

# The Advantages of Kato Katz Polkessin Against Floatation Technique in Detection of STH in School-Age Children in The Martapura Riverbank Area, South Kalimantan, Indonesia

Rifqoh, Jujuk Anton Cahyono, Yayuk Kustiningsih

Department of Medical Laboratory Technology, Banjarmasin

rif.mayasin@gmail.com

Abstract. Soil-transmitted helminths (STH) still remain a public health problem in developing countries. In Indonesia, STH is most prevalent in school-age children, especially with a lack of water sanitation and hygiene in riverbank areas. To elevate STH elimination in these areas, its need a highly sensitive method for STH detection. The Kato-Katz Polkessin was an innovation of the modified Kato-Katz method with low-cost materials consideration. This study aims to determine the advantage of Kato-Katz Polkessin against the Flotation technique for STH detection in school-age children in the Martapura riverbank area. An observational study in the population of elementary school children aged 6-13 years old. The total number of stool samples was 166 conducted by duplicated Kato-Katz Polkessin stool preparations that were examined for STH egg detection against the duplo flotation technique method. Both preparations used microscopy examination by two trained technicians. There were cost benefits of Kato-Katz Polkessin that it only uses a simple disposable stool template and the simple clearing process by glycerol than the relative expense of conical tube and the clearing steps by multiple centrifugations The microscopy examining results showed that the mean of STH egg per gram (EPG) by Kato-Katz Polkessin was 46 (range 23-253) and by Flotation technique was only 4 (range 1-36). The Wilcoxon Signed Rank Test result reveals that there was a significantly different EPG result by Kato-Katz than by the Flotation technique, with a p-value of 0.001, which is less than the significance level ( $\alpha$ ) of 0.05. In conclusion, there are advantages of Kato-Katz Polkessin against the Flotation technique in STH detection in school-age children in the Martapura riverbank area as follows it has higher numbers of EPG than the Flotation technique, it's easier, and it's less expensive. It is recommended for further study to compare the sensitivity and specificity of both methods with PCR.

Keywords: Advantages of Kato Katz, STH Detection

T. Triwiyanto et al. (eds.), *Proceedings of the 6th International Conference of Health Polytechnic Surabaya (ICoHPS 2023)*, Advances in Health Sciences Research 72, https://doi.org/10.2991/978-94-6463-324-5\_52

#### 1 Introduction

Soil-transmitted helminth (STH) infection remains a public health problem in the world. There are four species of STH that common infected human: Ascaris lumbricoides, Trichuris trichiura, Ancylostoma duodenale and Necator americanus. The previous study reported that Indonesia has a number of cases of hookworm (60 million) and the highest number of ascariasis and trichuriasis (more than 90 million cases of each) [1–3]. The STH prevalence-related factors include weather, humidity, mass treatment, water, minimal access to water-sanitation-hygiene (WASH), and non-use of footwear besides low education, and other socioeconomic characteristics. In 2019, the prevalence of STH infection in several areas in Indonesia was between 2.5% and 62%. Banjar Regency with its wide riverbank area was third of five regencies in South Kalimantan with a higher prevalence of STH infection that has 1.07% and Balangan Regency has the highest number of STH cases with a prevalence of 1.60%[3–7].

STH infection causes loss of carbohydrates and proteins and blood loss, it related to anemia. In Indonesia anemia was the same prevalent as STH infection. It impacts a decrease in the health condition, nutrition, intelligence, and productivity of sufferers then reducing the quality of human resources. STH in children has the highest burden, despite the fact that adults can also have the disease. As people age, they become more resistant to worm diseases that are already present. When someone consumes infected food or unwashed product, STH eggs that have been living in damp soil find their way into the host and start their life cycle[8–11].

