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Prevalence of asymptomatic malaria in selected communities in Benue state, North Central Nigeria: A silent threat to the national elimination goal

Celina Aju-Ameh, Samuel Awolola, Georgina Mwansat, Hayward Mafuyai

ABSTRACT

Aims: The objective of this study is to determine the prevalence of asymptomatic malaria in six of our study communities. A cross sectional survey of 272 apparently healthy symptomless children and adults age 18 months to 55 and above were included in the survey. Rapid diagnostic tests (RDTs), conventional microscopic examination of blood stained films as well as the polymerase chain reaction (PCR) based technique were used to detect the presence of malaria parasites. Subjects who tested positive were given age appropriate course of artemether/lumefantrine (Combiart) free of charge. Methods: Venous blood was collected in specimen tubes with ethylene diamine tetra acetic acid (EDTA) tubes and used for RDT, microscopy and PCR test. Polymerase chain reaction diagnosis was carried out using dry blood spot (DBS) on Whatman filter paper (GE Healthcare, UK, Grade 3 MM CHR CAT No: 3030–861). Results: The prevalence of asymptomatic malaria recorded for rural ranged between 24.9 and 40.1%; urban was 8.8%. There was a significant (p = 00.3 RDT, p = 0.001 microscopy) difference in asymptomatic malaria prevalence between rural and urban malaria as well as with age (p = 0.02). Conclusion: This study has demonstrated that absence of clinical symptoms and the frank disease does not equate absence of malaria. A community is still at risk of malaria because asymptomatic infections contribute to maintaining transmission of the pathogen. In scaling up to both national and international target, a multifaceted control intervention which should include targeting the parasite reservoir will be needed to actualize the elimination goal in both high and low transmission areas.

Keywords: Asymptomatic carriers, Elimination, Malaria, Prevalence

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INTRODUCTION

According to the World Health Organization 2016 report [1], an estimated 212 million cases of malaria occurred worldwide (UI: 148–304 million), most of the cases were in the WHO African Region (90%), followed by the WHO South-East Asia Region (7%) and the WHO Eastern Mediterranean region (2%). It was estimated that 429,000 deaths from malaria occurred globally (UI: 235,000–639,000), most of which were estimated to have occurred in the WHO African region (92%), followed by the WHO South-East Asia region (6%) and the WHO Eastern Mediterranean region (2%). Sub-Saharan Africa continues to carry a disproportionately high share of the global malaria burden. Some 15 countries, mainly in sub-Saharan Africa, account for 80% of malaria cases and 78% deaths globally. Since 2000, the decline in malaria incidence in these 15 countries (32%) has lagged behind that of other countries globally (53%) [2]. In the West African sub-region among malaria endemic countries, 15 are focused on malaria control, while Cape Verde is in the pre-elimination programme phase and Algeria in the elimination phase. A review of trends in 186 hospitals in Nigeria between 2005 and 2013 indicated an increase, or no change, in confirmed malaria cases, admissions and deaths for all age groups, and a stable slide positivity rate (SPR) (59%) [2].

The Carter Center in 2012 [3] reported that approximately 20–30% of all African malaria cases occur in Nigeria and Ethiopia. Malaria is endemic in Nigeria, with seasonal peaks during the rainy season. As much as 90% of the population is at risk of malaria with year-round transmission unlike the unstable transmission pattern observed in Ethiopia. It is responsible for high rates of school and work absences, which have important short-term and long-term social and economic impacts. It also reduces agricultural output as serious illness from malaria usually takes place during the late rainy season coinciding with peak farming season. Highly malarious countries are among the very poorest in the world, and typically have very low rates of economic growth [3]. Unacceptably, Nigeria now bears the greatest of malaria disease burden than any country in the whole world. A clearer picture of the said trend was given by Nigeria demographic and health survey (DHS) in 2008. The DHS reported that in Nigeria, malaria accounts for approximately 60% of outpatient visits, 30% of all hospitalizations, and up to 11% of all maternal mortality, 25% of all infant mortality and 30% of under-five mortality [4].

