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The prevalence and density of asymptomatic plasmodium infections among children in a rural community of South- Western Nigeria.

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Abstract

Asymptomatic malaria is highly prevalent in endemic areas of Africa and it is a new challenge for the malaria elimination agenda. In recent years, the number of hospitalizations due to malaria cases in sub-Saharan Africa has steadily decreased. However, there are indications that this positive trend could suffer a setback due to asymptomatic malaria cases, which may serve as reservoir of infections for vulnerable populations. This cross-sectional study examined the prevalence of asymptomatic malaria in school-aged children living in a rural community in South- West, Nigeria. The study was conducted from August to September 2020, involving 300 randomly selected school children. Histidine rich protein II rapid diagnostic test kits (HRP2) were used to screen participants for malaria. All blood samples were further examined microscopically using 3% Giemsa-stained blood smears for confirmation, speciation, and quantification of malaria parasites. The anaemia status of the children was determined by measuring the haematocrit. Statistical analysis of the data was done using the chi-square test, student t-test, Mann Whitney U test and Kruskal-Wallis rank sum tests to determine the association between variables and the difference in mean/median with a significance level $p \leq 0.05$. The results showed a high prevalence of asymptomatic malaria of 41% by microscopy. The prevalence of asymptomatic malaria was statistically significantly higher among children aged 6 to 11 years (52%), compared to children >12years (37%), and < 5years old (13%), (p=0.00001). *Plasmodium falciparum* was responsible for 96% of the infection while the remaining 4% had multiple infections with *Plasmodium falciparum and Plasmodium ovale*. This study confirms that the prevalence of asymptomatic cases of malaria among children in malaria endemic areas is high and they will benefit from malaria chemoprophylaxis.

Keywords: Asymptomatic malaria, Anaemia, Children, Plasmodium, malaria

Introduction

Malaria remains an important and potentially life-threatening disease with pregnant women and under-five year old children being the most vulnerable. It is estimated that there were 241 million malaria cases and 627 000 malaria deaths worldwide in 2020, with 95% of malaria cases (228 million) and 96% of deaths (602 000) worldwide occurring in the WHO Africa region. Nigeria contributed about 32% of global malaria deaths as at the time of this report (WHO, 2020). In 2015, the national prevalence of malaria among children under five years of age was 27% (via microscopy) (NMEP, 2016). However, there are wide geographical differences, with the percentage of children under five with malaria (via microscopy) as high as 64 percent and 63 percent in Kebbi and Zamfara States in the Northwest and as low as five percent in some southern states of Nigeria (NMEP, 2016). In recent times, global investment in malaria control has resulted in reductions in the disease burden but countries such as Nigeria require more targeted interventions to reach the required target reductions in deaths and morbidity from malaria (Ochwedo *et al.,* 2021).

Recently, the WHO updated international guidelines on malaria control with new recommendations on reducing the burden of disease through chemoprevention in at-risk groups (WHO, 2022). These regulations address the variations in the distribution of malaria risk in endemic settings and the contribution of asymptomatic infections to disease burden. Asymptomatic malaria infection is defined by the presence of asexual (trophozoites) and sexual (gametocytes) stages of the parasite in the blood in the absence of clinical symptoms (WHO, 2016). These asymptomatic infections result in morbidity associated with malaria and are an important driver of transmission as reservoirs of infection (Niang *et al.,* 2017; Ochwedo *et al.,* 2021). With the new WHO guidelines, countries such as Nigeria are able to tailor interventions to populations and locations that are at risk. To support this, it is important to determine existing trends in asymptomatic infection burden to identify priority areas for new interventions or intensification of existing strategies. In this paper, we present results of a cross-sectional survey aimed at determining the prevalence asymptomatic malaria among school children living in South- West, Nigeria.

Materials and Methods

Study design, population and setting

This cross-sectional study was conducted in Omuo-Ekiti, South West Nigeria, about 400km from Lagos, the commercial capital of Nigeria. Omuo Ekiti enjoys a tropical climate with two distinct seasons: the rainy season, between April and October, and the dry season, between November and March and has tropical forests and savannas.

