Medical Laboratory Technology Journal
10(2), 2024, 152-160
Received 2024-11-21; Revised 2024-12-22; Accepted 2024-12-25
Available online at : http://ejurnal-analiskesehatan.web.id

Kato Katz against Floatation Technique Comparison for Intestinal Helminth Detection of Elementary School Children in Swamp Wetland Area, South Kalimantan, Indonesia

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Abstract: Intestinal helminth infections are the most widespread of the world's neglected tropical diseases, primarily affecting morbidity in school-age children. Early and accurate intestinal helminth detection is important to determine an effective treatment for reducing morbidity. This research objective is to compare intestinal worm infections in elementary school children in swampy wetland areas using the Kato-Katz method and Flotation techniques. Two hundred ninety-six elementary school children in Danau Panggang Swamp-wetland, 6-13 years old, were examined for intestinal helminth eggs in no-preservative feces using microscopies Kato Katz and Floatation. The result of Kato Katz was a total egg counting per gram feces of *Ascaris lumbricoides* 23-92/gr feces *Trichuris trichiura* 23-207/gr feces and *Fasciolopsis buski* 23-69/gr feces, while the results of Floatation only *Ascaris lumbricoides* 10-50/gr feces *Trichuris trichiura* 20-90/gr feces. The percentage of intestinal helminth infection by Kato Katz was 31,76% (94 samples), which is higher than by the Floatation was 25,34% (75 samples). The Wilcoxon statistical test obtained a significance value of 0.001, which shows $p<\alpha$ ($\alpha=0.05$); there is a significant difference between the results of the quantitative examination of intestinal worm eggs between the Kato Katz and Floatation technique. It is recommended that Kato Katz's performance testing be continued compared to PCR methods and a wider sample area.
Keywords: Elementary school children; floatation; intestinal helminths detection; Kato-Katz.

INTRODUCTION

Intestinal helminth infection remains a public health problem in the world, especially among people who live in poverty. The World Health Organization recognized intestinal helminthiasis as one of 17 Neglected Tropical Diseases (NTDs) caused by parasites, a significant global health issue. These infections are particularly prevalent in poor sanitation and hygiene regions, affecting the most deprived communities. Soil-transmitted helminths (STH) are among the most common diseases worldwide, with an estimated 1.5 billion people, or 24% of the global population, infected. The main species causing these infections include *Ascaris lumbricoides* (roundworms), *Trichuris trichiura* (whipworms), and hookworms species *Necator americanus* and *Ancylostoma duodenale* (World Health Organization, 2023).

These infections can lead to various health issues, including nutritional and physical impairments, anemia, and increased risk of maternal and infant mortality. Intestinal helminthiasis infection causes loss of carbohydrates and proteins and blood loss. Therefore, it causes a decrease in the health condition, nutrition, intelligence, and productivity of sufferers then, reducing the quality of human (Hotez & Ehrenberg, 2010, Savioli dkk., 2018)

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The highest prevalence of intestinal helminthiasis is reported in tropical and subtropical regions, particularly in sub-Saharan Africa, China, South America, and Asia. Indonesia has several cases of hookworm (60 million) and the highest number of ascariasis and trichuriasis (more than 90 million cases of each). The prevalence of Intestinal helminth infection in Indonesia remains high, especially in the underprivileged population with poor sanitation. The prevalence of Intestinal helminth infection in several areas in Indonesia in 2019 was between 2.5% - 62% (Lee & Ryu, 2019).

The prevalence of intestinal helminth infections in Indonesia remains a significant public health concern, particularly in certain high-risk areas. A recent study indicates that the prevalence of helminth infections in primary school children in several provinces ranges from 61% to 79%, while for all age groups, it is around 41% to 59%1. The most common species found include *Ascaris lumbricoides*, *Trichuris trichiura*, hookworms, *Enterobius vermicularis Hymenolepis nana*, *Taenia saginata*, *Taenia solium*, *Schistosoma japonicum, Strongyloides stercoralis* (Rahayu et al., 2019).

World Health Organization (2019) released the guidelines for helminthiasis control, including 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) or elementary school children by lowering the prevalence of moderate-to heavy-intensity infections to <1%. The Indonesian government deworming program in 2019 implemented MDA for deworming based on surveillance by Kato Katz quantitative microscopy and provides albendazole treatment to school children biannually. Treatment for intestinal helminthiasis is provided in public health facilities for patients who come for treatment and positive helminth eggs fecal microscopic test results. Accurate diagnoses are important to determine an effective treatment for reducing morbidity, especially in elementary school children (Permenkes, 2017; Rifqoh et al., 2021).