In the strategic plan of Federal Ministry of Health 2014–2020, reduction of the malaria disease burden is the major focus. Nigeria has now moved from control mode to elimination mode and that is why the name of the national programme was changed from National Malaria Control Programme (NMCP) to National Malaria Elimination Program (NMEP) [5]. Beyond the change in nomenclature there is need for realistic changes on the complicated terrain in the fight against the malaria scourge. An aspect of malaria transmission that requires further research and appropriate intervention is asymptomatic malaria. Lindblade et al. [6] draw attention to the fact that even though there is a substantial decline in global malaria morbidity and mortality, current evidence cannot lead to malaria elimination in most malaria-endemic areas and that additional strategies need to be considered. One of such consideration would be in the use of antimalarial drugs to target the reservoir of malaria infection to reduce the transmission of malaria between humans and mosquito vectors. Several studies acknowledge the relationship between the reservoir of parasite, malaria transmission and elimination programs [7–10].

Several diagnostic methods are currently in use for detecting the malaria parasite each with various degrees of specificity and sensitivity. We have used here two of the commonly used methods in the study sites, the rapid diagnostic test (RDT) and microscopy. The polymerase chain reaction method was also employed on a few samples as a confirmatory test because of its sensitivity and specificity compared to microscopy the gold standard and RDT [11].

MATERIALS AND METHODS

Study area

Six communities in both Gboko and Otukpo local government areas of Benue State, north central Nigeria were selected for the study. Four of the study communities were rural and the inhabitant subsistence farmers while two were classified urban where the inhabitants engage more in secondary economic activities. Benue State is named after the Benue River and lies within longitude 7°47’ and 10°0’ East and Latitude 6°25’ and 8°8’ north. Based on Koppen climate classification, Benue State lies within the AW (tropical wet dry) climate and experiences two distinct seasons, the wet season and the dry season. The wet (rainy) season lasts from April to October with annual rainfall in the range of 100–200 mm. The dry season begins in November and ends in March. Temperatures fluctuate between 21–37°C in the year. Benue occupies a landmass of 34,059 square kilometres with a population of about 4,253,641 in 2006 census [12].

Study population

The sample population included willing participants of all ages irrespective of occupation, marital status, and educational background, social class, religious and cultural affiliation. Consequently, a mixed population of 272 individuals were screened and the survey was conducted at the end of September into the beginning of October 2015.

Ethical consideration

The study proposal was adequately reviewed (Project Number IRB/15/289); under its reviewed state ethical approval was given by the Institutional Review Board (IRB) of the Nigerian Institute for Medical Research (NIMR), Yaba, Lagos. The protocol and safety guidelines satisfy the conditions of NIMR-IRB policies regarding experiments that use human subjects. Also advocacy meetings with community and family heads were held and verbal consent gotten prior to the commencement of the survey. Subjects who tested positive were giving age appropriate course of artemether/lumefantrine (combiart) free of charge.

Blood collection, rapid diagnostic tests, microscopy and polymerase chain reaction

Blood collection: Blood samples were collected under sterile condition by swabbing the area with 70% alcohol and air dried. Venous blood was collected from study subjects using 5 ml capacity disposable syringes fitted with needles and dispensed into specimen tubes. The specimen tubes were pre-lined with ethylene diamine tetra acetic acid (EDTA) tube, gentle mixing of the EDTA and blood was ensured. The EDTA tubes were labelled and samples transferred to the General Hospital laboratory and used to prepare the various components for the RDT, microscopy and PCR test.

Rapid diagnostic tests: Sixty microliters of blood was used for the RDT test and results read and recorded after about 15 to 20 minutes.

Microscopy: Thick and thin smears were prepared and stained with 10% [13] and examined under x100 oil immersion objective and paired x10 oculars. Slide validation was done by a staff of the University of Jos Teaching Hospital.

Polymerase chain reaction: Dry blood spot (DBS 60 µl each) was soaked onto Whatman filter paper (GE Healthcare, UK, Grade 3 MM CHR CAT No: 3030–861) and air dried then kept in individual sealable sachets of ziplock plastic bags alongside desiccants. They were then hand carried to The molecular laboratory at the Nigerian Institute for Medical Research, Yaba, Lagos, Nigeria. Using the method by Bereczky et al. [14] DNA was extracted and PCR performed according to Snounou [15] nested protocol. The positive control was a standard 3D7 strain supplied by the laboratory. Primers used were Plasmo1: 5'GTT AAG GGA GTG AAG ACG ATC AGA-3' and Plasmo 2: 5' AAC CCA AAG ACT TTG ATT TCT CAT AA-3'.The PCR products were visualized with a transilluminator (UVP) after 1.5% agarose gel electrophoresis in 0.5 X Tris borate EDTA buffer and ethidium bromide (biohazard) staining.

Statistical analysis

All categorical variables were analyzed using chi-square (χ^2) test and a probability value (p-value) of $p< 0.05$ was regarded as significant.