The study was conducted from August to September 2020 and included 300 randomly selected school children as subjects. After permission was obtained from the village head to conduct the study, the village announcer was assigned the role of informing the community about the study. Information was also shared at the local school by the researchers and teachers. Parents of children attending the schools were invited to provide a written consent for their child to participate in the study. Inclusion criteria into the study were; school children, age up to 15years old, residence in the community for at least 6 months, written informed consent from parents/guardians as well as an assent for children aged $\geq$ 11years. Eligible children were given information forms to take home and where the parents were satisfied, they returned a signed copy of the consent form. Children not meeting the eligibility criteria or whose parents did not return or refused to provide consent were excluded. Before collection of samples, information regarding the study was explained to the parents and children and written consent was obtained for participation in the study.

Sample size:

The sample size was determined using the formula for estimating single population proportion (Charan and Biswas, 2013) based on the prevalence of 25.6% from similar setting (Ojurongbe *et al.,* 2011).

Sample collection and Laboratory investigation

Venous blood sample was withdrawn into an EDTA bottle labeled with study identification number of each consenting child. Histidine rich protein 2 RDT kit (Standard Diagnostics, Bioline Malaria Ag P.f, Korea) was used were used to screen samples obtained from participants for malaria at the field for rapid detection of malaria following the manufacturer's manual. The remaining blood samples were transferred to the Laboratory for thick and thin blood films for microscopic detection of malaria parasites and pack cell volume (PCV) estimation. Microscopy being the gold standard for malaria diagnosis, here we lay emphasis on the results obtained by microscopy using 3% Giemsa stain.

For the thick and thin films, three drops and one drop of blood was used to prepare the thick and thin films respectively on the same slide. Simultaneously, a plain Hawksley capillary tube was filled to a pre-determined mark to estimate the PCV.

The thick and thin blood film were allowed to air dry on flat surface away from flies and ants after which they were stained in 3% Giemsa stain on a rack for 45 minutes. After 45 minutes, the slides were blued in water, thereafter the slides were positioned in a slant position on a rack and allowed to dry. The slides were examined

independently by two Laboratory Scientists microscopically using at x100 oil immersion objective lens, discordant results were resolved by a third microscopist. At least 100 high power microscopic fields of the tick films were examined before declaring a slide negative. Where parasites were seen, the parasites were counted against 200 white blood cells (WBC). However, where 500 parasites were counted before reaching 200 WBC in the thick blood smear, the counting would end. The parasitaemia density, which is the parasite/µL of blood, was enumerated by dividing the malaria parasite counted by the total number of WBC counted in the thick smear and multiply by 8000. This is assuming that an average individual would have 8000 leukocytes/µL of blood (Shute, 1988).

Plain capillary tubes were filled up to three quarter of the tube and spun for 5 minutes at 11,000g in a microhaematocrit centrifuge (Hawksley and Sons Ltd, Sussex, UK), and the PCV values were measured with a haematocrit reader. Anaemia status was determined according to normal PCV ranges with reference to age and gender.

Data management and analysis

All data were double- entered into Microsoft Excel spreadsheets version 2016 and cross-checked for errors. Data were processed and analyzed using the SPSS.20 statistical software package (IBM Corp. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY). Data was summarized as proportions and differences in participant characteristics and the relationship between malaria parasitaemia and covariates such as age group and geographical areas was assessed using Chi-squared ($\chi 2$) or Fisher's exact tests. The student t-test was used to assess the statistical significance of the mean difference between male and female while the ANOVA test was used to determine the statistical significance of the mean difference between age groups. The Mann Whitney U and Kruskal-Wallis rank sum tests was used to assess the statistical significance of the difference in median between gender and age groups, respectively. The level of statistical significance for all tests p was ≤ 0.05.

Ethical consideration:

Ethical clearance for this study was obtained from the Federal Ministry of Health Ado Ekiti ethical review committee (MOH/EKHREC/EA/U/06)

Results and Discussion

Overall, the prevalence of asymptomatic malaria parasitaemia was 41% by microscopy and 42% by HRP2 test kit. Three hundred children were enrolled, females were 158 (53%), the mean age was 9.83 ± 3.391 years, and the mean temperature was $36.8^{0c} \pm 0.32$. The age group 6-11years had the highest proportion of participants in this study (151/300, 50%), followed by the age group >12 (109/300, 36%), and the least proportion of participants were children in ≤ 5 years (40/300, 13%). The age groups were evenly distributed among male and females, figure 1.

