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RESEARCH PAPER

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The Effect Of Time Variation and Sodium Chloride (NaCl) Concentration In The Sedimentation Method On Soil-Transmitted Helmints (STH) Examination

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ABSTRACT

Soil-transmitted helminths (STH) remain a significant public health concern, particularly in regions with inadequate sanitation. While the sedimentation method is routinely employed for STH diagnosis, the effects of procedural variables such as incubation time and sodium chloride (NaCl) concentration on recovery efficiency remain inadequately explored. This study aimed to evaluate the influence of variations in sedimentation time and NaCl concentration on the detection and morphological integrity of STH eggs in stool samples. This contributes to more reliable diagnostic results in laboratory settings, supports epidemiological surveillance, and enhances the accuracy of STH monitoring in endemic areas. A controlled experimental design was implemented, utilizing 48 treatment combinations that varied in NaCl concentration (2%, 4%, 6%, and 8%) and sedimentation duration (5, 10, 15, and 20 minutes). Stool samples naturally infected with *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm were processed and examined microscopically for egg count, morphology, and sedimentation quality. Data normality was assessed using the Shapiro–Wilk test, and differences among treatment groups were analyzed with the Kruskal–Wallis test followed by post-hoc pairwise comparisons. Results demonstrated that both NaCl concentration and sedimentation time significantly influenced egg recovery ($p < 0.05$), with 2% NaCl and 30 minutes yielding the highest recovery rate and optimal morphological preservation. Morphological assessment revealed that prolonged sedimentation (>40 minutes) resulted in noticeable egg deformation, potentially compromising identification accuracy. These findings highlight the critical importance of optimizing procedural parameters in routine STH diagnostics. Adjusting NaCl concentration and sedimentation time can enhance both quantitative recovery and qualitative morphological assessment, thereby improving diagnostic reliability in clinical and epidemiological settings. In conclusion, the study provides evidence-based recommendations for sedimentation protocols, bridging a methodological gap and offering practical guidance for parasitology laboratories aiming to enhance the accuracy of STH examinations.

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1. INTRODUCTION

The recognition of electromyography (EMG) is crucial for understanding the entry of parasitic worms into the human body, also known as a worm infection. Worms, including eggs, cysts, or larvae found in the soil, can enter thw body through pores and wounds in the skin. Worm infections can lead to a decline in the body's health and nutritional status, thus reducing the quality of human resources [1]. *Soil-Transmitted Helminths* (STH) are intestinal parasites that infect humans through contact with contaminated soil containing infective eggs or larvae. The most common species include *Ascaris lumbricoides, Trichuris trichiura, and hookworms* (*Ancylostoma duodenale and Necator americanus*) [2]. Infection with STHs can lead to malnutrition, anemia, impaired cognitive development, and reduced school and work performance, highlighting the significant public health burden they impose. Accurate diagnosis is crucial for effective treatment and control programs. These parasites pose a significant public health concern, particularly in tropical and subtropical regions, where inadequate sanitation and hygiene facilitate their transmission [3]. STH infections can cause nutritional

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deficiencies, anemia, growth retardation, and decreased cognitive performance, especially in children [4]. *Soil-transmitted helminths(STH)* are parasitic worms of the nematode (intestinal worm) class that are transmitted through soil. STH infect people who frequently come into direct contact with or come into contact with the soil. Types of STH worms that infect humans are roundworms (*Ascaris lumbricoides), whipworms (Trichuris trichiura), and hookworms*. To identify diseases caused by worms, laboratory tests are required on the worms or on the feces of an infected person [5]. The method of worm testing depends on the type of worm present or causing the infection. Examination of worm eggs in feces can be done using one of the methods, namely, sedimentation [6]. The sedimentation method is an alternative to the native method (gold standard). The sedimentation method uses a solution that causes worm eggs to settle at the bottom with the aid of a centrifuge [7]. The sedimentation method is quite often used in parasitology examinations, because it does not require a long time, is easy to perform in the examination, and is quite affordable and has advantages in sensitivity and specificity in identifying the presence of worm eggs in feces [8]

