Prevalence of Asymptomatic Malaria and Intestinal Helminthiasis Co-infection among Children Living in Selected Rural Communities in Ibadan Nigeria

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Abstract Malaria and Intestinal helminth parasites co-exist in the tropics due to prevailing climatic conditions and poor sanitary practices. These parasites have adverse effects on cognitive development, educational performance and school attendance of children. The epidemiology of these parasites and their co-infection among children have not been fully documented in Nigeria, community-based studies are limited. This study aims to highlight the burden of malaria parasites and intestinal helminths among children living in rural areas. A community-based cross-sectional study involving children aged 6 months - 14 years was carried out in six rural communities. Single stool and finger prick blood samples were collected. Wet mount and formol-ether techniques were employed to process stool samples for microscopy while Giemsa-stained thick blood smears were used to screen for *Plasmodium. falciparum* parasites. Overall prevalence of *Plasmodium. falciparum* asexual parasites, Intestinal helminth infections and malaria intestinal helminth co-infection were 52.3%, 35.9%, and 57.1% respectively. *Ascaris lumbricoides* was the only intestinal helminth species identified among the children. The prevalence of asexual *Plasmodium.falciparum* parasites significantly decreased with age (χ^2 =15.05, p<0.001) and was highest among children aged 5 years and below (χ^2 = 15.97, p<0.0001). Odds of Intestinal helminth infection were significantly higher among children above 5 years of age compared to those aged 5 years and below (OR = 1.97, CI = 1.03, 3.76). Prevalence of co-infection was least among the early adolescents (11-14 years) compared to the other age groups (χ^2 = 16.16, p<0.0001). Intestinal helminth and its co-infection with malaria constitute a major health burden among children in rural Nigeria, however with differentials in age.

Keywords: *malaria, intestinal helminth, co-infections, children, prevalence*

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1. Introduction

Malaria and soil-transmitted helminth infections are widely distributed in tropical and subtropical areas and are both of public health concern. About 85% of malaria deaths occur in children under five years of age and every 30 seconds a child dies from malaria [1]. According to the latest world malaria report, 102 countries are still endemic for malaria with an estimated 219,000,000 cases of malaria and 660,000 deaths. About 25% of these deaths occur in Africa. The Democratic Republic of the Congo and Nigeria account for over 40% of the total estimated malaria deaths and 40% of the estimated malaria cases globally [1]. In Nigeria malaria leads to 60% outpatient visits and 30% of hospitalizations among children under

five years of age and has more reported deaths due to malaria than any other country in the world [2].

Soil-transmitted helminths affect approximately 29% of the world's population with the highest prevalence in sub-Saharan Africa, Southern America, China and East Asia [3]. In Nigeria, intestinal helminth infections with *Ascaris lumbricoides*, *Trichuris trichiuria* and hookworm, have remained prevalent. Majority of those affected are young children between the ages of 5 and 14 years living in rural areas and urban slums [4]. *Nigeria has the greatest number of people infected with Neglected Tropical Diseases such as the intestinal helminth infections, schistosomiasis, and lymphatic filariasis (LF) among all the countries in Africa* [5].

Children are particularly susceptible to the adverse effects of these two infections [malaria and intestinal helminthiasis] due to their incomplete physical development

and their greater immunological vulnerability, high mobility and lower standards of hygiene [6]. Soil transmitted helminth in tropical regions of the world have always indicated at least 10% occurrence among any study population. School children remain the age group most vulnerable. The prevalence of ascariasis among school children aged 1-16 years ranges between 28.6% to 75.6% being influenced by climatic conditions and socio economic factors such as poverty, poor sanitation, poor water supply, poor hygiene, and lack of protective clothing as well as limited access to preventive measures and health care [7-14].

In the past decade there have been an increasing number of studies on co-infections between worms and malaria. The results of interaction of this co-infection have been reported differently at times to be protective or to exacerbate incidence of acute malaria or its clinical outcome [15,16,17,18]. Despite the implications of similar environmental and socio-economic conditions in the distribution of malaria and intestinal helminths, as well as the susceptibility of children; few studies have explicitly focused on the burden of malaria and intestinal helminthiasis on children in Nigeria. In this study we explored the occurrence of Malaria and IH, as well as their co-infection among children living in rural communities in Southwest Nigeria.