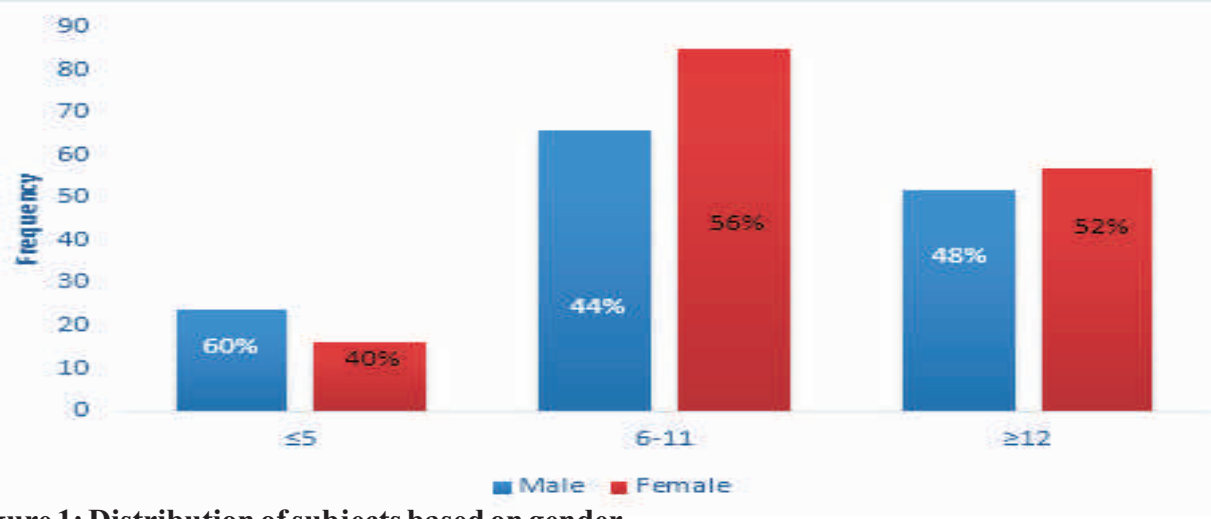

Figure 1: Distribution of subjects based on gender

Plasmodium falciparum was responsible for 96% of the infection while the remaining had multiple infections with *Plasmodium falciparum and Plasmodium ovale*. The prevalence of asymptomatic malaria was highest in age group 6-11year, 78/151 (52%), followed by ≥12, 40/109 (37%) and the least occurrence was in in ≤5 years, 5/40(13%); the association was statistically significant (p< 0.00001). However, there was no statistically significant association between gender and the occurrence of asymptomatic malaria children (p < 0.77369) (Table 1).

Table1: Th e distribu ion of asym otomatic malar a by age group and gender.

		HRP2			Microscopy		
		No. examined	No. infected (%)	P-value	No examined	No. infected (%)	P-value
Age	≤5	40	6(16)		40	5(13)	
	6 – 11	151	80(53)		151	78(52)	
	≥12	109	40(37)	.00001	109	40(37)	.00001
	Total	300	126(42)		300	123(41)	
Gender	Male	142	62(44)		142	60(42)	
	Female	158	64(41)	.66783	158	63(40)	.77369
	Total	300	126(42)		300	123(41)	

The median parasite density was 160 (IQR 80-480). There was no association between parasite densities and age groups (0.2369), or gender (0.9452), in asymptomatic malaria cases. Table 2.

Table 2: Parasite densities by age groups and gender

Characteristics	Median parasite density	IQR	p-value
Age (years)			
≤5	448	192-480	0.2369*
6-11	168	80-544	
≥	96	64-376	
Sex			0.9452**
Male	176	72-480	
Female	160	80-544	

*Kruskal Wallis test; **Mann Whitney U rank sum test

There was a statistically significant mean difference in PCV among participants' age groups (p < 0.001). In contrast, it was not statistically significant by gender (P=0.952). (Table 3).

