Malaria and Soil-TransmittedHelminth Co-InfectionAmong School-Aged Children in Mkpat Enin Local Government Area, Akwa Ibom State, Nigeria

Comment [A1]: Revise- topic to improve clarity

ABSTRACT

Malaria, caused by *Plasmodium falciparum*, and soil-transmitted helminths (STHs), caused by *Ascaris lumbricoides*, *Trichuris trichiura*, and *Ancylostoma duodenale*, arehighly prevalent parasitic infections of ~~significant~~ public health significance ~~concern~~ in Nigeria. Coinfection with these parasites ~~can intensify~~exacerbates health challenges, particularly ~~in~~among children. Understanding the prevalence and impacts of such co-infections is ~~essential for developing~~crucial for designing effective public health strategies. This cross-sectional study ~~investigated~~assessed the prevalence of malaria and soil-transmitted helminth co-infections among primary schoolchildren in Mkpat Enin Local Government Area (LGA), Akwa Ibom State. Between May and July 2024, finger-prick blood and stool samples were randomly collected from 348 children aged 4–15 years across ~~from~~ four primary schools. Malaria parasites were identified ~~detected~~ using thin-film microscopy of air-dried blood smears, ~~whereas~~while stool samples were analysed for STH ova using the formalin-ether concentration ~~method~~technique. The prevalence of *Plasmodium falciparum*was 21.5%, while the prevalence of *Ascaris lumbricoides*, *Trichuris trichiura*, and *Ancylostoma duodenale* was ~~21.5%,~~ 9.5%, 5.7%, and 2.6%, respectively. The overall ~~rate of~~ co-infection rate of ~~with~~ malaria and STHs was 7.8%, ~~and~~with significant associations ~~were observed~~ between these infections ($p < 0.05$). Malaria prevalence was slightly higher in males (21.8%) ~~than in~~compared to females (21.3%), whereas STH infections were more common ~~prevalent~~ in females (19.6%) than in males (15.8%).~~However,~~ the~~se~~differences in prevalence between sexes were not statistically significant ($p > 0.05$). Age-specific prevalence ~~wise,~~showed that children ~~older than~~aged 14 yearsand older had~~exhibited~~ the highest malaria prevalence (29.4%), ~~whereas~~while those aged 9–13 years had the highest prevalence of STH infections (19.4%). This study highlights ~~underscores~~ the ~~substantial~~considerable burden of malaria and STH infections among primary school children in Mkpat Enin LGA, with ~~notable~~ variations in prevalence influenced ~~infection rates based on~~by age and sex. This ~~ese~~ findings underscore ~~highlight~~ the need ~~to importance of~~ targeted~~d~~public health interventions that address parasitic infections,~~while~~ considering ~~the~~ demographic factors that influence~~ing~~ their distribution~~prevalence~~.

Comment [A2]: Simplify and clarify sentences to improve readability.
Be consistent in the usage of terminology for methods and findings
See suggestion made to integrate into abstract highlighted on red ink, and those to be deleted crossed through with a line.

Keywords: Malaria, Soil-transmitted helminths, Co-infection, Prevalence, Akwa Ibom State.

INTRODUCTION

Concomitant parasitic infections occur when two or more parasites coexist within a host, potentially altering ~~the~~ immune response to individual parasites and modifying the clinical outcomes. For instance, helminths can influence malaria severity by either mitigating or exacerbating its clinical manifestations(Ojurongbe *et al.*, 2010)~~[1]~~ Malaria, a febrile disease caused by protozoan parasites of the genus Plasmodium, is transmitted via the bite of infected female Anopheles mosquitoes (Udofia *et al.*, 2021)~~[2]~~.

Comment [A3]:
1)Endeavour to follow journal prescribed in-text citation format. Include author(s) surnames and publication year consistently as shown here. Apply this throughout the text.
2) Update references with recent related or similar studies
3) Verify all cited sources are listed in the reference section and formatted according to APA guideline.