2. Methods

Study area: This study was carried out in Ona-ara Local Government Area (LGA) which lies southeast of Ibadan, the capital of Oyo state. Malaria is endemic in the area with perennial transmission. Its wet season runs from March through October, November to February forms the dry season. The LGA is divided into 11 wards - 3 urban, 2 peri-urban and 6 rural wards. The rural wards were selected purposefully for this study, considering possibility of greater spread of helminthiasis and malaria in rural environments. Six communities were selected (one from each of the six rural wards) based on largest population size, accessibility of road and the informed consent and assistance of the village heads.

2.1. Study Period and Population

The study period was from February 2011 to June 2011. Children between the ages of 6 months to 14 years, who have been residing in the community for up to six months or more with no symptoms of malaria or any apparent illness as at the time of recruitment and whose caregiver provided written or verbal informed consent were enrolled into the study.

2.2. Collection of Samples

Research assistants visited all households with eligible children in the six rural communities selected. In case no one was available for the survey or no one was at home when a research assistant visited, or there was no eligible child or the household head refused to give consent, the research assistant proceeded to the next household.

For each child recruited, firstly, data such as age, sex and axillary temperature were obtained from the head of household/caregiver of the child and recorded in a structured questionnaire. Then, capillary blood from a finger prick was used for the preparation of thick blood films and filling of a heparinized capillary tube for the determination of packed cell volume. Thick blood smears were made on slides which were air-dried and taken to the research Laboratory in the Institute of Advanced Medical Research and Training, College of Medicine, University of Ibadan for microscopy. A clean and well labeled stool container was given to the caregiver with instructions on how to collect the child's stool sample the next morning. The research assistants returned very early in the mornings [between 8.00 and 10.00am] to collect the stool samples which were transported in cool-packs to the parasitology laboratory of the University College Hospital, Ibadan for microscopy.

2.3. Laboratory Diagnosis

Processing of blood films and microscopy

Thick blood films were stained with 10% Giemsa and examined for malaria parasites by two experienced microscopists and results were provided to caregivers the following morning. A sample was considered negative if no asexual blood stage parasite either ring forms or gametocytes was seen in 100 oil-immersion fields (x1000 magnification) after reading through 200 fields. In case of discrepancies, the slides were re-read again by a senior investigator (COF).

2.4. Processing of Stool Samples

Stool samples were processed using the wet mount identification technique and formal ether concentration technique as described by Cheesbrough [20] and examined for the presence of intestinal helminth ova. The presence of any helminth ova was considered positive; the absence of it, negative. Children who had parasitaemia were treated with artemether-lumefantrine (AL) while those diagnosed with intestinal helminths were treated with Pyrantel pamoate (Combantrin) at standard dosages.

2.5. Determination of Packed Cell Volume

Filled microhematocrit tubes were centrifuged in a microhematocrit rotor for 5 min at 10,000 g. PCV values $\leq$ 31% were considered as anaemia, which was further classified as mild (21–30%), moderate (15-20%), or severe ($\leq$15%) [9,19].

2.6. Data Analysis

The data was entered in EPI data 3.1 statistical package and analysed using Statistical Package for the Social Sciences (SPSS) version 16.0. The outcome variables measured included malaria parasitaemia, helminthiasis and co-infection with malaria and intestinal helminths. Descriptive statistics such as frequencies, proportions, means and confidence intervals were used to summarize the data. Categorical data were compared using the Chi-square test or the Fisher's exact test, as appropriate, a p-value of < 0.05 was considered statistically significant.

3. Results

3.1. Characteristics of the Study Population

Three hundred and seventy children satisfied the inclusion criteria and were enrolled into the study. The mean age of the study population was 1.5 ± 0.02. There were 195 (52.7%) females and 48.1% of participants was from the 0-5 years old. For malaria tests, 365 participants gave assent for blood sample collection, 167 participants submitted samples for stool analysis while 105 submitted both samples.

3.2. Prevalence of Anaemia with Respect to Age and Sex

More than half (60.3%) of the study population were anaemic, among these; 183 (82.0%), 33(15.0%), 7 (3.0%) had mild, moderate and severe anaemia respectively. There was a significant association between anaemia and age (χ^2 = 6.35, p = 0.012). Children aged ≤ 5 years were two times more likely to be anaemic compared to those > 5 years (OR = 2.02, 95% CI = 1.20, 3.40). Table 1below shows the prevalence of anaemia and anaemic levels with respect to age groups. No association was observed between anaemia and sex.