The laboratory in the Public Health Centre detects STH infection using the wet mount feces method only as a qualitative detection. The laboratory in the public health center is not equipped with the proper tools, even though Kato Katz, as WHO's recommended method for intestinal helminth infection diagnosis at the community level, has been listed in Indonesia's health ministry regulation. Previous studies by Sofia R. (2018) and Gunasari et al. (2022) compared the qualitative direct slide and Kato-Katz techniques, resulting in the Kato-Katz technique having higher sensitivity than the direct slide technique. There are differences in this study from previous studies, such as comparing the qualitative technique, and this study compares the quantitative technique between the Kato-Katz and Flotation techniques. The previous study used a small number of samples, but this research conducted huge samples and involved the children in swamp areas, which is endemic Fasciolopsis. This research objective is to compare intestinal helminth infection by Kato-Katz in elementary school children in the Swamp wetland area against the floatation technique.

MATERIALS AND METHODS

This research was a cross-sectional observational analytic study. A total number of 296 Elementary School Children 7-12 years old in Danau Panggang Swamp Wetland who had no MDA deworming treatment for at least 5 months. Children's non-preservative feces samples were examined for intestinal helminth infection eggs using both microscopies, Kato-Katz, and Floatation techniques. The

Kato Katz equipment used in this study was modified by Rifqoh et al. (2021) and was appropriate to WHO standards. The template of the modified Kato Katz is made from acrylic resin material with 2 mm a hole thickness, and 6 mm in diameter, which is stool weight average is 43,22 mg. The floatation technique refers to Bench Aids for the diagnosis of intestinal parasites (World Health Organization, 2019).

The Flotation technique allows the separation of parasitic elements from the coarsest organic debris, using a high specific density flotation solution. Eggs, cysts, and oocysts, with a specific density lower than the flotation solution, will rise to the top of the suspension of sodium chloride as flotation solutions. Add about 3 g of stools to 10 mL of formalin 5–10 %, mix well, and leave to rest for at least 30 minutes. Filter the suspension through a sieve or double layer of gauze and pour it into a conical test tube remaining about 1 cm below the rim. Centrifuge for 3 minutes at 1500 g and discard the supernatant. Resuspend the sediment in saline with a pipette and add the floatation solution. Resuspend the sediment in 10 mL of flotation solution with a pipette and centrifuge for 5 minutes at 800–1000 g. Remove the tube from the centrifuge and add a few drops of flotation solution until a meniscus forms. After about 10 minutes, harvest the upper part of the meniscus by placing a coverslip over it, then place it face down on a slide and examine it using a microscope (World Health Organization, 2019).

Two trained laboratory technicians conducted microscopic intestinal helminth infection egg detection. Both Kato-Katz and Floatation preparation and examination were done in sequence, and the Intestinal helminth infection eggs 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 intestinal helminth infection eggs, while the negative was an absence. The species identification of intestinal helminth infections were identified based on the distinctive features of Intestinal helminth infection eggs and eggshell morphology, i.e., *Ascaris lumbricoides, Trichuris trichiura,* and Hookworm (*Ancylostoma duodenale* and *Necator americanus*) (World Health Organization, 2019).

Statistical analysis descriptively and analytically the data. A Wilcoxon was conducted to compare eggs per gram number of two techniques by Kato Katz against the floatation technique. Approval for this study was obtained from the Poltekkes Kemenkes Banjarmasin Ethics Commission no.252/KEPK-PKB/2023.

RESULTS AND DISCUSSION

Children, particularly vulnerable to intestinal helminth infections, were the target of the deworming program in Indonesia. In 2019, the Mass Drug Administration (MDA) for deworming was the implementation of Permenkes No. 15 of 2017 with target identification for children aged 1 to 12 years through schools and other health facilities. The intestinal helminth surveillance in Permenkes No. 15 of 2017 recommends the Kato Katz technique as a quantitative detection. Another quantitative technique for intestinal helminth surveillance includes sedimentation and floatation techniques.

This study was conducted to compare intestinal helminth infection by Kato-Katz against the floatation technique in 296 elementary school children in Danau Panggang Swamp-wetland. The characteristics of all children can be seen in Table 1 and the results of microscopic examination of intestinal worm eggs using Kato Katz can be seen in Figure 1.

