PREVALENCE OF MALARIA INFECTION AND MOLECULAR PLASMODIUM SPECIATIONIN SUBJECTS ATTENDING SECONDARY HEALTH CENTRES IN ADAMAWA STATE, NIGERIA

ABSTRACT.

BACKGROUND: Malaria remains a disease of concern as it continues to plunge many into economic difficulty and much suffering, claiming lives especially children under 5 years old. Thestudy investigatedprevalence of malaria infection and molecular identificationof Plasmodium species in Adamawa state, Nigeria.

METHODS: Health facility-based cross-sectional study was conducted from March, 2021 to August, 2021 on subjects attending 15 secondary health facilities distributed across 3 zones in Adamawa State, Nigeria. The prevalence of Plasmodium infection was measured by light microscopic and polymerase chain reaction (RT-PCR) targeting P. falciparum 18S rRNA gene. Infection in relation to location, age, sex and socio-economic status was investigated. The ownership and usage of insecticidal treated mosquito nets (ITNs) were also assessed.

RESULTS:Prevalence by mcroscopic analysis was 39.08% with total parasite density 1633048/µl, while PCR assay amplifying 18S small-subunit ribosomes RNA (SSU rRNA) gene of Plasmodium confirmed only 15.7% of isolate as asymptomatic malaria infections. *Plasmodiumfalciparum* was the only species found in the study area. Infection by geographycal locations, age, sex, socio-economic status varied significantly (P=.05). Children under 5 years of age and female subjects recorded highest infection rate by both microscopy and PCR. Shockingly only 52.3% of subjects without ITNs were infected.

CONCLUSION: The results indicated the need for scale-up interventions to curb the high prevalence of P. falciparum infection in the study area with special attention to children and female subjects in the population.

Key words: Malaria infection, socioeconomic status, molecular identification, Adamawa state.

INTRODUCTION

Malaria, though preventable and treatable remained a life-threatening disease caused by protozoan parasite species (*Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae and Plasmodium knowlesi*), transmitted through the bite of an infected

female Anopheles mosquito (1). It is often referred to as disease of the poor due to its socioeconomic relevance in distribution. (2, 3& 4). Low socioeconomic households suffer high malaria infection due to their inability to prevent and treat malaria, while keeping them struggling to improve their socioeconomic status (5). The population at risk of contracting malaria and developing severe diseases than others are infants, children under 5 years of age, pregnant women and patients with HIV/AIDS as well as non-immune migrants. Symptoms of severe ill-health from malaria include headache, fever, tiredness and fatigue, convulsion, difficulty in breathing, dark or bloody urine, jaundice and abnormal bleeding (6).

Globally, there has been a rise in malaria cases from 216 million cases (in 91 countries) reported the year 2018 to 247 million cases in 2021 (across 84 malaria endemic countries). Most of the increase was in WHO Africa region and was attributed to the covid-19 era (7-8. Among four countries that accounted for half of the world malaria cases Nigeria leads with 27% followed by the Democratic Republic of Congo 12%, Uganda 5% and Mozambique 4%. (7). Hence, it is of no surprise that 97% of Nigerians are at risk of contracting malaria (9).

Plasmodium falciparum malaria has been reported as the leading cause of malaria deaths and sufferingburden in sub-Saharan Africa. Falciparum malaria case in Nigeria recently rose from 1.521.566 in 2018, 3.659.170 in 2020 to 3.828.757 in 2021 (7). WHO in 2019 included 'high political will to reduce malaria' as one of the key elements of high impact approach. Nigeria, in her effort to end malaria by 2030 launched 'End Malaria Council' to assist the Malaria Elimination Program achieves its goal in the country(Reference 10).

Preventive measures such as the sleeping under long lasting insecticidal nets (LLINs), insecticide treated mosquito nets (ITNs) and the use of indoor residual spraying (IRS) to control vectors and chemoprevention cannot be overemphasized as effective tools (Reference 6). Across the 21 LGAs in Adamawa State about one million children (aged 3-59 months) benefited from the Seasonal Malaria Chemoprevention (with sulfadoxine-pyremethamine and Amodiaquine) progamme with support from WHO and funding from Global Funds through the National Malaria Elimination Programme (NMEP) in 2022(11). In 2021, WHO recommended the use of RTS,S/AS01 vaccine for children as it has been found to give protection against malaria infection. About 2 million children have been reached in Kenya, Malawi and Ghana since 2019 with the RTS.S/AS01. In 2023R21Matrix M was approved as the second vaccine for malaria WHO as it has shown to be effective.(12). Nigeria grants approval for RTS/S vaccine in 2023 which is expected from Oxford (13).

To report recent findings that will pave way for evidence-based decision on effort to achieve goal of ending malaria sufferings in Nigeria, this study investigated the malaria parasitaemia and molecular identification of Plasmodium species in Adamawa State.

METHODS

STUDY AREAS

Adamawa state is located in North Eastern part of Nigeria. It was curved out of the former Gongola state in 1991 with its headquarters in Yola. It is bordered by Borno and Yobe states in the North, Gombe state in the West, Taraba state in the South and Republic of Cameroun from the East (along Nigerian international border). It has 3 zones (North, Central and South) and 21 local government areas with total population of 3178950 based on the last census conducted in2006, the projected population in 2022 was 4902100. The minimum and maximum temperatures are 15.2°C to 43°C respectively (Reference14-15). The annual rainfall ranges from 79mm to 179mm within the period of May to September.. Its coordinates are 9 20′N 12□30′E and it has landmass of 36917km²(Reference 15-16).. The topography is made up of mountainous land crossed by river valleys of Benue, Gongola and Yadserem. The valleys of Cameroun , Mandara and Adamawa mountains form part of the landscape(References 17)..

