

# HAEMOGLOBIN CONCENTRATION DIFFERENCES BETWEEN SINGLE AND MIXED SOIL - TRANSMITTED HELMINTH INFECTIONS IN SCHOOL - AGE CHILDREN

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## ABSTRACT

Soil-transmitted helminths (STHs) remain among the most prevalent parasitic infections worldwide, particularly in developing countries. Persistent infections can lead to malnutrition, anemia, and impaired cognitive development. However, limited research has examined differences in hemoglobin concentration between children with single versus mixed STH infections. This study aims to compare the hemoglobin levels in school-age children at Al-Jam'iyatul Washliyah Orphanage, Medan, with single and mixed STH infections. A cross-sectional design was employed, involving elementary school children. Stool samples were analyzed using the Kato-Katz method to identify STH species and classify infection type, while hemoglobin levels were measured with a portable analyzer. Data were analyzed using independent t-tests, with statistical significance set at  $p < 0.05$ . A total of 150 children participated, with an overall STH infection prevalence of 18.8%; of these, 76.9% had single infections and 23.1% had mixed infections. Mean hemoglobin concentration was significantly lower in children with mixed infections compared to those with single infections ( $XX \pm SD$  g/dL vs  $XX \pm SD$  g/dL,  $p < 0.05$ ). Mixed STH infections were associated with greater reductions in hemoglobin levels than single infections. Targeted deworming and nutritional interventions are essential to reduce anemia risk among vulnerable child populations.

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## 1. INTRODUCTION

Soil-transmitted helminths (STH) are among the most common causes of parasitic diseases and remain a major public health concern in Indonesia. The principal species, *Ascaris lumbricoides*, *Trichuris trichiurid*, and hookworms (*Necator americanus* and *Ancylostoma duodenale*), are transmitted through contact with contaminated soil. These infections can lead to chronic blood loss, gastrointestinal disturbances, nutritional deficiencies, stunted growth, and persistent anemia. Globally, more than 1.5 billion people are affected, including approximately 600 million school-age children and 270 million preschoolers [1] [2]

Soil-transmitted helminth (STH) infections remain highly prevalent in Indonesia. Studies report that children, particularly those aged five to ten, experience ascariasis rates of 40–60% and trichuriasis rates as high as 90% [3]. Data from the North Sumatra Provincial Health Office indicate that 87% of primary school children in Deli Serdang tested positive for helminths, corresponding to an estimated 32% of the population being affected [4]. Consistent earlier findings in Medan, infection rates among school-age children exceeded 70%, with *Trichuris Trichiura* identified as the most prevalent species [5].

In addition to impairing nutrient absorption, helminth infections cause substantial blood loss, particularly in cases of hookworm infestations, which can result in daily losses ranging from 2–100 mL, depending on disease severity [6]. Children with chronic infections often experience anemia, malnutrition, and reduced academic performance [7]. Anemia remains highly prevalent in developing countries, with the highest rates observed among children under five in Southeast Asia and Africa [8]. In Indonesia, approximately 29% of school-age children are affected.

Numerous studies have demonstrated a clear correlation between hemoglobin levels and helminth infections. Research conducted in North Sulawesi and Manado found that children with soil-transmitted helminth (STH) infections had significantly lower hemoglobin concentrations compared to non-infected peers [9]. Furthermore, mixed infections generally result in greater reductions in hemoglobin levels than single infections [10]. For example, children infected solely with *Ascaris lumbricoides* had mean hemoglobin values of 10 g/dL, whereas those with mixed infections involving hookworms, *Ascaris*, *Entamoeba histolytica*, and *Trichuris Trichiura* recorded levels as low as 9.4 g/dL. Given the high prevalence of helminthiasis and its strong association with anemia among Indonesian children, further research is needed to clarify the differences in hemoglobin concentrations between single and mixed helminth infections, especially in vulnerable school-aged populations.

## 2. RESEARCH METHOD

To compare hemoglobin levels between primary school students with single and mixed soil-transmitted helminth (STH) infections, this study employed a cross-sectional design. The study was carried out in August at the Al-Jam'iyatul Washliyah Orphanage and Elementary School in Pulo Brayan, Medan, Indonesia. The accessible population consisted of students who met the eligibility criteria at the study site, while the target population included all students who had been diagnosed with STH infection.