Accurate diagnosis of STH infections is essential for effective control and treatment programs. One of the laboratory techniques commonly used for detecting STH eggs in fecal samples is the sedimentation method [9]. This method relies on the difference in specific gravity between parasite eggs and the surrounding solution, allowing the eggs to settle at the bottom for microscopic examination [10]. However, several factors influence the efficiency of this method, including the duration of sedimentation and the concentration of the solution used, such as sodium chloride (NaCl) [1]. Traditional diagnostic methods, such as the sedimentation technique, rely on physically separating parasite eggs from stool samples. However, recovery efficiency and morphological preservation can be influenced by procedural variables, including the concentration of sodium chloride (NaCl) used in the solution and the duration of sedimentation or centrifugation. Although several studies have evaluated sedimentation methods, there is limited consensus on the optimal NaCl concentration and sedimentation time, resulting in variability in diagnostic sensitivity and accuracy [11]. This gap in standardization underscores the need for systematic investigation to optimize procedural parameters for reliable STH detection.

Centrifugation time can be relied upon to yield the desired number of eggs and achieve the desired morphological results. This is because the long centrifugation time can cause the sedimented worm egg cells not to form properly or the sample to lyse. The research that will be conducted aims to determine whether there is a comparison of the results of the effect of centrifugation time and NaCl concentration that are varied on the number of worm eggs obtained and how the morphology of STH eggs is seen using the NaCl sedimentation method with variations in centrifugation time of 5, 10, 15, 20 minutes at 2000 rpm and variations in NaCl concentration of 2%, 4%, 6%, and 8% [5]. Sodium chloride (NaCl) is the main cation in extracellular fluid and plays a crucial role in regulating osmotic pressure. It is often used as an infusion solution with other electrolyte solutions. NaCl is known as table salt, a substance with a high osmotic pressure.(6)0.9% NaCl is an isotonic fluid that is physiological, non-toxic and does not cause hypersensitivity, and can protect tissue granulation from dry conditions [12]. NaCl solution is used as a chemical solution to precipitate worm eggs and affects worms depending on the species and concentration used. The use of NaCl in STH examination using the sedimentation method is based on the fact that NaCl solution can clarify worm eggs and does not damage the eggs [13]. Therefore, this study was conducted to analyze the effect of time variation and Sodium Chloride (NaCl) concentration in the sedimentation method on the recovery and morphology of Soil-Transmitted Helminths (STH) eggs. The primary objective of this research is to identify the optimal combination of NaCl concentration and sedimentation time that yields the most consistent and reliable recovery of STH eggs.

The contribution of this study is to provide an improved and standardized sedimentation protocol that enhances diagnostic accuracy in parasitology laboratories, particularly in low-resource settings. The findings are expected to help laboratories refine their diagnostic procedures, ensuring more reliable monitoring of helminth infections and supporting global efforts to deworm. The variation in time and NaCl concentration can affect the recovery rate and clarity of parasite eggs observed under the microscope. Insufficient time may result in incomplete sedimentation, while excessive time may cause distortion or degradation of eggs [14]. Similarly, inappropriate NaCl concentration can lead to changes in osmotic pressure that affect the morphology or flotation behavior of helminth eggs [15]. Therefore, optimizing both parameters is crucial to obtaining accurate and reproducible diagnostic results. The present study aims to assess the effect of time variation and NaCl concentration in the sedimentation method on the recovery rate and morphological integrity of STH eggs in stool samples. By systematically varying these parameters and analyzing their impact on both quantitative egg counts and qualitative morphology, this research seeks to establish evidence-based recommendations for sedimentation protocols. The outcomes are expected to enhance laboratory diagnostic accuracy, contribute to standardized parasitological practices, and support global efforts in STH control and epidemiological surveillance.

2. MATERIALS AND METHOD

A. Design, Place, and Time

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This study employed an experimental design with a post-test control group consisting of 16 treatments and three replications, yielding a total of 48 treatments. The study used a Completely Randomized Design (CRD). This study will be conducted over a five-month period, from January to May 2025, at the Pharmaceutical Biology Laboratory, Anwar Medika University, Sidoarjo.