Soil-transmitted helminthiasis (STHs), one of the most prevalent neglected tropical diseases (NTDs) in Nigeria, remains a significant global health challenge, particularly in impoverished and underserved communities where control measures are difficult to implement ~~[3,4,5 & 6].~~(Ref)

According to the 2023 World Malaria Report, there was approximately 249 million cases of malaria in 2022,~~and~~ a slight increase from 244 million in 2021. Malaria-related deaths were estimated at 608,000 in 2022, compared to 610,000 in the previous year ~~[7]~~. Globally, approximately 1.7 billion people are infected with one or more STHs. A 2003 survey estimated ~~reported~~ that over 1.2 billion individuals were infected with *Ascaris lumbricoides*, with more than half of these cases occurring in China. Additionally, the prevalence of *Trichuris trichiura*~~is~~was estimated at 795 million, and hookworm infections affect ed approximately 740 million people ~~[8, 9]~~. Despite increasing interest in understanding the dynamics of malaria-helminth co-infections, particularly their prevalence, clinical implications, and risk factors, longitudinal~~,~~ community-based studies remain scarce. This is especially true in rural areas~~,~~ such as Mkpat Enin Local Government Area, Akwa Ibom State, Nigeria. This study aimed to address this gap by exploring the prevalence and interactions of malaria and helminth co-infections in this region ~~[2, 10].~~

2. MATERIALS AND METHODS

2.1 Study Area

This study was conducted in four primary schools in the Mkpat Enin Local Government Area, Akwa Ibom State. Mkpat Enin is located at longitude 8° 30' and latitude 5° 30' in the Southern region of Nigeria,~~it~~ covering an area of 42km^2 (Fig.1). The population primarily consists of ~~Most of the people living in Mkpat Enin are~~ civil servants and farmers.

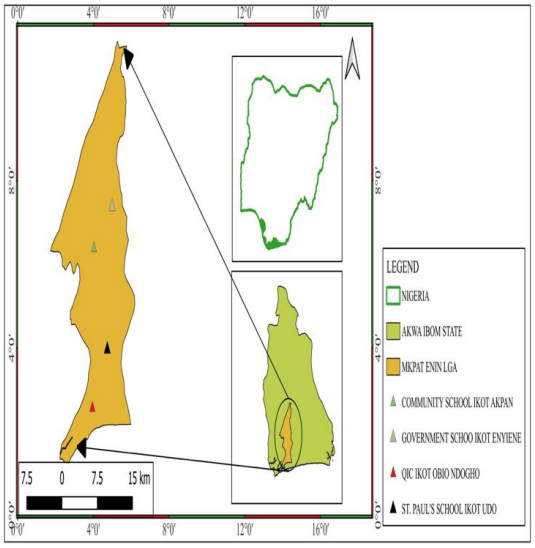

Figure 1 : Map of Mkpat Enin Local Government Area, showing the study location.

Comment [A4]: Place in-text citation in APA-style format. Take note of all grammatical error and effect correct all through the text.

Comment [A5]: This should read 2.0

Comment [A6]: Ensure uniformity in numbering and presentation of subsections.

Comment [A7]: Study design (2.1) should come before study area (2.2). Also consider eliminating unnecessary repetitions for improved sentence structure. See suggested revision on highlight.

Comment [A8]: Write in full

2.2: Study Design

This study was a cross-sectional, school-based investigation involving 348 pupils recruited over three months using a multistage sampling technique. The sample size was proportionately distributed across the four selected schools, considering the total pupil population in each school. Participants were selected through simple paper balloting, where students picked slips marked "YES" or "NO"; those who picked "YES" were included in the study. A total of 348 stool and blood samples were collected from pupilsin~~of~~ the four schools,~~Specifically~~, with 87 stool samples ~~were~~ collected from each school. The schools randomly selected for the studyincluded:~~were~~ Community School, Ikot Akpan, ~~and~~ St. Paul's School, Ikot Udo, Government School, Ikot Eyiene, and QIC School, Ikot Obio Ndoho.

