ARTÍCULO ORIGINAL

# Comparison of Kato Katz and Quantitative Polymerase Chain Reaction Methods in Diagnosing Helminth Infection in Pregnant Women in Enrekang District, Indonesia

Comparación de los Métodos de Kato Katz y de la Reacción en Cadena de la Polimerasa Cuantitativa en el Diagnóstico de la Infección por Helmintos en mujeres embarazadas del Distrito de Enrekang, Indonesia

Tuty Erawaty<sup>1a\*</sup>, Ansariadi<sup>2b</sup>, A. Arsunan Arsin<sup>3b</sup>, Ridwan A<sup>4b</sup>, Stang<sup>5c</sup>, Anwar<sup>6d</sup>, Zulkifli<sup>7e</sup>

## SUMMARY

**Background:** Pregnant women are very vulnerable to diseases, one of which is worm infection. Worm infections in pregnant women affect the fetus's condition, such as the risk of prematurity, low birth weight, and perinatal mortality. The STH target set by the World Health Organization (WHO) to be achieved by 2030 is to establish an efficient STH control program in adolescents, pregnant, and lactating women in the context of elimination, so a sensitive diagnostic is needed to detect worm infections. This study aims to determine the comparison of examination using the Kato Kats (KK) and Quantitative Real-Time Polymerase Chain Reaction (qPCR) methods in pregnant women in Enrekang Regency. **Method:** The study was conducted using a cross-sectional design. Samples were selected using a purposive sampling technique according to the criteria set by the researcher as many as 84 respondents. Fecal specimens were collected and examined using the Kato Katz and qPCR methods. Analysis was used to determine the value of sensitivity and specificity with diagnostic tests and kappa values for the value of suitability with a confidence interval of 95 %. **Results:** From the fecal examination, the sensitivity value of Kato Katz and qPCR for Hookworm was 41.67 % and 45.45 %. And for the specificity of Kato Katz and qPCR for

DOI: https://doi.org/10.47307/GMC.2023.131.4.3

ORCID: https://orcid.org/0000-0002-9692-6136<sup>1</sup> ORCID: https://orcid.org/0000-0003-3311-6686<sup>2</sup> ORCID: https://orcid.org/0000-0003-2712-6773<sup>3</sup> ORCID: https://orcid.org/0000-0003-2884-8188<sup>4</sup> ORCID: https://orcid.org/0000-0001-6438-1154<sup>5</sup>

<sup>a</sup>Master's Programme, Department of Epidemiology, Faculty of Public Health, Hasanuddin University, Indonesia

Recibido: 4 de octubre 2023 Aceptado: 27 de octubre 2023 <sup>b</sup>Department of Epidemiology, Faculty of Public Health, Hasanuddin University, Indonesia

- <sup>e</sup>Department of Biostatistics, Faculty of Public Health, Hasanuddin University, Indonesia
- <sup>d</sup>Department of Environmental Health, Faculty of Public Health, Hasanuddin University, Indonesia
- <sup>e</sup>Megarezky University Makassar and Faculty of Public Health, Hasanuddin University, Indonesia
- Corresponding author: Tuty Erawaty, E-mail: thoetheet77@gmail. com tutyerawaty1309@gmail.com

Hookworm of 91.67 % and 90.41 %. Conclusion: *qPCR* is more sensitive than Kato Katz, so Kato Katz should be used for screening while *qPCR* is used to assess elimination status.

Keywords: STH, Kato Katz, qPCR.