The samples used in this study were suspensions of Ascaris lumbricoides, Trichuris trichiura, and Hookworm eggs obtained at the Surabaya Health Laboratory Center. The tools used in the study of variations in centrifugation time and NaCl concentration using the sedimentation method were masks, hand scoops, centrifuges, test tubes or centrifuge tubes, stirring rods, beakers, object glasses, cover glasses, dropper pipes, and microscopes. The materials used in this study were suspensions of Ascaris lumbricoides, Trichuris trichiura, and Hookworm eggs, NaCl, and distilled water.

B. Work procedures

STH worm egg examination using 2%, 4%, 6% and 8% NaCl solution is done by making NaCl solution first by weighing 10 g, 20 g, 30 g, and 40 g NaCl and each dissolved in 500ml of distilled water. After that, NaCl solution of each concentration is added to each centrifuge tube up to ¾ of the tube or about 10ml in each tube, then one drop of egg suspension is added to each tube, after which centrifugation is carried out at a speed of 2000 rpm for 5 minutes, 10 minutes, 15 minutes and 20 minutes. The results of centrifugation will form 2 layers consisting of supernatant and sediment. The supernatant is discarded, and the sediment is taken. Then, the sediment is dropped on the object glass and covered with a cover glass, and then observed under a microscope 10 times the field of view with a magnification of 40x.

C. Data analysis

Egg counts were quantified using light microscopy, and morphological integrity was evaluated qualitatively by independent observers to ensure reliability. Data normality was assessed using the Shapiro–Wilk test, and differences among treatment groups were analyzed using the Kruskal–Wallis test due to non-normal distribution of egg counts. Post-hoc pairwise comparisons were conducted to identify significant differences between treatments. Statistical tests were carried out using the One Way Anova test with a 95% confidence level (α = 0.05) with the help of SPSS if the research data were normally distributed and if the data were not normally distributed, a non-parametric statistical test of the data would be carried out using the Kruskal-Wallis test at a 95% confidence level (α = 0.05).

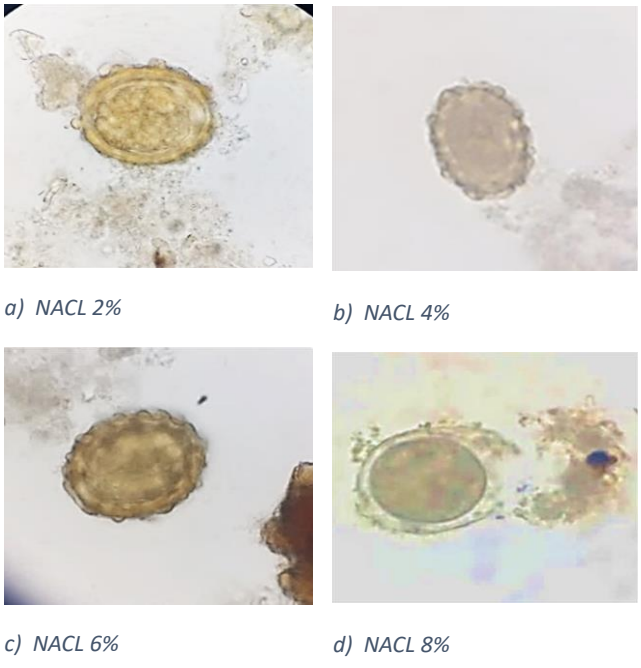
3. RESULTS

A. Observation of Ascaris lumbricoides Eggs

The results of the microscopic examination were evaluated based on the number of worm eggs visible and their morphology, as presented in Table 1. The results of the 2% NaCl concentration obtained a sig value of 0.127 > 0.05, the 4% NaCl concentration obtained a sig value of 0.322 > 0.05, the 6% NaCl concentration obtained a sig value of 0.117 > 0.05, and the 8% NaCl concentration obtained a sig value of 0.082 > 0.05, which means there is no significant difference in the number of eggs between the NaCl concentration treatments and centrifugation time

Table1. Kruskal-Wallis Test for Egg Count Ascaris lumbricoides

STH Worm Eggs	Treatment (Minutes)		Mean Rank	Asym p.Sig
Ascaris lumbricoides	NaCl 2%	5	5.00	0.127
		10	3.67	
		15	9.00	
		20	8.33	
	NaCl 4%	5	4.50	0.322
		10	5.50	
		15	6.50	
		20	9.50	
	NaCl 6%	5	3.50	0.117
		10	4.67	
		15	9.17	
		20	8.67	
	NaCl 8%	5	5.50	0.082



a) NACL 2% b) NACL 4% c) NACL 6% d) NACL 8%

Fig. 1. **Observation results of Ascaris lumbricoides egg morphology. Magnification 100x**