The major occupation of the people is farming as reflected in the two vegetation zones; Sudan and Northern Guinea Savannah. The common crops cultivated are groundnuts, cotton, maize, guinea corn, millet, cassava, yam and rice. Communities along the river banks engage in fishing while most of the Fulanis practice nomadic farming (17-18.

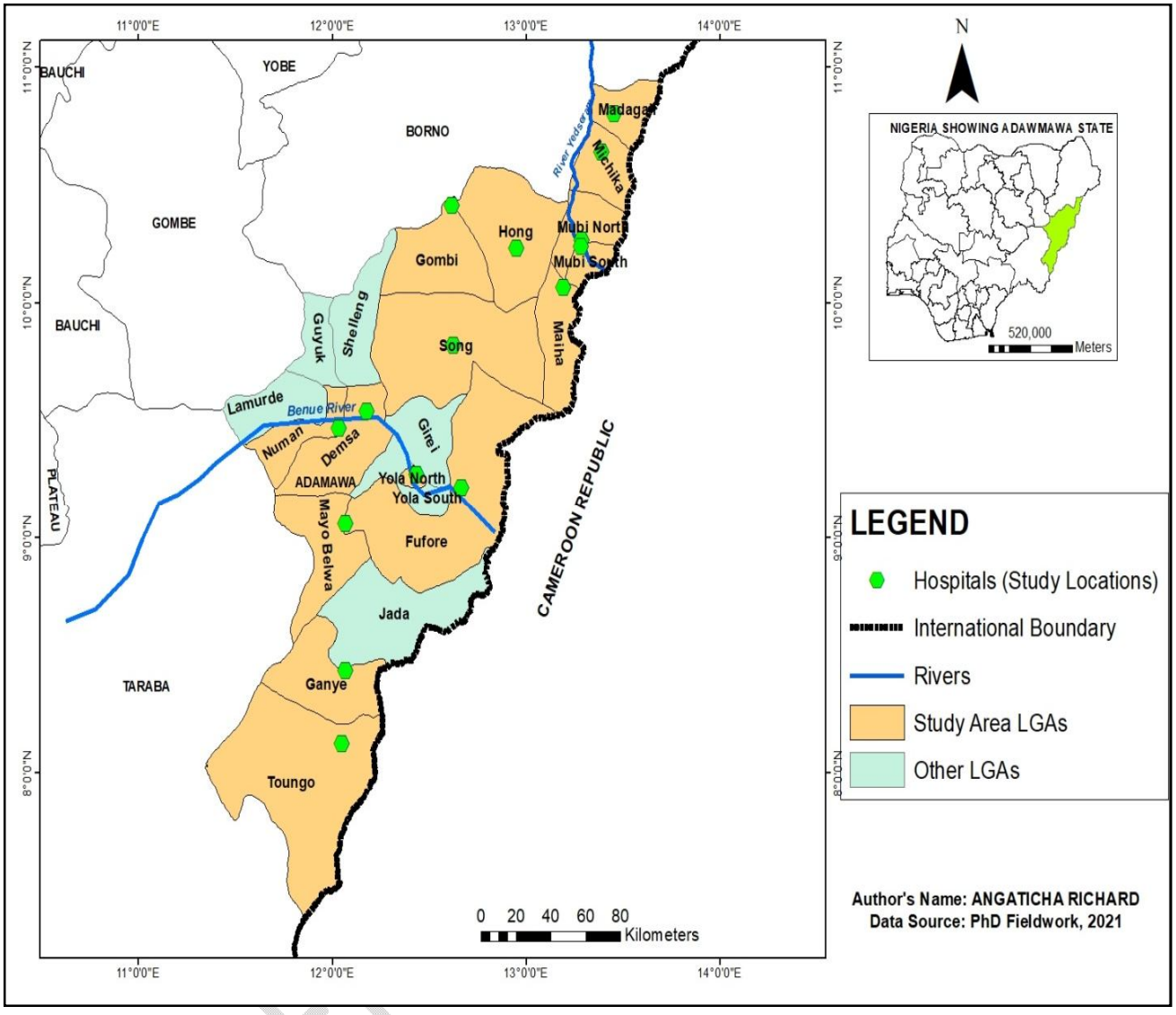

Figure 1: Map of Nigeria showing study areas. Source:

Source: Department of Geography, University of Port Harcourt.

STUDY DESIGN

SAMPLING TECHNIQUE

Random sampling technique was used. The study area was further stratified into 3 Zones (North, Central and South) in terms of location. The Nothern zone consist of Madagali LGA, Michika LGA, Mubi North LGA, Mubi South LGA, Maiha LGA while Central zone consist of Hong LGA, Gombi LGA, Song LGA, Yola North and Fufore LGA. Selected from South were Numan LGA, Mayo-Belwa LGA, Demsa LGA, Ganye LGA and Toungo LGA. Total of 15 secondary

health facilities were selected; 5 health facilities from each zone. Sample size was determined by Yamane's formula: $n=N/1+N(e)^2$ Where n=sample size, N=population size, e= level of precision. The confidence level at 95% and level of precision used was 0.05. The population size of the 3 zones of Adamawa State was used to determine the total sample size.For example: Adamawa North; population size by 2022 projection is 1051900 and by 2006 census is 682026; applying the Yamane's formula you will have 400. And so, for other zones. Now 400 x 3=1200 (17). A total of 1200 samples were collected.