2.3: Study population and Sample size estimation

The study population consisted of ~~All~~ children aged 4 -15 years whoprovided assent and whose parents or guidians gave written informed consent.~~to parent/guardian consent were included in this study.~~

The sample size ~~A sample size of 348~~ was determined usingthe formula:

$n = z2pq / d^2$

where n=required sample size,

Z=confidence interval at 95% (standard value=1.96),

P = estimated prevalence(24%, i.e., 0.24),

q (1−p)= expected non-prevalence,~~ and~~

d=margin of error at 5% (0.05)

Based on this formula, the minimum calculated sample size was 343 participants. To account for non-response, 348 participants were recruited~~. or the maximum tolerable error. An estimated prevalence of 24% (P = 0.024) was used. The minimum number of participants in the study was 343.~~

2.4 Collection of Blood Samples and Microscopic Examination

Blood samples were collected using sterileconditions~~procedures~~ by a trained laboratory technician. After cleaning the middle or ring finger with a 70% alcohol-moistened swab, the finger was dried with clean cotton and pricked with a ~~disposable~~sterile lancet. ~~The collected~~Blood smears were prepared as follows: ~~was examined for the presence of malaria parasites using blood smear microscopy [10]. For the~~Thin blood film:A small drop of blood was placed at one end of a clean slide and spread using another slide held at an angleto create a thin film.~~, ensuring that the blood ran along the edge of the spreader. The slide was then pushed forward to spread blood into a thin film. For a~~Thick film:Three drops of blood were placedon~~at~~ the opposite end of the slide and spread evenly with an applicator.

Slides were air-dried and transported to the laboratory in a slide box. Fixation was performed ~~using~~with methanol, and the slides were stained~~was performed~~ with 10% Giemsa solution for 10 minutes. After staining ~~The~~ slides~~was washed~~were rinsed with distilled water, air-dried, and examined under a microscope using a x100 oil immersion lens ~~[11].~~Ref.

Comment [A9]: Did you seek assent from the pupils? Necessary for ethical issues

Comment [A10]: Revise sample size calculation for clarity. See suggestion

Comment [A11]: See suggested correction for consideration

2.5 Collection and Examination of Stool Samples

Stool samples were collected ~~from participants~~ in clean, leak-proof, sterile containers~~,~~ and preserved in 10% formalin. The samples were processed using the formalin-ether concentrationtechnique~~method~~.as follows:~~Specifically,2 ml~~Two millilitres of preserved stool was added to a clean conical centrifuge tube containing 7 mL of 10% formalin water. The mixture was filtered through a sieve into a 15 mL conical centrifuge tube. ~~followed by the addition of 4 mL~~Four millilitres of diethyl etherwere added to the filterate, and the tube was ~~. The contents were~~ centrifuged at 300rpm for 1 minute. ~~, and~~The supernatant was discarded, andsmears were prepared from the sediment onto sterile slides.~~with~~Three slides were prepared ~~created~~ for each participantand ~~[12]. The slides were~~ examined microscopically by two independent experts using 10x and 40x objective lenses to identify ~~the~~ helminth ova and cysts(Ref).

Comment [A12]: Provide reference for stool examination technique

2.6 Data Analysis

~~The~~Collected data were stored in Microsoft Excel and validated before the analysis. Statistical analyses were performed using IBM SPSS version 21 (IBM SPSS Inc., Chicago, IL, USA). Descriptive statistics, including frequencies and percentages, were used to summarize ~~the~~ variables. Chi-square tests were applied ~~used~~ to assess ~~the~~ associations between malaria and helminth co-infections and ~~their~~ related risk factors. ~~Additionally,~~ Pearson's correlation test was ~~used~~employed to evaluate the strength of ~~the~~ associations between variables. Statistical significance was set at $p < 0.05$.

