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Comparison Of Sensitivity And Specificity Between Sheather's Sugar Flotation Method And Saturated NACL Flotation Method For Detecting Helminth Eggs in Fecal Samples

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ABSTRACT

Soil-Transmitted Helminth (STH) infections remain a common public health problem, so diagnostic methods with high sensitivity and accuracy are needed to detect helminth eggs in fecal samples. This study aimed to compare the sensitivity and specificity of Sheather's sugar solution and saturated NaCl solution in detecting helminth eggs. Using an observational design, 30 fecal samples were examined by both flotation methods, and the results were compared to determine sensitivity and specificity. The results showed that Sheather's sugar solution had 100% sensitivity and 100% specificity, with 10 positive samples and 20 negative samples detected. Saturated NaCl solution showed 80% sensitivity and 100% specificity, with 8 positive samples, 20 negative samples, and 2 false-negative results. These findings indicate that Sheather's sugar method is superior to saturated NaCl in detecting helminth eggs. Sheather's sugar solution is considered more effective because it produces clearer preparations, separates eggs from debris, and preserves egg morphology, making microscopic identification easier. In contrast, saturated NaCl solution may produce a less clear field of view due to crystal formation, which can reduce observation quality and diagnostic sensitivity. Therefore, Sheather's sugar solution is recommended as a more sensitive alternative for diagnosing helminth infections in fecal samples.

INTRODUCTION

Soil-Transmitted Helminth (STH) infections remain a major public health problem, particularly in tropical and subtropical regions where sanitation, hygiene, and access to clean water are still suboptimal. The main helminths involved are Ascaris lumbricoides, Trichuris trichiura, and the hookworms Ancylostoma duodenale and Necator americanus. Transmission occurs when eggs or larvae contaminating soil, food, or water are ingested or penetrate the human body. STH infections may be asymptomatic, but high-intensity infections can lead to gastrointestinal disturbances, anemia, malnutrition, and impaired growth and development, especially in children (Word Health Organization, 2023; Chen et al., 2024). Therefore, accurate laboratory methods capable of detecting helminth eggs are essential to support diagnosis and control of helminthiasis.

Microscopic examination of stool is one of the most widely used methods for diagnosing STH infections because it is relatively simple, easy to implement, and

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does not require complex equipment. Several techniques can be employed, including direct smear, sedimentation, and flotation. The flotation method is based on the difference in specific gravity between helminth eggs and the flotation solution. When the specific gravity of the solution is higher than that of the eggs, the eggs float to the surface and can be collected using a coverslip for microscopic observation (Muhrija et al., 2021; Anggraini et al., 2020). The success of this method is influenced by the type of solution, its specific gravity, flotation time, sample homogenization, and the quality of the resulting preparation. Studies indicate that helminth eggs generally have a specific gravity ranging from 1.05 to 1.23; therefore, effective flotation solutions typically have a specific gravity between 1.20 and 1.30 (Boava et al., 2025).

Saturated sodium chloride (NaCl) solution is one of the most commonly used flotation solutions because it is easy to prepare, inexpensive, and widely available in laboratories. Saturated NaCl has a specific gravity of approximately 1.20 and is frequently used as a standard in many laboratories (Astuti, 2018; Ghofur et al., 2025). However, this solution may produce crystals or less clear preparations if evaporation occurs during the examination. Such conditions can interfere with microscopic observation and lead to failure in identifying helminth eggs. In addition to saturated NaCl, Sheather's sugar solution, which is based on sucrose, is also used as a flotation medium. Sheather's sugar solution has a specific gravity of approximately 1.27, enabling it to float various nematode eggs (Shusterman et al., 2021). The relatively stable nature of this solution also helps preserve egg morphology and produces a clearer field of view.

Previous studies have evaluated the use of various flotation solutions, including Sheather's sugar, sucrose, magnesium sulfate, zinc sulfate, and NaCl. Shusterman et al., (2021) reported that Sheather's sugar and sucrose solutions yielded better detection of Trichuris eggs compared with several other solutions and did not cause significant morphological distortion of the eggs. A study by Khadka et al., (2020) in Nepal also demonstrated the effectiveness of Sheather's sugar flotation in detecting helminth eggs. Ghofur et al., (2025) compared saturated NaCl, $ZnSO_4$, and $MgSO_4$ and found that, descriptively, $ZnSO_4$ with a 40-minute incubation time detected the highest number of eggs, although the difference was not statistically significant. These findings indicate that the effectiveness of the flotation method strongly depends on the characteristics of the solution and the examination procedure used.

However, studies specifically comparing the diagnostic performance of Sheather's sugar solution and saturated NaCl for detecting helminth eggs in human stool samples remain limited, particularly those assessing the sensitivity and specificity of both methods simultaneously. Most previous studies have only compared the number of eggs detected, the type of solution used, or the duration of flotation (Ghofur et al., 2025; Shusterman et al., 2021) Other studies were conducted on animal samples, so their results cannot be directly applied to describe the performance of both methods in human stool. Thus, there is a research gap in the lack of direct comparison of sensitivity and specificity between Sheather's sugar flotation and saturated NaCl flotation for detecting STH eggs in human fecal samples.

This study is important because stool examination results for helminth eggs can influence diagnostic accuracy and subsequent management decisions for helminth

infections. The uniqueness of this research lies in the direct comparison of two flotation methods Sheather's sugar solution and saturated NaCl by evaluating the sensitivity and specificity of each method using microscopic examination as the reference. This study is expected to provide information on the more effective method for detecting helminth eggs in stool samples and to serve as a consideration for laboratories in selecting a flotation solution that is sensitive, specific, simple, and suitable for routine examination.