## RESUMEN

Antecedentes: Las mujeres embarazadas son muy vulnerables a las enfermedades, una de las cuales es la infección parasitaria. Las infecciones parasitarias en las embarazadas afectan al estado del feto, como el riesgo de prematuridad, el bajo peso al nacer y el riesgo de mortalidad perinatal. El objetivo fijado por la Organización Mundial de la Salud (OMS) para 2030 es establecer un programa eficaz de control de las enfermedades parasitarias en adolescentes, embarazadas y mujeres lactantes en el contexto de la eliminación, por lo que se necesita un diagnóstico sensible para detectar las infecciones parasitarias. El objetivo de este estudio es determinar la comparación del examen mediante los métodos Kato Katz (KK) y Reacción en Cadena de la Polimerasa en Tiempo Real Cuantitativo (qPCR) en mujeres embarazadas de la regencia de Enrekang. Método de investigación: El estudio se llevó a cabo mediante un diseño transversal. Las muestras se seleccionaron mediante la técnica de muestreo intencional, de acuerdo con los criterios establecidos por el investigador, hasta un total de 84 encuestados. Se recogieron muestras fecales y se examinaron mediante los métodos Kato Katz y qPCR. Se utilizaron análisis para determinar el valor de sensibilidad y especificidad con pruebas diagnósticas y valores kappa para el valor de idoneidad con un intervalo de confianza del 95%. Resultados: A partir del examen fecal, el valor de sensibilidad de Kato Katz y qPCR para Anquilostoma fue de 41,67 % y 45,45 %. Y para la especificidad de Kato Katz y qPCR para Anguilostoma de 91,67 % y 90,41 %. Conclusión: la qPCR es más sensible que el Kato Katz, por lo que el Kato Katz debería utilizarse para el cribado, mientras que la qPCR se utiliza para evaluar el estado de eliminación.

Palabras clave: STH, Kato Katz, qPCR.

# INTRODUCTION

Soil Transmitted Helminth (STH) infections are transmitted through soil contaminated with human feces. Transmission of intestinal worm infections commonly occurs in areas with poor hygiene and sanitation. Human infection can occur after coming into contact with contaminated soil, objects, or surfaces, or by ingesting food or drink contaminated with parasite eggs or larvae (1). Worm infections affect food intake, digestion, absorption, and metabolism. Cumulatively, worm infections can result in loss of nutritional requirements due to calorie and protein depletion and blood loss. This can hinder physical development, intelligence, and work productivity and reduce the body's immunity, making it vulnerable to other diseases (2).

Another loss due to worm infections is productive time calculated using the Daily Adjusted Life Years (DAILY) method. Based on the DAILYs calculation, the productive time lost for worm infections caused by *Ascaris lumbricoide* was between 1.2 and 10.5 million, between 1.8 and 22.2 million for *Trichuris trichiura* and between 1.6 and 6.4 million for *Necator americanus* and *Ancylostoma duodenale* (3).

Pregnant women are highly susceptible to diseases, one of which is worm infection. Worm infections in pregnant women affect the fetus's condition, such as the risk of prematurity, low birth weight, and perinatal death. This is because pregnant women experience anaemia due to iron loss which results in the disruption of haemoglobin formation due to decreased food intake and malabsorption of nutrients (Apriyadi, Umasugi and Fitriasari, 2022).The STH target set by the World Health Organization (WHO) to be achieved by 2030 is to establish an efficient STH control program in adolescents, pregnant and lactating women.

Determining the prevalence of helminthiasis infection is done through fecal examination. Kato Katz is a very cost-effective diagnostic method in identifying where to conduct mass drug administration (*MDA*), the frequency of MDA and assessing the progress of program goals, but compared to the molecular testing method qPCR, the sensitivity of Kato Katz is lower (5)

The microscopic Kato-Katz technique is a relatively simple and low-cost method recommended by the WHO for the detection of STH and other helminth eggs in faecal samples (6,7). Consequently, it is widely used in randomized controlled trials (RCTs), epidemiological surveys, and surveillance studies to determine the impact of STH interventions. Yet, the technique has considerable shortcomings. There is substantial variation in the readings, resulting from uneven distribution of eggs within a single stool sample (within sample variation), day-to-day fluctuations of egg excretion (between sample variations), and ultimately results depending on the readers' skills and experience (1,4,8). Most importantly, the Kato-Katz method may particularly miss lowintensity infections leading to underestimation of the actual prevalence, but in the case of efficacy trials artificially inflated cure rates (CRs) from undetected residual low-egg count infections post-treatment (8). Moreover, expertise in microscopy is increasingly rare (9,10).

A study conducted by Heredia et al. showed that qPCR showed significantly greater sensitivity (p<0.05) with the ability to detect at least 5 EPGs for all three STH species, compared to 50 EPGs by KK and FF. These results suggest that the diagnostic performance of qPCR should be considered for use in confirmation of transmission interruption and discontinuation of preventive chemotherapy/MDA in areas with low STH prevalence (11).