INCLUSION CRITERIA

The study was delimited to subjects of all ages that presented at the selected secondary health centres irrespective of their social status and those whose parents/guardian signed the consent forms for under children after clear explanation of study objectives both in English and Hausa languages.

EXCLUSION CRITERIA

Subjects who are on malaria treatment and visitors (e.gtravellers) who report to health facilities and thereafter will leave the study areas were excluded.

QUESTIONNAIRE ADMINISTRATION

The questionnaire was administered through face-to-face interview, however, those that could not express themselves in English language were interviewed in Hausa (Most residents speak the language). Subjects' bio-data and information on socioeconomic status, ownership and usage of ITNs and other variables associated with symptomatic infection were obtained. Each sampling points coordinates was obtained by GPS Application by Ketan computers (20).

SAMPLE COLLECTION AND STORAGE

For laboratory investigation 5ml of blood was collected from each subject with assistance of the licencesd laboratory scientist working in the secondary health facilities, blotted in triplicate onto Whatman 3 paper and allowed to air dry at room temperature. Whatman paper was individually placed in asealed plastic bags marked with subject's study numbers and date of collection before being transported to the Nigerian Institute of Medical Research (NIMR) Lagos, Nigeria. The 108

samples for molecular identification were randomly selected across the study areas. For microscopy, films were made at the laboratories of the health facility stained and transported to laboratories in General Hospitals (GH) Mubi and Ganye for investigation.

PROCESSING OF BLOOD SMEARS/ MICROSCOPY FOR PARASITE STATUS AND DENSITY.

Thick (stained with Field stain A and B) and thin (stained with 3% Giemsa) blood films were prepared and examined by oil immersion microscopy. Thick films are for detection of parasitaemia and thin films for species differentiation. Results obtained was recorded against each questionnaire and compiled in record book.Parasitaemia was calculated using the formular;

Parasite count × 8000/set range of WBC=parasite /µl (21).

Questionnaire Administration

-The questionnaire was administered through face-to-face interview However, those that could not express themselves in English language were interviewed in Hausa (Most residents speak the language). Patient's ownership and usage of LLIN and other variables associated with symptomatic infection

-Processing of blood smears/ Microscopy for parasite status and density/ Processing of blood smears

-Parasite DNA isolation/ Plasmodium speciation by species-specific nested PCR.

Sample collection and storage.

DATA ANALYSIS

Chi-square test, analysis of variance and descriptive statistics were used for comparison of malaria parasiteamia in the study areas; the package used was statistical package for social science (SPSS) version 2022.

DNA EXTRACTION

Parasite DNA was extracted from the dried blood spot according to the manufacturer's instructions. The prepared samples were kept at −20ºC until use.

PLASMODIUM SPECIATION BY SPECIES-SPECIFIC NESTED PCR

The standard method for molecular identification of *Plasmodium*species carried out relies on ribosomal RNA gene targets in the 18s subunit and requires a nested PCR (22).

Two genus-specific primers rPLU5 and rPLU6 were used for the first cycle amplification. An aliquot of the product thus obtained was used for second amplification cycle in which the individual parasite species was detected separately using the species-specific primers (Table 1).

Table 1: Details of primer and reaction conditions used for the nested PCR analysis

Primer	**Sequence (5'-3')**	**Cycling condition**	**Product size**
Nest 1	rPLU5 CTTGTTGTTGCCTTAAACTTC	94°C-3mins;{95°C-30s; 49°C-30s; 72°C-90s}X30; 72°C-5mins	1.2kb
	rPLU6 TTAAAATTGTTGCAGTTAAAACG		
Nest 2	rFAL1 TTAAACTGGTTTGGGAAAACCAAATATATT	94°C-3mins;{95°C-30s; 60°C-30s; 72°C-90s}X30; 72°C-5mins	205bp
	rFAL2 ACACAATGAACTCAATCATGACTACCCGTC		
Nest 2	rMAL1 ATAACATAGTTGTACGTTAAGAATAACCGC	94°C-3mins;{95°C-30s; 49°C-30s; 72°C-90s}X30; 72°C-5mins	144bp
	rMAL2 AAAATTCCCATGCATAAAAAATTATACAAA		
Nest 2	rVIV1 CGACTTCCAAGCCGAAGCAAAGAAAG	94°C-3mins;{95°C-30s; 49°C-30s; 72°C-90s}X30; 72°C-5mins	120bp
	rVIV2 TCCTTACTTCTAGCTTAATCCACATAACTGATAC		
Nest 2	rOVA1 TGTAGTATTCAAACGCAGT	94°C-3mins;{95°C-30s; 49°C-30s; 72°C-90s}X30; 72°C-5mins	800bp
	rOVA2		

	TATGTACTTGTTAAGCCTTT	

A master mix (Taq polymerase enzyme, buffer, magnesium chloride) was prepared for one 12.5µl PCR reactions and reagents were added in order presented in Table 2. Amplification was done following conditions in Table 1. After amplification of DNA template for Nest 1, Nest 2 reagents were added to PCR tube as provided in Table 2. The mixture was gently mixed and amplification done following condition stated in Table 1. The PCR product (DNA amplified) obtained were subjected to amplicon separation in a 1.5% agarose gel (with ethidium bromide stain) electrophoresis.