The study sample comprised schoolchildren who fulfilled the inclusion criteria:

- a Enrolling in grades 1–6 of the orphanage elementary school,
- b Laboratory-confirmed STH infection based on the Kato-Katz technique, and
- c Willingness to undergo hemoglobin testing through blood sampling.

Children with non-STH parasitic infections or pre-existing hematological conditions that could affect hemoglobin status were excluded from participation. To ensure sufficient statistical power for detecting differences in hemoglobin levels between single and mixed STH infection groups, the minimum sample size was calculated using the unpaired numerical clinical trial formula [11].

The unpaired numerical clinical trial sample size formula was used to determine the sample size [11].

$$n_1 = n_2 = 2 \left\{ \frac{(Z\alpha + Z\beta)}{(X_1 - X_2)} \right\}$$

$Z\alpha = 1.96$  (Type I error set at 5%, two-way hypothesis) [11]

$Z\beta = 1.282$  (Type II error set at 10%) [11].

$X_1 - X_2 = 1$  (Minimum difference that is considered meaningful) [12].

Standard deviation = 1.4 [12]

$$n_1 = n_2 = 2 \left\{ \frac{(1,96 + 1,282)1,4}{1} \right\}$$

$$n_1 = n_2 = 2 \left\{ \frac{(3,242 \cdot 1,4)}{1} \right\}$$

$n_1 = 41$  Worm infestationSingle STH

$n_2 = 41$  Worm infestationSingle STH  $n_1 + n_2 = 41 + 41 = 82$

Based on the sample size formula above, the total number of samples was 82 people.

Data analysis was conducted using SPSS statistical software version 19.0. A 95% confidence interval (CI) and a significance level of  $p < 0.05$  were applied. For normally distributed data, an independent sample t-test was used to assess differences in hemoglobin concentration between single and mixed soil-transmitted helminth (STH) infections; when distributional assumptions were not met, the Mann–Whitney U test was employed. The study protocol was reviewed and approved by the Health Research Ethics Committee (KEPK), Faculty of Medicine, Universitas Sumatera Utara, in cooperation with Haji Adam Malik General Hospital, Medan. Informed consent was obtained from all participants or their guardians, and ethical clearance was secured to ensure compliance with biomedical research standards.

### 3. RESULTS

#### 3.1 Results of the Study

This study was conducted at Al-Jam'iyatul Washliyah Orphanage and Elementary School of Pulo Brayan, Medan, in August 2015. A total of 150 children were initially screened, of whom 8 did not return stool samples. The remaining 142 participants underwent parasitological examination, which revealed that 92 (64.8%) were infected with Soil-Transmitted Helminths (STH), while 50 (35.2%) were uninfected. Among the infected group, 49 had single STH infections, and 43 had mixed infections.

From this pool, 85 children met the inclusion criteria, and 82 provided blood samples for analysis of hemoglobin concentration and erythrocyte morphology. The final study population comprised 41 children with single soil-transmitted helminth (STH) infections, defined as infection with *Ascaris lumbricoides*, *Trichuris Trichiura*, or hookworm, and 41 children with mixed infections involving one or more of these helminths.

In accordance with the study design, participants were divided into two groups (single vs. mixed infection). The single-infection group included 24 male children (58.5%), while the mixed-infection group comprised 29 male children (70.7%). The mean age of children in the single-infection group was 11.54 years, compared to 10.81 years in the mixed-infection group. Average body weight and height were 26.83 kg and 126.29 cm, respectively, in the single-infection group, and 21.98 kg and 125.81 cm, respectively, in the mixed-infection group.

Table 1: Demographic Characteristics

Demographic Characteristics	Worm infestation in Single STH (n=41)	Worm infestation in Mixed STH (n=41)
Gender, n (%)		
Male	24 (58.5)	29 (70.7)
Female	17 (41.5)	12 (29.3)
Age, mean (SB), years	11.54 (1.65)	10.81 (1.98)
Body mass, mean (SB), kg	26.83 (6.40)	21.98 (4.85)
Height, mean (SB), cm	126.29 (12.98)	125.81 (12.12)
Nutritional Status, n (%)		
High	12 (29.3)	1 (2.4)
Poor	11 (26.8)	35 (85.4)
Normal	18 (43.9)	5 (12.2)

As shown in Table 1, the distribution of helminth infections among the study subjects was as follows: *Ascaris lumbricoides* in 26 children (31.7%), *Trichuris Trichiura* in 15 children (18.3%), combined *A. lumbricoides* and *Trichuris Trichiura* in 38 children (46.3%), and triple infection with *A. lumbricoides*, *Trichuris Trichiura*, and hookworm in 3 children (3.7%). The most common infection pattern was mixed infection with *A. lumbricoides* and *Trichuris Trichiura*, affecting 38 children (46.3%). Regarding nutritional status, the single-infection group was predominantly characterized by normal nutritional status (18 children; 43.9%), whereas in the mixed-infection group, the majority (35 children; 85.4%) presented with poor nutritional status.

