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Association Between Soil Transmitted Helminth Infections, Hemoglobin Levels, and Eosinophil Counts in Elementary School Children

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ABSTRACT Soil-Transmitted Helminths (STH) remain a major public health concern among school-aged children, particularly in regions with limited sanitation and inadequate hygiene practices, where chronic parasitic exposure may impair hematological health. This study investigates the extent to which STH infection is associated with reduced hemoglobin levels and elevated eosinophil counts in elementary school children, focusing on the potential hematological and immunological consequences of infection. A cross-sectional analytical design was applied to 44 participants selected by consecutive sampling. Fecal samples were examined using the 33% zinc sulfate ($ZnSO_4$) flotation method to detect helminth eggs or larvae, while venous blood samples from infected participants were analyzed with an automated hematology analyzer to obtain hemoglobin concentration and eosinophil percentages. Normality testing was conducted using the Shapiro–Wilk test, and associations between variables were assessed using Spearman's rho correlation. The findings indicate that 10 of the 44 children (22.7%) were positive for STH infection. Among these infected subjects, 70% exhibited hemoglobin levels below the normal threshold, while 60% showed eosinophil counts exceeding the reference range. Statistical analysis demonstrated a significant negative correlation between STH infection and hemoglobin levels ($p = 0.040$), and a significant positive correlation between STH infection and eosinophil counts ($p = 0.037$). These results indicate that STH infections contribute to anemia and eosinophilia, suggesting measurable disruption of hematological function. The study concludes that implementing routine deworming, blood screening, and hygiene-centered health education within school-based programs is essential to reduce STH prevalence and safeguard children's physiological well-being and cognitive development in endemic areas.

INDEX TERMS Soil-Transmitted Helminths, Hemoglobin, Eosinophils, Anemia, School-Age Children

I. INTRODUCTION

Soil-transmitted helminth (STH) infections remain a major neglected tropical disease, causing substantial morbidity in low- and middle-income countries. Recent global estimates suggest that around 1.5 billion people are infected, with the highest burden occurring in tropical and subtropical regions where access to safe water, sanitation, and hygiene is limited [1]–[4]. School-age children are disproportionately affected due to frequent contact with contaminated soil, immature immune systems, and inadequate hygiene practices, which together increase the risk of chronic intestinal parasitism, growth impairment, and educational underperformance [5]–[7].

In Indonesia, STH infections are still frequently reported among schoolchildren and remain closely linked to poor environmental sanitation, slum housing, and limited health literacy [5], [8], [9]. Local data from Bojonegoro Regency indicate that basic sanitation coverage and clean and healthy living behavior (Perilaku Hidup Bersih dan Sehat, PHBS) have not yet been optimized, with a high proportion of households relying on semi-permanent latrines and reporting diarrhea as a common health problem, which may reflect ongoing helminth transmission.

A growing body of evidence shows that STH infections are strongly associated with anemia through chronic intestinal blood loss, impaired iron absorption, and systemic inflammation, leading to reduced hemoglobin levels and altered red blood cell indices [6], [10]–[13]. Meta-analyses and recent field studies in various endemic settings have consistently demonstrated that infected children have significantly lower hemoglobin levels and higher odds of anemia than their uninfected peers [6], [11], [12], [14]. At the same time, helminth infections elicit eosinophilia as part of the Th2-type immune response, and elevated eosinophil counts are frequently observed in children with ascariasis, trichuriasis, and hookworm disease [12], [15]–[17].

State-of-the-art diagnostic approaches for STH include stool microscopy using flotation or concentration techniques, such as zinc sulfate ($ZnSO_4$) centrifugal flotation, which remains a sensitive and widely applicable method for detecting ova and larvae in resource-limited laboratories [18]–[20]. Recent comparative studies confirm that 33% $ZnSO_4$

solution offers good performance for STH diagnosis and often yields more eggs than alternative flotation solutions [19]. In parallel, automated hematology analyzers provide rapid and reliable measurements of hemoglobin and differential leukocyte counts, including eosinophils, which are crucial for assessing the hematological impact of parasitic infections [11], [21].

