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# The Prevalence and Effects of Asymptomatic Malaria and Intestinal Helminths Co-Infection among School-Aged Children in Calabar, Nigeria

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## ABSTRACT

The study aimed to determine the co-occurrence of intestinal helminths and *Plasmodium* parasites and its effect in school children living in Ikot-Omin, a rural community in Nigeria.In a cross-sectional study, 578 school children aged 5-15 years old were enrolled. Both thick and thick blood films were made and examined by two microscopists independently with a light microscope using x100 objective lens. The stool samples were examined using the brine floatation concentration technique for the presence of eggs and larvae of worms within 6 hours of collection. Anaemia status was determined according to normal pack cell volume rangeswith reference to age and gender.Overall, 207 (35.8%) of the participants had *Plasmodium* parasitaemia of which *Plasmodium falciparum* was 99.5%.Ninety-two (15.9%) participants had only malaria infection, 138 (23.9%) had intestinal helminths infection only while 115 (19.9%) had malaria and intestinal helminths co-infection. Anaemia varied by the status of infections and had the highest proportion of 58% among participants with helminth infection alone ($p < 0.0001$).School children will benefit from mass deworming and the use of Long Lasting Insecticide Treated Nets in addition to educational intervention.

**KEYWORDS:** Anaemia; Co-infection; Helminthiasis; Malaria

## 1. Introduction

Malaria parasite infection is one of the most challenging obstacles facing people's health in Africa south of Sahara (WHO, 2018). It is greatly co-prevalent with intestinal helminth infections, particularly among children that are inhabitants of the endemic areas of Africa south of Sahara, thus leading to an increased co-morbidity of both infections (Spiegel *et al.,* 2003; Ojurongbe *et al.,* 2011).

Both malaria and intestinal helminths are diseases of poverty that thrive more in tropical low and middle income countries (LMIC) with anaemia as one of the possible outcomes (Hotez *et al.,* 2004; Ojurongbe *et al.,* 2011).

Surveillance studies of the world's population have shown that close to half of the population especially people inhabiting the tropics and subtropics suffer from infection caused by parasitic helminths and *Plasmodium* parasite of different species (Snow *et al.,* 2005).

Recent reports by the Nigeria Centre for Diseases Control (NCDC, 2020) and the WHO (2018), show that there is a steady decline in malaria cases globally, however, Nigeria with an estimated population of 200 million people still accounts for 25% of the global burden of malaria. Furthermore, studies show that the prevalence of intestinal helminths in Nigeria is about 54%and confirmed that the prevalence could still be as high as it was in the 20th century (Ekundayo *et al.,* 2007; Karshiman, 2018).While Intestinal helminths are neglected tropical diseases affecting mostly people living in the rural communities and the urban slums, almost everyone living in malaria endemic area is at risk of malaria (Hotez *et al.,* 2004; Relman and Choffnes, 2011; Dada-Adegbola, 2013; WHO, 2018).

There are over a billion cases of intestinal helminths in the world, accounting for almost half of global morbidity (Finkelstein *et al.,* 2008). Most of the people infected with these helminths are children who live in sub - Sahara Africa, and about 90% of these children are at risk of malaria. One of the most prevalent intestinal helminths are the hookworm species such as *Necatoramericanus* and *Ancylostomaduodenale*, with both causing blood loss in the gastrointestinal tract. Thushookworm is one of the major causes of hypoferric anaemia in children living in sub-Sahara Africa (Hotez *et al.,* 2004). It is also widely accepted that malaria parasite infection is an important contributor to anaemia especially in children and pregnant women (Noland *et al.,* 2014).

There are conflicting reports about the correlation between malaria and SHTs infections (Mutapi *et al.,* 2007).While some studies reported that tin malaria/helminth co-infection, parasite density is lowered (Spiegel *et al.,* 2003), others reported to the contrary (Nacher *et al.,* 2002). This study aimed to determine the co-occurrence of intestinal helminths and *Plasmodium* parasites and its effect in school children living in Ikot-Omin, a rural community in Calabar, Nigeria.

## 2. Materials and Methods

This is a prospective cross-sectional study conducted in public primary and secondary schools in Ikot Omin; a suburb of Calabar in Cross River State, Southern Nigeria. Ikot Omin is located at latitude $5^0$3’ 32” N and longitude $8^0$21’10” E. This area is mostly plantation settlement located at the tropical rain forest belt with average temperature and humidity of 28°C and 80.5% respectively. The study area has two seasons, rainy and dry seasons. The rainy season lasts for about 7-8 months (March to October).