Table 2: Constituents of reaction mixture for Nest 1 and Nest 2 reactions of *P. falciparum, P. malariae, P. vivax* and *P. ovale*

Nest 1		**Nest 2**	
Reagent	**X1 (µl**	**Reagent**	**X1 (µl)**
Double-distilled H_2O	8.0	Double-distilled H_2O	8.25
Pre-mix with BSA (X1)	2.5	Pre-mix with BSA (X1)	2.5
rPLU5 (0.4µM)	0.5	rFAL1/rMAL1/rVIV1/rOVA1 (0.3µM)	0.375
rPLU6 (0.4µM)	0.5	rFAL2/rMAL2/rVIV2/rOVA2 (0.3µM)	0.375
DNA template	1.0	Nest 1 product	1.0
TOTAL	12.5		12.5

The electrophoresis was allowed to run for 45 minutes and the gel was later viewed under UV light using gel documentation machine.

RESULTS

TABLE 3: *PLASMODIUMFALCIPARUM* INFECTION BY LOCATIONS IN ADAMAWA STATE.

S/No.	Locations	No. examined	No. infected(%)	statewide % infected	PD(µl)	Coordinates
1.	CH Gulak Madagali LGA.	80	22(27.5)	1.83	75656	10º48'17.8"N 13º27'04.5"E
2.	GH Maiha Maiha LGA.	80	39(48.8)	3.25	124824	10º03'54.3"N 13º11'22.3"E
3.	GH Michika Michika LGA.	80	31(38.8)	2.58	174808	10º38'29.9"N 13º23'03.9"E
4.	NMLC Mubi Mubi North LGA.	80	44(55)	3.67	139360	10º16'07.8"N 13º17'02.2"E
5.	GH Mubi Mubi South LGA.	80	46(57.5)	3.83	9000	10º15'43.7"N 13º16'12.2"E
6.	CH Fufore Fufore LGA.	80	29(36.3)	2.42	138728	9º12'56.55204"N 12º39'49.63896"E
7.	GH Garkida Gombi LGA.	80	25(31.3)	2.08	63996	10º24'40.8"N 12º33'56.7"E
8.	CH Hong Hong LGA.	80	28(35)	2.33	59080	10º14'06.0"N 12º56'44.3"E
9.	CH Song Song LGA.	80	20(25)	1.67	99128	9º49'10.0"N 12º37'15.2"E
10.	SH Yola Yola North LGA.	80	55(68.8)	4.58	208896	9º16'40.6"N 12º26'47.6"E
11.	GH Borrong Demsa LGA.	80	35(43.8)	2.92	88428	9º32'29.4"N 12º10'23.3"E
12.	GH Ganye Ganye LGA.	80	18(22.5)	1.5	95456	8º26'10.1"N 12º03'47.1"E
13.	GH Numan Numan LGA.	80	28(35)	2.33	138068	9º28'06.6"N 12º0156.1"E
14.	CH Mayo-Belwa Mayo-Belwa LGA.	80	25(31.3)	2.08	105716	9º03'34.7"N 12º03'52.2"E

15.	CH Toungo Toungo LGA.	80	24(32.5)	2.0	111904	8º07'23.9"N 12º02'48.4"E
	Total	**1200**	**469(39.08)**		**1633048**	

GH= General hospital, CH= Cottage hospital, SH= Specialist hospital PD= Parasite density, S/No= Serial number, %=percentage. No positive and % infected:P>0.05, PD: *P*=.05.

The result in Table 3 shows infection in study locations (hospitals) with their respective Local government areas; the highest infection was recorded in Yola North at Specialist hospital 68.8% with statewide infection of 4.58% followed by Mubi South at General hospital Mubi 57.5% with statewide infection of 3.83% while the least infection was recorded at General hospital Ganye 22.5% with statewide infection of 1.5%. In terms of Parasite density; Yola North had 208896/µl followed by Michika 174808/µl and Mubi-North 139360/µl while the least's parasite density was recorded in Hong 59080/µl and Mubi-South 9000/µl. Statistical difference for number infected and overall percentage infected P>0.05 whereas parasite density *P*=.05.

Table 4 shows infection by zone; in the North it was 45.5% with statewide 15.17%, in the central it was 39.25% with statewide 13.08% and south 32.5% with statewide 10.83%. The difference in infection was statistically significant as *P*=.05. Highest parasite density was recorded in the Central 569828µl followed by South 539572/µl and least in the North 523648/µl.

Table 4: Malaria infection by geographical zone in Adamawa state.

S/No	Zone	No. examined	No. infected	% infected	statewide % infected	PD(µl)
1.	North	400	182	45.5	15.17	523648
2.	Central	400	157	39.3	13.08	569828
3.	South	400	130	32.5	10.83	539572
	Total	1200	469	39.08	39.08	1633048