Nevertheless, research gaps remain at the local level. Many studies in Indonesia have focused on prevalence or nutritional outcomes separately, without simultaneously quantifying correlations between STH infection status, hemoglobin concentration, and eosinophil count within specific school populations. Evidence from Bojonegoro Regency, where environmental risk factors and STH exposure are still prominent, is particularly limited. This restricts the ability of local health authorities to prioritize integrated interventions that combine deworming, nutritional support, and hematological monitoring.

Therefore, the present study aims to analyze the correlation between STH infection, hemoglobin levels, and eosinophil counts among elementary school children in Bojonegoro Regency. Building on current evidence and contextualizing it in a high-risk rural setting, this work offers several contributions:

1. it provides updated, school-based parasitological and hematological data from an endemic area;
2. it quantifies the strength and direction of the association between STH infection, anemia, and eosinophilia using robust non-parametric statistics; and
3. it generates actionable recommendations for integrating routine deworming, hygiene promotion, and hematological screening into school health programs aligned with current WHO guidance [2], [22], [23].

The remainder of this article is structured as follows. Section II details the study design, sampling strategy, laboratory procedures, and statistical methods. Section III presents the prevalence of STH infection and the distribution of hemoglobin and eosinophil values, as well as the correlation analysis. Section IV discusses these findings in relation to the existing literature on anemia, eosinophilia, and STH control. Section V concludes with key implications for public health practice and outlines priorities for future research in similar endemic settings.

II. METHODS

This study employed an observational analytic design with a cross-sectional approach to determine the correlation between Soil-Transmitted Helminth (STH) infection, hemoglobin concentration, and eosinophil levels among elementary school children. The cross-sectional design allowed all variables to be measured at a single time point without intervention or treatment, providing an efficient method to evaluate associations within a defined population [28].

A. STUDY LOCATION AND DURATION

The research was conducted at SDN Sobontoro 2, Bojonegoro Regency, East Java, Indonesia. Data collection was carried out over a two-month period from February to March 2025, coinciding with the academic semester to ensure stable student attendance. The parasitological and hematological analyses were performed in a certified clinical laboratory associated with the Department of Medical Laboratory Technology.

B. STUDY POPULATION AND SAMPLING METHOD

The target population consisted of all active students enrolled at SDN Sobontoro 2. The sampling technique employed was consecutive sampling, wherein eligible participants were recruited sequentially until the required sample size was achieved. The inclusion criteria were as follows:

1. Registered elementary school students aged approximately 7–12 years;
2. Obtained signed informed consent from parents or guardians;
3. Willingness to provide both stool and venous blood samples.

Exclusion criteria were:

1. Incomplete biological sample submission;
2. Refusal to participate at any stage of data acquisition;
3. Presence of diagnosed hematological disorders unrelated to STH infection.

A total of 44 children met the criteria and were included in the analysis. This sample size aligns with similar cross-sectional parasitological studies in resource-limited field conditions [29], [30].

C. ETHICAL CONSIDERATIONS

Ethical approval was granted by the institutional ethics committee. Guardians received full disclosure explaining the study objectives, procedures, potential risks, and confidentiality measures. Participation was strictly voluntary, and test results were communicated confidentially to parents for appropriate follow-up.

D. SPECIMEN COLLECTION PROCEDURES

1) Stool Collection for STH Identification

Participants were provided with sterile stool containers and instructed on proper morning-stall sample collection using clean technique to reduce contamination. Each specimen was labeled with coded identifiers instead of names to preserve anonymity.

2) Blood Collection for Hemoglobin and Eosinophil Analysis

Children testing positive for STH underwent venous blood sampling. Approximately 2–3 mL of blood was collected using a standard 22–24 gauge needle and stored in EDTA-coated tubes to prevent coagulation. The sampling adhered to pediatric phlebotomy standards to minimize procedural discomfort [31].

