

The Effect of Variations in Centrifugation Time and Speed on the Quantity of Soil Transmitted Helminth (STH) Eggs

Abdul Ghofur¹, Kurniasih Wijayanti^{2*}

^{1,2}Program Studi DIII Tenaga Laboratorium Medis, Akademi Analis Kesehatan Pekalongan, Jawa Timur, Indonesia

*Corresponding author:

E-mail: kurniasihwijayanti28@gmail.com

Abstract.

Soil Transmitted Helminth (STH) infection is a significant health problem in Indonesia with a prevalence of 28.12%. Diagnosis of worm infections requires accurate detection, one of which is by the sedimentation method. The results of this sedimentation method are influenced by variations in centrifugation time and speed. This study aims to determine the effect of centrifugation time of 3, 5, and 10 minutes at a speed of 2500 rpm on the quantity of STH eggs. This study design is quasi-experimental using STH positive fecal samples from the Parasitology Laboratory of the Pekalongan Health Analyst Academy. The results showed a total of 12 eggs at 3 minutes, 18 eggs at 5 minutes and 24 eggs at 10 minutes, the Friedman test results ($p = 0.264 > 0.05$) mean there is no significant effect between variations in time and centrifugation speed on the quantity of STH eggs.

Keywords: Centrifugation Speed, Egg Count, Sedimentation Method, Soil-Transmitted Helminths and Time Variation.

I. INTRODUCTION

Soil Transmitted Helminths (STH) are a group of parasitic worms which can be transmitted through soil contaminated by human feces infected with infective eggs or larvae. Worms that are included in the STH group are *Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus* and *Ancylostoma duodenale* [1].

STH infections remain a major public health problem in developing countries like Indonesia, with a national prevalence reaching 28,12% and serious impacts such as anemia and growth and developmental disorders in school-age children [2]. The main types of worms include *Ascaris lumbricoides*, *Trichuris trichiura*, *Ancylostoma duodenale* and *Necator americanus*, which are transmitted through soil contaminated with eggs or larvae due to poor sanitation, open defecation habits, and poor hygiene such as not washing hands or walking barefoot [3]. In Central Java, data from the Health Department recorded a prevalence of worm infestation of 26%, while in Semarang it reached 38% in children aged 5-14 years, and even 80% in areas with poor sanitation [4].

According to the World Health Organization (WHO), more than 1,5 billion people or 24% of the world's population will be infected with STH by 2022, with 270 million preschool children and 600 million school children requiring ongoing preventive treatment [2]. In Indonesia, parasitological epidemiology shows that this infection has been stable for the last 50 years, mainly due to soil contaminated with human feces as the main source [5].

STH detection relies on qualitative (sedimentation, flotation, native, Suzuki, Harada-Mori, Baermann) or quantitative (Stoll, mini-FLOTAC, Kato-Katz) microscopic examination of feces, where the sedimentation method utilizes gravity or centrifugation to separate worm eggs based on differences in specific gravity [4]. The advantage of sedimentation is time efficiency compared to flotation, but its effectiveness is affected by variations in centrifugation time and speed, which can reduce the number of eggs detected or damage the morphology if not optimal [3]. Onesiforus and Kusuma's (2022) [3] research found optimal results at 2000 rpm/10 minutes (7 eggs) and 3000 rpm/5 minutes (6 eggs), while Saragih (2022) [5] reported 12 eggs at 2000 rpm/5 minutes.

This issue is crucial because variations in centrifugation parameters affect the quality of early detection, which is essential for STH control in endemic areas such as Indonesia [5]. Short time fails to completely settle the eggs, while excessive time or speed damages the morphology of eggs, interfering with

microscopic analysis [3]. Previous studies were limited to speeds of 1500-3000 rpm, leaving a gap at 2500 rpm for 3, 5, and 10 minutes, so new studies are needed to optimize laboratory procedures [5].

This study aims to determine the effect of time variations (3, 5, 10 minutes) and centrifugation speed of 2500 rpm on the quantity of STH eggs using the sedimentation method, specifically testing the effectiveness of each combination for optimal results [3]. The urgency lies in increasing the accuracy of early detection of STH, supporting public health programs such as PHBS and Health Service control in high endemic areas, considering that prevalence remains high despite global interventions [2]. The novelty of the 2500 rpm speed test—not previously explored—provides new baseline data for parasitology laboratory standards, complementing the studies of Onesiforus-Kusuma (2022) [3] and Saragih (2022) [5] and contributing to the Academy of Health Analysts literature.

II. METHODS

This study used an experimental laboratory design with a post-test only control group design. Three experimental groups were used: Group 1, time 3 minutes, speed 2500 rpm (P1), Group 2, time 10 minutes, speed 2500 rpm (P2), and Group 3 as a control using the gold standard sedimentation method with a time of 5 minutes, speed 2500 rpm (K0). This study used 3 repetitions in each experimental group.

Sample preparation

Homogenize positive samples of STH worm eggs before testing, the samples are validated to determine that the sample is a positive sample by direct examination to find worm egg cells.

Principle of sedimentation method

The sedimentation method utilizes the principle of sedimentation which relies on the difference in specific gravity of parasite eggs and fecal debris, where centrifugal force accelerates the settling of *Ascaris lumbricoides*, *Trichuris trichiura*, and *hookworm* eggs to the bottom of the tube, while lighter particles are suspended in the supernatant [8]. The theoretical framework is based on Stokes' law for sedimentation, which states that the settling rate is proportional to the square of the particle radius and the difference in density, with rpm speed and time duration as key factors; too high a speed damages the thin walls of *Trichuris* eggs, while a short time results in incomplete sedimentation [9].