World Health Organization (WHO) guidelines for STH control include Mass Drug Administration (MDA) Programs based on prevalence measurements, aiming at reducing morbidity in children by lowering the prevalence of moderate to heavy intensity infections to <1%. The Deworming Program in Indonesia implemented MDA based on surveillance by Kato Katz quantitative microscopy and provides albendazole treatment to school children biannually. An accurate technique of STH detection was important to the surveillance and evaluation of treatment. Indonesia's health ministry regulation recommends the Kato Katz technique for STH eggs detection at the community level survei base data to MDA policy[12–14].

Study by [15] developed the modified of Kato Katz kit namely Kato Katz Polkessin from abbreviation of Health Polytechnic of Banjarmasin. Further study in 2022 continue this study reveal that Kato Katz technique better than direct wet mount for detects helminth eggs in fresh stool, it percentage of STH was about 10% higher than by direct [16]. Other quantitative concentration technique were stool centrifugation or floatation based. The both technique has been microscopy-based approaches, remain as the same as the Kato Katz as main technique particularly in resource-poor regions where these STH still prevalent[17].

This research aimed determine the advantage of Kato Katz Polkessin against the Flotation technique in the detection of STH in school-age children in the Martapura riverbank area. The Kato Katz equipment used in this study was the Kato Katz Polkessin [15] which was appropriate with WHO standards and the Floatation technique used the Bench Aid for intestinal parasite by WHO procedures[17].

### 2 Materials And Method

A total number was 166 of School Children 6-13 years old in Martapura Riverbank Area. The inclusion criteria were the children who had no MDA deworming treatment for at least 5 months and had informed consent. This research was a microscopy STHs egg detection by two trained laboratory technician. Feaces samples of 166 were examined for STHs egg detection using both microscopies modified Kato-Katz and Floatation technique methods. The Kato Katz Polkessin thats modified by [15] 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. Therefore the modified Kato Katz could take more stool specimens because of the bigger volume than the original (stool weight average is 43,22 mg while the original 41,7 mg)

One hundred and sixty-six Kato-Katz thick smear stool preparations were done by duplication. The modified Kato-Katz template was placed on the microscope slide, filled the hole with the amount of sieved faeces then remove the template. Place over the faeces sample one piece of adhesive strips that 24 hours soaked in methylene blue 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, One hundred and sixty-six faeces floatation technique preparation were done by duplication too. 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, then repeat step 3. Resuspend the sediment in 10 mL of floation 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 floation 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 using a microscope[17].

Both Kato-Katz and Floatation technique method preparation and examination refer to the procedure in WHO bench aid of intestinal parasiteswere 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 search for eggs of helminths is classically done using the 100x magnification. The entire preparation is examined. To accomplish this, one should work systematically. Always start at a corner of the coverslip and work in a straight line from the chosen corner towards the opposite side. Once there, move one row aside

and work back until the entire preparation has been examined. Always proceed by looking at the next microscopic field with a small overlap: when a field has been examined, an object in this field is chosen, and is brought towards the opposite side of

the field. This second field is then examined. When parasitic structures are found, details are examined at 400x magnification.

The species of STHs egg 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*)

Data collected and analyzed by statistical analysis used descriptive and the Wilcoxon Test. Approval for this study was obtained from the Ethics Commission of RSDI No.00123/KEPK-RSDI/02/2023

#### 3 Results

This study was based on one hundred and sixty six faeces samples collected from school children aged 6–13 years. The study was conducted in elementary school at Martapura riverbank area. At a mean age of 10 years, 92 from 166 children (55.42 percent) were male and 74 (44.58 percent) were female. The characteristics of the study subjects were shown in Table 1.

Chara	cteristic	n (%)
Sex	Male	92 (55.42)
	Female	74 (44.58)
Age	6	18 (10.84)
	7	15 ( 9.04)
	8	26 (15.66)
	9	25 (15.06)
	10	22 (13.25)
	11	28 (16.87)
	12	18 (10.84)
	13	14 ( 8.43)
		166 (100)

Table 1. Characteristic of School Aged Children

The average time for duplicate Kato Katz preparation was 25 minutes againts the double floatation preparation with triple centrifuged purification was 73 minutes. The total cost for a single Kato-Katz Polkessin thick smear was \$0.33, for a duplicate was \$0.66, and for the floatation single and double were \$2.35 and \$2.83, respectively.



