PREDICTION OF HEMOGLOBIN LEVELS AND EOSINOPHIL COUNTS DUE TO SOIL TRANSMITTED HELMINTHS (STH) INFECTION IN ELEMENTARY SCHOOL STUDENTS IN THE RIVER BANKS AREA, SOUTH KALIMANTAN, INDONESIA

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ABSTRACT

Introduction: Soil-transmitted helminths (STH) infections in School-Age Children still exist as a public health problem in the River Banks Area. Elementary school students are one of the vulnerable populations to STH infection. This research purposed to determine the prediction of hemoglobin levels and eosinophil counts due to STH infection in elementary school students in the River Banks Area, South Kalimantan, Indonesia.

Methods: One hundred thirty-three kato katz stool preparation of three elementary school students in the river banks area, 68 were male and 65 were female, aged between 7 and 12 years old, were conducted for egg per gram (EPG) STHs eggs count Kato Katz method microscopic examination, and hematology analyzer blood examination to determine hemoglobin levels and eosinophil counts.

Result: Linear regression analysis revealed that EPG of STHs was associated with a hemoglobin level of 13.937 with a 0.031 fold decrease in risk and with an eosinophil count of 140.204 with a 4.156 fold increase in risk, (95% confidence interval; p<0.001).

Conclusion: Although EPG of STHs can be used to predict the risk of decreased hemoglobin levels and increased eosinophil counts, EPG is not the only factor to predict both of them in elementary school children.

Keywords: prediction, hemoglobin, eosinophil, epg of sths, river banks area.
PREVISÃO DE NÍVEIS DE HEMOGLOBINA E CONTAGEM DE EOSINÓFILOS DEVIDO À INFECÇÃO POR HELMINTOS TRANSMITIDOS PELO SOLO (STH) EM ESTUDANTES DO ENSINO FUNDAMENTAL NA ÁREA DE RIVER BANKS, SOUTH KALIMANTAN, INDONÉSIA

Introdução: As infecções por helmintos transmitidos pelo solo (STH) em crianças em idade escolar ainda existem como um problema de saúde pública na área de River Banks. Os estudantes do ensino fundamental são uma das populações vulneráveis à infecção por HST. Esta pesquisa objetivou determinar a previsão dos níveis de hemoglobina e contagem de eosinófilos devido à infecção por HST em estudantes do ensino fundamental na região de River Banks, Kalimantan do Sul, Indonésia.

Métodos: Cento e trinta e três kato katz preparação das fezes de três estudantes do ensino fundamental na área de margens do rio, 68 eram do sexo masculino e 65 eram do sexo feminino, com idades entre 7 e 12 anos, foram realizados para ovos por grama (EPG) STHs ovos contar exame microscópico método Kato Katz, e exame de sangue analisador de hematologia para determinar os níveis de hemoglobina e contagens de eosinófilos.

Resultado: A análise de regressão linear revelou que o EPG de HSTs foi associado a um nível de hemoglobina de 13,937 com diminuição de risco de -0,031 vezes e com contagem de eosinófilos de 140,204 com aumento de risco de 4,156 vezes (intervalo de confiança de 95%; p<0,001).

Conclusão: Embora o EPG das HSTs possa ser usado para prever o risco de diminuição dos níveis de hemoglobina e aumento das contagens de eosinófilos, o EPG não é o único fator para prever ambos em crianças do ensino fundamental.

Palavras-chave: previsão, hemoglobina, eosinófilo, epg de sths, área de margens de rios.

1 INTRODUCTION

Soil-transmitted helminths (STH) infection is one of the Neglected Tropical diseases (NTDs) that remains a public health problem among people who live in poverty in the world. Ascaris lumbricoides, Trichuris trichiura, Ancylostoma duodenale, and Necator americanus are the species of STH that infected to humans. The previous study reported that Indonesia has a high number of cases of hookworm and the highest number of ascariasis and trichuriasis (more than 90 million cases of each). The prevalence of STH infection in 2019 was different in several areas of Indonesia between 2.5-62%. STH commonly infects school children, especially in rural areas, where the previous study revealed there were 57.24% of school children in agricultural areas of North Sumatera, Indonesia, positive for one or more species of STH (Lee & Ryu, 2019; Pasaribu et al., 2019).
The STH prevalence in Indonesia remains high in the underprivileged population, with poor sanitation. STH is not only transmitted by soil, there were water, sanitation, and hygiene (WASH) as the main factors in STH transmission. Contamination of water sources by STH could be by human sewage disposal that directly goes to sewers and rivers. There were 18-55% of households in Indonesia practice open defecation. Previous study shows that STH prevalence in school children in river basin areas is about 30.83% (Gray et al., 2019, Kurscheid et al., 2020, Adiguna et al., 2022, Rifqoh et al., 2023).

The study of preschool-age children in Nangapanda, Ende, Nusa Tenggara Barat by Djuardi et al. (2021) shows a high STH infection prevalence with the predominant species being *Ascaris lumbricoides* and *Trichuris trichiura*.