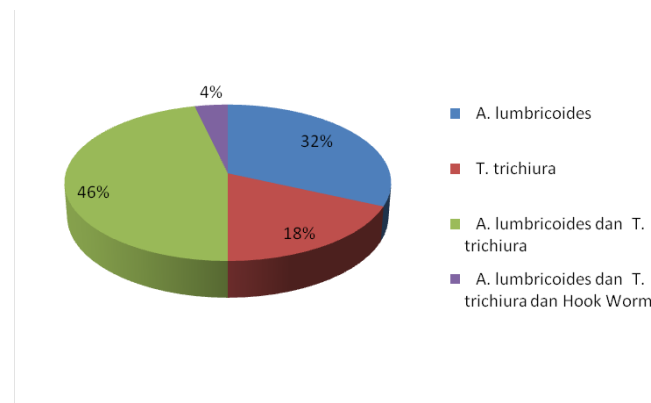


Figure 1: The Proportion of Worm Infection Incidences

Table 2: Types of Worm Infestation in Single and Mixed STH

Types of STH worms	n=82
Single, n (%)	= 41
A. lumbricoides	26 (63.4)
Trichuris Trichiura	15 (36.6)
Mixture, n (%) = 41	
A. lumbricoides and Trichuris Trichiura	38 (92.7)
A.lumbricoides, T.trichiura and Hookworm	3 (7.3)

Among children with single helminth infections, *Ascaris lumbricoides* was the predominant species, identified in 26 cases (63.4%). In the mixed-infection group, the majority were co-infected with *A. lumbricoides* and *Trichuris Trichiura*, affecting 38 children (92.7%).

Table 3: Differences in Hematology Profiles based on Worm Infestation in STH

Hematology Profile	Worm infestation in Single STH (n=41)	Worm infestation in Mixed STH (n=41)	P
Leukocytes (/mm <sup>3</sup> )	8296.51 (3641.45)	8947.79 (2359.52)	0.260 <sup>a</sup>
Erythrocytes (million/mm <sup>3</sup> )	4.67 (0.30)	4.65 (0.37)	0.715 <sup>b</sup>
Hemoglobin (g/dl)	13.28 (0.83)	12.21 (1.32)	0.0001 <sup>b</sup>
Hematocrit (%)	36.42 (5.81)	35.30 (2.39)	0.003 <sup>a</sup>
MCV (fL)	79.70 (3.02)	76.06 (6.77)	0.030 <sup>a</sup>
MCH (pg)	27.83 (4.74)	26.34 (3.64)	0.023 <sup>a</sup>
MCHC (g/dl)	36.24 (3.11)	34.62 (2.19)	0.020 <sup>a</sup>
RDW (%)	12.97 (1.65)	14.48 (3.08)	0.015 <sup>a</sup>
Platelets (/mm <sup>3</sup> )	350.76 (58.83)	346.93 (65.97)	0.782 <sup>b</sup>
Eosinophils (%)	6.94 (3.92)	10.12 (6.42)	0.031 <sup>a</sup>

Based on the research results, there were significant differences in the mean of hemoglobin, hematocrit, MCV, MCHC, RDW, and eosinophils between groups of subjects with single and mixed STH infections ( $p < 0.05$ ).

Table 4: Differences in Hemoglobin Levels in the Groups of Worm infestation in Single and mixed STH

	Worm infestation in Single STH (n=41)	Worm infestation in Mixed STH (n=41)	p
Hemoglobin (g/dL), mean (SD)	13.28 (0.83)	12.21 (1.32)	< 0.001

The mean hemoglobin level in the single-infection group was 13.28 g/dL, which was slightly higher than the mean level in the mixed-infection group (12.21 g/dL). Independent sample t-test analysis revealed a statistically significant difference between the two groups ( $p < 0.001$ ).