## METHODS

## Design

This study was conducted in the working areas of Baroko, Baraka, and Malua Health Centers with the consideration that Baroko Health Center (Puskesmas) with a high endemicity level (66.67 %), Barako Health Center with a moderate endemicity level (25%) and Health Center Malua free (0%) based on the results of the 2018 survey in June - July 2023. The research used a crosssectional study design.

#### **Sample Size Calculation**

The population in this study were pregnant women who were registered at the Arok, Baraka, and Malua Health Centers in the period January - April 2023 as men 165. Sample calculation using the formula:

$$\begin{aligned} &\frac{Z_{1-\frac{\alpha}{2}}^{2}P(1-P)N}{d^{2}(N-1)+Z_{1-\frac{\alpha}{2}}^{2}P(1-P)} \\ &=\frac{(1,96)^{2}(0,47)(0,53)(165)}{(0,05)^{2}(165)+(1,96)^{2}(0,47)(0,53)} \end{aligned}$$

n = 115

The sample selection was carried out by purposive random sampling according to the criteria made by the researcher, the exclusion criteria were not returning the distributed pots. The number of samples collected was 84 respondents (Figure 1).



Figure 1. Sample Flowchart.

# Data collection on respondent characteristics and faecal samples

An explanation of the study was given during the distribution of the stool pots to pregnant women. The collection of women's faeces was done independently after receiving directions given by the research team assisted by the village midwife. The requirement for specimen collection was that the faeces were not contaminated with liquid materials and soil. Faeces were collected using equipment prepared by the researcher, namely a screw cap plastic container equipped with a spatula. Fecal samples were collected by respondents by bringing them to the village midwife or the community health center. When the fecal pots were returned, data were collected, and consent was signed. Data collected included characteristics of age, education, occupation, gestational age, number of pregnancies, and Hb levels.

This research involves the examination of stool samples using the Kato-Katz and qPCR methods on the respondents. The steps include meetings with relevant parties for research purposes, distributing stool collection containers, and explaining to pregnant women. The next steps involve village midwives distributing the stool containers to pregnant women, and providing explanations about the purpose of stool sample collection, the collection procedure, storage, and the return schedule. Respondents return the stool containers to the village midwife or the local health center, indicating their consent to participate and signing an informed consent form.

# Kato Katz examination

Preparation of Kato Katz specimens was carried out by researchers at the Baroko and Baraka Health Center laboratories. Examination of specimens using the Kato Katz method was carried out by laboratory personnel from the Health Centers of Baroko, Baraka, and Malua and cross-checked directly by the FilCa Program Manager of the South Sulawesi Provincial Health Office.

The Kato-Katz Solution involves the assembly of materials, namely 100 mL of aquadest, 100 mL of glycerol, and 1 mL of either a 3 % malachite green or 3 % methylene blue solution. The 3 % malachite green or methylene blue solution is prepared by meticulously weighing 3 grams of malachite green, introducing it into a glass beaker, gradually adding aquadest, and stirring until a homogeneous 3 % solution is achieved. Subsequently, the Kato-Katz solution is formulated by pouring 100 mL of aquadest into a small plastic container, incrementally introducing 100 mL of glycerol, and 1 mL of the 3 % malachite green or 3 % methylene blue solution, followed by thorough stirring to yield a 201 mL Kato-Katz solution.

The process of Soaking and Applying Cellophane begins with the preparation of a small plastic container, approximately 15x15x15 cm in size, where the Kato-Katz solution is poured. Cellophane tape, measuring 30x25 mm, is then submerged in the Kato-Katz solution for a duration exceeding 24 hours. When ready for use, tweezers are employed to delicately position the soaked cellophane onto the fecal smear. It is noteworthy that cellophane soaked in the Kato-Katz solution can be utilized over an extended period, provided it remains in a suitable condition and is stored within a sealed container.