Table 3: Mean pack cell volume by Age group and gender of the included children

Age (Years)	N (%)	Mean PCV (±)	P-value	Gender	N (%)	Mean PCV (±)	P-value
<=5	40 (13.3)	31.03 (5.46)	0.001	Male	142 (47)	33.18 (4.67)	0.952
6 – 11	151 (50.3)	32.68 (4.00)		Female	158 (53)	33.15 (4.10)	
>=12	109 (36.3)	34.61 (3.94)					
Total	300 (100)				300 (100)		

Furthermore, the mean PCV of the positive asymptomatic malaria children was lower (32.3% ± 3.96%) than the mean PCV of children who were negative for malaria (33.7% + 4.56%), however, the difference was not significant statistically (p=0.006).

Discussion:

The prevalence of malaria in asymptomatic children was 41% in this study, a result higher than the national average. Our result was similar to (Yapi *et al.,* 2014), who reported 63% of asymptomatic malaria in Cote d'Ivoire, also above their national average. In addition, asymptomatic malaria was significantly more common in children over five years (P= 0.00001), however, there was no significant difference in the parasite densities among the age groups (p=0.2369). It is likely that the prevalence of asymptomatic malaria was lower in children under the age of five probably because they are the target of malaria control interventions such as use of long-lasting insecticide-treated net (LLITN) and chemoprophylaxis like intermittent preventive treatment with sulphadoxine pyrimethamine (IPTc-SP) in Nigeria (NMIS, 2011). Additionally, it is believed that adults and older children have had different episodes of malaria over time, and acquired some form of immunity to the parasites. Children under the age of five are more likely to carry asymptomatic infections (Zaw *et al.,* 2017).

As expected, *Plasmodium falciparum* was the most common *Plasmodium* species, with multiple infections with *Plasmodium ovale* occurred in only 4% of the participants. *Plasmodium falciparum* is the most pathogenic of all *Plasmodium* species responsible for about half a million deaths annually worldwide (WHO, 2017). One of the adverse effects of *Plasmodium falciparum* is anaemia because it lyses all types of red blood cells (Ojurongbe *et al.,* 2011). This is reflected in the moderately low mean PCV of the participants, however, other factors such as malnutrition, worm infections could also be contributing factors (Oduwole and Igwe, 2022). This study did not evaluate effect of other factors on the participants.

Furthermore, asymptomatic malaria cases in the study area may form a group of individuals called asymptomatic carriers and can become a reservoir of malaria parasites in the community, which is readily available for transmission by female *Anopheles* mosquitoes (Kimbi *et al.,* 2012). Asymptomatic parasitaemia represents a

significant threat to malaria control and in particular to its eradication, as it provides reservoirs for the parasites to reestablish the malaria transmission cycle, thus disrupting the efforts of national malaria control programs targeted at controlling malaria transmission (Gerardin *et al.,* 2016). The limitation of this study was that there was no follow-up of the asymptomatic cases to determine the proportion that will eventually develop malaria symptoms.

Conclusion

In conclusion, it is without a doubt that to eliminate the malaria, interventions must also target asymptomatic individuals who harbor the reservoirs of the *Plasmodium* parasite. Nigeria policy makers need to key into the updated WHO guidelines on malaria chemoprevention and elimination.

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References

Charan, J. & Biswas, T. (2013). How to calculate sample size for different study designs in medical research?. *Indian Journal of Psychological Medicine*; ***35*(2)**: 121-126. doi:10.4103/0253-7176.116232.

Gerardin, J., Ouédraogo, A.L., McCarthy, K.A., Eckhoff, P.A. & Wenger, E.A. (2015). Characterization of the infectious reservoir of malaria with an agent-based model calibrated to age-stratified parasite densities and infectiousness. *Malaria Journal*; ***14*(1)**:1-13. doi:10.1186/s12936-015-0751-y.

Kimbi, H.K., Keka, F.C., Nyabeyeu, H.N., Ajeagah, H.U., Tonga, C.F., Lum, E. & Lehman, L.G. (2012). An update of asymptomatic falciparum malaria in school children in Muea, Southwest Cameroon. *Journal of Bacteriology Parasitology*; ***3*(154)**:2. doi:10.4172/2155-9597.1000154.

