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Quantitative Comparison of *Soil Transmitted Helminths* (STH) Eggs Flotation Method with Different Solutions and Times

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ABSTRACT

Background & Objective: Soil-Transmitted Helminths (STH) infections are among the most common communicable diseases worldwide and have a significant impact on public health. These infections are caused by several types of intestinal worms, including *Ascaris lumbricoides, Trichuris trichiura, Strongyloides stercoralis, Necator americanus*, and *Ancylostoma duodenale*. Children and individuals working in agriculture are particularly at high risk of infection. The flotation method is commonly used to detect helminth eggs in stool samples. This study aimed to assess the effectiveness of the flotation method using different solutions—NaCl, $ZnSO_4$, and $MgSO_4$—at incubation times of 30 minutes and 40 minutes in detecting the number of STH eggs. **Method:** This study used an experimental design, where the researchers applied different flotation solutions and incubation times. The results were statistically analyzed using the MANOVA test. **Results:** The MANOVA test showed that all significance values were > 0.05, indicating no statistically significant effect of the different flotation solutions and incubation times on the quantitative detection of STH eggs. However, descriptive analysis revealed that the $ZnSO_4$ solution with 40 minutes of incubation was more effective than NaCl and $MgSO_4$, as it detected a higher number of STH eggs. **Conclusion:** It can be concluded that there is no statistically significant effect of the flotation method using different solutions and incubation times on the quantitative detection of Soil-Transmitted Helminth eggs. Nonetheless, $ZnSO_4$ with 40 minutes of incubation showed relatively better descriptive results.

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Introduction

Soil-Transmitted Helminth (STH) infections are among the most common communicable diseases worldwide. An estimated 1.5 billion people, or 24% of the global population, are affected. These infections primarily impact the poorest communities in tropical and subtropical regions with inadequate access to clean water and sanitation, with the highest prevalence in Sub-Saharan Africa, China, South America, and Asia (WHO, 2023). According to data from the Indonesian Ministry of Health in 2015, the prevalence of helminth infections among children aged 1–12 years in several Indonesian provinces ranged from 30% to 90% (Kemenkes, 2022).

Intestinal worm infections are common in communities but often receive insufficient attention. These infections are caused by a group of worms known as Soil-Transmitted Helminths (STH), which include *Ascaris lumbricoides* (A. lumbricoides), *Trichuris trichiura* (T. trichiura), Strongyloides stercoralis, Necator americanus, and Ancylostoma duodenale (A. duodenale), all of which require soil as part of their life cycle. School-aged children and agricultural workers are considered high-risk groups for STH infections (Novianty et al., 2019).

Stool examination for helminth infections is performed to detect the presence of eggs or infective larvae. This examination can be qualitative—detecting the presence of helminth eggs without counting them—or quantitative—determining the number of eggs per gram of stool (Regina et al., 2018).

Various methods are used to detect helminth eggs, including direct smear (direct slide), flotation techniques, adhesive tape methods, thick smear techniques, and sedimentation methods for routine examination. The most commonly used techniques for diagnosing nematode infections are flotation and sedimentation (Islamiati et al., 2021). In the flotation method, stool samples are floated using solutions such as saturated sodium chloride (NaCl), which is commonly used for samples containing a low number of eggs (Nurhidayanti & Indah Sari, 2023). The advantages of the flotation method include its ability to detect light infections and to separate stool debris from helminth eggs, making identification easier (Islamiati et al., 2021). Other flotation solutions that can be used include $ZnSO_4$, sucrose, $MgSO_4$, and $NaNO_3$ (Steinbaum et al., 2017). The effectiveness of the flotation method is influenced by factors such as specific gravity, type of solution, homogeneity of the solution, and flotation incubation time (Wasilah et al., 2024).

Previous studies related to this topic include research by Alma Tiara Rahayu et al., which compared flotation methods using NaCl, $ZnSO_4$, and $MgSO_4$ solutions with incubation times of 10 and 20 minutes. They found *Ascaris lumbricoides* eggs (fertile, infertile, and decorticated) across 12 positive stool samples. For $ZnSO_4$ 33%, they found 53 eggs at 10 minutes and 29 eggs at 20 minutes. For $MgSO_4$ 33%, 16 eggs were found at 10 minutes and 39 at 20 minutes. For NaCl 33%, 10 eggs were found at 10 minutes and 19 at 20 minutes. At a 43% concentration, $ZnSO_4$ yielded 34 eggs at 10 minutes and 29 at 20 minutes; $MgSO_4$ had 17 and 26 eggs; and NaCl yielded 11 and 1 egg respectively. These results indicated that the optimal flotation conditions were

$ZnSO_4$ 33% for 10 minutes, $MgSO_4$ 33% for 20 minutes, and NaCl 33% for 20 minutes (Rahayu et al., 2021).