### 2 THEORETICAL FRAMEWORK

STH infection causes loss of carbohydrates, proteins, and blood loss which is caused by STH physical friction or direct sucking on intestine mucosa which could decrease the hemoglobin level. Therefore it impacts a decrease in the health condition, nutrition, intelligence, and productivity of sufferers then reducing the quality of human resources.

The impact of decreasing hemoglobin levels in human blood affected the weaker immune system. Therefore, STH infection induces a T-helper type 2 (Th2) in the immune response in the host. Th2 can cause severe tissue damage and fibrosis if chronic. Helminth parasites are well known for their capacity to modulate the parasite-directed host immune response (De Amici et al., 2023).

### 3 METHODOLOGY

This research was a quantitative microscopy STH egg detection using Kato Katz Methods. 133 school-age children 7-12 years old in three elementary schools in river banks of Banjar district were involved in blood and stool examinations by two trained laboratory technicians. Stool samples were examined for STH egg counting using the microscopy Kato-Katz method.

One hundred and thirty-three Kato-Katz thick smear stool preparations were done in duplication. The modified Kato-Katz template was placed on the microscope slide, filled the hole with the amount of sieved SAC’s stool then removed the template. Place over the stool sample one piece of adhesive strips that 24 hours soaked in malachite green.
aqua-glycerol. Press gently the strips with another microscope slide to spread the stool. Place the slide with the strips upward, and keep the slide for 45 minutes at room temperature to clear the stool material.

Kato-Katz method preparation and examination refer to the procedure in WHO bench aid of intestinal parasites (2019) were done in sequence and the STH egg were identified microscopically using Olympus CX33 trinocular light microscope at 100x and 400x magnification. The positive result of this study was determined based on the presence of STH egg while the negative was an absence. The species of STH egg were identified based on eggshell morphology and the distinctive features of STH eggs, i.e., *Ascaris lumbricoides*, *Trichuris trichiura*, and Hookworm (*Ancylostoma duodenale* and *Necator americanus*).

Statistical analysis was performed by SPSS 21 Software (IBM Corporation, Armonk, NY, USA). Descriptive statistics were expressed as percentages, minimum, maximum, and range. Predicting value assessed by linear regression test. Approval for this study was obtained from the Ethics Commission of RSDI No.00123/KEPK-RSDI/02/2023.

### 4 RESULTS AND DISCUSSION

The results of this study, show characteristics in elementary school children as in Table 1.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>68</td>
<td>51.1</td>
</tr>
<tr>
<td>Female</td>
<td>65</td>
<td>48.9</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>9.0</td>
</tr>
<tr>
<td>8</td>
<td>29</td>
<td>21.8</td>
</tr>
<tr>
<td>9</td>
<td>30</td>
<td>22.6</td>
</tr>
<tr>
<td>10</td>
<td>29</td>
<td>21.8</td>
</tr>
<tr>
<td>11</td>
<td>30</td>
<td>22.6</td>
</tr>
<tr>
<td>12</td>
<td>3</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>133</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 1 shows characteristics (sex and age) based on identity information 68 of 133 children (51.1 percent) were male and 65 (48.9 percent) were female. The 7-12-year-old SAC in primary school is one of the target groups for MDA in Indonesia, they receive the MDA biannually. The nutritional impairments caused by STH infection are one of
many issues that could interfere with children’s development and health such as stunting (Permenkes, 2017; Aarts et al., 2020).

This results study, shows EPG STHs and number of species of STH in elementary school children in Table 2 as follow:

<table>
<thead>
<tr>
<th>EPG</th>
<th>Species of STH</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Ascaris lumbricoides</td>
<td>101</td>
<td>75.9</td>
</tr>
<tr>
<td>24</td>
<td>Trichuris trichiura</td>
<td>8</td>
<td>6.0</td>
</tr>
<tr>
<td>48</td>
<td>Ascaris lumbricoides</td>
<td>5</td>
<td>3.8</td>
</tr>
<tr>
<td>72</td>
<td>Trichuris trichiura</td>
<td>4</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>Mix</td>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>133</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Source: Prepared By the Author (2023)

Table 2 shows a 75.9% (101/133) students were found to be negative and only 24.1% (32/133) students were found to be positive for STH infection. The levels of Egg per gram (EPG) STHs eggs count Kato Katz method was 0-72, 10.6% with Ascaris lumbricoides, 12% with Trichuris trichiura and 1.5% with mix infection.

The descriptive data of EPG STHs values, hemoglobin levels and eosinophil counts, of minimum, maximum, mean and standard deviation values as shown in Table 3.

<table>
<thead>
<tr>
<th>EPG, Hemoglobin, Eosinophil</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPG</td>
<td>0</td>
<td>72</td>
<td>9.92</td>
<td>20.06</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>38.85</td>
<td>433.29</td>
<td>181.45</td>
<td>116.18</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>11.0</td>
<td>14.8</td>
<td>13.03</td>
<td>.98</td>
</tr>
</tbody>
</table>

Source: Prepared By the Author (2023)

The levels of EPG STHs, eosinophil and Hemoglobin among the students are presented in Table 3. The mean EPG level was $9.92 \pm 20.06$, eosinophil level was $181.45 \pm 116 \mu/l$ and hemoglobin level was $13.03 \pm 0.98 \text{ g/dl}$.