3.0 RESULTS

Comment [A13]: Be consistent in your numbering

A total of 348 stool and ~~348~~ blood samples were collected from pupils in ~~of~~ four primary schools~~,for examination, of which~~ comprising 184 ~~were~~ female(52.6%) and 165 ~~were~~ males(47.4%). The sociodemographic characteristics of the participants are ~~presented~~summarized in Table 1. The prevalence of ~~soil-transmitted helminths and malarial parasites is shown in Table 2.~~*Plasmodium falciparum*was~~had the highest prevalence~~75(21.6%, *P*=.01).Three species of soil-transmitted helminths(STHs) were detected~~in the study:~~*Ascaris**lumbricoides*, (33, 9.5%),*Trichuris**Trichiura* (20, 6.0%), and *Ancylostoma duodenale*, (9, 2.6%), resulting in a total STH prevalence of ~~and total helminth infection,~~ 62(17.8%, p = .00). The prevalence ofmalaria and helminth infections varied~~coinfection~~ among ~~the four~~ schools ~~is shown in~~(Table 3).~~where~~ St Paul School, Iket Udo had the highest ~~prevalence of~~ malaria prevalence at 25 (28.7%) and the highest ~~rate of~~ coinfectionrate(9.2%), while~~(~~Government School, Ikot Eyenene~~),~~ ~~had~~recorded the highest STH prevalence at ~~rate of helminth infection~~ 21 (24.1%).

Comment [A14]: Specify p-values in parentheses. Percentages should be mentioned after the respective frequencies for easier comparison.

~~The rates of co-infection by age are~~As shown in Table 4, coinfections of*Ascaris* and *Plasmodium*were the most common, with a prevalence of ~~infection had the highest rate of coinfection, among others~~ 14(4.0%). ~~;~~Children aged 4-9years had the highestcoinfection rates of *Ascaris* and *Plasmodium*~~coinfection~~(6, 4.7%), while children aged 10-15years had the highest rates of *Trichuris* and *Plasmodium*coinfection (5, 2.3%). ~~The prevalences of malaria and helminth infections are shown in Table 4.5.~~ Malaria wasslightly more prevalent among males (36, 21.8%) than females (39, 21.3%), ~~and there was~~with no significant sex difference ~~between males and females~~(p = 0.92). Conversely, STH infections were more prevalent in females (36, 19.6%) than males (26, 15.8%), though the difference ~~and there~~ was not statistically~~significant difference in coinfection between males and females~~ (p = 0.54) (Table 5).

Figure 1 illustrates the rate of coinfection by sex. Females exhibited a higher prevalence~~is shown in Figure 1, there was a high occurrence~~ of *Plasmodium*, *Trichuris* and *Ascaris* coinfection ~~in females~~(4, 2.2%), compared to males, who had the highest prevalence of *Trichuris* and *Plasmodium*coinfection (4,

2.4%).

Comment [A15]: Rephrase sentence for improved readability, as suggested in red highlight

Table1: Showing the socio-demographic variables of primary school children sampled for the study

Comment [A16]: Consider including risk factor analysis (eg., exposure, hygiene practices) if available

Comment [A17]: Use column headers that are specific and self-explanatory. (eg., "total number" examined instead of "total examined")

Parameters		No. Examined
Sex	Female	183 (52.6%)
	Male	165 (47.4%)
Age	4-9	129 (37.1%)
	10-15	219 (62.3%)
Schools	COMMUNITY SCHOOL IKOT AKPAN	87 (25.0%)
	ST PAUL'S SCHOOL IKOT UDO	87 (25.0%)
	GOVERNMENT SCHOOL, IKOT EYIENE	87 (25.0%)
	QIC IKOT OBIO NDOHO	87 (25.0%)

Table 2: Prevalence of malaria parasites and soil-transmitted helminths

Comment [A18]: Figures and tables should have concise captions that summarize the main findings. Double-check the accuracy of all values (eg., some row total did not match description

Parasite	Number examined	*Plasmodium falciparum*	*Ascaris lumbricoides*	*Trichuris trichiura*	*Ancylostoma duodenale*	Total Helminths
Positive	348	75	33	20	9	62
Negative	348	273	268	327	339	286
Total	348	75 (21.6%)	33(9.5%)	20(6.0%)	9(2.6%)	62(17.8%)
P value		0.01*	0.003*	0.004*	0.0001*	0.002*

Comment [A19]: Sum 33+268=301?