Fig. 1. The STH eggs in preparation by Kato Katz and Floatation

Fig.1. shows *Trichuris trichiura* eggs in faeces preparation by Kato Katz (left) compared by Floatation technique (right) microscopic examination in 400x magnification. The microscopic identification in Kato Katz preparation shows the eggs absorb the methylene blue despite that showing obvious morphology of *Trichuris trichiura* eggs which are specific features of its eggshell, however, the eggs were easily identified. In the preparation by floatation technique show the swollen *Trichuris trichiura* eggs because the saturated zinc sulfate solution effect. The *Ascaris lumbricoides* infertil eggs in the Kato Katz had not found in the floatation because the specific weight more than the solution [17].



Fig. 2. The number of STH eggs per gram faeces samples by both method

STH positively by Kato Katz was 24.7% vs Floatation was only 19.9%. Fig.2. shows the mean number of STH egg per gram (EPG) by Kato Katz was 46 (range 23-253) vs by Floatation was only 4 (range 1-36). The Wilcoxon Test has a significantly different EPG (p-value = 0.001).

#### 4 Discussion

This study was conducted in 166 children of elementary school at Martapura riverbank area, as shown in Table 1, 92 from (55.42 percent) were male and 74 (44.58 percent) were female. The results of previous study by [18] showed that household variability together with socio-demographic factors including age, gender, and education are crucial in predicting an individual's degree of STH knowledge.

Identification in Kato Katz preparation in Fig.1 obviously contrasts with the background because of the eggshell's thickness so it couldn't absorb the methylene blue. In the Floatation technique, 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 lightweight debris could covered and cause the eggs unidentified. Hookworm eggs have a gravity of 1.055, it floats to the surface and stick to a coverslip due to the difference in densities with the zinc sulfate solution solution[19–21].

The Floatation technique method only uses zinc sulfate solution solution as the reagent, while Kato Katz uses the methylene blue in aqua-glycerol. The lower specific gravity of the eggs relative to that of the flotation solutions was used in the theory behind the fecal flotation of parasite eggs. Depending on the formulation, the different solution have different specific gravities that can range from 1.18 to 1.27. The majority of parasite eggs have a specific gravity between 1.05 and 1.20, which makes them float while larger, denser fecal detritus sinks to the bottom. The flotation technique allows separation of parasitic elements from the coarsest organic debris, using a high specific density flotation solution. Helminths eggs with a specific density lower than the flotation solution, will rise to the top of the suspension. The specimen can be fresh or fixed stool[17, 19, 22].

Glycerol in the Kato Katz reagent functions as a clearing agent, therefore the Kato Katz preparation has more clearly background than the Floatation technique preparation with no clearing agent in it, so it's full of dirt, debris, etc. The floatation technique uses triple centrifugation and washing with tap water for purification. This step needs more preparation time than Kato-Katz procedure[23]. The average time for duplicate Kato Katz preparation was 25 minutes againts the double floatation preparation with triple centrifuged purification was 73 minutes.

Before microscopic examination Kato Katz preparation was incubated for at least 30 minutes at room temperature, according to [15, 24], the clearing process was increased by incubation with glycerol. In this study Kato Katz reagent is 1 ml methylene blue 3% solved in 100 ml aqua destilata and 100 ml glycerol, hence it has almost 50% glycerol.

The total cost for a single Kato-Katz Polkessin thick smear was \$0.33, for a duplicate was \$0.66, and for the floatation single and double were \$2.35 and \$2.83, respectively. Several previous study [23, 25, 26] reveal that the Kato Katz has less expensive than FLOTAC, Mini Flotac and sentrifugated purification flotation technique and the overall cost of any strategy was most heavily influenced by others factors include salaries.