E. PARASITOLOGICAL EXAMINATION OF STOOL

The stool samples were examined using the 33% zinc sulfate ($ZnSO_4$) centrifugal flotation method. This method provides high diagnostic sensitivity for detecting eggs of Ascaris lumbricoides, Trichuris trichiura, and hookworm species [19], [28]:

1. Approximately one green-pea-sized amount of feces was mixed with 33% $ZnSO_4$ solution in a centrifuge tube to half volume;

2. The suspension was homogenized and covered;
3. Centrifuged at 2500 rpm for 5 minutes;
4. Additional $ZnSO_4$ solution was added to fill the meniscus;
5. A coverslip was placed for 10 minutes for ova adherence;
6. The coverslip was transferred to a glass slide and examined microscopically at 10× and 40× magnifications.

This method is widely recommended for field diagnosis due to its affordability and reproducibility [32].

F. HEMATOLOGICAL EXAMINATION

Blood samples were analyzed using an automated hematology analyzer capable of measuring hemoglobin concentration and differential leukocyte counts. Automated analyzers minimize operator variability and support efficient screening for anemia and eosinophilia [33]. Normal reference values for interpretation:

1. Hemoglobin: 11.0–16.0 g/dL for school-aged children;
2. Eosinophil percentage: 1–3%.

Values outside these ranges were categorized as abnormal hematological indices.

G. DATA MANAGEMENT AND STATISTICAL ANALYSIS

Data were entered into SPSS software for statistical processing. The Shapiro–Wilk test assessed normality due to the small sample size ($n < 50$). The results indicated that the data were non-normally distributed ($p < 0.05$). Therefore, Spearman's rho a non-parametric correlation test was used to analyze associations between:

1. STH infection status (positive/negative),
2. Hemoglobin level (continuous),
3. Eosinophil count (continuous).

Interpretation rules:

1. $p < 0.05$ indicated statistically significant correlation;
2. Correlation coefficient (r) values:
   a. 0.00–0.19: very weak;
   b. 0.20–0.39: weak;
   c. 0.40–0.69: moderate;
   d. 0.70–0.89: strong;
   e. 0.90–1.00: very strong [34].

Both the negative correlation of hemoglobin vs. STH and positive correlation of eosinophils vs. STH were considered biologically plausible findings consistent with helminth pathology [35].

III. RESULT

The results of the study were taken from primary data obtained by conducting an examination to identify worms, larvae, or eggs of Soil Transmitted Helminths (STH) in the feces of children at SDN Sobontoro 2, Bojonegoro Regency. The study was conducted in February-March 2025 with a total of 44 feces samples. Samples that tested positive for STH infection underwent venous blood tests to measure hemoglobin levels and the number of eosinophil cells.

Based on FIGURE 1, the results of stool examination of 44 elementary school children respondents using the 33% $ZnSO_4$ Flotation method, 10 respondents were found to be positive for Soil Transmitted Helminths infection and 34 respondents were negative for STH infection.