Another study by Nurul Azmah Nikmatullah et al. compared $ZnSO_4$ and sucrose solutions with a 10-minute incubation for detecting helminth eggs. Among 35 samples, $ZnSO_4$ 33% detected 13 STH eggs, while sucrose 33% detected only 1 egg. A Kolmogorov-Smirnov Z test confirmed that $ZnSO_4$ 33% was significantly more effective than sucrose 33% (Nikmatullah et al., 2023).

Based on the literature, the present study aims to evaluate the effectiveness of the flotation method using NaCl, $ZnSO_4$, and $MgSO_4$ solutions with extended incubation times of 30 and 40 minutes. This differs from previous research that used shorter incubation times of 10 and 20 minutes.

Objective

To determine the effect of the flotation method using different solutions and incubation times on the quantitative detection of Soil-Transmitted Helminth (STH) eggs.

Method

This study is experimental in nature, involving researcher-controlled interventions on the samples. The focus is on using different flotation solutions and incubation times to assess their impact on the quantity of detected helminth eggs. The research was conducted in January 2025 at the Parasitology Laboratory of the Health Analyst Academy (AAK) in Pekalongan. The samples used were stool specimens known to be positive for STH eggs.

Each sample was tested using one solution and incubation time, repeated three times. With three solutions and two incubation durations, the study involved a total of 18 treatments.

Data were obtained from both primary and secondary sources. Primary data came from the flotation tests, while secondary data were sourced from relevant books and journals. Statistical analysis was performed using the MANOVA test to determine the effect of multiple independent variables (types of solutions and incubation times) on the dependent variable (quantity of STH eggs).

Results

Based on stool sample examinations using saturated NaCl, $ZnSO_4$, and $MgSO_4$ solutions with incubation times of 30 and 40 minutes, conducted at the Parasitology Laboratory of the Health Analyst Academy (AAK) in Pekalongan, the results are presented as follows:

TABLE 1. Quantitative results of STH eggs using saturated NaCl solution with 30-minute and 40-minute incubation times.

Test	Solution	Incubation Time and Species Type			
	NaCl	30 min	Species	40 min	Species
1	Egg count	1	*T.trichiura*	1	*T.trichiura*
2	Egg count	2	*A.lumbricoides*	2	*A.lumbricoides*

Test	Solution NaCl	Incubation Time and Species Type			
		30 min	Species	40 min	Species
3	Egg count	2	*A.lumbricoides, T.trichiura*	1	*A. duodenale*
Total		**5**		**4**	

Based on table 1, it is known that the examination of STH worm eggs using the flotation method using saturated NaCl solution with 3x repetition tests with an incubation time of 30 minutes, found 5 STH worm eggs with details: 2 *Trichuris trichiura* and 3 *Ascaris lumbricoides*, while with an incubation time of 40 minutes, 4 STH worm eggs were found with details: 1 *Trichuris trichiura*, 2 *Ascaris lumbricoides*, and 1 *Ancylostoma duodenale*.

TABLE 2. Quantitative results of STH helminth eggs using saturated $ZnSO_4$ solution with incubation time of 30 min and 40 min

Test	Solution $ZnSO_4$	Incubation Time and Species Type			
		30 min	Spesies	40 min	Species
1	Egg count	3	*A.lumbricoides, T.trichiura*	3	*A.lumbricoides, T.trichiura*
2	Egg count	1	*T.trichiura*	6	*A.lumbricoides, T.trichiura*
3	Egg count	1	*A.lumbricoides*	2	*T.trichiura*
Total		**5**		**11**	

Based on table 2, it is known that the examination of STH worm eggs using the flotation method using a saturated $ZnSO_4$ solution with 3x repetition tests with an incubation time of 30 minutes, found 5 STH worm eggs with details: 3 *Trichuris trichiura* and 2 *Ascaris lumbricoides*, with an incubation time of 40 minutes found 11 STH worm eggs with details: 7 *Trichuris trichiura* and 4 *Ascaris lumbricoides*.