The minimum sample size to detect a significant difference for malaria parasite infection, intestinal worm infection and malaria parasite and intestinal worm co-infection were 314, 384 and 63 respectively. The sample size with the highest numerical value was adopted as the sample size and a 10% attrition of the minimum sample size was added to account for missing data (Araoye, 2004).

*Exclusion criteria:* Children aged less than5 years and more than 15 years, less than 6 month’s residents in the community, history of antimalaria or anthelminthic drug usage in less than 2 weeks or 6months respectively, and refused consent of parents/guardians and assent by children.

*Collection of samples and preparation of blood films:* Blood samples were collected from the participants by finger prick for detection, speciation and malaria parasites count and for haematocrit (PCV). Also, a clean wide-mouthed plastic container was given to the participant after blood collection to collect freshly passed stool sample the following day. About 5.0g of freshly passed stool samples were collected from each of the participants

Thick blood film was made with approximately 8.0µl of blood spread out to obtain a diameter of approximately 10mm while 2.0µl of blood was used to make the thin film. The prepared films were allowed to dry in the air, and thin film fixed in absolute methanol. Both the thin and unfixed thick films were stained using 3% Giemsa’s stain prepared with a phosphate buffer solution of pH 7.2 for 30 minutes. After staining, they were both rinsed in the buffer solution, stood vertically to dry in the air and were examined by two microscopists independently with x100 objective lens of light microscope using immersion oil. The thin film was used for speciation

of *Plasmodium* while the thick film was used for detection and quantification of parasitaemia in each subject. The malaria parasite density was determined by counting the number of asexual parasites against 200 white blood cell in the blood film and using the method described by another study (Shute, 1988).

Blood samples were simultaneously collected into a heparinized microheamatocrit tubes and were spun for 5 minutes at 10,000g in Hawksleymicrohaematocrit centrifuge and the PCV level determined with the Hawksley reader (Hawksley and Sons Ltd, Sussex, UK) Anaemia status was determined according to normal PCV ranges with reference to age and gender by Dacie and Lewis (2001).

***Detection of intestinal helminths***

The stool samples were examined macroscopically for consistency, presence of blood stain, mucus, adult worms, segments and larvae of worms within 6 hours of collection. For the microscopic examination, about 1.0 g of each of the freshly passed stool samples was emulsified in about 5.0 ml. of physiological or normal saline. An aliquot of the homogenized solution was placed on one end of a glass slide, and covered with a coverslip. Another aliquot was placed on the opposite end of the same glass slide and a drop of Lugol's iodine added to it, mixed and coverslip. Both normal saline and iodine smears were examined with a binocular microscope using x10 and x40 objective lenses respectively for the presence of intestinal parasites. Furthermore, brine floatation concentration technique was used to ensure there was no false negative result. About 1.0 g of each stool sample was emulsified in about 5.0 ml of a saturated solution of sodium chloride (brine) that has the specific gravity of 1.2. The brine was added to the brim of universal plastic container used for the emulsification of the stool sample and a glass slide placed on top without leaving a space between the slide and container. The preparation was left for 15 - 20 minutes. Thereafter, the slide was carefully and quickly inverted without allowing a drop of the solution to fall off and examined with the x10 objective lens of the microscope.

Questionnaire was administered to the participants to obtain demographic data and to determine their knowledge of malaria and intestinal helminths

***Statistical analysis***

Statistical Package for Social Sciences (SPSS) version 20.0 was used for statistical analysis of the data. Chi-squared ($\chi^2$) and Z scores were used to compare proportions while t-test was used to determine means of parasite density in malaria mono-infection and co-infection with helminth

## 3. Results

A total of 578 children participated in the study. Males 296 (51.2%) were slightly more than females 282 (48.8%). More than half of the participants (56%) were between 9-12 years old, 28% were 5-7years old, and 16% were 13-16 years old.

Table 1 shows the distribution of malaria infection, intestinal helminth infections, and malaria-helminths co-infection by age. Overall, 207 (35.8%) of the participants had plasmodium parasitaemia of which *P.falciparum* was 99.5% and the youngest age group 5-8 years appeared to have the highest prevalence of malaria parasitaemia although this was not statistically significant (p = 0.271).Ninety-two (15.9%) participants had malaria infection only, 138 (23.9%) had intestinal helminths infection only, while 115 (19.9%) had malaria and intestinal helminths co-infection.