RESULTS

Malaria prevalence in respect to settlement as given in Table 1 within Gboko and Otukpo LGA, Benue State, shows significant difference in prevalence of malaria only in RDT and microscopy test, while no significance was observed in malaria prevalence rate based on RDT/microscopy positive cases and PCR tests. For the RDT, the highest prevalence was observed in the rural settlement 59 (24.8%) while the least in urban 3 (8.8%), in microscopy tests, rural settlement still had the highest malaria prevalence 95 (39.9%) against urban settlement 3 (8.8%). The PCR method even though carried out on only 15 RDT negative samples (Figure 1) due to funding challenge, was the most sensitive at detecting the presence of the *Plasmodium falciparum* malaria parasite shown in Figure 2. There was a significant (p = 00.3 RDT, p = 0.001 microscopy) difference in asymptomatic malaria prevalence between rural and urban malaria. Prevalence of malaria in respect to sex (Table 2 and Figure 3) showed no significant difference (p = 0.88) while there was a significant difference (p = 0.02) in respect to age (Table 2 and Figure 4).

DISCUSSION

This study provides a dataset that shows clearly a significant (p = 0.003 RDT; p = 0.001 microscopy) difference in asymptomatic malaria prevalence between the urban and rural dwellers in the study communities.

Table 1: Malaria prevalence in respect to settlement within Gboko and Otukpo LGA, Benue State

Settlement	No. Examined	RDT (%)	MicroT(%)	RDT & MicroT (%)	PCR (%)
Rural	238	59(24.8)	95(39.9)	32(13.4)	2(0.8)
Urban	34	3(8.8)	3(8.8)	1(2.9)	-
TOTAL	272	62(22.8)	98(36.0)	33(12.1)	2(0.7)
p-value		0.003	0.001	0.078	0.826

NS: No significant difference in columns where p>0.05

Table 2: Prevalence of asymptomatic malaria with sex, age groups in the selected communities

Variables	Category	N	Positive
Sex			
Male	144	31 (21.5)	
Female	128	29 (22.7)	
Age group (years)	2–10	109	35 (32.1)
	11–20	57	15 (26.3)
	21–30	26	5 (19.2)
	31–40	15	0 (0)
	41–50	19	2 (10.5)
	51+	35	5 (14.3)

Sex: $\chi 2 = 0.01$, $p = 0$ ns Age: $\chi 2 = 12.94$, $p = 0.02$*
*Statistically significant ($p<0.05$) and ns = not significant where $p>0.05$

Asymptomatic malaria prevalence (32.1%) was highest with the youngest age grouping (2–10). This is similar to findings by Salako et al. [16] and agrees with the declaration of World Health Organization [2] that children are among some of the vulnerable population groups at higher risk of malaria. Another study [17] concluded that malaria in urban areas differed from the rural environment in the heterogeneity and complexity displayed, which has important implications for malaria control.

We focused here on asymptomatic malaria because of the importance of these symptomless and untreated group that are a silent threat to the national elimination programme. They act as the reservoir for infection, re-infection and malaria resurgence [18]. Alves et al. [19]

