 EPG was 16 (6.32 percent), 46 EPG was 9 (3.56 percent), 69 EPG was 7 (2.77 percent) and co-infection with *Ascaris lumbricoides* 23 EPG was 1 (0.39 percent) and 46 EPG was 1 (0.39 percent).

Table 4. Cross-tabulation of STHs Microscopic Examination by Kato Katz and Direct

Direct Wet Mount	Modified Kato Katz		Total
	Positive N (%)	Negative N (%)	N (%)
Positive	49	1	50
N (%)	(19.37)	(0.39)	(19.76)
Negative	29	173	203
N (%)	(11.46)	(68.38)	(80.24)
Total	78	175	253
N (%)	(30.83)	(69.17)	(100)

Table 4 shows a cross-tabulation of microscopic results for STHs Diagnostic by Modified Kato Katz and Direct Wet Mount Stool. The number of positive STHs by Direct Wet Mount Stool was 50 (19.76 percent) and 203 (80.24 percent) negative STHs. The microscopic examination by the modified Kato Katz method resulted in 78 (30.83 percent) positive STHs and 175 (69.17 percent) negative STHs. The number of positive STHs by Kato Katz was 11.07 percent higher than Direct. This different STHs positivity result by both methods was in line with the previous study by Sofia R (2018); there were 94.82 percent positive STHs by Kato Katz or about 10.24 percent higher than by Direct, which was 84.48 percent in 58 stool specimens of SAC in Lapang 4 Elementary School Aceh Utara[11]. Another study by Gunasari et al (2022) has a similar result which was a ten percent gap between both methods. There were 45.71 percent positive STHs by Kato Katz and about 35.71 percent by Direct in 140 preserved stool specimens[5].

To confirm the STHs examination result by Direct with the modified Kato-Katz method, both results of the STHs diagnostic by modified Kato-Katz and the direct statistically tested by Cohen's Kappa. The statistical result is shown in Table 5.

Table 5. Cohen's Kappa Microscopic for STHs diagnostic by Kato Katz and Direct

		Value	Asymp.Std. Error	Approximate Tb	Approximate Significance
Nominal by Nominal	Contingency Coefficient	.579			.000
Measure of Agreement	Kappa	.682	.051	11.293	.000
N of Valid Case		253			

Table 5 shows a concordance between STHs infection prevalence by Kato Katz and the Direct method. The concordance of both methods' results was significantly moderate based on Cohen's kappa (ķ=0.682). It's confirmed that STH infection positively was similar by both methods, even though the result by Kato Katz was about 11 percent higher than by direct. It becomes more large difference if the result was interpreted based on Kemenkes RI (2017), which was a principal deworming policy in Indonesia, classifies the STH prevalence as follows high, which is ≥ 50%; moderate,≥ 20% to <50%; and low, <20%. The STHs prevalence confirmed by the Kato Katz method was 78 (30.83 percent), interpreted as moderate prevalence; thus, by direct 50 (19.76 percent) as low prevalence[12].

This study result shows a different level between STHs diagnostic by Kato Katz, which was moderate, then by Direct, with a low prevalence. The STHs prevalence level differences affected to deworming policy. WHO (2002) and Kemenkes RI (2017) have taken action based on deworming policy by preventive chemotherapy two times annually Mass Drug Administration (MDA) to control STHs in school-age children[10,12].

To control STHs helminth intensity in children, we should reveal STHs prevalence by an accurate diagnostic method. The pathologies caused by STHs' effect on children's growth and cognition were not only equal to STH's presence thus also by a large number of STHs presence. Only moderate and heavy intensity of STHs infection affects the anemia or stunting physical growth. Therefore, if the prevalence and intensity of helminthiasis do not conduct in a better method, it could be an accurate interpretation and affect deworming, preventive deworming, and STHs control[13].

Other research Calvopina et al, (2018) revealed the Kato-Katz technique has the best performance compared to three other techniques, spontaneous sedimentation technique in the tube, formalin-ether concentration technique, and direct wet mount as the lowest sensitivity technique[14]. Results study by Cools et al (2019) showed that in low endemicity of *Clonorchis sinensis* worm infection, the limited number of samples could underrate its prevalence. Therefore, at least two stool samples should have multiple stool smears of Kato-Katz preparation[15].

The limitation of this research is that none of all SAC's specimen stool consisted of the hookworm eggs, which is one of the STHs eggs. Thus it has yet to compare both methods' microscopic results for hookworm eggs since it was fragile with glycerol exposure. This study result was a different number of STHs prevalence with two different

interpretations of STHs prevalence classification, which by Kato Katz was 50 (19.76 percent) as moderate prevalence while by Direct method was 78 (30.83 percent) as low prevalence. The concordance of both STHs prevalence by Kato Katz against Direct based on Cohen's kappa (ķ=0.682) was significant.

CONCLUSION

The study concluded the STH infection prevalence in elementary school children in West Martapura River Banks confirmed by Kato-Katz was higher than by the direct. Even though by statistic Cohen's Kappa value shows the significant concordance between both methods, it was two different interpretations of STHs prevalence classification, which by Kato Katz was moderate prevalence while by Direct method low prevalence only. It is recommended to the Kato-Katz method be used in further study, especially for STHs diagnostic in low STHs infection prevalence in the field survey.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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