Table 5: Mean of Hemoglobin Levels Based on Type of Worm Infestation

Type of Infection	n	Hb Level, Mean, g/dL
<i>Single</i>		
A. lumbricoides (AL)	26	13.47 (0.89)
Trichuris trichiura (TT)	15	12.95 (0.61)
<i>Mixed Infection</i>		
AL and TT	38	12.40 (1.15)
AL, TT, and Hookworm (HW)	3	9.80 (1.00)

The mean hemoglobin level in the single-infection group was 13.47 g/dL for children with *Ascaris lumbricoides* and 12.95 g/dL for those with *Trichuris Trichiura*. In the mixed-infection group, children co-infected with *A. lumbricoides* and *Trichuris Trichiura* had an average hemoglobin level of 12.4 g/dL, while those with triple infections involving *A. lumbricoides*, *Trichuris Trichiura*, and hookworm had the lowest mean level at 9.8 g/dL. ANOVA testing revealed a statistically significant difference in mean hemoglobin levels across the four infection types ( $p < 0.001$ ), with the lowest values observed in the triple-infection group.

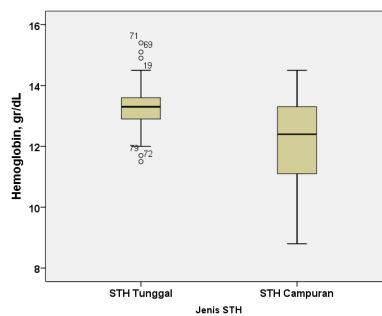


Figure 2: Boxplot Graph of Differences in Hemoglobin Levels between Groups of Worm infestation in Single STH and Worm infestation in Mixed STH

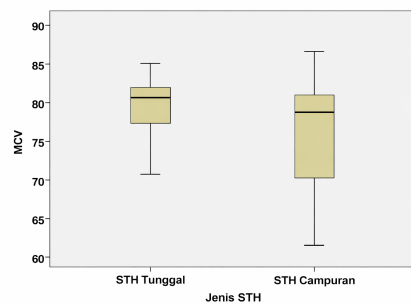


Figure 3: Boxplot Graph of MCV Difference between Worm infestation in Single STH and Worm infestation in Mixed STH

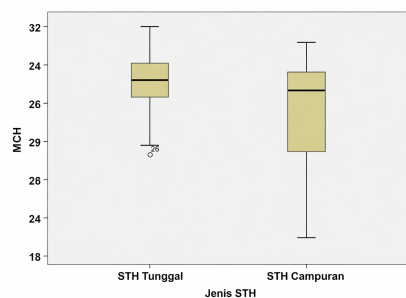


Figure 4: Boxplot Graph of the Difference in MCH between Worm infestation in Single STH and Worm infestation in Mixed STH

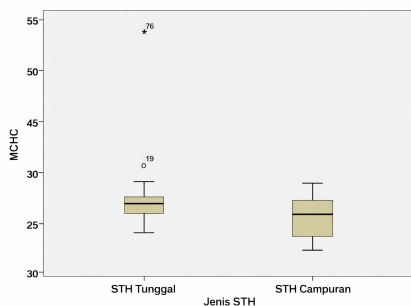


Figure 5: Boxplot Graph of the MCHC Difference of Worm Infestation in Single and Mixed STH

Table 6: Prevalence of Anemia

Anemia Status	n=82
Anemia	13 (18.8)
No Anemia	69 (81.2)

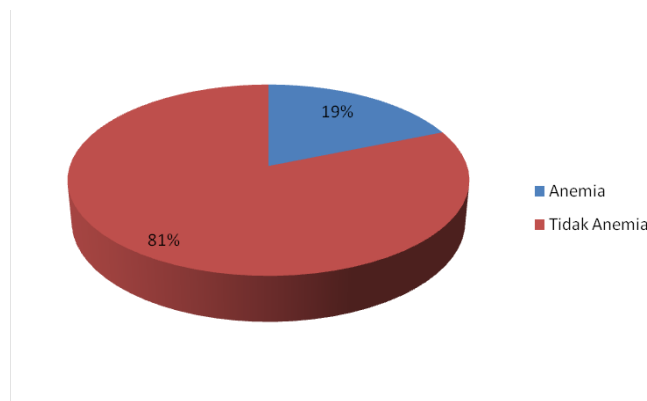


Figure 6: Anemia Prevalence

The prevalence of anemia in this study was 19% or 13 subjects out of 82 subjects studied.