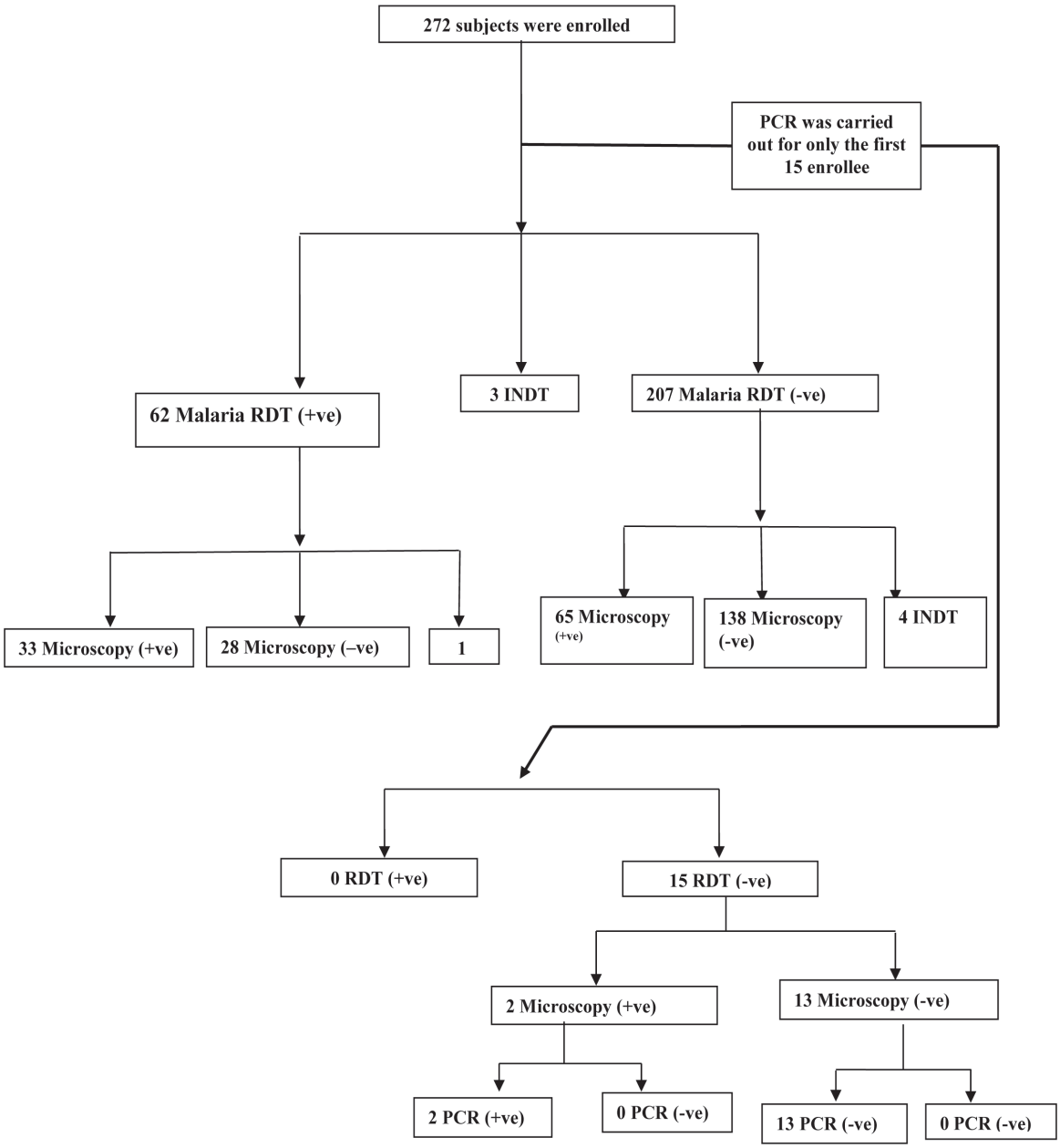

Figure 1: Study flow chart showing patients enrolled and diagnostics carried out.
Abbreviation: INDT-Indeterminate

found that prevalence of asymptomatic infections is 4–5 times higher than symptomatic infections among Brazilian natives and that although, the asymptomatic group infected mosquitoes at a much lower rate, these patients remain infective longer than treated symptomatic patients.

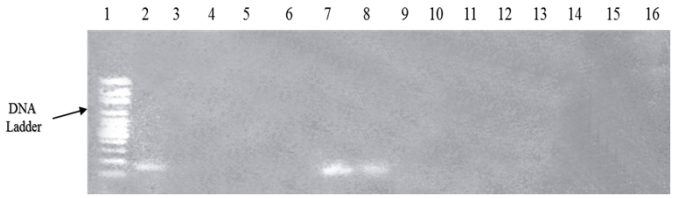

Figure 2: Detection and Identification of Plasmodium *falciparum* by PCR Amplification.
Lane 1: 100bp DNA ladder
Lane 2: 3D7 positive control
Lane 3: No template control
Lanes 4, 5, 6, 9, 10, 11, 12, 13, 14, 15 and 16: Plasmodium-negative samples
Lanes 7 and 8: Plasmodium-positive samples