The Preparation of Faecal Smears involves the utilization of personal protective equipment (PPE) to minimize the risk of infection. Identification of the sample is marked on a microscope slide using a waterproof marker, ensuring it corresponds with the information on the fecal container. A sheet of wax paper, measuring 10x10 cm, is placed on the table, and a small quantity of feces is deposited on it. Straining of the feces is achieved by positioning a sieve wire on top of the feces and applying pressure with a spatula until the feces has been sufficiently strained. Using a spatula, the strained feces are gathered. A perforated cardboard or plastic piece is placed on a slide, and the strained feces is inserted into the perforation. Carefully lifting the perforated cardboard, the feces is covered with the cellophane that has been soaked in the Kato-Katz solution. Uniform flattening is then performed with a rubber bottle cap, and the slide is allowed to rest for approximately 20 to 30 minutes.

The Microscopic Examination of Intestinal Parasites entails positioning the prepared fecal smear on a microscope slide, ensuring it rests on a level surface. Examination is conducted using a light microscope with either a 10x or 40x objective lens. A comprehensive evaluation of the entire field of view is undertaken, with the counting of eggs by species. This counting facilitates the determination of eggs per gram (EPG) for each identified species, by the appropriate formula.

Eggs EPG = ----- x 1 000 (mg)

Fecal Weight (41,7 grams)

# qPCR examination

Specimen preparation with the qPCR method was carried out by Rise Laboratory Staff of the Faculty of Public Health, Hasanuddin University, and the examination was carried out by Rise Laboratory Staff of the Faculty of Public Health, Hasanuddin University at the Makassar Health Laboratory Centre (BBLK) using Biorad CFX 96 equipment. In the DNA extraction process, 250 mg of fresh feces is taken into an Eppendorf tube. Then, 200 µL of GT buffer and 20 µL of Proteinase K are added, followed by the disruption of tissue using a Microprestel, and subsequent incubation at 60°C for 30 minutes. After that, 200 µL of GB buffer is added and vortexed for 5 seconds, followed by another incubation at 60°C for 20 minutes to ensure complete lysis. Following this, 200 µL of absolute ethanol is added, and the mixture is vortexed for 10 seconds. The next step involves preparing a GD column and transferring all the lysate into it, followed by centrifugation at 16,000 G for 2 minutes. The liquid collected in the collection tube is discarded, and 400 µL of W1 buffer is added to the GD column, then centrifuged again at 16,000 G for 30 seconds.

The liquid in the collection tube is once more discarded, and 600  $\mu$ L of Wash buffer is added to the GD column, followed by centrifugation at 16,000 G for 30 seconds. The GD column is then centrifuged at 16,000 G for 3 minutes to dry it. Subsequently, the GD column is moved to a new Eppendorf tube. Next, 100  $\mu$ L of preheated elution buffer is added directly to the matrix. This is allowed to stand for 5 minutes, after which it is centrifuged at 16,000 G for 30 seconds. The eluted DNA in the Eppendorf tube is now ready for PCR analysis.

## **Data Analysis**

The study data were analyzed using Stata Version 14 (StataCorp, 4905 Lakeway Drive College Station, Texas 77845 USA) serial number: 1069939313. The type of test for sensitivity and specificity with the diagnostic test and the suitability value using the kappa coefficient value. Each test used has a Confidence interval (CI) value of 95 % and a p-value <0.05.

## RESULTS

## **Univariate Analysis**

Table 1 shows that the largest age group of respondents in this study was the age group 20-30 years as many as 60 respondents (71.43 %) and the lowest at the age of <20 years as many as 4 respondents (4.76 %). Respondents based on the latest education had more high school education as many as 48 respondents (57.14 %) and the lowest percentage of education levels were at the elementary level as many as 1 respondents (1.19 %). Respondents based on occupation. more as housewives namely 73 respondents (86.90 %) and the lowest profession as civil servants (PNS) as many as 5 respondents (5.95 %).

