Original Research Article

MALARIA INFECTION AND MOLECULAR CHARACTERISATION OF PLASMODIUM SPECIES IN ADAMAWA STATE, NIGERIA.

Comment [h1]: The authors should reconsider the title of the manuscript. There is characterization of Plasmodium species but identification

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 characterization of Plasmodium species in Adamawa state, Nigeria.

METHODS: Fifteen secondary health facilities from 15 Local government areas (LGAs) were selected, which was further stratified into three zones consisting 5 LGAs each. Questionnaires were distributed to informed consented subjects and 5mls of venous blood collected from each.Total of 1200 blood samples were examined by oil immersion microscopy. Molecular characterization of *Plasmodium* species was carried out using dry blood spot.

RESULTS: Both microscopy and molecular analysis detected *Plasmodiumfalciparum* as the only species in Adamawa state. Out of the 1200 blood samples examined, 469(39.1%) was infected with total parasite density of 1633048/µl. By location, Yola North LGA recorded highest infection 68.8% with state wide infection 4.6% $P<0.05$ and parasite density of 208896/µl $P<0.05$. Infection by zones; Adamawa North had 45.5% with state wide 15.2% and parasite density 523648/µl $P<0.05$. Infection in relation to age groups, sex and socioeconomic status was $P<0.05$. Parasitaemia by possession and frequency of sleeping under ITN was $P<0.05$.

CONCLUSION: Variation in intensity of infection by location and other characteristics in this study call for data driveninterventions.

Comment [h2]: The authors should conclude based on the objectives of the study

Key words: Malaria infection, socioeconomic status, molecular characterization, 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 across the world (2, 3& 4). Low socioeconomic

Comment [h3]: The authors should reconsider the comma and the parenthesis in this sentence.

Comment [h4]: Malaria is tropical and sub-tropical areas disease.

households suffer high malaria infection due to theirinability to prevent and treatmalaria, whilekeeping 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%. It is disturbing; Nigeria is also among four countries that accounted for over half of global malaria deaths with 31%, trailed by the Democratic republic of Congo 13%, United Republic of Tanzania 4% and Mozambique 3.8% (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 suffering 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).

Preventive measures such as the sleeping under long lasting insecticidal nets (LLINs), insecticide treated mosquito nets (ITN) and the use of indoor residual spraying (IRS) to control vectors and chemoprevention cannot be overemphasized as effective tools (Reference). In 2021, WHO recommended the use of RTS,S/AS01 vaccine for children as it has been found to give protection against malaria infection.

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

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 districts (North, Central and South) and 21 local government areas with total population of 3178950 based on 2006 census. The

Comment [h5]: The authors should revisit their information whether they were any covid-19 outbreak before December 2019

Comment [h6]: Kindly remove this section because of repetition

Comment [h7]: The authors should replace by "burden"

Comment [h8]: The authors should update the statistics till 2024

Comment [h9]: The authors should confirm the types of insecticide treated nets distributed to the population of study areas.

Comment [h10]: The authors should shed more light on the implementation of malaria vaccine "mosquiris" in Cameroon, kenya,Bukina Faso, Mali and Nigeria and its potency

Comment [h11]: The authors should identify the gap in knowledge they want to address

Comment [h12]: The authors attest whether they are referring to senatorial districts?

Comment [h13]: The readers will like to know recent data on the population census.

minimum and maximum temperatures are 18□C and 42□C respectively(Reference). The annual rainfall ranges from 700mm to 1600mm within the period of April to October. Its coordinates are 9□20′N 12□30′E and it has landmass of 36917km²(Reference).. 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(Reference)..

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 (10-11).