National Malaria Elimination Programme (NMEP). National Population Commission of Nigeria (NPCN), National Bureau of Statistics (NBS), & ICF International. Nigeria malaria indicator survey 2015. Abuja, Nigeria and Rockville, Maryland, USA: NMEP, NPCN, NBS, & ICF International. 2016; Retrieved from https://dhsprogram.com/pubs/pdf/MIS20/MIS20.pdf Accessed November 12, 2022.

Niang, M., Thiam, L. G., Sane, R., Diagne, N., Talla, C., Doucoure, S. & Toure-Balde, A. (2017). Substantial asymptomatic submicroscopic Plasmodium carriage during dry season in low transmission areas in Senegal: Implications for malaria control and elimination. *PloS One*.; ***12*(8)**, e0182189 doi:10.1371/journal.pone.0182189.

Nigeria Malaria Indicator Survey. (NMIS) (2010). "National Population Commission, National Malaria Control Programme and ICF International". *Demographic and Health Survey*; Available from https://dhsprogram.com/methodology/survey/survey-display-392-cfm. Accessed November 5, 2022.

Ochwedo, K.O., Omondi, C.J., Magomere, E.O., Olumeh, J.O., Debrah, I., Githeko, A.K., Kazura, J.W., Mukabana, W.R. & Guiyan, Y. (2021). Hyper-prevalence of submicroscopic Plasmodium falciparum infections in a rural area of western Kenya with declining malaria cases. *Malaria Journal*; **20**: 1-5. doi:10.1186/s12936-021-04012-6.

Oduwole, O.A. & Igwe, E.M. (2022). Hyperendemicity of intestinal parasitic infections among children in a rural community in Ondo State, *Nigeria. Journal of Basic and Applied Medical Science*; **2(1)**: 1-5.

Ojurongbe, O., Adegbayi, A.M., Bolaji, O.S., Akindele, A.A., Adefioye, O.A., & Adeyeba, O.A. (2011). Asymptomatic falciparum malaria and intestinal helminths co-infection among school children in Osogbo, Nigeria. *Journal of Research in Medical Sciences: The Official Journal of Isfahan University of Medical Sciences*; ***16*(5)**:680 -686.

Shute, G.T. (1988). The microscopic diagnosis of malaria. In: Werndorfer, W. H & McGregor L. (eds) *Malaria Principle and practice of Malariology*. Edinburg: Churchill Livingstone; Pp 23.

World Health Organization. (2017). *World Malaria Report*; Available at http://www.who.int/malaria/publications/world-malaria-report-2017/report/en/. Retrieved November 12, 2022.

World Health Organization. Malaria terminology. Geneva: World Health Organization, 2016; https://www.who.int./malaria/publications/atoz/malaria-terminology/en/. Accessed November 5, 2022.

World Health Organization. Updated WHO recommendations for malaria chemoprevention and elimination. 2022; Available from https://www.who.int/news/item/03-06-

2022-updated-who-recommendations-for-malaria-chemoprevention-and-elimination. Retrieved November 12, 2022.

World Health Organization. World malaria report 2020: 20 years of global progress and challenges. 2020; Available from: https://www.who.int/publications/i/item/9789240015791. Accessed November 10, 2022.

Yapi, R.B., Huerlimann, E., Houngbedji, C. A., Ndri, P.B., Silue, K.D., Soro, G. & Raso, G. (2014). Infection and co-infection with helminths and Plasmodium among school children in Côte d'Ivoire: results from a national cross-sectional survey. *PLoS Neglected Tropical Diseases*; ***8*(6)**: e2913. doi: 10.1371/journal.pntd.0002913.

Zaw, M.T., Thant, M., Hlaing, T.M., Aung, N.Z., Thu, M., Phumchuea, K. & Sattabongkot, J. (2017). Asymptomatic and sub-microscopic malaria infection in Kayah State, eastern Myanmar. *Malaria Journal*; ***16*(1)**: 1-7. doi:10.1186/s12936-017-1789-9.

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