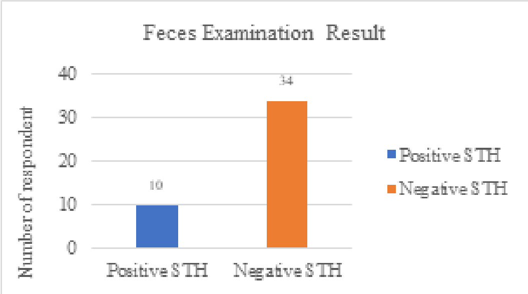

FIGURE 1. The Result of Feces Examination in Elementary School Children

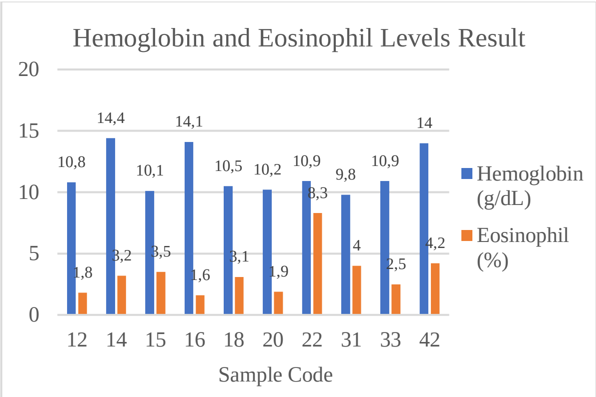

FIGURE 2. The Result of Examination Hemoglobin Levels and Eosinophil Counts

Based on PICTURE 2, the results of the examination of hemoglobin levels and eosinophil counts in 10 respondents who were positive for STH infection, the results of hemoglobin levels below normal were 7 respondents (70%) with an average hemoglobin level of 10.4 g / dL. The normal value of hemoglobin levels is 11.0-16.0 g / dL. While the number of eosinophil cells obtained results above normal, namely 6 respondents (60%) with an average number of eosinophils of 4.3%. The normal value of the number of eosinophil cells is 1-3% in the blood.

TABLE 1
The Result of Spearman's rho Correlation test statistical analysis

	Correlation Coefficient	N	Sig
STH with hemoglobin levels	-0,655	10	0,040
STH with eosinophil cell count	0,663	10	0,037

After the research data were collected, the Shapiro-Wilk test was performed to assess the normality of the variables. The test revealed that the data for STH infection, hemoglobin levels, and eosinophil counts were not normally distributed ($p < 0.05$). In this study, a nonparametric statistical approach was considered appropriate. Therefore, the Spearman rho correlation test was used to examine the relationship between STH infection and hemoglobin levels and eosinophil cell counts. The following is the Spearman's rho Correlation Test hypothesis:

a. H_0: If the Sig. (2-tailed) value is > 0.05, there is no relationship
b. H_1: If the Sig. (2-tailed) value is < 0.05, there is a relationship

Based on TABLE 1, the results of the Spearman's rho Correlation test statistical analysis of hemoglobin levels with STH, the Sig. (2-tailed) value is < 0.05, which is 0.040. While the results of the number of eosinophil cells with STH obtained the Sig. (2-tailed) value < 0.05, which is 0.037. Thus, it means that there is a relationship between hemoglobin levels and the number of eosinophil cells with Soil Transmitted Helminths (STH) infection. The correlation coefficient value obtained is 0.655, which means that the level of relationship strength is strong, and has a negative value at hemoglobin levels. This shows that the relationship is not unidirectional or inverse, the higher the STH infection, the lower the hemoglobin level. While the number of eosinophil cells has a positive value because the correlation coefficient is unidirectional, the higher the STH infection, the higher the number of eosinophil cells.

IV. DISCUSSION

A. INTERPRETATION OF RESULTS AND PHYSIOLOGICAL MECHANISMS

The present study found that 10 out of 44 students (22.7%) tested positive for intestinal helminth infections, and those infected displayed both reduced hemoglobin levels and elevated eosinophil counts. Among infected children, 70% showed hemoglobin levels below normal, while 60% exhibited elevated eosinophils. These findings strongly indicate that active STH infection is linked to detectable hematological disturbances, particularly anemia and eosinophilia.

The association between STH infection and decreased hemoglobin seen in this study reflects the well-established parasitic mechanisms of blood and iron loss. Hookworms (Ancylostoma duodenale and Necator americanus) actively consume host blood by attaching to the intestinal epithelium using chitinous mouthparts, causing chronic micro-hemorrhages. Meanwhile, Ascaris lumbricoides and Trichuris trichiura indirectly contribute to anemia by reducing nutrient absorption through mucosal inflammation and intestinal permeability. The inverse correlation between infection status and hemoglobin suggests a physiological burden exceeding normal hematopoietic compensation capacity, particularly in children whose iron reserves are often marginal [36], [37].