Table 7: Differences in Anemia Incidence Based on Type of STH Infestation

Type of STH	Anemia (n=13)	No Anemia (n=69)	p
Single	0 (0)	41 (100)	< 0.001
Mixed	13 (31.7)	28 (68.3)	

The group of children with a single STH worm infestation did not exhibit anemia. In contrast, 13 individuals (31.7%) in the mixed STH worm infection group suffered from anemia. Differences in anemia incidence according to the kind of STH worm infestation were discovered using the Chi-Square test ( $p < 0.001$ ).

Table 8: Differences in Anemia Incidence Based on TypeWorm infestation STH

Type of STH Worms	Anemia (n=13)	No Anemia (n=69)	p
<i>Single</i>			
A. lumbricoides (AL)	0 (0)	26 (31.7)	< 0.001
Trichuris trichiura (TT)	0 (0)	15 (18.3)	
<i>Mixed</i>			
AL and TT	10 (76.9)	28 (100)	< 0.001
AL, TT, and Hookworm (HW)	3 (23.1)	0 (0)	

The group of children with a single STH worm infestation did not exhibit anemia. In contrast, 13 individuals (31.7%) in the mixed STH worm infection group suffered from anemia. Differences in anemia incidence according to the kind of STH worm infestation were discovered using the Chi-Square test ( $p < 0.001$ ).

Table 9: Erythrocyte Morphology by Type of Worm Infestation in STH

Erythrocyte Morphology	Single STH (n=41)	Mixed STH (n=41)
Normocytic	29 (70.7)	20 (48.8)
Hyperchromic normocytic	7 (17.1)	3 (7.3)
Normocytic hypochromic	2 (4.9)	9 (22.0)
Normocytic microcytic	1 (2.4)	1 (2.4)
Microcytic normochromic	2 (4.8)	0 (0)
Hypochromic microcytic	0 (0)	7 (17.1)
Microcytic hypochromic normocytic	0 (0)	1 (2.4)

The findings showed that erythrocyte morphology in the single-infection group was predominantly normocytic. Similarly, most children in the mixed-infection group also exhibited normocytic morphology. Microcytic hypochromic erythrocytes, a specific abnormality associated with helminth infection, were not observed in the single-infection group. However, seven children (17.1%) in the mixed-infection group presented with microcytic hypochromic morphology

Table 10: Morphology of Microcytic Hypochromic Erythrocytes Based on Type of STH Worm Infestation

Erythrocyte Morphology	Single STH (n=41)	Mixed STH (n=41)	p
Microcytic hypochromic	0 (0)	7 (17.1)	0.012
Not microcytic hypochromic	41 (100)	34 (82.9)	

Morphological investigation revealed no microcytic hypochromic in the group of participants with a single STH worm infection, but seven subjects (17.1%) had a mixed STH worm infection. The type of erythrocyte shape differed significantly between the groups of people with single STH worm infection and mixed STH infection, according to the results of the study using Fisher's exact test ( $p=0.012$ ).

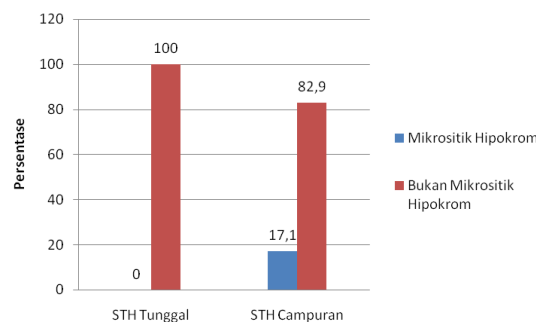


Figure 7: Bar Graph of Microcytic Hypochromic Frequency in Single and Mixed STH Infection Groups

### 3.2 Discussion

Soil-transmitted helminth (STH) infections remain a major cause of anemia and malnutrition among children, particularly those in elementary school who are more likely to come into contact with contaminated soil during outdoor activities. In this study, 68.8% of children at Al-Jam'iyatul Washliyah Orphanage in Pulo Brayon, Medan, were found to have helminth infections, with *Ascaris lumbricoides* and *Trichuris Trichiura* being the most common etiological agents. Mixed infections with *A. lumbricoides* and *Trichuris Trichiura* accounted for the largest proportion (46.3%), while hookworm infections were relatively rare. The high burden of disease is likely attributable to poor environmental sanitation, unhygienic school facilities, unsafe food vendors near the school, and limited awareness of personal hygiene practices such as handwashing. Boys were more frequently infected than girls, which may be linked to lower adherence to hygiene practices and greater involvement in outdoor activities without footwear