## 5 Conclusion

The study concluded, there are advantages of Kato Katz Polkessin against the Flotation technique in the detection of STH in school-age children in the Martapura riverbank area as follows it is higher numbers of EPG than the Flotation technique, is easier, and is less expensive. It is recommended for further study to compare the sensitivity and specificity of both methods with PCR.

## `References

- Pullan, R.L., Smith, J.L., Jasrasaria, R., Brooker, S.J.: Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. Parasit. Vectors. 7, 37 (2014). https://doi.org/10.1186/1756-3305-7-37.
- Hotez, P.J., Bottazzi, M.E., Strych, U., Chang, L.-Y., Lim, Y.A.L., Goodenow, M.M., AbuBakar, S.: Neglected tropical diseases among the Association of Southeast Asian Nations (ASEAN): overview and update. PLoS Negl. Trop. Dis. 9, e0003575 (2015). https://doi.org/10.1371/journal.pntd.0003575.
- Lee, J., Ryu, J.-S.: Current Status of Parasite Infections in Indonesia: A Literature Review. Korean J. Parasitol. 57, 329–339 (2019). https://doi.org/10.3347/kjp.2019.57.4.329.
- Rahayu, N., Suryatinah, Y., Mellyanie, G., Juhairiyah, J., Annida, A., Fahrizal, D.: Laporan Penelitian Tahun 2019: Evaluasi Program Penanggulangan Kecacingan di Provinsi Kalimantan Selatan. (2019).
- Kurscheid, J., Laksono, B., Park, M.J., Clements, A.C.A., Sadler, R., McCarthy, J.S., Nery, S. V, Soares-Magalhaes, R., Halton, K., Hadisaputro, S., Richardson, A., Indjein, L., Wangdi, K., Stewart, D.E., Gray, D.J.: Epidemiology of soiltransmitted helminth infections in Semarang, Central Java, Indonesia. PLoS Negl. Trop. Dis. 14, e0008907 (2021).
- Ackley, C., Elsheikh, M., Zaman, S.: Scoping review of Neglected Tropical Disease Interventions and Health Promotion: A framework for successful NTD interventions as evidenced by the literature. PLoS Negl. Trop. Dis. 15, e0009278 (2021).
- Neto, A.F.R., Di Christine Oliveira, Y.L., de Oliveira, L.M., La Corte, R., Jain, S., de Lyra Junior, D.P., Fujiwara, R.T., Dolabella, S.S.: Why Are We Still a Worm World in the 2020s? An Overview of Risk Factors and Endemicity for Soil-Transmitted Helminthiasis. Acta Parasitol. 1–15 (2023).
- 8. Djuardi, Y., Lazarus, G., Stefanie, D., Fahmida, U., Ariawan, I., Supali, T.: Soil-

transmitted helminth infection, anemia, and malnutrition among preschool-age children in Nangapanda subdistrict, Indonesia. PLoS Negl. Trop. Dis. 15, e0009506 (2021).