Co-infection was lowest among age group 9-12 (10.7%) although, this was not statistically significant (p = 0.640). Only hookworm infection showed a trend to be higher among 13 to 15 year-old group otherwise the differences in the distribution of the intestinal helminths infection were uniform across the age groups and not statistically significant.

Male participants also had a higher prevalence of malaria parasitaemia which was also not statistically significant (p = 0.165). The sex distribution of the intestinal nematodes showed that male children were more likely to be infected with Hookworm than the females (p = 0.048). There was no statistically significant difference in

Table 1: Distribution of malaria parasitaemia, helminths infection and co-infection by age

| **Age group (years)** | **No. (%) co- infected d** | **No. (%) infected with malaria parasite** | **No. (%) infected with *Ascaris*** | **No. (%) infected with Hook worm** | **No. (%) infected with *Trichuris*** | **No. (%) examined** |
|---|---|---|---|---|---|---|
| 5-8 | 28 (17.4) | 66 (41) | 51 (31.7) | 30(18.6) | 3 (1.9) | 161(27.9) |
| 9-12 | 67 (10.7) | 109 (33.7) | 106(32.8) | 74(22.9) | 10 (3.1) | 323(55.9) |
| 13-16 | 20 (21.3) | 32 (34) | 28 (29.8) | 29(30.9) | 2 (2.1) | 94 (16.3) |
| Total | 115 (19.9) | 207(35.8[a]) | 185 (32) | 133(23) | 15 (2.6) | 578 (100) |
| P-value | NS | NS | NS | NS | NS | |

the sex distribution of *Ascarislumbricoides* and *Trichuristrichiura*. Similarly, the prevalence of malaria /helminth co-infection was not statistically different in males and females; 20.3% and 19.5% respectively, (p = 0.450), (Table 2).

The prevalence of anaemia among the participants was 45.3% irrespective of their infection status. Anaemia varied by the type of infections and had the highest proportion of 58% among participants with helminth alone and the lowest proportion of 31.3% among participants who had neither malaria parasitaemia nor helminth infection (Table 3a). The difference was statistically significant (p < 0.0001). Boys (50.7%) were more likely to be anaemic compared to girls (39.7%; p = 0.010, Table 3b). Similarly, the age group 13-15 years showed the highest proportion of anaemia 58 (61.7%; p < 0.0001, Table 3c). We also found that 31% of the children who were not infected with either malaria or helminths were anaemic.

Table2: Distribution of malaria parasitaemia, helminths infection and co-infection by sex

| Gender | No. (%) infected with *Ascaris* | No.(%) infected with Hookworm | No. (%) Infected with *Trichuris* | No. (%) Infected with malaria parasite | No. (%)co-infected | No. (%) examined |
|---|---|---|---|---|---|---|
| Male | 98 (33.1) | 77 (26.0) | 8 (2.7) | 114(38.5) | 60 (20.3) | 296(51.2) |
| Female | 87 (30.9) | 56 (19.9) | 7 (2.5) | 93(33.0) | 55 (19.5) | 282(48.8) |
| Total | 185 (32.0) | 133 (23.0) | 15 (2.6) | 207(35.8) | 115(19.9) | 578(100) |
| P-value | NS | 0.048 | NS | NS | NS | |

NS- not significant

The mean parasite density in the malaria mono-infection was 899.3 (± 910.86) compared to 393.7 (± 70.67) in the malaria/helminth co-infection; mean difference (MD) 505.5; (95% CI 337.52-673.50; P< 0.0001).