Conversely, increased eosinophil counts among infected children reflect an immunological response characteristic of helminth exposure. Eosinophils participate in type-2 immune responses, activated by interleukin-5 and IgE mechanisms, and are involved in antibody-dependent cell-mediated cytotoxicity (ADCC). Elevated eosinophils therefore serve not only as a diagnostic marker but as a direct indicator of continual immune engagement against parasitic antigens [38].

The findings from this research also demonstrate a strong negative correlation coefficient between STH and hemoglobin, and a positive one with eosinophils, supporting a coherent biological pathway: parasitic infection → mucosal and hematological disruption → altered blood indices. These effects are especially pronounced in school-age children who are physiologically vulnerable due to ongoing growth, and behaviorally at risk due to frequent soil exposure.

B. COMPARISON WITH OTHER SIMILAR STUDIES

The results align with other research in Indonesia and globally. A study by Muhlisin et al. (2024) reported that children infected with STH universally showed hemoglobin levels below normal and that infection prevalence correlated with anemia severity [4]. Similar findings were also presented in Ethiopia, where Gemechu et al. (2024) documented a statistically significant association between STH presence and anemia prevalence among schoolchildren [29]. Furthermore, Lubis et al. (2025) concluded that STH-positive children demonstrated depressed erythrocyte indices and depleted iron stores, reinforcing similar hematological consequences [12].

Regarding eosinophils, Pratama et al. (2023) found that 65% of infected Indonesian children exhibited eosinophilia, which closely parallels the 60% observed in this study, suggesting that eosinophil elevation is a consistent immunological marker across settings [14]. Bria et al. (2025) additionally found increased eosinophils in Ascaris-infected children, strengthening the association between parasite-induced tissue migration and immune cell activation [15].

However, there are also studies showing less pronounced hematologic shifts, particularly in low-intensity infections. A study by Nuryati and Astuti (2024) demonstrated that mild STH infections do not immediately depress hemoglobin but may instead induce subclinical iron deficiency without overt anemia [10]. This contrast suggests that hematological outcomes may vary depending on worm burden, nutritional status, and infection duration.

Environmental and behavioral risk factors identified in the field also reinforce prior observations. Barefoot activity, hand-to-mouth soil contact, and inconsistent handwashing remain leading routes of transmission [3], and these hygiene behaviors were also frequently observed among children in Bojonegoro as noted in field observation.

Therefore, this study's findings are broadly consistent with international and regional literature, reinforcing that STH infection reliably predicts hematological dysfunction in children. The unique contribution of this work is contextualizing these associations within the specific environmental and sociodemographic conditions of Bojonegoro, where sanitation weaknesses and semi-permanent latrine use continue to facilitate environmental contamination.

C. STUDY LIMITATIONS AND IMPLICATIONS OF FINDINGS

Despite its strengths, this study has inherent limitations that should be acknowledged when generalizing the findings.

1) Sample Size and Representativeness

The final sample consisted of 44 students from a single primary school. While this number aligns with similar single-site cross-sectional studies, a broader population across multiple schools would permit stronger inferential power and more representative conclusions [39].

2) Cross-sectional Design
The study's cross-sectional approach captures associations at a single time-point but cannot establish causality or temporality. While biologically plausible, it remains uncertain whether anemia developed as a result of infection or predated it due to malnutrition or other comorbidities [40].

3) Lack of Infection Intensity Quantification
The study identifies presence or absence of infection, but does not differentiate infection intensity (light, moderate, heavy) based on WHO egg-per-gram criteria. Infection intensity may directly influence blood-loss magnitude and thus anemia severity [41].

4) Nutritional and Dietary Variables Not Assessed
Hemoglobin levels are affected not only by helminth infection, but also by iron intake, protein consumption, and micronutrient status. Not controlling for diet-related factors may introduce cofounding variability [42].