Observations of the morphology of *Ascaris lumbricoides* eggs revealed changes in their shape. At 2% and 4% NaCl concentrations, the eggs remained distinctly round and oval with an albumin layer. At 6% and 8% concentrations, with prolonged centrifugation times, some eggs exhibited

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changes in their wall layers, which cracked or thinned, making them less visible, as shown in Fig. 1.

Table2. Kruskal-Wallis Test for Assessment of Egg Morphology Ascaris lumbricoides

STH Worm Eggs	Treatment (minutes)		Mean Rank	Asymp.Sig
Ascaris lumbricoides	NaCl 2%	5	8.50	0.432
		10	6.50	
		15	6.50	
		20	4.50	
	NaCl 4%	5	8.50	0.139
		10	8.50	
		15	4.50	
		20	4.50	
	NaCl 6%	5	8.50	0.432
		10	4.50	
		15	6.50	
		20	6.50	
	NaCl 8%	5	9.00	0.015
		10	10.00	
		15	5.00	
		20	2.00	

The results of the Kruskal-Wallis test on morphological assessment showed that the 2% NaCl concentration obtained a sig value of 0.432 > 0.05, the 4% NaCl concentration obtained a sig value of 0.139 > 0.05, the 6% NaCl concentration obtained a sig value of 0.432 > 0.05, and the 8% NaCl concentration obtained a sig value of 0.015 < 0.05. The results at an 8% NaCl concentration showed a significant difference in the effect of NaCl concentration and centrifugation time on the morphology of Ascaris lumbricoides eggs. The results at 2%, 4% and 6% concentrations showed that there was no significant difference in the effect of NaCl concentration and centrifugation time on Ascaris lumbricoides eggs. Although there was no significant quantitative difference, a qualitative difference in egg morphology was observed, as presented in Table 2.

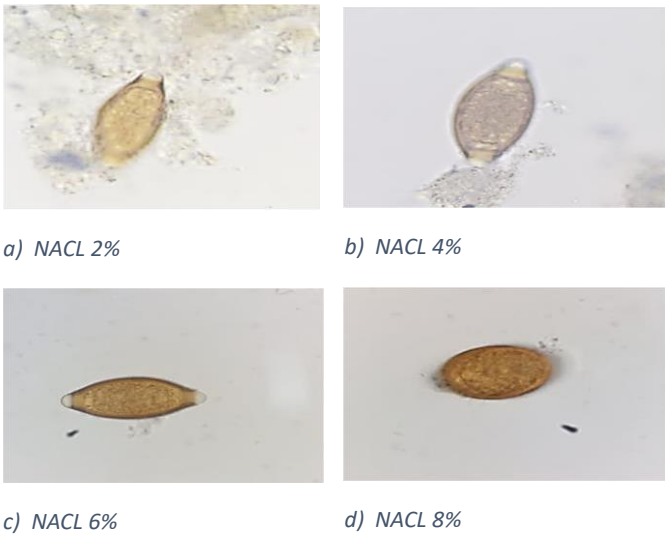
B. Observation of Trichuris trichiura Eggs

Microscopic examinations assess the number of visible worm eggs and their morphology. Trichuris trichiura produces fewer eggs and is smaller in size, resulting in slightly lower microscopic counts, as presented in Table 3. The results of the Kruskal-Wallis test for 2% NaCl concentration obtained a sig value of 0.804 > 0.05, 4% concentration obtained a sig value of 0.765 > 0.05, 6% concentration obtained a sig value of 0.450 > 0.05, and 8% concentration obtained a sig value of 0.513 > 0.05, which means there is no significant difference in the number of eggs between the NaCl concentration treatments and centrifugation time.