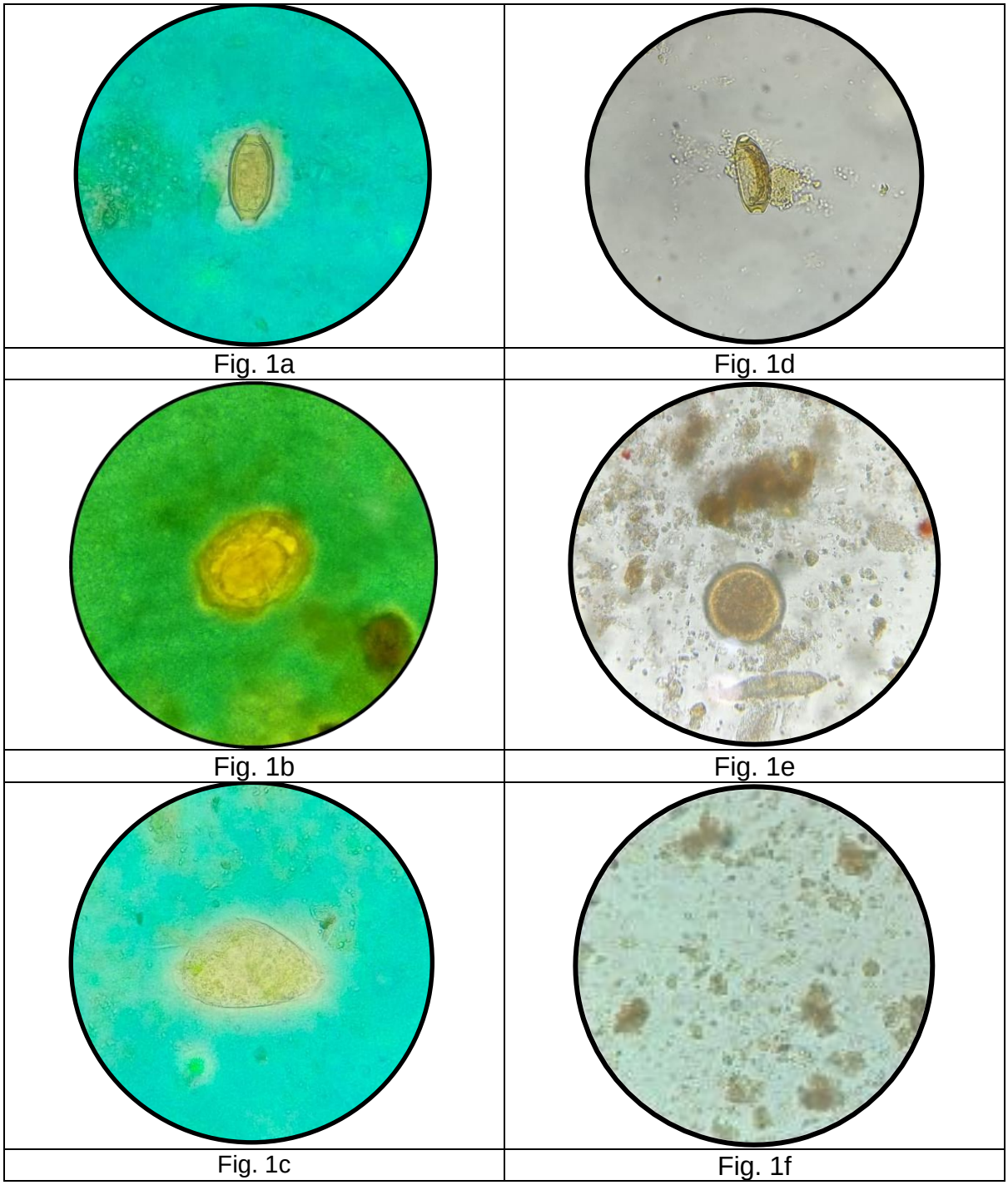

Fig. 1a | Fig. 1d

Fig. 1b | Fig. 1e

Fig. 1c | Fig. 1f

Figure 1. Intestinal Helminth Eggs Microscopic Examination Result in 1000x Magnification by Kato Katz (Fig. 1a: *Trichuris trichiura,* Fig.1b *Ascaris lumbricoides*, Fig.1c *Fasciolopsis buski*) and by Floatation Technique (Fig.1d *Trichuris trichiura,* Fig.1e *Ascaris lumbricoides,* and Fig.1f None of helminth's egg).