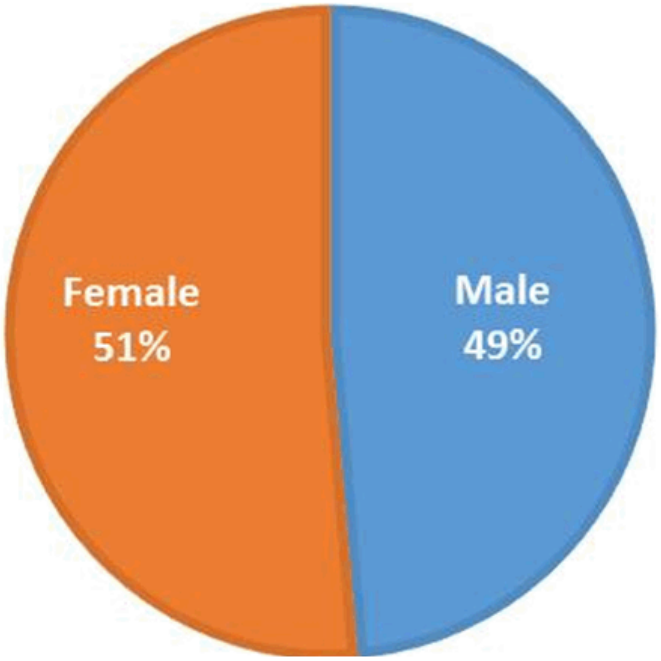

Figure 3: Malaria prevalence in respect to sex in Otukpo and Gboko local government area of Benue State.

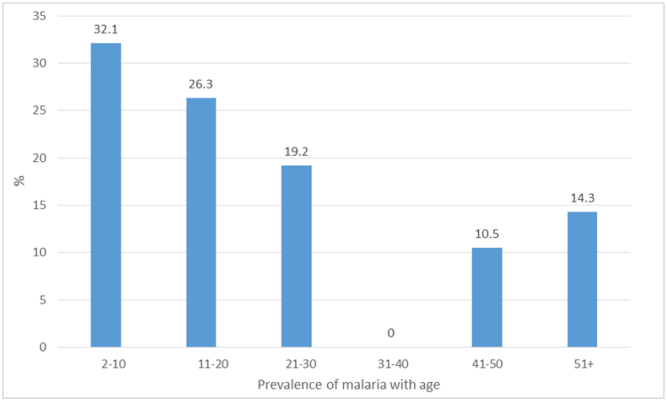

Figure 4: Malaria prevalence in respect to age in Otukpo and Gboko local government area of Benue State.

The choice of the most appropriate test for malarial diagnosis is predicated on a number of factors some of which are the level of malaria endemicity (including species), the urgency of diagnosis, availability of personnel and financial resources. Conventional microscopy remains the gold standard for malaria diagnosis, although it requires highly-skilled personnel and may have a lower sensitivity than the more recent molecular techniques. It is, however, inexpensive and reliable. Rapid assays are expensive but are quick and convenient. Molecular techniques are better suited to research laboratories to check for development of drug resistance and relapse, and can be useful for species identification when counts are very low or samples have undergone some deterioration [20].

Even though asymptomatic parasite carriers are common, detecting the parasite is difficult and poses a problem for malaria control [21]. We included the PCR method as a confirmatory test tool for a subset of the sample population and as with other studies [22, 23] found it to be more sensitive, specific at detecting the presence or absence of the parasite. Morassin et al. [24] found the PCR to be a useful tool as a second-line when the results of conventional techniques were negative in patients presenting a syndrome consistent with malaria, as well as yielding an accurate species identification. Bousema et al. [25] has also put forward argument for the wider deployment of molecular diagnostic tools needed to provide adequate insight into the epidemiology of malaria and infection dynamics to aid elimination efforts.

This investigation is deemed important because it highlights:

- The danger of focusing on only vector control and symptomatic patients and neglecting the reservoir of the malaria parasite- the asymptomatic carriers
- The need to focus on rural malaria studies and intervention
- The performance, applicability and importance of the PCR method in epidemiological surveys

As a summary we re-echo here the suggestion made by Wilson [26], over eight decades back that there is a real need to carry out multidimensional study of malaria in African villages.

CONCLUSION

We suggest that policy makers and malaria control units explore asymptomatic carriers and locate parasite foci for effective malaria control and attainment of the national elimination goal.

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Author Contributions
Celina Aju-Ameh – Substantial contribution to concept and design, Acquisition of data, Group 2: Drafting article, Revising it critically for important intellectual content, Final approval of the version to be published
Samuel Awolola – Substantial contribution to concept and design, Acquisition of data, Drafting article, Final approval of the version to be published
Georgina Mwansat – Substantial contribution to concept and design, Acquisition of data, Drafting article, Final approval of the version to be published
Hayward Mafuyai – Substantial contribution to concept and design, Acquisition of data, Drafting article, Final approval of the version to be published

Guarantor
The corresponding author is the guarantor of submission.

Conflict of Interest
Authors declare no conflict of interest.

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