Despite these limitations, the study has key implications. Implications for Clinical Monitoring and School Health SystemsThe findings support integrating stool testing and blood analysis into routine school-based health evaluations, especially in high-risk communities. Regular screenings could permit early identification of children at risk of anemia, enabling timely medical treatment and nutritional supplementation [43]. The significant correlation between STH and hematological changes underscores the importance of:

a. periodic deworming,
b. sanitation improvement,
c. hand hygiene education, and
d. footwear use campaigns.

WHO recommends regular anthelmintic administration for children in endemic regions, and this study reinforces the appropriateness of that guidance for Bojonegoro [22], [36]. Subsequent studies could:

1. Employ multi-site sampling
2. Incorporate quantitative parasitology (EPG counting)
3. Include serum ferritin and iron-binding capacity to separate infection-induced vs. diet-induced anemia
4. Track children longitudinally to determine causal progression
5. Examine co-infections (e.g., protozoa, malaria)
6. Understanding both parasitic and non-parasitic determinants of child anemia would refine disease management and policy interventions [44], [45].

IV. CONCLUSION

This study aimed to analyze the correlation between Soil-Transmitted Helminth (STH) infections, hemoglobin concentration, and eosinophil counts among elementary school children in Bojonegoro Regency. The findings of this research demonstrated that 10 of the 44 examined children (22.7%) were infected with STH, and among these infected participants, 70% exhibited hemoglobin levels below the normal reference range (mean value: 10.4 g/dL), while 60% presented with elevated eosinophil counts (mean value: 4.3%). Statistical analysis using Spearman's rho confirmed a significant negative correlation between STH infection and hemoglobin levels ($p = 0.040$) and a significant positive correlation between STH infection and eosinophil counts ($p = 0.037$), confirming that helminth infections are associated with hematological changes indicative of anemia and eosinophilia. These results reinforce existing evidence that parasitic infections can impair hematologic health and contribute to chronic developmental and cognitive risks in children residing in sanitation-limited environments. Looking forward, future studies should expand sample size across multiple schools, incorporate quantitative egg-counting methods to determine infection intensity, and include nutritional and socioeconomic variables to distinguish infection-related anemia from nutritionally induced anemia. Longitudinal designs would further enable tracking of hematological recovery following treatment interventions and hygiene improvements. The outcomes of this research highlight the importance of integrating parasitological screening, regular deworming programs, and hematological monitoring into school-based health initiatives, and underscore the urgency of improving hygiene education, sanitation infrastructure, and community health literacy to reduce infection transmission and support healthier child development trajectories.

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DATA AVAILABILITY

No datasets were generated or analyzed during the current study.

AUTHOR CONTRIBUTION

All authors contributed substantially to this work. Shella Nur Laily was responsible for conceptualizing the study, designing the methodology, conducting specimen analysis, and drafting the manuscript. Retno Sasongkowati provided supervision, ensured procedural accuracy, and contributed to data interpretation and critical review of the text. Evy Diah Woelansari performed statistical analysis, verified results, and supported relevant literature integration and manuscript refinement. Museyaroh facilitated administrative processes, coordinated ethical compliance, and managed field logistics and participant engagement. All authors reviewed and approved the final manuscript and are accountable for the integrity of the work.

DECLARATIONS

ETHICAL APPROVAL

This study was approved by the Research Ethics Committee of Poltekkes Kemenkes Surabaya with approval code. All procedures involving children were conducted in accordance with recognized ethical standards for human subject research.

CONSENT FOR PUBLICATION PARTICIPANTS.

Written informed consent was obtained from all parents or legal guardians of the participating children prior to their involvement in this study. Participants were free to withdraw at any time without consequence.

COMPETING INTERESTS

The authors declare that they have no known financial or personal conflicts of interest that could have influenced the findings or interpretations presented in this article.

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