Comment [A20]: Sum 327 negative parasite added to 20 positive parasite =347 instead of 348.

Table 3: Prevalence of soil-transmitted helminths among pupils according to school

Name of school	Total number examined	Total number infected with Malaria	Total number infected with any STHs	Mixed Infection (Malaria & Helminth)
Community school Ikot Akpan	87	19 (21.8%)	14 (16.1%)	5 (5.7%)
St Paul's School, Ikot Udo	87	25 (28.7%)	11 (12.6%)	8 (9.2%)
Government School Ikot Eyennene	87	18 (20.7%)	21 (24.1%)	7 (8.0%)
QIC Ikot Obio Ndoho	87	13 (14.9%)	16 (18.4%)	5 (5.7%)

Table 4: Showing the prevalence of co-infection by age

Age	No. examined	Ascaris + Plasmodium	Ascaris + Trichuris + Plasmodium	Trichuris + Plasmodium	Ancylostoma + Ascaris

Age	No. examined	Ascaris + Plasmodium	Ascaris + Trichuris + Plasmodium	Trichuris + Plasmodium	Ancylostoma + Ascaris
4-9	129	6 (4.7%)	2 (1.6%)	1 (0.8%)	0
10-15	129	8 (3.7%)	3 (1.4%)	5 (2.3%)	2 (0.9%)
Total	348	14 (4.02%)	5 (1.4%)	6 (1.7%)	2 (0.6%)
P-value		0.74	0.75	0.74	0.75

Table 5 : Showing the prevalence of parasites by sex and age

Sex	No. examined	*Plasmodium*	*Ascaris lumbricoides*	*Trichuris trichiura*	*Ancylostoma duodenale*
Female	183	39 (21.3%)	19 (10.4%)	11 (6.0%)	6 (3.3%)
Male	165	36 (21.8%)	14 (8.5%)	9 (5.5%)	3 (1.8%)
Total	348	75 (21.6%)	33 (9.5%)	20 (5.7%)	9 (2.6%)
P-value		0.92	0.53	0.82	0.38

Comment [A21]: Ensure uniform spacing and alignment within all tables

Sex	No. examined	*Plasmodium*	*Ascaris lumbricoides*	*Trichuris trichiura*	*Ancylostoma duodenale*
Age					
4-9	129	26 (20.2%)	15 (11.69%)	11 (8.5%)	3 (2.3%)
10-15	219	49 (22.4%)	18 (8.2%)	9 (4.16%)	6 (12.7%)
P-value		0.63	0.73	0.75	0.74