Table 1. PREVALENCE OF ANAEMIC LEVELS BY AGE GROUPS

AGE GROUP	ANAEMIC LEVELS		
	MILD N (%)	MODERATE N (%)	SEVERE N (%)
≤ 5 YEARS	85 (38.1)	20 (9.0)	7 (3.1)
> 5 YEARS	98 (43.9)	13 (5.8)	0 (0)
TOTAL	183 (82.0)	33 (14.8)	7 (3.1)

3.3. Prevalence of P. falciparum Asexual Parasites with respect to Sex, Age and Anaemic Status

Among 365 participants tested for malaria parasites, 191 tested positive, this gave an overall *Plasmodium falciparum* prevalence of 52.3%. Prevalence in males was similar to that in females. A higher prevalence of *P.falciparum* parasites was recorded in the age group ≤ 5 years (58.0%) compared to that among the > 5 years age group, this difference was significant (χ^2 = 4.312, p = 0.038). The prevalence of malaria parasite according to sex, age group and anaemic status is shown in Table 2.

Table 2. PREVALENCE OF P.FALCIPARUM BY SEX, AGE GROUP AND ANAEMIC STATUS

CHARACTERISTIC	n (%)	p
*Sex**		0.68
Male	93 (53.8)	
Female	98 (51.0)	
*Age group**		0.00
0 - 5 years	102 (58.0)	
5.5 - 10 years	77 (54.2)	
11 - 14 years	12 (25.5)	
*Anaemic **		0.14
Yes	109 (49.3)	
No	49 (59.8)	

*n = 365; **n = 303

3.4. Prevalence of Intestinal Helminthiasis with Respect to Sex, Age and Anaemic Status

One hundred and sixty seven (167) stool samples were analyzed for the detection of any intestinal helminth. *Ascaris lumbricoides* was the only intestinal helminth species detected among the stool samples tested. Overall prevalence of *Ascariasis* was 35.9% and was highest (51.4%) among the growing children (> 5 – 10 years) than in the other age groups. Similar prevalence was detected among both sexes. The association between children's characteristics and intestinal helminth test result are shown in Table 3.

Table 3. PREVALENCE OF HELMINTHIASIS BY SEX, AGE GROUP, ANAEMIC STATUS

CHARACTERISTIC	n (%)	p
*Sex**		0.30
Male	35 (40.2)	
Female	25 (31.2)	
*Age group**		0.06
≤ 5years	22 (27.8)	
> 5years	38(43.2)	
*Anaemic***		0.33
Yes	36 (36.7)	
No	20 (45.5)	

*n = 167; **n = 142.

3.5. Prevalence of Co-infection with Malaria Parasites and Intestinal Helminth

Among the one hundred and five children who submitted both blood and stool samples, 45 (42.9%) tested positive for malaria and helminth parasites. Also, a significant association existed between the age groups and co-infection (χ^2 = 6.73, p = 0.034), more than half (56.8%) of the co-infected children were in the 5.5 - 10 years age group. Children in this age group were about two times more likely to be co-infected with malaria and helminthiasis compared to under-fives (OR = 2.3, 95% CI = 0.19, 0.95). There was significant relationship between anaemic status and co-infection status. Table 4 shows the association between children's characteristics and co-infection status.

Table 4. PREVALENCE OF CO-INFECTION BY SEX, AGE GROUP, ANAEMIC STATUS

CHARACTERISTIC	n (%)	p
*Sex**		0.32
Male	27 (48.2)	
Female	18 (36.7)	
*Age group**		0.11
≤ 5 years	19 (34.5)	
>5years	26 (52.0)	
*Anaemic **		0.93
Yes	27 (45.0)	
No	49 (48.4)	

*n = 105; **n = 91.

4. Discussion

Findings from this study demonstrate that prevalence of asymptomatic malaria parasitaemia, intestinal helminth infections, their co-infections and anaemia among children in the rural communities studied is of public health concern. The prevalence of anaemia in the study area is higher when compared with a similar study carried out

earlier in another part of Southwest Nigeria [9] and lower to the prevalence reported by Abanyie *et al* [21] in a recent publication. Also higher prevalence of anaemia recorded among the under fives compared to the older children was observed in previous studies in Nigeria [22,23] and Angola [24].

With an overall falciparum malaria prevalence of 52.3%, the study area has high transmission and is therefore hyper- endemic for malaria; this is in line with the descriptions of high transmission areas according to the WHO policy brief on malaria [25] and is similar to previous results observed in Nigeria and Gambia [21,26,27]. This high prevalence of asymptomatic malaria observed in the dry season calls for attention. It implies numerous potential reservoirs for malaria transmission and probably one of the reasons why malaria prevalence remains hyper-endemic in the study area. This data therefore could be helpful in predicting the probable force of infections when malaria seasons approach.