### Table 1

## Frequency Distribution Based on Demographic Characteristics of Pregnant Women in Enrekang Regency in 2023

Demographic Characteristics	n=84	%	
Age Group			
< 20 Years	4	4.76	
20 - 30 Years	60	71.43	
> 35 Years	20	23.81	
Education			
SD	1	1.19 -	
SECONDARY SCHOOL	14	16.67	
HIGH SCHOOL	48	57.14	
D3	4	4.76	
S1 / Equivalent	17	20.24	
Jobs			
Housewife	73	86.90	
Civil Servant	5	5.95	
Employee	6	7.14	

Source: Primary Data. 2023

Based on Table 2, the highest gestational age of respondents was trisemester as many as 3 respondents (44.05 %) and the lowest was

in trisemester 1 as many as 12 respondents (14.29 %). The highest number of pregnancies or gravida respondents was multigravida as many as 67 respondents (79.76 %). And respondents with non-anaemia status based on hemoglobin levels were 63 respondents (75 %).

The diagnosis of worm infection in respondents is shown in Table 3 for the types of *Ascaris lumbricoides* (roundworm) and *Trichuris trichiura* (whipworm) was not found using either the Kato Katz or qPCR method, only Hookworm (hookworm) was found in the Kato Katz method at 13.10 % and in the qPCR method at 14.29 %.

#### Table 2

Frequency Distribution Based on Characteristics of Pregnant Women in Enrekang Regency in 2023

Characteristics of Pregnan	%	
Pregnancy Age		
Trisemester 1	12	14.29
Trisemester 2	35	41.67
Trisemester 3	37	44.05
Gravida		
Primigravida	17	20.24
Multigravida	67	79.76
Anaemia		
Lightweight	16	19.05
Medium	5	5.95
Weight	0	0.00
Not anaemic	63	75.00

Source: Primary Data. 2023

#### Table 3

Frequency Distribution Based on Diagnosis of Worm Infection by Kato Katz and qPCR Methods in Enrekang District Year 2023

Diagnosis of Worm Infection	Positive		Negative		
	n	%	n	%	
Kato Katz					
Ascaris lumbricoides	0	0.00	0	0.00	
Trichuris trichiura	0	0.00	0	0.00	
Hookworm	11	13.10	73	86.90	
qPCR					
Ascaris lumbricoides	0	0.00	0	0.00	
Trichuris trichiura	0	0.00	0	0.00	
Hookworm	12	14.29	72	85.71	

Source: Primary Data. 2023

## **Bivariate Analysis**

In this study. the STH species Ascaris lumbricoides and Trichuris trichiura were not found so the sensitivity and specificity values were only for Hookworm. Using the Kato Katz method the sensitivity was 42 % (95 % CI) and specificity was 92 % (95 % CI) for Hookworm. While the estimated Positive Predictive Value (PPV) was 45 % (95 % CI) and the Negative Predictive Value (NPV) was 90 % (95 % CI) for Hookworm. While using the qPCR method; the sensitivity was 45 % (95 % CI) and the specificity was 90 % (95 % CI) for Hookworm; while the estimated Positive Predictive Value (PPV) was 42 % (95 % CI) and Negative Predictive Value (NPV) was 92 % (95 % CI).

The kappa value of 0.34 (p-value=0.0008) indicates that the strength of agreement/reliability between the Kato Katz and qPCR methods in the diagnosis of Hookworm infection in pregnant women is minimal.

## ERAWATY T, ET AL

#### Table 4

## Diagnostic Value of Kato Katz and qPCR Method Test in Pregnant Women in Enrekang Regency in 2023

	Kato Katz		qPCF	R		
	Sensitivity (%) (95% CI)	Specificity (%) 95% CI)	Sensitivity (%) 95% CI)	Specificity (%) (95% CI)		
Ascaris lumbricoides Trichuris trichiura Hookworm	0(0.00) 0(0.00) 42(31.52)	0(0.00) 0(0.00) 92(86.98)	0(0.00) 0(0.00) 45(35.56)	0(0.00) 0(0.00) 90(84.97)		

Source: Primary Data. 2023

Table 5

Kappa values of Kato Katz and qPCR methods for each STH species in pregnant women in the Enrekang district Year 2023

	<b>qPCR</b> (+)	<b>qPCR</b> (-)	Kappa (p-value)	
Ascaris lumbricoides				
Kato Katz (+)	0 (0.00 %)	0 (0.00 %)		
Kato Katz (-)	0 (0.00 %)	0 (0.00 %)		
Trichuris trichiura				
Kato Katz (+)	0 (0.00 %)	0 (0.00 %)		
Kato Katz (-)	0 (0.00 %)	0 (0.00 %)		
Hookworm				
Kato Katz (+)	5 (5.95 %)	6 (7.14 %)	0.34 (0.0008<0.05)	
Kato Katz (-)	7 (8.33 %)	66 (78.57 %)		