Inspection procedures

The procedure begins with sample validation via native examination: drop 2% eosin on a glass object, mix enough feces, close the cover glass, observe at 10x/40x magnification to confirm intact worm egg cells [3,5]. Next, the sedimentation method is carried out: take 1 ml of feces into a test tube, add 0.85% NaCl to $\frac{3}{4}$ of the tube, homogenize and centrifuge at 2500 rpm for 3, 5 and 10 minutes respectively, discard the supernatant, take 1-2 drops of sediment onto a glass object, cover the glass deck, observe microscopically at 10x/40x magnification, and count the eggs in a zigzag manner in the entire field of view [6,7]. All steps are performed at stable room temperature to prevent egg dehydration or excessive solution viscosity, with photographic documentation if morphology is in doubt [8,9].

III. RESULTS AND DISCUSSIONS

Observation Results of the Effect of Variations in Centrifugation Time and Speed on the Quantity of STH Eggs

Table 1. Observation Results of the Effect of Centrifugation Time Variations on the Quantity of STH Eggs

Repetition	Quantity of worm eggs		
	3 minutes	5 minutes	10 minutes
1	7	3	8
2	3	10	7
3	2	5	9
Total	12	18	24

Based on Table 1, the research results showed a total of 12 eggs at minute 3, 18 eggs at minute 5, and 24 eggs at minute 10. Descriptively, these data indicate an increase in the number of eggs detected as centrifugation time increases.

Table 2. Effect of Variations in Centrifugation Time and Speed (2500 rpm) on the Quantity of *Soil*

Test	Worm Species	Time (Minutes)		
		3	5	10
1	<i>Ascaris lumbricoides</i>	6	12	15
2	Hookworm	6	3	3
3	<i>Trichuris trichiura</i>	0	3	6
Total		12	18	24

Transmitted Helminth (STH) Eggs Based on Species

The results of the 3 minute time variation obtained a total of 12 eggs, consisting of 6 *Ascaris lumbricoides* eggs and 6 *hookworms* in all three tubes. The 5 minute time variation obtained a total of 18 eggs, consisting of 12 *Ascaris lumbricoides* eggs, 3 *hookworms* and 3 *Trichuris trichiura* eggs. Meanwhile, the 10 minute time variation obtained the largest total of 24 eggs, consisting of 15 *Ascaris lumbricoides* eggs, 6 *hookworms* and 3 *Trichuris trichiura* eggs.

Friedman Test Results

Table 3. Friedman Test Results

Test Statistics	
N	3
Chi-Square	2,667
Df	2
Asymp. Sig.	.264

The Friedman test results showed a sig. (Asymp. Sig) value of 0.264 ($p > 0.05$), meaning there was no significant difference between the 3, 5, and 10 minute sedimentation times on the quantity of STH eggs.

IV. DISCUSSION

The findings of this study indicate that variations in centrifugation time of 3 minutes, 5 minutes, and 10 minutes at a speed of 2500 rpm descriptively indicate an increase in the quantity of eggs found. However, the results of statistical tests indicate that the significance value is 0.264 ($p > 0.05$), meaning that variations in centrifugation time do not significantly affect the quantity of STH eggs in the sedimentation method. In other words, changes in centrifugation duration within this range do not cause an increase or decrease in the number of eggs detected. The absence of this effect is thought to be because at a speed of 2500 rpm the centrifugal force generated is sufficient to sediment the eggs from the initial minutes of the centrifugation process. The time range of 3–10 minutes is in the stable sedimentation phase, so that increasing the duration no longer increases the efficiency of egg sedimentation.

The lack of effect can be explained by the physics of sedimentation. At 2500 rpm, the centrifugal force generated is sufficient to separate eggs from fecal debris within a short time. Once the eggs have settled, increasing the time no longer increases the sedimentation rate. This explanation is also proposed by Handayani (2020) [12] and Saragih (2022) [5], who stated that the process reaches a plateau after the initial minutes.

Descriptively, this study does show differences in the average scores between the 3, 5, and 10-minute time periods, but these differences are not large enough to exceed the statistical significance level. Saragih (2022) [5] also explained that fluctuations in descriptive scores cannot be directly interpreted as effectiveness without inferential testing (statistical testing). Therefore, the correct interpretation is that all three time periods are equally effective. This provides the basis for answering the specific research questions.

Based on the entire discussion, it can be confirmed that variations in centrifugation time did not affect the quantity of STH eggs ($p = 0.264$). Consequently, no duration was proven to be optimal, so the specific problem formulation was answered through the results of the influence test.

V. CONCLUSION

This study found that variations in centrifugation time of 3, 5, and 10 minutes at 2500 rpm did not significantly affect the quantity of *Soil Transmitted Helminth* (STH) eggs using the sedimentation method, as shown by the Friedman test results ($p = 0.264 > 0.05$), although descriptively, it showed an increase from 12 eggs (3 minutes) to 24 eggs (10 minutes) with a predominance of *Ascaris lumbricoides*. This finding is consistent with Saragih (2022) [5] and Putri and Rahman (2021) [11], who confirmed that at this speed, the sedimentation process reaches a stable phase from the beginning, so that additional duration does not increase the efficiency of *Trichuris trichiura* or *hookworm* egg detection. Practically, these results recommend using a minimum time of 3 minutes to optimize parasitology laboratory efficiency without sacrificing accuracy, supporting STH early detection programs in endemic areas such as Central Java.

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