MATERIALS/METHOD

A quantitative analysis was used as the research methodology, with an observational design and a cross-sectional perspective. The study population consisted of stool samples from individuals suspected of Soil-Transmitted Helminth (STH) infection at the Parasitology Laboratory, Politeknik Kesehatan Kemenkes Surabaya, who met the inclusion criteria between October 2025 and May 2026. A total of 30 samples were collected using a consecutive sampling technique. Inclusion criteria were individuals suspected of STH infection based on complete blood count results (elevated eosinophils and decreased hemoglobin) or clinical symptoms (pallor, weakness, fever, and allergic manifestations). Exclusion criteria included individuals not suspected of STH infection, samples with inadequate volume or quality (urine contamination or insufficient quantity). The research was conducted at the Parasitology Laboratory, Department of Medical Laboratory Technology, Politeknik Kesehatan Kemenkes Surabaya, between October 2025 and May 2026. Ethical approval was obtained from the Research Ethics Committee (No.EA/4100/KEPK-Poltekkes_Sby/V/2026). Primary data collection methods were employed in this study. Stool samples were preserved in 10% formalin and examined using two flotation methods: Sheather's sugar solution (specific gravity 1.27) and saturated NaCl solution (specific gravity 1.20). Each sample was examined three times on consecutive days for both methods. For each method, 5 g of stool was mixed with flotation solution in a reaction tube until nearly full, a coverslip was placed on top and left for 45 minutes, then transferred to a glass slide and examined under microscope at 10× and 40× magnifications. Data were sorted and collected using an observation sheet. The data on the observation sheet was then transferred into a master table format. The data obtained were presented in tabular form. Sensitivity and specificity were calculated using 2×2 contingency tables comparing both flotation methods against direct microscopy as the reference standard.

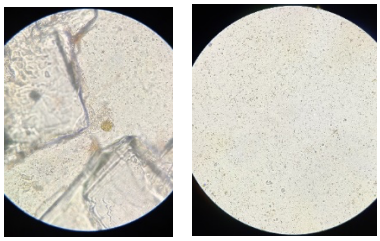
RESULTS AND DISCUSSION

Thirty stool samples were examined using both Sheather's sugar flotation and saturated NaCl flotation methods. Direct microscopy identified 10 positive and 20 negative samples for STH eggs. Sheather's sugar method detected all 10 positive samples correctly with no false negatives, yielding a sensitivity of 100% and specificity of 100%. Saturated NaCl method detected 8 positive samples but missed 2 samples (false negatives), resulting in a sensitivity of 80% and specificity of 100% (Table 1).

Table 1 Diagnostic performance of both flotation methods (n=30)

Method	True Positive	False Negative	True Negative	False Positive	Sensitivity	Specificity
Sheather's sugar	10	0	20	0	100%	100%
Saturated NaCl	8	2	20	0	80%	100%

Microscopic field quality differed between methods. Sheather's sugar preparations showed clearer fields with minimal debris and no crystal formation. Saturated NaCl preparations exhibited crystal formation and more debris, potentially interfering with egg identification.



(a) (b)

Figure 1 (a) saturated NaCl field of view, (b) sheather's sugar field of view

This study directly addresses the research objective of comparing diagnostic performance between Sheather's sugar and saturated NaCl flotation methods for STH egg detection. The superior sensitivity of Sheather's sugar (100% vs 80%) demonstrates its enhanced capability to detect true positive cases, which is critical for accurate diagnosis and treatment decisions. The 20% sensitivity gap between methods can be explained by several factors. Sheather's sugar solution (specific gravity 1.27) provides better separation of eggs from fecal debris compared to saturated NaCl (specific gravity 1.20). The higher specific gravity allows more efficient flotation of eggs within the 1.05-1.23 range typical of STH eggs. Additionally, the non-ionic nature of sucrose-based solutions preserves egg morphology without causing plasmolysis or distortion, facilitating easier identification.

The crystal formation observed in NaCl preparations represents a significant practical limitation. As the solution evaporates during the 45-minute flotation period, salt crystals form and obscure the microscopic field. This phenomenon likely contributed to the two false-negative results, where eggs may have been present but not visualized due to poor field clarity. In contrast, Sheather's sugar solution remains stable without crystallization, maintaining clear visualization throughout the examination period. Both methods achieved 100% specificity, indicating that neither method produces false-positive results. This finding is clinically important, as it confirms that positive results from either method can be trusted for diagnosis. However, the higher sensitivity of Sheather's sugar makes it the preferred choice, particularly in cases with low egg counts where missing a diagnosis could delay treatment.

These findings align with previous reports on sugar-based flotation solutions. Studies have documented superior egg recovery with sucrose solutions

compared to salt-based solutions, attributed to better preservation of egg integrity and reduced interference from fecal debris. The practical advantage of Sheather's sugar extends beyond diagnostic accuracy—its ability to produce cleaner preparations reduces examination time and technician fatigue, potentially improving laboratory workflow efficiency. The main limitation of this study is the relatively small sample size (n=30), which may affect the precision of sensitivity and specificity estimates. Additionally, all samples were preserved in formalin, which may not reflect performance with fresh specimens. Future studies with larger sample sizes and varied specimen types would strengthen these findings.

CONCLUSIONS

Sheather's sugar flotation method demonstrates superior diagnostic performance compared to saturated NaCl for STH egg detection in stool samples. With 100% sensitivity versus 80% for NaCl, Sheather's sugar should be considered as an alternative flotation solution, particularly in settings where accurate detection of low-intensity infections is critical.

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