There was a significant association between the children's age group and malaria ($p < 0.05$). Children under 5 years had the highest prevalence (58%) of malaria; this supports existing propositions [28, 29, 30] and agrees with the fact that in an area of stable malaria, immunity develops progressively from early childhood to adolescence [9,13].

An overall prevalence of 35.9% was observed for intestinal helminth infections among the children in this study. This shows a decline in the prevalence, when compared to the values reported in 2005 in the same area and during the same season [31]. The decline in prevalence of helminth infections could be due to the community-based mass distribution of Ivermectin in the study area. This anti-helminthic campaign is being carried out to reduce onchocercal microfilaria worm loads and has been noted in previous studies to also reduce the prevalence of other helminth infections [48,49].

However, this result is comparable to a prevalence of 36.1% for a rural area in an earlier study conducted in Ife, Nigeria [32] and is still relatively high according to WHO manual for preventive chemotherapy in human helminthiasis [33]. The study area falls under the Moderate-risk areas ($\geq 20\%$ and $< 50\%$ prevalence); thus the recommended frequency of once a year re-treatment should be adhered to. The relatively high prevalence of helminth infections in this study population could be due to of poor environmental sanitation/hygiene and improper sewage disposal in the area.

A higher proportion (43.2%) of those infected with intestinal helminth were children aged 5 years and above, this could be traced to greater mobility and outdoor behaviors characteristic of this age group. They are more likely to walk to the bush; they are known to be more active and frequently make contact with soil and other contaminated materials (exposing them to the ingestion of ova as food contaminants) compared to the under fives who, though make contact with soil are still better protected by their carers. Similar conclusion has been drawn from existing review articles [34,35,36].

The prevalence of co-infection (48.2%) observed in this study is higher than that (4.3%) from a previous study in Osun state, Nigeria [9] but lower to a baseline prevalence (55%) in a randomized control trial conducted in the same area [21]. This could be related to the study age groups, study periods and designs of the studies. A previous study in Cameroun observed a 24.3% prevalence of co-infection [19]; another in Tanzania [37] detected a 16.5% prevalence rate.

In this study, children without intestinal helminth infections were about two times more likely to test positive for malaria parasite compared to children with helminth infection (OR = 2.46, CI 1.21, 5.02). This does not agree with the findings of Ojurongbe *et al* [9] and Abanyie *et al [21]* in Osun state, Nigeria and previous related studies in Thailand and Ethiopia[38, 39] where, a positive and statistically significant association between geohelminth and malarial infection were reported. Reasons for these are not very clear and calls for further research. However, in support of the findings of this study, a prior research conducted in rural area of Ghana reported helminth infection to be associated with increased levels of Interleukin- (IL-) 10 [40], an anti-inflammatory cytokine known to inhibit the protective immune responses against malaria parasites and to be involved in exacerbating parasitemia during Plasmodium infection [41,42,43,44,45]. Results from the Ghanaian study, suggest that helminth infections may alter the antimalarial immune response through suppression of proinflammatory activity. Also animal models [46,47] have shown that helminth infections can lead to the induction of regulatory T cells that have the ability to inhibit both Th1 and Th2 responses directed to other pathogens. This buttresses the fact that more research is still needed to demystify the actual course and role of helminths in their interactions with malaria parasite, this will be very relevant for a more scientific and evidence-based approach in the prevention, control and management of malaria-helminth co-infections.

5. Conclusion

This study showed that malaria, helminthiasis and co-infection of both are common among children in rural areas of Ona-ara LGA. Most importantly, it confirmed that age is an independent risk factor for *both parasitic infections.* These findings stand to guide future research on prevention and control of parasitic infections among children living in rural areas of Nigeria; it has also provided evidence-based information to plan and design effective interventions for malaria case management among children of different age groups. Community-based malaria and intestinal helminth integrated control programmes among children and health education for caregivers is strongly advocated.

Ethical Consideration

Permission was obtained from the local government secretariat and head of the communities. The ethical approval for the study was obtained from the State Ethical Review Committee, Oyo state, Nigeria.

Authors' Contributions

Authors IOA and CA contributed to the conception of the study. IOA, CA and COF designed and developed the

study protocol. CA was instrumental in data collection and supervision of research assistants. COF supervised malaria microscopy. HOD supervised stool sample collection and microscopy. CA and IOA undertook the analysis. CA and IOA produced the draft and all authors thereafter reviewed it several times over till the final draft was produced. All authors read and approved the final manuscript.

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Conflict of Interest Statement

The authors declare that they have no conflict of interests.

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