PD= Parasite density, µl= microliter, χ^2=14.204, P=0.001

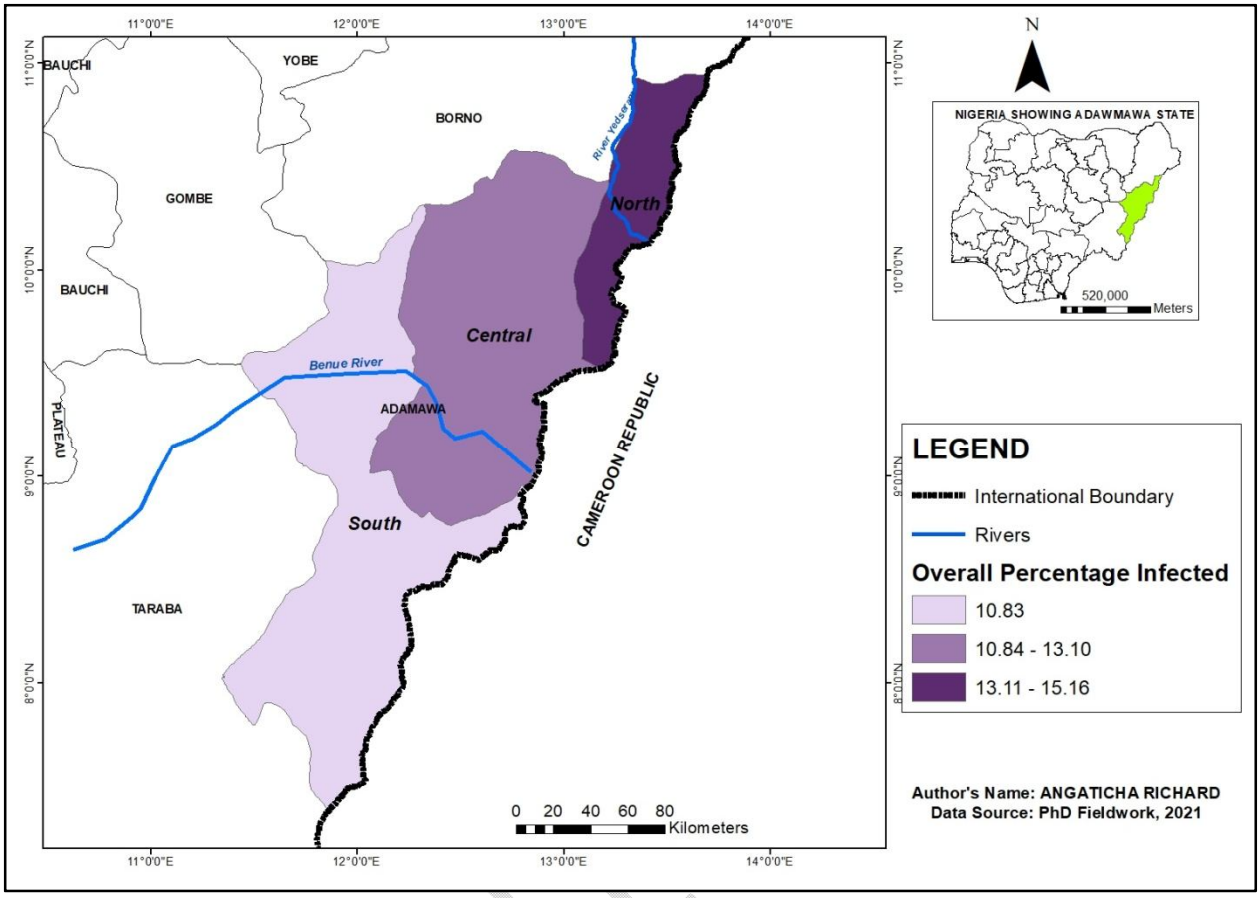

Figure 2: Map of the Adamawa State showing Malaria overall percentage infection by Zones.

Sourece: Department of Geography, University of Port Harcourt.

MOLECULAR ANALYSIS

Out of the 108 blood samples examined by molecular analysis 17 were infected with *Plasmodiumfalciparum* as the only species found in Adamawa state by this research. The fragment size detection of 205bp shows the presence of *Plasmodium falciparum* (plate 1). Age group 0-5 years old had the highest infection with 26.1% followed by age groups 16-20 years with 22.2% and 6-10 years 18.8% *P*=.05 Table 5. In relation to sex, female was higher with 17.9% infection and male had 13.5% *P*=.05. The total infection was 15.7% Table 6.

Table 5: *Plasmodium falciparum* malaria detected by molecular characterization in relation to age in Adamawa State.

S/No.	Age group(years)	No. examined	No. infected	% infected	P-value
1.	0-5	23	6	26.1	
2.	6-10	16	3	18.8	
3.	11-15	6	0	0	
4.	16-20	9	2	22.2	
5.	21-25	20	2	10	0.000
6.	26-30	11	2	18.2	
7.	31-35	6	0	0	
8.	36-40	6	1	16.7	
9.	41&above	11	1	9.1	
	Total	108	17	15.7	

S/No = serial number, % = percentage.

Table 6: *Plasmodium falciparum* malaria detected by molecular characterization in relation to sex in Adamawa State.

S/No.	Sex	No. examined	No. infected	% infected	P-value
1.	Female	56	10	17.9	
2.	Male	52	7	13.5	0.021
	Total	108	17	15.7	

S/No = serial number, % = percentage.