Comment [A21]: Ensure uniform spacing and alignment within all tables

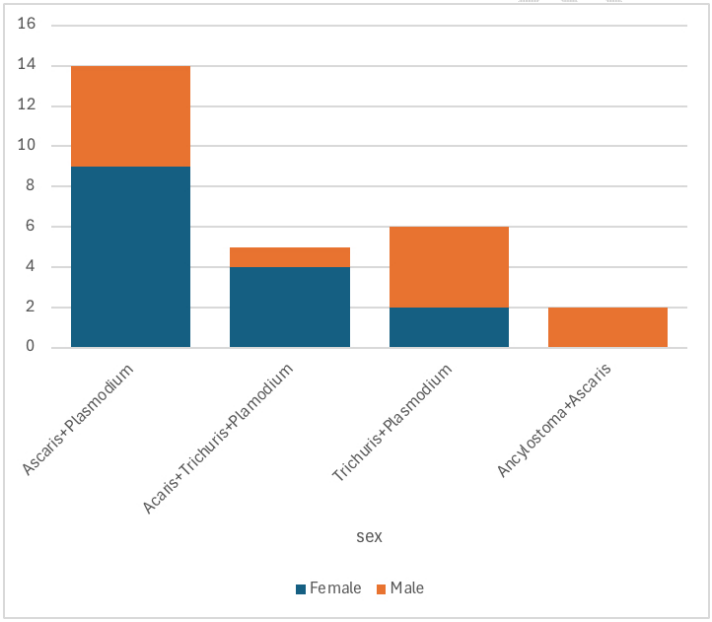

Figure 2: A graph showing the rate of coinfection by sex

Comment [A22]: The figure legend do not match the information in the text

Comment [A23]: 1) Discuss how the observed significance could impact public health interventions
2) Expand on why certain age groups or sex showed differences in prevalence of susceptibility.
3) Reference specific interventions (eg., sanitation or deworming campaign)in the study area to strengthen the discussion.

See other suggestion for consideration.

4.0 Discussionnot 5

Children in endemic regions, such as Nigeria, frequently~~often~~ experience co-infections with malaria

and soil-transmitted helminths(STHs), driven by ~~various factors that contribute to high transmission rates. Key among these factors are the prevailing~~ environmental and climatic conditions condusive to the transmission ~~that favour the spread~~ of both infections (Ref)[13].~~The co-infection rate for malaria and STH~~In this study, the coinfection rate for malaria and STH was 7.8%. This rate is higher than the 5.9% reported by (Ref)[14] in Osogbo, Nigeria, but lower than the 9.49% reported by [9]. A significant association ~~was found~~ between malaria and STH co-infectionwas observed in this study (p =.05).

The prevalence of malaria parasites was 21.5%, ~~which is~~ lower than the 27.3% reported by [15] in Ajagba, Southwestern Nigeria, but higher than the 11.1% prevalence reported ~~by [16]~~ in Ethiopiaby Author et al.(year). ~~The prevalence of malaria showed~~No significant variation was found between males (21.8%) and females (21.3%) (p =0.92), consistent with previous studies [17-18]. Thesefinding indicates ~~suggests~~ that both sexes are equally exposed to malaria factors, suggesting~~influencing malaria infections, confirming~~ that sex is not a determinant of malaria susceptibility [19].Children aged > 10 years had the highest prevalence of malaria (22.4%), which aligns with studies linking older children to increased outdoor activities that heighten exposure to mosquito vectors (Ref)

Soil-transmitted helminthiasis ~~(STH)~~ caused by *Ascaris lumbricoides*, ~~hookworms, and~~*Trichuris trichiura*, and hookworms *w*as also documented ~~observed~~ in this study, consistent with findings from ~~other~~previous researchers [5,20,21]. The overall prevalence of STH infections ~~was~~(17.8%)~~, which~~ was lower than the 45.3% reported by [22] in Itu Local Government, Akwa Ibom State, but slightly higher than the 17.44% reported in Akwa North LGA, Anambra State [9]. Additionally, the prevalence was ~~This is also~~ lower than the 24.2% ~~recorded~~observed in Eastern Obolo, Akwa Ibom State [13]. The relatively low prevalence of parasitic infections ~~observed~~ in this study may be attributed to improved ~~environmental~~ sanitation, mass distribution of insecticide-treated mosquito nets, routine use of anti-helminthic drugs, and government-led mass-deworming campaigns. Among the detected ~~three~~ helminths ~~detected~~, *Ascaris lumbricoides* was likely related to ~~the most prevalent, accounting for 53.2% of all the infections. Trichuris trichiura, followed by 32.3%, whereas hookworms were the least common (14.5 %). The high prevalence of A. lumbricoides may be due to the community's~~ poor water supply and inadequate sanitation facilities, which facilitates ~~create favourable conditions for~~ its transmission. Children aged 4–9 years had the highest prevalence of STHinfections (22.5%), consistent with studies indicating thatyounger children are more likely to play in contaminated environments and have poor ~~may lack awareness of proper~~ hygiene practices, increasing their susceptibility (Ref). Females demonstrated a higher prevalence of STH infections ~~risk of STH infection. Conversely, STH infection was more prevalent in females~~ (19.6%) compared to ~~than in~~ males (15.8%). This ~~finding~~ aligns with the ~~reported~~ higher prevalence ratesreported among females (51.9%) compared to ~~than~~ males (45.3%) in Ethiopia [23]. The observed differences could be influenced by~~higher prevalence among females could be attributed to~~ behavioural, biological, or social factors, although the differences in this study~~that make them more susceptible to infection although there~~ was not statistically significant ~~difference in this study~~ (p =0 .73)