The correlation of EPG with hemoglobin levels and eosinophil counts based on statistic test shown in Table 4.
This study result reveal at Table 4, Adjusted R square = 0.512, meaning that EPG is able to influence the number of eosinophils by 51.2% and the rest is influenced by other factors. Adjusted R square = 0.409, meaning that EPG is able to influence the hemoglobin levels by 40.9% and the rest is influenced by other factors.

Study results in Table 5. shows the linear regression coefficient of EPG with the number of eosinophils.

<table>
<thead>
<tr>
<th>Eosinophil</th>
<th>R</th>
<th>R Square</th>
<th>Adjusted R Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophil</td>
<td>.718</td>
<td>.515</td>
<td>.512</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>.643</td>
<td>.414</td>
<td>.409</td>
</tr>
</tbody>
</table>

Source: Prepared By the Author (2023)

This study result reveal at Table 5, Prediction of number of eosinophils follows the equation: Number of eosinophils = 140.204 + 4.156 (EPG) For example, the EPG value is 96, then the hemoglobin level = 140.204 + 4.156 (96) = 539.2 /µl. Figure 1 shows the relationship between EPG STHs and the number of eosinophils. The relationship shows a positive value, meaning that the higher the EPG value of STHs, the number of eosinophils increases.

![Figure 1. Correlation EPG vs eosinophil counts](image_url)

Source: Prepared By the Author (2023)
The results of the study are in Table 6. shows the linear regression coefficient of EPG with hemoglobin levels.

<table>
<thead>
<tr>
<th>Dependent Variable: hemoglobin levels</th>
<th>Unstandardized Coefficients</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Constant)</td>
<td>13.339</td>
<td>.000</td>
</tr>
<tr>
<td>EPG</td>
<td>-0.031</td>
<td>.000</td>
</tr>
</tbody>
</table>

Source: Prepared By the Author (2023)

This study result reveal at Table 6 Prediction of hemoglobin levels follows the equation: Hemoglobin level = 13.339 – 0.031 (EPG). For example, the EPG value is 96, then the hemoglobin level = 13.339 – 0.031 (96) = 10.4 g/dL. Figure 2 shows the relationship between EPG STHs and hemoglobin levels. The relationship shows a negative value, meaning that the higher the EPG value of STHs, the hemoglobin level decreases.

Despite EPG of STHs can be used to predict the risk of decreased hemoglobin levels and increased eosinophil counts, EPG is not the only factor to predict both of them in elementary school children. Eosinophilia was identified in 29/133 (21.8%) of the school children and the overall mean eosinophil level was 181.45 ± 116 µ/l. A linear regression model was used to describe the relationship of EPG STHs with increased eosinophil counts. Significantly associated with eosinophil counts (p<0.01).

While eosinophilia could be caused infection and malignancies, it is frequently caused by STH
infection. The eosinophils stimulate host defense against helminths, which occurred mainly from histologic images of eosinophils and parasites in tissue specimens and in vitro studies that recognized the anti-parasitic activities of eosinophil granule protein (Rosenberg HF, 2013, Simon D, 2010). Since the earliest descriptions of this enigmatic cell, eosinophils have been implicated in both protective and pathogenic immune responses to helminth infection.

A significant correlation between eosinophil count with EPG was present in all species of A. lumbricoides, T. trichiura, Hookworm, or mixed infections. The results of this study were in accordance with previous studies conducted in Medan and Honduras (Jiero, S., 2015, Gabrie, JA, 2016). This was because if EPGs were high, so eosinophils that needed to kill the parasites were high too. Eosinophils acted as effector cells drawn by specific IgE crosslinks bound to antigens on the surface of the worm's body through FceR1. The antibody-dependent cellular toxicity (ADCC) mechanism will invite eosinophils to release their granule content such as eosinophil protein granule (ESPG), matrix metalloproteinase (MMP), ROI, and protease whose function destroyed the worms into pieces and removed from the host body (Makepeace, BL, 2012).

Despite the STHs infection, only 21/133 (15.8%) of the school children had anemia and the overall mean hemoglobin level was 13.03 ± 0.98 g/dl. A linear regression model was used to describe the relationship of EPG STHs with decreased hemoglobin levels. Significantly associated with hemoglobin levels (p<0.01). Soil-transmitted helminthiasis, on the other hand, are known to cause blood loss leading to iron-deficiency anemia, a consequence of the parasites’ feeding on blood from vessels and mucosal tissues (World Health Organization 2011).

The hemoglobin of the children are not only affected by helminth infection. Helminth infection takes a long time to influence and reduce the Hb levels of the human host (Hayati Z, 2011). Besides, Anemia is also caused by several other factors, such as iron deficiency, impaired iron absorption, and improper diet patterns (Azizaturridha, A. 2016). Ascaris lumbricoides infection is commonly asymptomatic or may produce mild, non-specific symptoms (Jourdan PM, 2018).
5 CONCLUSION

Although EPG of STHs can be used to predict the risk of decreased hemoglobin levels and increased eosinophil counts, EPG is not the only factor to predict both of them in elementary school children.
REFERENCES


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