Source: Primary Data. 2023

#### DISCUSSION

This study compares the Kato-Katz and qPCR methods for detecting STH infections in a sample of 84 pregnant women. The examination results obtained using both methods revealed a sensitivity of 41.67 % and specificity of 91.67 % for Hookworms when using the Kato-Katz method, as compared to a sensitivity of 45.45 % and specificity of 90.41 % for Hookworms when using qPCR.

qPCR demonstrates higher sensitivity compared to Kato-Katz, albeit with a marginal difference in sensitivity of 3.78 %. In contrast, Kato-Katz exhibits higher specificity than qPCR, with a difference of 1.26 %.

In a study conducted by Benjamin-Chung et al. (5), which detected three STH species, Kato-Katz demonstrated sensitivities of 49 % for Ascaris lumbricoide, 52% for Trichuris trichiura, and 32 % for Hookworm. qPCR sensitivities were 79 % for Ascaris lumbricoide, 90 % for Trichuris trichiura, and 93 % for Hookworm, with specificities of 97 % for Trichuris trichiura and Hookworm, and 97 % for Ascaris lumbricoides (5) highly sensitive diagnostics are needed to detect STH infection. We compared double-slide Kato-Katz, the most commonly used copromicroscopic detection method, to multiparallel quantitative polymerase chain reaction (qPCR. Another study by Mationg et al., 2017, reported qPCR sensitivities of 89.9% for Ascaris lumbricoides and 72.3 % for Trichuris trichiura,

while Kato-Katz sensitivities were 30.3 % for *Ascaris lumbricoides* and 44.0 % for *Trichuris trichiura* (12).

A study by Keller et al. (8), indicated sensitivities of 45% for Kato-Katz and 100% for qPCR in detecting *Ascaris lumbricoides*, 52.30% for Kato-Katz and 91.61% for qPCR in detecting *Trichuris trichiura*, and 25.30% for Kato-Katz and 100% for qPCR in detecting Hookworm (8). Additionally, in a study by Dunn et al., 2020, combining Kato-Katz and qPCR results to achieve "true positive" outcomes, Kato-Katz sensitivities were 45.45% for *Ascaris lumbricoides*, 52.30% for Hookworm, with specificities of 100% for qPCR in detecting *Ascaris lumbricoides*, 91.61% for *Trichuris trichiura*, and 100% for Hookworm (13).

The sensitivity of Kato-Katz for Hookworm in this study, at 41.67 %, is higher than the results of Benjamin-Chung et al. (5), at 32 %, and Keller et al. (8) at 25.30 %. The specificity of Kato-Katz for Hookworm is nearly the same as in other studies, consistently exceeding 90 %. These findings suggest that the results of this study align closely with those of other research in assessing helminth infections using Kato-Katz.

Kato-Katz is commonly used for STH surveillance due to its cost-effectiveness and ease of implementation in resource-constrained settings. However, a significant limitation of this method is that samples must be examined within 30 minutes for Hookworm before the eggs disintegrate and become unobservable under a microscope. Moreover, differentiating between *Necator americanus* and *Ancylostoma duodenale*, which are both types of Hookworm, is not feasible using Kato-Katz due to their morphological similarities.

One of the reasons Kato-Katz is still employed in STH diagnostics is the lack of superior alternative diagnostic methods. Although other microscopic techniques like the McMaster method and FLOTAC exist, they, like Kato-Katz, are hampered by low sensitivity (5,11,14,15).

The lower sensitivity and specificity of Kato-Katz compared to qPCR may be attributed to variations in STH prevalence and infection intensity, different laboratory techniques, and the time lapse between fecal sample collection and Kato-Katz examination. Laboratory technicians' expertise in identifying STH under a microscope also influences the results. In the Enrekang District, the microscopists at community health centers have not received STH examination-related training in the past three years, which significantly impacts the examination results.