5.0 Conclusion

This study highlights the significant prevalence of malaria and STH infections among primary

Comment [A24]: Adjust all in-text citation to approved format

Comment [A25]: Attend to all suggestions on grammatical errors and styles to improve clarity and readability

Comment [A26]: Numbering should not exceed 3.0. see author(s) guide.

school children in Mkpat Enin Local Government Area. While Infection rates varied by age, no significant differences were observed between~~but not by~~ sexes. The predominant parasites ~~main~~ identified ~~parasites~~ were *Plasmodium falciparum, Ascaris lumbricoides*, *Trichuris trichiura*, and *Ancylostoma duodenale*. A coinfection rate of 7.8% was recorded among the 348 pupils examined, with*A. lumbricoides* (9.5%) being ~~was~~ the most prevalent helminth and~~while~~*P. falciparum*(21.6%) ~~was~~ the most common malariaparasite.

Consent

In accordance with ~~As per~~ international ~~or~~and university standards, ~~respondents'~~ written consent was obtained from all respondents and securely ~~collected and~~ preserved by the author(s).

Ethical consideration

Ethical clearance and approval (HREC, No. AKHREC/30/05/24/218) were obtained from the Department of Research and Education Ethical Committee of the State Ministry of Health~~,~~ toconduct the study involving the collection of specimens from primary school pupils in Mkpat Enin LGA. Prior to sample collection, written informed consent was obtained from the parentsor guardians of the pupils~~. before samples were collected.~~

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Comment [A27]: italicize

Comment [A28]: add "parasite" at the end of discussion.
*For actionable recommendation, suggest target interventions, such as hygiene education or aged-specific deworming programs.
*Highlight how findings can guide future research policies.

Comment [A29]: Be consistent with approved font style and size.
How was the informed consent process conducted? Was assent obtained from pupils?

Comment [A30]: Implement.

Comment [A31]: Be consistent with the approved font style(Times New Roman) and size(12)

Cited and listed References do not conform with APA referencing style format

Comment [A32]: Add comma

Comment [A33]: delete

Comment [A34]: delete

Comment [A35]: Journal should be italicized, all punctuations applied right. Please revise all referencesto conform with APA referencing style format.
See correction on ref.1 to 3.
1.Ojurongbe, O., Awe, F., Olowe, O., Okanlawon, B. & Adeyeba, O. (2010). Prevalence of soil-transmitted helminth infections in tertiary institution in Western Nigeria. *New York Science Journal*, 3(1), 1-5.
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... [1]

Comment [A36]: Delete column and replace with comma

Comment [A37]: Is this the name of the Journal?

Comment [A38]: italicize

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Please write a few sentences regarding the importance of this manuscript for the scientific community. A minimum of 3-4 sentences may be required for this part.	**1. Provides valuable insights into co-infection of malaria and STH among school-aged pupils in the study area which is a region with endemic prevalence of this infection.** **2. The use of well-structured methodologies, including multistage sampling and validated laboratory techniques, enhances the reliability and reproducibility of the findings.** **3. The integration of sociodemographic data and the exploration of factors influencing infection prevalence offer a comprehensive understanding of the local epidemiology, which is crucial for targeted public health interventions.** **4. While manuscript effectively highlights the key findings, more in-depth discussion on potential interventions and recommendations for mitigating co-infection would add more value.**	
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