TABLE 3. Quantitative results of STH eggs using saturated MgSO4 solution with incubation time of 30 minutes and 40 minutes

Test	Solution $MgSO_4$	Incubation Time and Species Type			
		30 min	Species	40 min	Species
1	Egg count	1	*A.lumbricoides*	2	*A.lumbricoides, T.trichiura*
2	Egg count	2	*A.lumbricoides, T.trichiura*	3	*A.lumbricoides*
3	Egg count	1	*A.lumbricoides*	1	*T.trichiura*
Total		**4**		**6**	

Based on Table 3, the examination of STH (Soil-Transmitted Helminths) eggs using the flotation method with saturated $MgSO_4$ solution and 3 repeated tests with 30-minute incubation found 5 STH eggs, consisting of 1 *Trichuris trichiura* and 3 *Ascaris lumbricoides*. At 40 minutes of incubation, 6 STH eggs were found, consisting of 2 *Trichuris trichiura* and 4 *Ascaris lumbricoides*.

Statistical analysis using MANOVA showed that at 30-minute incubation, all solutions had a significance value of 1, while at 40-minute incubation, the lowest significance value was 0.248. This means that statistically, there was no significant effect of the flotation method using various solutions and incubation times on the quantity of STH eggs.

Discussion

Based on the research conducted at the Parasitology Laboratory of the Health Analyst Academy (Akademi Analis Kesehatan) Pekalongan to determine the effect of the flotation method using various solutions and incubation times on the quantity of STH eggs, the following results were obtained:

This study showed that the flotation method using $ZnSO_4$, $MgSO_4$, and NaCl solutions with 30-minute incubation did not result in significant differences in the quantity of STH eggs. However, at 40-minute incubation, the use of $ZnSO_4$ solution descriptively yielded a greater number of eggs compared to $MgSO_4$ and NaCl solutions. Nevertheless, statistically, MANOVA test results showed a significance value > 0.05 for all solutions and incubation times, indicating no significant differences in STH egg quantity based on the flotation method using different solutions and incubation times.

The statistical findings indicate that there is no significant difference in the quantitative count of STH eggs between 30-minute and 40-minute incubation times using various flotation solutions. This could be due to the small sample size for each repetition, making the statistical test less valid.

Based on the data in Table 2, it can be seen that descriptively, $ZnSO_4$ solution with 40-minute incubation was more effective for flotation compared to NaCl and $MgSO_4$ solutions, as it resulted in the highest number of STH eggs—11 eggs. These results are consistent with previous studies by Alma Tiara Rahayu, Aldi Pratama, M. Wahyu Setiawan, Ma'rifatussolihat, and Nurul Azmah Nikmatullah (Rahayu et al., 2021), as well as by Nurul Azmah Nikmatullah, Wijiastuti, and Chintya Rahmadilla (Nikmatullah et al., 2023), which stated that $ZnSO_4$ is the most effective solution for detecting STH eggs using the flotation method.

The number of eggs found with a 40-minute incubation was higher than that with 30-minute incubation. This indicates that a 40-minute flotation incubation period remains optimal before the egg absorbs the solution maximally. This is important because prolonged flotation can damage the egg wall, causing it to absorb the solution, increase in specific gravity, and subsequently sink back to the bottom of the tube. A saturated flotation solution will produce different outcomes since increasing saturation changes the molecular weight and specific gravity (Rahayu et al., 2021).

Choosing the appropriate flotation solution is crucial, as differences in specific gravity can affect the egg count. A flotation solution must have a specific gravity higher than that of helminth eggs (approximately 1.10–1.20) to effectively separate and count them (Rusyda, 2023).

Conclusion

There is no statistically significant difference in the effectiveness of flotation methods using different solutions and incubation times in detecting the number of STH eggs. However, descriptively, $ZnSO_4$ solution with 40-minute incubation yielded the highest number of STH eggs compared to NaCl and $MgSO_4$. Suggestion for future

researchers: it is recommended to use a larger sample size to obtain more statistically valid results and to explore longer incubation times beyond 40 minutes.

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