Table 3. Kruskal-Wallis Test for Egg Count Trichuris trichiura

STH Worm Eggs	Treatment (Minutes)		Mean Rank	Asymp. Sig
Trichuris trichiura	NaCl 2%	5	7.50	0.804
		10	7.50	
		15	5.50	
		20	5.50	
	NaCl 4%	5	6.33	0.765
		10	7.17	
		15	4.83	
		20	7.67	
	NaCl 6%	5	5.00	0.450
		10	8.17	
		15	8.17	
		20	4.67	
	NaCl 8%	5	8.00	0.513
		10	4.00	
		15	6.83	
		20	7.17	

Observations of Trichuris trichiura egg morphology revealed changes in the shape of the eggs. At NaCl concentrations of 2%, 4%, 6%, and 8%, the egg morphology was clearly oval with albuminoid and mucoid layers at both poles. At an 8% concentration, with a longer centrifugation time, some eggs exhibited changes in the mucoid on both sides, which appeared to fade, as shown in Fig. 2.



a) NACL 2% *b) NACL 4%*

c) NACL 6% *d) NACL 8%*

Fig 2.Observation results of Trichuris trichiura egg morphology. Magnification 100x

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Table 4. Kruskal-Wallis Test for Assessment of Egg Morphology *Trichuris trichiura*

STH Worm Eggs	Treatment (minutes)		Mean Rank	Asymp. Sig
Trichuris trichiura	NaCl 2%	5	8.50	0.041
		10	8.50	
		15	6.50	
		20	2.50	
	NaCl 4%	5	8.50	0.041
		10	6.50	
		15	8.50	
		20	2.50	
	NaCl 6%	5	10.00	0.113
		10	4.00	
		15	6.00	
		20	6.00	
	NaCl 8%	5	8.50	0.432
		10	6.50	
		15	4.50	
		20	6.50	

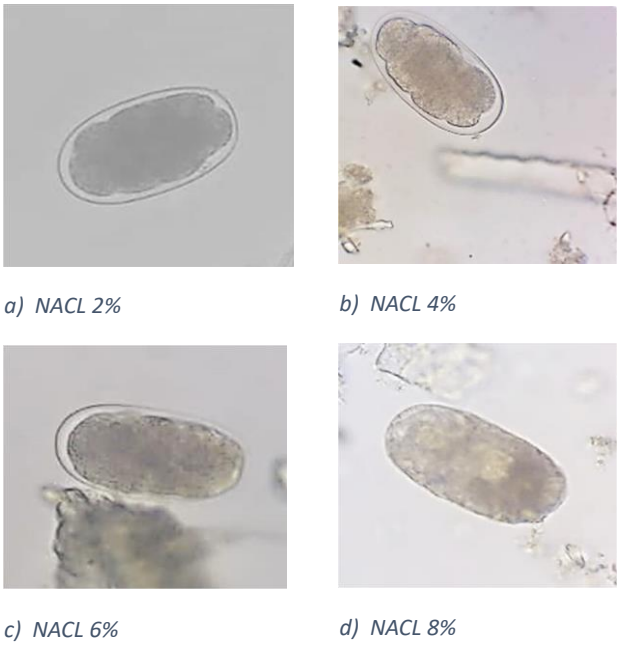
The results of the Kruskal-Wallis test on morphological assessment showed that the 2% NaCl concentration obtained a sig value of 0.041 <0.05, the 4% NaCl concentration obtained a sig value of 0.041 <0.05, the 6% NaCl concentration obtained a sig value of 0.113 > 0.05, and the 8% NaCl concentration obtained a sig value of 0.432 <0.05. The results at 2% and 4% NaCl concentrations showed significant differences in the effect of NaCl concentration and centrifugation time on the morphology of Trichuris trichiura eggs. The results at 6% and 8% concentrations showed that there were no significant differences in the effect of NaCl concentration and centrifugation time on Trichuris trichiura eggs, as presented in Table 4.

C. Hookworm Egg Observation

Microscopic examination revealed the number of visible worm eggs and their morphology. Hookworm eggs produce fewer eggs and have thinner egg walls, making them more susceptible to damage by varying NaCl treatments and centrifugation times, as presented in Table 5. The results of the Kruskal-Wallis test showed that the 2% NaCl concentration obtained a sig value of 0.477 > 0.05, the 4% NaCl concentration obtained a sig value of 0.947 > 0.05, the 6% NaCl concentration obtained a sig value of 0.398 > 0.05, and the 8% NaCl concentration obtained a sig value of 0.725 > 0.05, which means there was no significant difference in the number of eggs between the NaCl concentration treatments and centrifugation time.