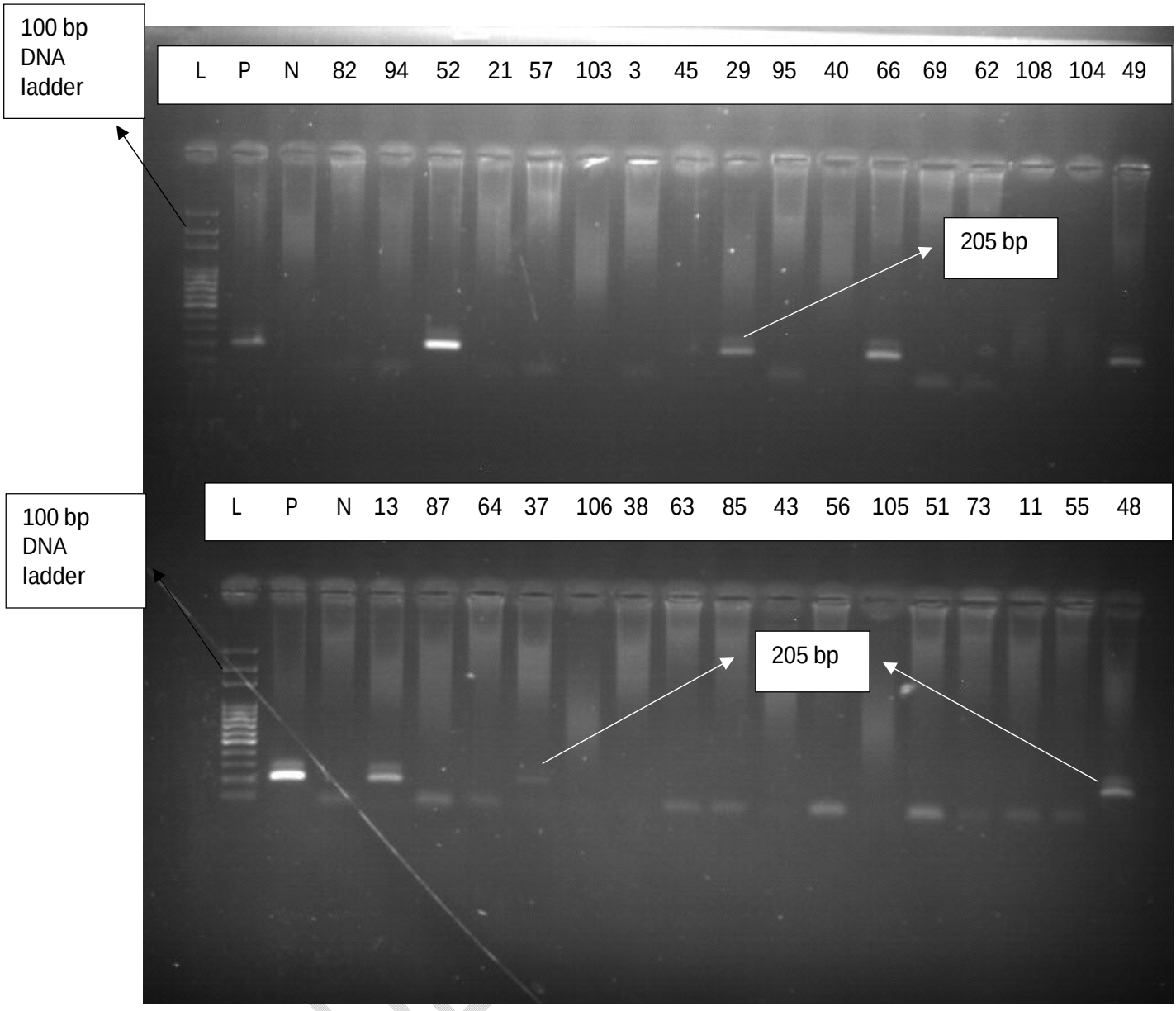

Plate 1:Electrophoresis after Plasmodium-specific PCR amplification: L= DNA ladder (100 base pair), P=Positive control; N=Negative control. Lanes 52, 29, 66, 49, 13, 37, and 48 are positive.