Microscopic examination result by Kato Katz found the *Trichuris trichiura, Ascaris lumbricoides,* and *Fasciolopsis buski,* despite the Flotation technique only found *Trichuris trichiura and Ascaris lumbricoides.* Based on heavy eggs, such as those of Fasciola or infertile Ascaris eggs, are not efficiently concentrated with this technique. In addition, eggs and cysts tend to lose their typical shape after 40–60 minutes.

Table 1. Characteristics of Elementary School Children

Characteristic		N (%)
Sex	Male	102 (34.46)
	Female	194 (65.54)
Age	6	6 (2.03)
	7	43 (14.53)
	8	46 (15.54)
	9	49 (16.55)
	10	53 (17.91)
	11	51 (17.23)
	12	37 (12.50)
	13	11 (3.72)
Total		296 (100)

Table 1 shows characteristics (sex and age) based on identity form. 102 of 296 elementary school children (34.46 percent) were male and 194 (65.54 percent) were female.

Table 2. Intestinal Helminth Infection in Kato Katz and Floatation Microscopic Examination Result

Species Intestinal Helminth Infection	Kato Katz N (%)	Range of EPG*	Floatation N (%)	Range of EPG*
Negative	202 (68.24)	0	221 (74.66)	0
Ascaris lumbricoides	37 (12.50)	23-92	32 (10,81)	10-50
Trichuris trichiura	29 (9.80)	23-207	26 (8,78)	20-90
Ascaris lumbricoides *Trichuris trichiura*	23 (7.77)	23-115	17 (5.74)	20-70
Fasciolopsis buski	5 (0.34)	23-69	0 (0.00)	0
Total Number (%)	296 (100)	23-207	296 (100)	10-90

*EPG = Egg Per Gram Feces

Table 2 shows intestinal helminth infection eggs qualitatively by Kato Katz and Floatation based on microscopic results. The number of negative intestinal helminth infections was 202 (68.24 percent) by Kato Katz and 221 (74.66 percent) by floatation. The total number of intestinal helminth infections by Kato Katz was 94 (31.76 percent) which the species were *Ascaris lumbricoides* 37 (12.50 percent), *Trichuris trichiura* 29 (9.80 percent), co-infection *Ascaris lumbricoides* and *Trichuris trichiura* was 23 (7.77 percent), *Fasciolopsis buski* 5 (1.69 percent). The total number of intestinal helminth infections by floatation was only 75 (25.34 percent) which the species were *Ascaris lumbricoides* 32 (10.81 percent), *Trichuris trichiura* 26 (8.78 percent), co-infection *Ascaris lumbricoides* and *Trichuris trichiura* was 17 (5,74 percent) and no *Fasciolopsis buski.*

Table 2 shows the intensity of *Ascaris lumbricoides* was only as light based on the numbers of eggs per gram by Kato Katz as follows: *Ascaris lumbricoides* eggs with 23 EPG, 46 EPG, and 1169 EPG and co-infection with *T*richuris *trichiura* 23 EPG and 46 EPG. The intensity of *Ascaris lumbricoides* and *Trichuris trichiura* as light intensity based on all specimen numbers in EPG by Kato Katz was between 1-999 EPG for *Trichuris trichiura* eggs and 1-4449 EPG for *Ascaris lumbricoides*. In this study, we used the Kato-Katz template modified by Rifqoh et al. (2022) with a template hole 6 mm in diameter and 2 mm in thickness. Which is 56,52 mm^3 in volume or equivalent to 43,22 mg of feces; thus, the factor multiplied for egg per gram (EPG) was 23 (1 gram or 1000 mg divided by 43,22 mg). Number of EPG results that have been calculated and then used to classify the infection intensity as light, moderate, or heavy infection. According to the World Health Organization (2020), the severity of Intestinal helminth infections 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.

Kato Katz's method was quantitative, and the intensity of Intestinal Helminth infection was interpreted by the total number of Intestinal Helminth infection eggs per gram feces (EPG) of each species. EPG was calculated based on the factor and the number of Intestinal Helminth infection eggs in Kato Katz's preparation. A factor is a number that was calculated from 1 gram or 1000 mg divided by the average weighing of feces in all holes of Kato Katz templates calibration (World Health Organization, 2019).