When asked about their knowledge of malaria and intestinal helminths, 68% of the participants have good knowledge about malaria transmission while only 17% have a good knowledge of intestinal helminths (Z score = 5.474, p < 0.00001, Table 4)

Table 3a: Comparative prevalence of anaemia in co-infection, independent infections of malaria and helminth

| Status of infection | No. Infected | No (% anaemia) |
|---|---|---|
| Co-infection | 115 | 63(54.8) |
| Malaria alone | 92 | 46(50.0) |
| Helminth alone | 138 | 80(58.0) |
| Not- infected | 233 | 73(31.3) |
| Total | 578 | 262(45.3) |

Chi-square = 32.28, p < 0.0001

Table 3b: Prevalence of anaemia by gender

| Gender | Total | No (%) anaemic |
|---|---|---|
| Male | 296 | 150 (50.7) |
| Female | 282 | 112 (39.7) |
| Total | 578 | 62 (45.3) |

$\chi^2 = 6.999$, P = 0.010

## 4. Discussion

Our study confirms that there is a high prevalence of asymptomatic malaria in children living in an endemic area (Ojurongbe *et al.,* 2011). While younger children were more susceptible to asymptomatic malaria, there was no difference across all the age groups of the school children. Overall, there was no statistical association between gender and malaria mono-infection and co-infection with helminths, similarly no association between the age of the children and their infection status. However, our study observed that there was a statistically significant association between male gender and hookworm infection (p = 0.048). We cannot specifically state what is responsible for this because it was not assessed. It may be because boys exhibit a higher risk profile for worm infestation such as playing barefooted in contaminated soil compared to girls (Humphries *et al.*, 2013).

This study found that boys were more likely to be anaemic compared to girls. This finding is in congruence with the findings in this study that hookworm prevalence was higher among boys and it is a major cause of anemia in this setting. Similarly, the age group 13-15 years had significantly the highest proportion of anaemia compared to other age groups. This is also likely to be attributable to the highest rate of hookworm infections among this age group as shown in the analysis above. Furthermore, this study did not observe any significant association between malaria and anaemic status of the participants unlike what was observed in children infected with helminths. While we agree that malaria can lead to severe anaemia in infected cases, the association was not statistically significant in this study. it is believed that, the higher the age of the malaria cases, the lower the effect of parasitaemia on the haemoglobin (Brooker *et al.,* 2006). Over 70% of the particpants in this study were older than 8years.It was also observed that, the proportion of children with anaemia was higher in the helminth mono-infection compared to other groups, contrary to the report from Kimbi *et al.* (2012) where anaemia was higher in children with malaria/helminth co-infection. High prevalence of anaemia in this group could be largely due to the hookworm infection.[10] Not surprising, because hookworms are largely responsible for significant blood loss in the gastrointestinal tract of infected children[4]. Additionally, intestinal helminths cause malnutrition which is a risk factor for anaemia (Rahman *et al.,* 2019).

This study has equally shown that participants with only malaria parasite infection of which *P. falciparum* was over 99%, recorded more parasite densities than subjects with malaria-intestinal parasites co-infection. Helminth has been widely reported as a modulator of the immune system in the presence of malaria co-infection thus, inhibiting malaria parasite proliferation and providing reduced susceptibility to malaria parasite (Murray *et al.*, 1977).The underlying mechanisms for this phenomenon require further studies. There has been conflicting reports about the effect of helminths on malaria parasite burden, our study showed that helminth infection reduced malaria parasite density significantly compared to malaria mono-infection. Our study supports findings from another study conducted in Tanzania (Kinung'hi *et al.*, 2014). The limitation of our study was that brime floatation technique was used, thus we may have missed eggs of other helminths that do not float in saturated saltwater.

Table 4: Demographic information and evaluation of socio-economic risk factors

| | Assessment of awareness of infections | | | |
|---|---|---|---|---|
| **Infection status** | **Good (%)** | **Fair (%)** | **Poor (%)** | **Total (%)** |
| Malaria parasite infection | 394 (68.1) | 101 (17.4) | 83 (14.3) | 578 (100) |
| Intestinal nematodes infection | 100 (17.3) | 296 (51.2) | 182 (31.4) | 578 (100) |

Z score = 5.474, $p < 0.00$

Furthermore, children had better knowledge of malaria transmission compared to their poor knowledge of intestinal helminth infection. Improving their level of knowledge on intestinal helminth infection may be an important starting point in achieve a successful and lasting social and behavioural change communication. To successfully eliminate intestinal helminth, an integrated approach may be useful that includes the educational intervention of the public in addition to mass drug administration.

## 5. Conclusion

Overall, almost half of the participants were anaemic irrespective of their infection status and will benefit from mass deworming program and the use of long-lasting insecticide-treated nets (LLITN). Also, there is a need for educational intervention for preventing malaria and helminth infections targeted at children living in endemic communities. Additionally, we propose further study should be carried out that will study the interaction between malaria parasites and intestinal helminths and how they influence each other

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