- Scott, M.E., Koski, K.G.: Soil-Transmitted Helminths: Does Nutrition Make a Difference? BT - Nutrition and Infectious Diseases : Shifting the Clinical Paradigm. Presented at the (2021). https://doi.org/10.1007/978-3-030-56913-6\_12.
- Gildner, T.E., Cepon-Robins, T.J., Urlacher, S.S.: Cumulative host energetic costs of soil-transmitted helminth infection. Trends Parasitol. 38, 629–641 (2022). https://doi.org/10.1016/j.pt.2022.04.007.
- Okwuonu, E.S., Aguzie, I.O.N., Atama, C.I., Ani, C.P.: A REVIEW ON GEOHELMINTHS AND PHARMACEUTICALS. J. Biol. Res. Dev. Vol. 1, 92 (2020).
- 12. Organization, W.H.: Guideline: preventive chemotherapy to control soiltransmitted helminth infections in at-risk population groups. World Health Organization (2017).
- 13. RI, K.: Peraturan Menteri Kesehatan Republik Indonesia Nomor 15 Tahun 2017 tentang Penanggulangan Cacingan. Jakarta Kemenkes RI. (2017).
- Keiser, J., Utzinger, J.: Community-wide soil-transmitted helminth treatment is equity-effective. Lancet. 393, 2011–2012 (2019). https://doi.org/10.1016/S0140-6736(18)32981-7.
- 15. Rifqoh, R., Kustiningsih, Y., Cahyono, J.A.: Development of Modified Kato Katz Kit as Laboratory Scale Prototype for Early Detection of Soil-Transmitted Helminths (STHs) Infection. Med. Lab. Technol. J. 7, 201–208 (2021).
- Rifqoh, R., Cahyono, J.A., Kustiningsih, Y.: Confirmation of Soil-Transmitted-Helminths Infection Prevalence by Kato-Katz Kit Diagnostic in Elementary School Children in West Martapura River Banks, South Kalimantan Indonesia. Trop. Heal. Med. Res. 5, (2023).
- 17. Organization, W.H.: Bench aids for the diagnosis of intestinal parasites. World Health Organization (2019).
- Lee, P., Kurscheid, J.M., Laksono, B., Park, M.J., Clements, A.C.A., Lowe, C., Stewart, D.E., Gray, D.J.: Model validation for a knowledge and practices survey towards prevention of soil-transmitted helminth infections in rural villages in Indonesia. Sci. Rep. 13, 1444 (2023).
- Zajac, A.M., Johnson, J., King, S.E.: Evaluation of the importance of centrifugation as a component of zinc sulfate fecal flotation examinations. J. Am. Anim. Hosp. Assoc. 38, 221–224 (2002).
- 20. Amoah, I.D., Singh, G., Stenström, T.A., Reddy, P.: Detection and quantification of soil-transmitted helminths in environmental samples: A review of current state-of-the-art and future perspectives. Acta Trop. 169, 187–201 (2017).
- 21. Agid, N., Sjöqvist, E.: Prevalence of hookworm infection evaluated with Willis flotation and Formal Ethyl Acetate concentration: A field study in Da Nang, Vietnam, (2016).
- 22. Mbong Ngwese, M., Prince Manouana, G., Nguema Moure, P.A., Ramharter, M., Esen, M., Adégnika, A.A.: Diagnostic techniques of soil-transmitted helminths:

impact on control measures. Trop. Med. Infect. Dis. 5, 93 (2020).

- Turner, H.C., Bettis, A.A., Dunn, J.C., Whitton, J.M., Hollingsworth, T.D., Fleming, F.M., Anderson, R.M.: Economic considerations for moving beyond the Kato-Katz technique for diagnosing intestinal parasites as we move towards elimination. Trends Parasitol. 33, 435–443 (2017).
- Wisetmora, A., Artchayasawat, A., Laummaunwai, P., Pitaksakulrat, O., Wattanawong, O., Boonmars, T.: Formalin-fixed Stool Improves Performance of the Kato-Katz method. (2023).
- Speich, B., Knopp, S., Mohammed, K.A., Khamis, I., Rinaldi, L., Cringoli, G., Rollinson, D., Utzinger, J.: Comparative cost assessment of the Kato-Katz and FLOTAC techniques for soil-transmitted helminth diagnosis in epidemiological surveys. Parasit. Vectors. 3, 1–11 (2010).
- Assefa, L.M., Crellen, T., Kepha, S., Kihara, J.H., Njenga, S.M., Pullan, R.L., Brooker, S.J.: Diagnostic accuracy and cost-effectiveness of alternative methods for detection of soil-transmitted helminths in a post-treatment setting in western Kenya. PLoS Negl. Trop. Dis. 8, e2843 (2014).

**Open Access** This chapter is licensed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (http://creativecommons.org/licenses/by-nc/4.0/), which permits any noncommercial use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made.

The images or other third party material in this chapter are included in the chapter's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the chapter's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.