Table 3. Cross-Tabulation of Intestinal Helminth Infection Microscopic Examination by Kato Katz and Floatation

Floatation	Modified Kato Katz		Total
	Negative N (%)	Positive N (%)	N (%)
Negative N (%)	198 (66.89)	23 (7.77)	221 (74.66)
Positive N (%)	4 (1.35)	71 (23.99)	75 (25.34)
Total N (%)	202 (68.24)	94 (31.76)	296 (100.00)

Table 3 shows a cross-tabulation of microscopic results for Intestinal helminth infection Diagnostic by Modified Kato Katz and Floatation. The number of positive Intestinal helminth infections by floatation was 75 (25,34 percent), and 221 (74,66 percent) negative Intestinal helminth infections. The microscopic examination by the modified Kato Katz method resulted in 94 (31.76 percent) positive Intestinal helminth infection and 202 (68.24 percent) negative Intestinal helminth infection. The number of positive Intestinal helminth infections by Kato Katz was 6.32 percent higher than floatation. This different Intestinal helminth infection positivity result by both methods was in line with the previous study by Sofia, R. (2018), there was 94.82 percent positive Intestinal helminth infection by Kato Katz or about 10.24 percent higher than by floatation, which was 84.48 percent in 58 Feces specimens of SAC in Lapang 4 Elementary School Aceh Utara. Another study by Gunasari et al. (2022) had a similar result, which was a ten percent gap between both methods. There were 45.71 percent positive Intestinal helminth infections by Kato Katz and about 35.71 percent by floatation in 140 preserved feces specimens.

To confirm the Intestinal helminth infection examination result by floatation with the modified Kato-Katz method, both results of the Intestinal helminth infection diagnostic by modified Kato-Katz and the floatation were statistically tested by Wilcoxon. Wilcoxon statistical test obtained a significance value of 0.001, which shows $P<\alpha$ ($\alpha=0.05$), meaning that there is a significant difference between the results of a quantitative examination of intestinal worm eggs between the Kato Katz method and floatation.

This study result shows a different prevalence level between Intestinal helminth infection diagnostic by Kato Katz, which was moderate, and intestinal helminth infection diagnostic by floatation, which only had a low prevalence. The prevalence level differences affected the deworming policy. World Health Organization (2020) and Kemenkes (2017) have taken action based on a deworming policy by preventive chemotherapy two times annually Mass Drug Administration (MDA) to control Intestinal helminth infection in school-age children.

Despite efforts by the government, including the target of reducing the prevalence of intestinal worms to below 10% in each regency/city, it has not been fully achieved. This program is carried out periodically, once every 6 months, to ensure that worm infections can be effectively controlled and provides albendazole treatment to children biannually. The MDA Program, which is based on intestinal helminth surveillance, is an effort to give deworming to a large number of target groups simultaneously. The main goal is to reduce the burden of worm diseases in the community (Kemenkes, 2017).

To control the intensity of intestinal helminth infection in children, we should reveal the prevalence of intestinal helminth infection by an accurate diagnostic method. The pathologies caused by Intestinal Helminth Infection's effect on children's growth and cognition were not only equal to STH's presence but also to the presence of a large number of Intestinal Helminth Infections. There were only moderate and heavy-intensity Intestinal helminth infections that affected the anemia or stunted physical growth. Therefore, if the prevalence and intensity of helminthiasis are not conducted in a better method, it could be an accurate interpretation and affect deworming and preventive deworming and Intestinal helminth infection control (Majid et al., 2019).

The Kato Katz thick smear of feces specimens microscopy result was better than other quantitative concentration methods, which were based on feces centrifugation or floatation. Kato Katz technique was the gold standard of quantitative helminth egg counts in the feces. WHO recommends the Kato Katz technique to diagnose intestinal helminth infection intensity at the community level because it is simple, low cost, reproducible, and could be used in a field-based epidemiological survey (Montresor et al., 2020; World Health Organization, 2019; Savioli et al., 2018; Zendejas-Heredia et al., 2021).

The limitation of this research is that none of the feces specimens consisted of hookworm eggs, which are one of the Intestinal helminth infection eggs. Thus, neither method has compared microscopic results for hookworm eggs since they were fragile due to glycerol exposure.

CONCLUSION

The quantitative examination of intestinal worm eggs using the Kato Katz and Flotation techniques showed significant differences. Compared to PCR methods and a wider sample area, it is recommended that Kato Katz's performance testing continue.

ACKNOWLEDGEMENT

The author would like to thank Poltekkes Kemenkes Banjarmasin for facilitating this research.

CONFLICT OF INTEREST

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

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