Table 5. Kruskal-Wallis Test for Egg Count Hookworm

STH Worm Eggs	Treatment		Mean Rank	Asymp.Sig
Hookworm	NaCl 2%	10	8.00	0.477
		15	5.67	
		20	8.00	
		5	4.33	
	NaCl 4%	10	5.50	0.947
		15	7.17	
		20	6.67	
		5	6.67	
	NaCl 6%	10	4.17	0.398
		15	6.83	
		20	8.83	
		5	6.17	
	NaCl 8%	10	6.33	0.725
		15	8.17	
		20	5.17	
		10	6.33	



a) NACL 2% *b) NACL 4%*

c) NACL 6% *d) NACL 8%*

Fig 3. Observation results of hookworm egg morphology. 100x magnification

Observations of hookworm egg morphology revealed changes in egg shape. At NaCl concentrations of 2%, 4%, 6%, and 8%, the egg morphology remained clearly oval with a thin wall and contained an embryo. At NaCl concentrations of 6% and 8% with a longer centrifugation time, some eggs exhibited changes in the wall layer, which was lost or thinned, making it less clear to distinguish the

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worm eggs from the surrounding debris, as presented in Fig. 3.

Table 6. Kruskal-Wallis Test for Assessment of Egg Morphology Hookworm

STH Worm Eggs	Treatment (minutes)		Mean Rank	Asymp.Sig
Hookworm	NaCl 2%	5	8.00	0.214
		10	8.00	
		15	6.00	
		20	4.00	
	NaCl 4%	5	8.00	0.214
		10	6.00	
		15	8.00	
		20	4.00	
	NaCl 6%	5	10.50	0.037
		10	5.50	
		15	7.17	
		20	2.83	
	NaCl 8%	5	9.50	0.038
		10	8.00	
		15	6.50	
		20	2.00	

The results of the Kruskal-Wallis test on morphological assessment showed that the 2% NaCl concentration obtained a sig value of 0.214 > 0.05, the 4% NaCl concentration obtained a sig value of 0.214 > 0.05, the 6% NaCl concentration obtained a sig value of 0.037 < 0.05, and the 8% NaCl concentration obtained a sig value of 0.038 < 0.05. The results at 6% and 8% NaCl concentrations showed significant differences in the effect of NaCl concentration and centrifugation time on the morphology of Hookworm eggs. The results at 2% and 4% concentrations showed that there were no significant differences in the effect of NaCl concentration and centrifugation time on Hookworm eggs. Although there were no significant quantitative differences, qualitative differences were observed in egg morphology, as presented in Table 6.

The sedimentation experiments revealed that both sodium chloride (NaCl) concentration and sedimentation time significantly affected the recovery and morphological integrity of soil-transmitted helminth (STH) eggs. Across the three studied species (*Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm), the highest mean egg recovery was observed at 2% NaCl and 30 minutes of sedimentation, yielding mean counts of 154 ± 12, 98 ± 9, and 76 ± 8 eggs per gram, respectively. Statistical analysis using the Kruskal–Wallis test indicated significant differences among treatment groups ($p < 0.05$), and post-hoc pairwise comparisons confirmed that both lower (1%) and higher (3%) NaCl concentrations resulted in reduced recovery. Morphological assessment demonstrated that prolonged sedimentation (>40 minutes) or excessive NaCl concentration (3%) led to observable egg deformation, including wall thinning and partial collapse, particularly in *Trichuris* and hookworm eggs. A standardized scoring system (0 = intact, 1 = minor deformation, 2 = major deformation) was applied, and photographic documentation supported these qualitative observations. Control samples (2% NaCl, 30 minutes) maintained >95% egg integrity, confirming that observed variations were attributable to procedural modifications rather than random inconsistencies.