In this study, the prevalence of Hookworm was 14.29 % with the Kato-Katz method and 15.48 % with qPCR among pregnant women. This indicates that the prevalence of STH is higher when using qPCR compared to Kato-Katz.

From the examination results using both methods, a sensitivity of 42 % and specificity of 92 % was obtained for Hookworm using Kato Katz while a sensitivity of 45 % and specificity of 90 % for Hookworm using qPCR. The difference in sensitivity and specificity values for Hookworm between the Kato Katz and qPCR methods was not significantly different.

This is different from previous studies with sensitivity and specificity values that are much different between Kato Katz and qPCR and detect all three types of STH with Kato Katz sensitivity values for *Ascaris lumbricoide* by 49%. *Trichuris trichiura* (52%) and Hookworm by 32%. As for qPCR sensitivity for *Ascaris lumbricoide* (79%). 90% for *Trichuris trichiura* and 93% for Hookworm. Kato Katz specificity is 97% for *Ascaris lumbricoides* while qPCR specificity is 97% for *Ascaris lumbricoides*, *Trichuris trichiura* and Hookworm (5).

Furthermore, other studies also show that the sensitivity of KK to detect *Ascaris lumbricoides* (45 %), *Trichuris trichiura* (52.30 %), and Hookworm (25.30 %) while the sensitivity of qPCR to detect *Ascaris lumbricoides* (100 %), *Trichuris trichiura* (91.61 %) and for Hookworm is (100 %) (8).

The Kato-Katz method is widely used for STH surveillance because it is inexpensive and relatively easy to perform in resource-poor settings. A significant limitation of this method is that samples must be examined within half an hour for Hookworm before the eggs disintegrate and cannot be seen under a microscope (13).

Hookworm in pregnant women is the highest (78.16 %) which can cause pregnant women to

lose blood as much as 0.005-0.1 mL/day and cause anemia. thus affecting pregnancy due to lack of oxygen intake to the fetus which can cause abnormalities in the fetus (16).

In this study, the type of STH detected was *Hookworm* while *Ascaris lumbricoides* and *Trichuris trichiura were* not found. In line with the results of the study, Enrekang District has implemented *Mass Drug Administration* (MDA) since 2018 until now with coverage of > 80 %, so *Ascaris lumbricoides* and *Trichuris trichiura* are no longer found due to the effectiveness of albendazole during MDA.

A single dose of albendazole and mebendazole on days 14-21 had an average Egg erection rate (ERR) of 94 % and 87.4 % in *Ascaris lumbricoides*. 86.8 % and 40.8 % in Hookworm and 44.9 % and 23.8 % in *Trichuris trichiura*, respectively (14).

Efforts are needed to treat *Hookworm* infection by selective treatment of positive cases. So, screening is needed in Enrekang District through the ANC program. All pregnant women attending ANC should have their stool examined so that they can be treated with the WHO standard treatment for pregnant women in trimester 2 of pregnancy.

In addition, with low STH prevalence rates (<20 %) based on the results of this study in Enrekang District using both KK (13.10 %) and qPCR (14.29 %), highly sensitive diagnostics are needed before elimination and to detect re-infection. Although qPCR has a higher cost compared to Kato Katz in achieving elimination, the continued use of low-sensitivity diagnostics may hamper efforts to determine when STH transmission is interrupted which may not necessitate prolonging mass deworming. The cost of prolonged MDA likely outweighs the more expensive diagnostics.

## CONCLUSIONS

The sensitivity of qPCR is greater than that of Kato Katz, so for screening Kato Katz can be used, for assessing the effectiveness of MDA. It is necessary to examine using the qPCR method in areas with low prevalence rates.

# LIMITATIONS

Limited funds in qPCR examination so that the samples examined are not in all pregnant women. For further research to be carried out with 100 % coverage in pregnant women.