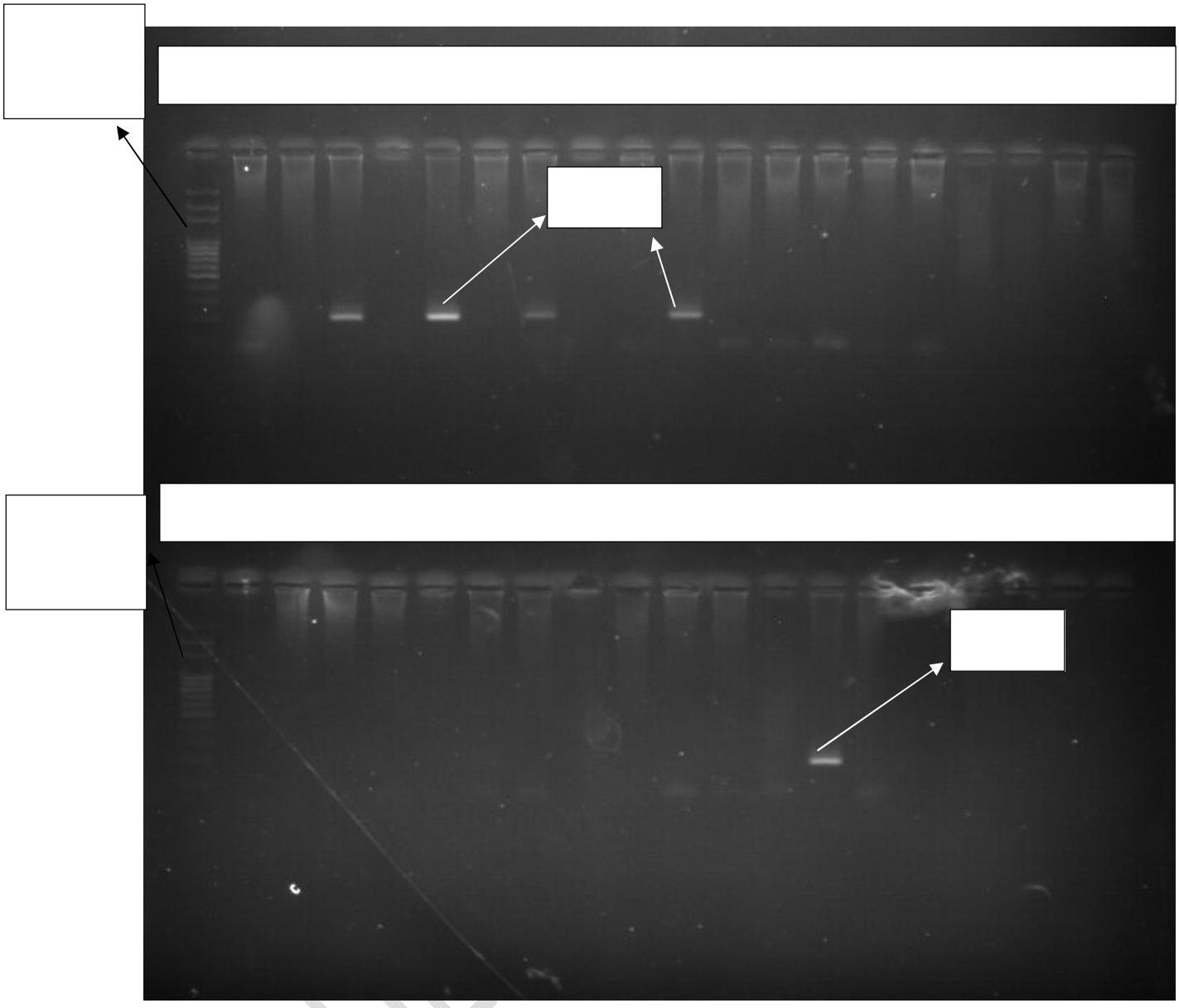

Plate 2 :Gel documentation detected picture showing fragment size detection of P. falciparum (205bp): L= DNA ladder (100 base pair), Lanes 65, 8, 6, 2, and 58 are positive.

The Adamawa State overall data results in relation to age groups shows age 0-5 years old had the highest infection with 55% followed by 6-10 years with 42.5% and the least was recorded in age group 16-20 years 35.4% *P=.05*. In terms of parasite density age group 41 and above year's old recorded 321600/µl while the least was recorded within age group 6-10 years recorded 100512/µl Table 7.

In relation to sex, females had 40.3% infection with parasite density of 967252/µl and males recorded 37.3% infection with parasite density 665796/µl *P*=.05 Table 8.

Table 7: Age related malaria parasitaemia in Adamawa State.

S/No	Age group(yrs)	No examined	No infected	% infected	Parasite density(µl)	P-value
1.	0-5	119	55	46.2	189184	
2.	6-10	80	34	42.5	100512	
3.	11-15	83	33	39.8	112472	
4.	16-20	144	51	35.4	162564	
5.	21-25	138	56	40.6	184612	0.000
6.	26-30	120	49	40.8	167236	
7.	31-35	149	53	35.6	188176	
8.	36-40	165	61	40	206692	
9.	41 & above	202	77	38.1	321600	
	Total	1200	469	39.1	1633048	

S/No= Serial number, yrs= years, %= percentage, µl= microliter

Table 8: Malaria parasitaemia in relation to sex in Adamawa State.

S/No	Sex	No examined	No infected	% infected	Parasite density(µl)	P-value
1.	Female	660	266	40.3	967252	
2.	Male	540	203	37.6	665796	0.000
	Total	1200	469	39.1	1633048	

S/No= serial number, %= percentage, µl= microliter

According to socioeconomic class, the middle class had the highest infection with 43% followed by the higher class 38.6% whereas the lower class recorded 38.1% infection *P*=.05. The highest parasite density was recorded in lower class 926603/µl and least in middle class with 338605/µl Table 9.

Table 9: Malaria parasitaemia in relation to socioeconomic status in Adamawa State.

S/No	Socioeconomic class	No examined	No infected	% infected	Parasite density(µl)	P-value
1.	Higher class	293	113	38.6	367840	
2.	Middle class	221	95	43	338605	0.000
3.	Lower class	686	261	38.1	926603	
	Total	1200	469	39.1	1633048	

%=percentage, µl=microliter.

Investigating malaria parasitaemia in relation to possession and frequency of sleeping under mosquito net, it was found that subjects who do not have mosquito net recorded highest infection 52.3% followed by those who do not sleep under mosquito net (because is stuffy) 50% whereas those reported to sleep under mosquito net regularly recorded 46.1% infection*P*=.05 . Those who reported to sleep under treated mosquito net regularly recorded 658708/µl parasite density and the 4 infected non-regular users of untreated mosquito net recorded least parasite density 12600/µl Table 10.

Table 10: Malaria parasitaemia in relation to possession and frequency of sleeping under mosquito net in Adamawa State.

S/No	Possession and frequency of sleeping under mosquito net.	No examined	No infected	% infected	P.D(µl)	P-value
1.	Do not have mosquito net	132	69	52.3	272964	
2.	Regular sleeping under mosquit net (ITN)	573	201	34.8	658708	
3.	Regular sleeping under mosquito net (non-ITN)	15	7	46.7	34332	
4.	Non-regular sleeping under Mosquito net (ITN)	451	183	40.6	621668	0.000
5.	Non-regular sleeping under Mosquito net (non-ITN)	14	4	28.6	12600	
6.	Do not sleep under mosquito net (is stuffy)	10	5	50	32776	
	Total	1200	469	39.1	1633048	

S/No= serial number, %= percentage, P.D= parasite density, %= percentage, µl= micrometer

DISCUSSION

This study investigated the prevalence of malaria in relation to socioeconomic status, treatment seeking behavior, mode of prevention practiced; in particular the use of mosquito nets in Adamawa state. . In this study the only species of Plasmodium found by both microscopy and molecular analysis was *Plasmodiumfalciparum* which agrees with report that falciparum malaria is responsible for 99.7% of estimated malaria cases in Africa (23). It also concurs with findings in Rivers state, Nigeria (24) and Kano, Nigeria (25). Out of the total 1200 samples examined by microscopy 469 (39.1%) were infected, the total parasite density was 1633048/µl, this is of utmost concern as reports also indicate the rise in malaria cases in Adamawa state (8).