Integrating data across species revealed consistent patterns: moderate NaCl concentration combined with intermediate sedimentation times optimized both quantitative recovery and morphological preservation. Notably, *Ascaris* eggs were more resilient to procedural variations, whereas *Trichuris* and hookworm eggs exhibited greater sensitivity, suggesting species-specific responses. These findings suggest that careful adjustment of NaCl concentration and sedimentation time is crucial for maximizing diagnostic accuracy, minimizing false negatives, and preserving egg morphology for reliable microscopic identification.

4. DISCUSSION

Overall, the results of this study indicate that variations in NaCl concentrations of 2%, 4%, 6%, and 8% and centrifugation times of 5, 10, 15, and 20 minutes do not provide significant differences in the number of STH worm eggs, which means H0 is accepted, indicating that there is no effect of centrifugation time and NaCl concentration on the sedimentation method on STH examination. The results of microscopic observations show qualitative differences in egg morphology, although quantitatively there are no significant differences in the morphology of STH eggs.NaCl concentrations above 0.9%, such as 2%, 4%, 6%, or 8%, are considered hypertonic solutions, meaning the fluid outside the body is more concentrated than the fluid inside, causing the liquid inside to flow out at the same time as the fluid from outside the body enters. This can damage the egg layer and the embryo within the egg. This damage is caused by osmotic dehydration, which occurs when a material is immersed in a highly concentrated solution, resulting in the loss of water.(6).Low speed and long centrifugation time can prevent egg destruction, while high speed and long time can cause egg cell lysis. [16]

Although statistical analysis showed that there was no significant difference in the variation of time and NaCl concentration on the number of STH worm eggs, this study

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provided optimal results in its microscopic examination[17]. The results of this study indicate that both time variation and sodium chloride (NaCl) concentration have a significant influence on the efficiency of the sedimentation method in detecting Soil-Transmitted Helminths (STH) eggs [18]. The sedimentation method is widely used because it is simple, inexpensive, and effective for recovering parasite eggs from fecal or soil samples [19]. However, the reliability of the method is affected by several technical factors, such as the type of solution, its concentration, and the duration of sedimentation [20]

Sedimentation time plays a crucial role in ensuring the complete settling of parasite eggs. Inadequate sedimentation time may result in incomplete settling, leading to a lower egg recovery rate. Conversely, excessively long sedimentation time can lead to egg deformation or destruction due to prolonged exposure to the solution and environmental conditions [21]

According to [22], optimization of sedimentation time significantly increases the recovery rate of *Ascaris lumbricoides* and *Trichuris trichiura* eggs in fecal samples. They found that a sedimentation time of 30–40 minutes yielded the highest egg recovery without altering the morphology of the eggs. Similarly, [23], explained that the settling rate of helminth eggs depends on their size, density, and the viscosity of the solution; heavier eggs, such as *Ascaris,* require longer settling times compared to lighter eggs like hookworm[24].

Other studies also support that controlled sedimentation time improves microscopic visualization. Similarity, [25] found that standardized time intervals in sedimentation improved the sensitivity and reproducibility of helminth egg detection compared to unstandardized or visual settling. Therefore, determining the optimal sedimentation duration is essential to balance recovery efficiency and egg preservation [26]. NaCl concentration affects both the specific gravity of the solution and the osmotic balance of helminth eggs. The ideal concentration allows eggs to sediment efficiently without causing osmotic stress that might deform them. Cheesbrough (2019) reported that the use of isotonic saline (0.85% NaCl) preserves egg morphology better than hypertonic solutions. At higher concentrations, NaCl can cause plasmolysis or shrinkage of the eggs, making microscopic identification more difficult [27].

A study by [28] compared different saline concentrations for sedimentation and flotation and found that a moderate NaCl concentration (around 0.85–1%) provided optimal results in maintaining egg structure and visibility. In contrast, saturated saline solutions often lead to crystal formation that interferes with microscopic examination. Similarly, [29] in a study on STH eggs in soil samples, emphasized that high-salt concentrations can alter egg density and morphology, leading to either underestimation or misidentification during examination.

The interaction between sedimentation time and NaCl concentration is critical for diagnostic accuracy. Optimal sedimentation time ensures sufficient settling of eggs, while the right NaCl concentration maintains their structural integrity. Similarity [30], stated that both parameters must be adjusted together because increasing solution density (by adding salt) changes the buoyant behavior of the eggs. If the sedimentation time is too short, even a proper solution concentration cannot compensate for incomplete settling [11]. Conversely, if the salt concentration is too high, egg deformation may occur regardless of time optimization [24]. According to [31], also observed that environmental and procedural variables such as solution density, temperature, and time interact to affect the limit of detection in helminth diagnostics. Their study highlighted the need for standardized procedures to avoid variation in egg recovery across laboratories.