## ETHICAL APPROVAL

This research has received ethical approval from the Ethics Committee of the Public Health Faculty, Hasanuddin University with ethics number: 4859/UN4.14.1/TP.01.02/2023

#### REFERENCES

- Chong NS, Hardwick RJ, Smith SR, Truscott JE, Anderson RM. A prevalence based transmission model for the study of the epidemiology and control of soil-transmitted helminthiasis. PLoS One. 2022;17(8 August):1-28.
- 2. NundrisariD. The Relationship Between Environmental Sanitation and Personal Hygiene and the Incidence of Soil-Transmitted Helminthes Infection in Garahan Kidul Plantation Workers. Thesis, Faculty of Medicine, Univ Jember. 2019.
- Montresor A, Mupfasoni D, Mikhailov A, Mwinzi P, Lucianez A, Jamsheed M, et al. The global progress of soil-transmitted helminthiases control in 2020 and World Health Organization targets for 2030. PLoS Negl Trop Dis. 2020;14(8):1-17.
- Apriyadi KR, Umasugi MT, Fitriasari E. Risk of Helminthiasis in Pregnant Women. J Multidisipliner Bharasa. 2022;1(2):82-91.
- Benjamin-Chung J, Pilotte N, Ercumen A, Grant JR, Maasch JRMA, Gonzalez AM, et al. Comparison of multi-parallel qPCR and double-slide Kato-Katz for detection of soil-transmitted helminth infection among children in rural Bangladesh. PLoS Negl Trop Dis. 2020;14(4):1-23.
- Bosch F, Palmeirim MS, Ali SM, Ame SM, Hattendorf J, Keiser J. Diagnosis of soil-transmitted helminths using the Kato-Katz technique: What is the influence of stirring, storage time, and storage temperature on stool sample egg counts? PLoS Negl Trop Dis. 2021;15(1):e0009032.
- Liu C, Lu L, Zhang L, Bai Y, Medina A, Rozelle S, et al. More poop, more precision: Improving epidemiologic surveillance of soil-transmitted helminths with multiple fecal sampling using the Kato–Katz technique. Am J Trop Med Hyg. 2017;97(3):870.

- 8. Keller L, Patel C, Welsche S, Schindler T, Hürlimann E, Keiser J. Performance of the Kato-Katz method and real-time polymerase chain reaction for the diagnosis of soil-transmitted helminthiasis in the framework of a randomised controlled trial: Treatment efficacy and day-to-day variation. Parasites and Vectors. 2020;13(1):1-12.
- 9. Zelger B, Eisendle K, Mensing C, Zelger B. Detection of spirochetal micro-organisms by focus-floating microscopy in necrobiotic xanthogranuloma. J Am Acad Dermatol. 2007;57(6):1026-1030.
- Caplan J, Niethammer M, Taylor II RM, Czymmek KJ. The power of correlative microscopy: multi-modal, multi-scale, multi-dimensional. Curr Opin Struct Biol. 2011;21(5):686-693.
- 11. Heredia PAZ, Colella V, Hii SF, Traub RJ. Comparison of the egg recovery rates and limit of detection for soil-transmitted helminths using the Kato-Katz thick smear, faecal flotation, and quantitative real-time PCR in human stool. 2021:1-18.
- 12. Mationg MLS, Gordon CA, Tallo VL, Olveda RM, Alday PP, Reñosa MDC, et al. Status of soil-transmitted helminth infections in schoolchildren in

Laguna Province, the Philippines: Determined by parasitological and molecular diagnostic techniques. PLoS Negl Trop Dis. 2017;11(11):1-16.

- 13. Dunn JC, Papaiakovou M, Han KT, Chooneea D, Bettis AA, Wyine NY, et al. The increased sensitivity of qPCR in comparison to Kato-Katz is required for the accurate assessment of the prevalence of soiltransmitted helminth infection in settings that have received multiple rounds of mass drug administration. Parasites and Vectors. 2020;13(1):1-11.
- 14. Olliaro PL, Vaillant MT, Diawara A, Speich B, Albonico M, Utzinger J, et al. Egg excretion indicators for the measurement of soil-transmitted helminth response to treatment. PLoS Neglected Tropical Diseases. 2022;16: e0010593.
- Paniker CJ, Ghosh S. Medical Parasitology. Seventh Ed. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd; 2013.
- 16. Gebrehiwet MG, Medhaniye AA, Alema HB. Prevalence and associated factors of soil-transmitted helminths among pregnant women attending antenatal care in Maytsebri Primary Hospital, North Ethiopia. BMC Res Notes. 2019;12(1):4-9.