By location Yola North recorded highest infection 68.75% with overall state infection of 4.58% and parasite density 208896/µl. This finding is slightly higher than reported 50.6% infection among children in Yola, but, agrees with reports that Yola North had the highest malaria cases (26). The high infection rate in Yola North could be attributed to, warm temperature, densely populated settlements and proximity to River Benue along which both rainy and dry season farming activities takes place; hence favourable conditions for Anopheles mosquitoes to breed and transmit infection at ease. Other factors could be lack of compliance to regular sleeping under mosquito net because of hot weather condition. Mubi South and Mubi North recorded 57.5% (with statewide 3.83%) infection and 55% (with statewide 3.66%) infection respectively. The presence of River and traditional dug well in houses and communities (as observed during field work) could serve as contact sites with mosquitoes amidst other factors like clustered city setting leading to high rate of infection in Mubi town. In addition, the indiscriminate dumping of refuse could serve as breeding sites for mosquito in Mubi town (27). Difference in number of infection by location in Adamawa state was not statistically significant as $P>0.05$ whereas parasite density was significant as $P<0.05 P=.05$. Infection by zones showed Adamawa North recorded 45.5% with statewide infection of 15.17% and total parasite density of 523648/µl this could not be unconnected with the presence of Anopheles mosquitoes (18) in addition to predominantly farming occupation. Most communities still use dug well even in houses that are without window/door nets hence exposing locals to mosquito bite before and after bed time (i.e if any mosquito net). Adamawa central recorded 39.25% infection with state wide infection of 13.08% and parasite density 569828/µl. This is not surprising because the breeding sites of mosquito along River Benue are very possible; rice and vegetable farmers, fishermen and pastoralists could easily be exposed to infection. Less infection was recorded in most parts of Adamawa South with exceptions to LGAs along the Benue River (Demsa and Numan); Adamawa south recorded 32.5% infection with statewide infection 10.83% and parasite density 539572/µl. A relative low malaria cases in Adamawa South was reported, especially around Toungo LGA and Ganye LGA possibly due to proximity to GashakaGumti National park which has low temperature and dense forestmaking it unfavourabe for mosquito breeding(28). The difference in percentage infection by zone was significant as $P=.05$.

The overall data obtained from Adamawa state in relation to age showed, the highest infection was recorded within age group 0-5 years old 46.2% with parasite density 189184/µl, same with molecular analysis 0-5 years recorded highest infection with 26.1% this concur with reports that most malaria deaths occur in children less than 5 years as they are among the vulnerable groups to infection due to low immunity(8& 29). The least infection was recorded among age group 16-20 years 34.5% with parasite density 162564/µl. The less infection could be due to acquired immunity, this finding is higher than the 26% infection reported in age group 11-21 years (30). Infection by age groups was statistically significant as $P<0.05$.

In relation to sex, females recorded higher infection 40.3% and males had 37.6% infection $P<0.05$. Parasite density by sex was females 967252/µl and males 665796/µl. Molecular analysis result also showed females had 17.9% infection, male 13.5% $P<0.05$. The higher infection in females may be gender related as women are known to engage in outdoor house chores at dawn and dusk a favourable moment for vector mosquito bite. Higher malaria infection in females than males was also reported in Yola (29) and in Emohua, Rivers state, Nigeria (24).

According to socioeconomic status, the middle class recorded highest infection 43% followed by higher class 38.6% $P=.05$. The lower class recorded highest parasite density 926605/µl followed by higher class 367840/µl. It was reported that high burden of malaria is directly related to poverty (23 31) which concurs with this finding in terms of parasite density.

Further study was carried out on infection in relation to possession and sleeping under mosquito net. It was found that subjects who do not have mosquito net recorded 52.3% infection followed by subjects who do not sleep under mosquito net (because is stuffy) with 50% infection whereas those who sleep under mosquito net (ITN) regularly had 34.8% infection. Access and ownership of mosquito net does not translate to its use, as revealed in this study, some findings reports the misuse of mosquito nets for other purposes such as fishing (24 32), hence ownership is not enough (25 33). There was a high parasite density 658708/µl among subjects who sleep under mosquito net which could be as a result of contact with mosquitos outdoors. There was statistical difference in infection related to possession and ownership of mosquito net as $P=.05$.

CONCLUSION

Considering the high infection of malaria in Adamawa state 39.1%, there is need for sustained interventions with insecticidal treated mosquito nets and chemoprevention, with rate of infection by locations in mind. Sustained awareness on importance of sleeping under mosquito net should be encouraged as possessing it does not necessarily translate into proper use. Other methods of prevention such as environmental sanitation and wearing protective clothingsis encouraged while awaiting the vaccine roll out for children as additional tool. Free malaria communities could lead to improved socioeconomic status as the infection is associated with economic difficulty.

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ETHICAL APPROVAL AND CONSENT

Clearance was obtained from University of Port Harcourt Research ethics committee in 2019 and Adamawa State Ministry of Health Research Ethical Committee in 2021 with approval number ADHREC 5/03/2021/043. All subjects and guardian of children under 18 years gave written informed consent to participate in providing blood sample. A verbal explanation of the objectives, benefits (such as interest groups' possible intervention to eliminate malaria which could improve standard of living in the study area) and potential risks associated with participation in the study were given to all subjects . Subjects' personal information was kept confidential.

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