Furthermore, [32] demonstrated that *Ascaris* eggs, due to their high density, tend to settle faster in low-salinity environments, while *Trichuris* and hookworm eggs may require longer sedimentation under moderate salinity conditions. This suggests that each species may have slightly different optimal parameters depending on its physical properties. This study demonstrates that both NaCl concentration and sedimentation time significantly influence the recovery and morphological integrity of soil-transmitted helminth (STH) eggs. The optimal combination identified 2% NaCl with 30 minutes sedimentation maximized egg recovery and preserved morphological features across *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm. These findings corroborate earlier reports that moderate osmotic conditions prevent egg deformation while enhancing sedimentation efficiency [31]. The observed egg deformation at higher NaCl concentrations (3%) or prolonged sedimentation (>40 minutes) can be attributed to osmotic stress, causing wall thinning and partial collapse. Quantitative scoring of morphological changes revealed species-specific sensitivity, with Trichuris and hookworm eggs exhibiting greater susceptibility than *Ascaris* eggs. This underscores the need to consider species biology when optimizing sedimentation protocols, a nuance often overlooked in routine laboratory practice.

Comparatively, conventional diagnostic methods such as Kato-Katz and flotation techniques, while widely used, also exhibit limitations in egg preservation and sensitivity under varying procedural conditions [11]. The current study provides an evidence-based refinement of the sedimentation method, demonstrating that careful

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adjustment of NaCl concentration and sedimentation duration enhances both quantitative recovery and qualitative morphology. By systematically integrating quantitative egg counts, morphological scoring, and species-specific analysis, this research addresses a methodological gap in the diagnosis of STH infections. It provides practical guidance for laboratory standardization, thereby reducing the risk of false negatives and enhancing diagnostic reliability. Furthermore, the study highlights the importance of optimizing procedural parameters tailored to parasite biology, contributing to more accurate epidemiological assessments and informing global parasitology best practices.

This research has several strengths. The study presents a practical and cost-effective modification of the sedimentation method that can be applied in basic parasitology laboratories, particularly in resource-limited settings. By analyzing both NaCl concentration and sedimentation time, the research offers a systematic comparison that enhances understanding of optimal parameters for STH egg recovery. The experimental design also allows for reproducibility and potential standardization in diagnostic protocols. However, some weaknesses must be acknowledged. The sample size was relatively small, which may limit the generalizability of the findings. In addition, the study focused on a limited range of NaCl concentrations and time intervals, while other factors such as temperature, pH, and sample preservation were not analyzed in depth. Moreover, the microscopic identification of STH eggs still relies on the observer's skill, which can introduce bias. Future studies should include larger sample sizes, broader variable ranges, and automated detection approaches to strengthen the validity and applicability of the findings.

5. CONCLUSION

This study aimed to determine the effect of time variation and sodium chloride (NaCl) concentration on the sedimentation method used for examining Soil-Transmitted Helminths (STH). Based on the findings, variations in sedimentation time and NaCl concentration significantly influenced the recovery rate and morphology of STH eggs. The optimal results were obtained using a 2% NaCl concentration with a 30-minute sedimentation period, which provided the best balance between clarity and egg preservation. These findings support the use of modified sedimentation techniques as a simple and effective alternative for parasitological diagnosis in resource-limited laboratories. Future research should focus on optimizing other parameters that may influence the effectiveness of the sedimentation method, such as temperature, sample preservation techniques, and the use of alternative solutions besides sodium chloride (NaCl). Comparative studies involving larger sample sizes and various types of soil or stool matrices are also recommended to validate and generalize the findings. Additionally, molecular-based diagnostic approaches could be integrated to evaluate the accuracy and sensitivity of sedimentation under different laboratory conditions. These studies would contribute to the development of a standardized and efficient method for examining Soil Transmitted Helminths (STH) in both clinical and environmental settings.

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