Comparable findings have been reported in Ethiopia [13], where poor hygiene, lack of footwear, and inadequate sanitation were associated with a high prevalence of STH infections (54.9%). Similarly, research in Bali [14] found that hookworm infections were more common in men, largely due to occupational exposure from farming without footwear. Gebnet et al. also confirmed that *Trichuris Trichiura* frequently co-infects with *Ascaris lumbricoides*, the most widespread STH globally. Strong correlations between *A. lumbricoides* and *Trichuris Trichiura* have likewise been documented in India, suggesting that fecal-oral contamination is a common transmission route. Local

studies in South Tapanuli further highlight that unsafe water supplies, inadequate sanitation facilities, and poor environmental hygiene substantially increase the risk of infection [15]

In this study, children with mixed soil-transmitted helminth (STH) infections had significantly lower hemoglobin (Hb) levels compared to those with single infections. The mean Hb concentration in the mixed-infection group was 12.21 g/dL, whereas the single-infection group averaged 13.28 g/dL. The lowest Hb value recorded was 8.8 g/dL, and 18.8% of children with mixed infections were classified as anemic. Anemia was particularly associated with triple infections involving hookworm and co-infections with *Ascaris lumbricoides* and *Trichuris Trichiura*. These findings align with previous evidence suggesting that nutritional competition, impaired iron absorption, and gastrointestinal blood loss are key mechanisms underlying STH-related anemia.

The majority of children exhibited normocytic erythrocyte morphology; however, seven children (17.1%) with mixed infections developed microcytic hypochromic anemia, indicative of iron deficiency. Iron deficiency anemia associated with helminthiasis is characterized by reduced Hb, Ht, MCV, MCH, and MCHC values, along with elevated RDW and eosinophil counts in both single and mixed infections. Hookworm infections, in particular, are known to cause chronic blood loss, with the severity of anemia depending on worm burden, duration of infection, and the individual's iron reserves [16].

Because heme synthesis is impaired, decreases in MCV and MCH are typically associated with iron deficiency anemia. In the early stages, the body's iron reserves may compensate; however, prolonged STH infections eventually deplete these reserves, leading to hypochromia and microcytosis [17]. Our findings demonstrate that mixed infections are associated with lower MCV and MCH values compared to single infections. Reduced MCHC levels, more pronounced in mixed infections, further indicate hypochromic anemia [9]. Elevated RDW values additionally suggest anisocytosis, a hallmark of iron deficiency anemia [18].

Both the single- and mixed-infection groups exhibited elevated eosinophil counts, with higher values observed in the mixed-infection group. This response reflects the immune system's reaction to helminth antigens and the associated inflammatory processes [19]. Taken together, these findings highlight the substantial hematological burden of STH infections in school-age children. Mixed infections, in particular, were more detrimental than single infections, as evidenced by reduced hemoglobin levels, impaired red cell indices, and increased rates of anemia. Preventive measures should therefore include regular deworming, nutritional interventions to address iron deficiency, and sustained health education to promote hygiene practices. Reducing transmission and morbidity in this vulnerable population also requires improved sanitation facilities, access to clean water, and reinforcement of handwashing habits.

#### 4. CONCLUSION

With a prevalence of 64.8%, this study confirms that soil-transmitted helminth (STH) infections remain a significant health concern among school-age children at Al-Jam'iyatul Washliyah Pulo Brayan Orphanage in Medan. Children with single infections had higher mean hemoglobin concentrations (13.28 g/dL) compared to those with mixed infections (12.21 g/dL). Notably, 31.7% of children with mixed infections were anemic, with 17.1% presenting microcytic hypochromic anemia. In contrast, anemia was not observed in children with single infections. These findings demonstrate that mixed STH infections exert a more detrimental impact on hematological status than single infections. To reduce the burden of STH-related morbidity in vulnerable populations, preventive strategies must be strengthened, including regular deworming, nutritional supplementation, hygiene education, and improvements in sanitation and access to clean water.

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