Comment [h14]: The vegetation zones are not tributary of farming but nature

Comment [h15]: The authors should take note of the excessive use of "it" and "it is" in the manuscript

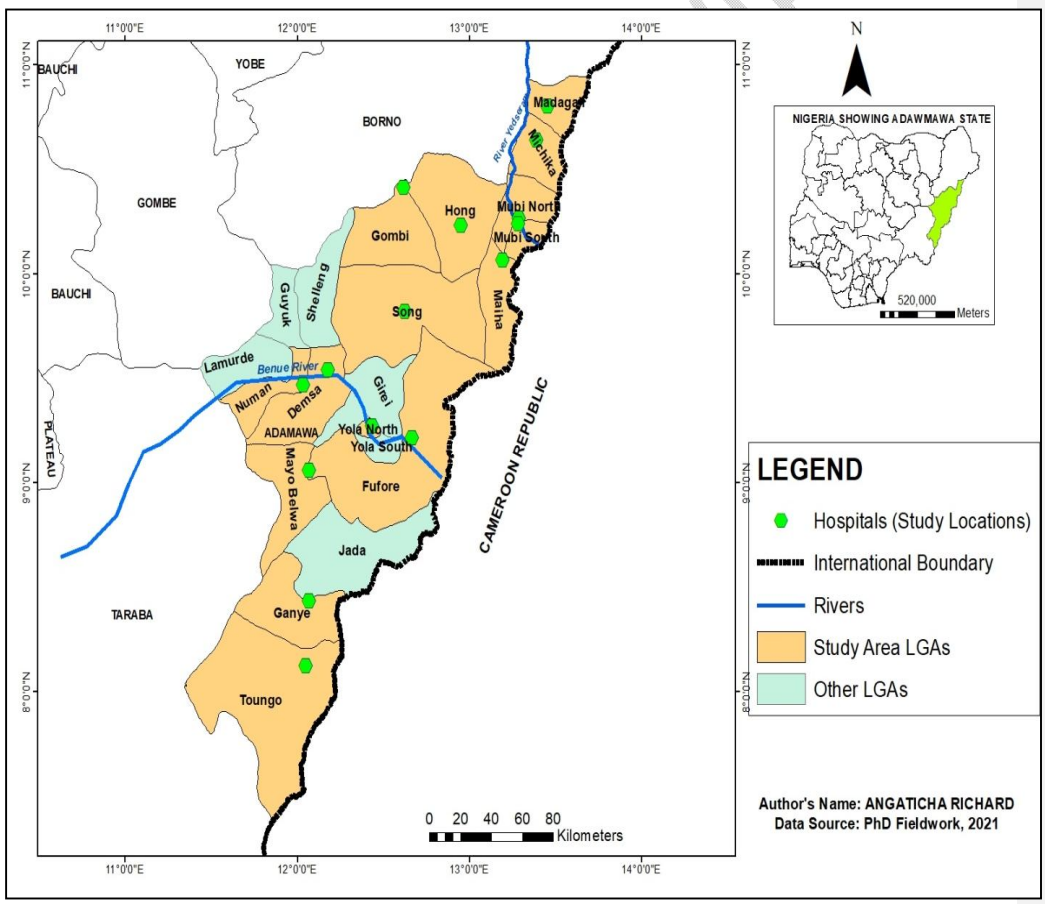

Figure 1: Map of Nigeria showing study areas.Source:

Comment [h16]: The authors should take note that the map does not specifically indicates the three zones under study

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. 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 (12).A total of 1200 samples were collected.

SAMPLE COLLECTION

After verbal explanation, questionnaireswere distributed to subjects who gave consent to collect bio-data, information about their socioeconomic level and prevention with mosquito nets; parents gave consent for subjects under 18 years of age. From each of the 15 selected health facilities 80 samples were collected.For laboratory investigation 5ml of blood was collected from each subject with assistance of the licensed laboratory scientists working in the health facilities. Each sampling points coordinates was obtained by GPS Application by Ketan computers (13)

INCLUSION CRITERIA

The subjects must be residents of the study areas, apparently healthy and not on anti-malaria prophylaxis.

EXCLUSION CRITERIA

Subjects that are on malaria treatment and visitors (e.g travellers) 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). 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

Comment [h17]: Please the sample size calculation was based on which prevalence. The authors should also mention the reference

Comment [h18]: The authors should mention the level of significance, the confidence level, the marginal error. and the adjustment level for nonresponse rate.

Comment [h19]: How was the questionnaire administered?

Comment [h20]: The authors should transfer this highlighted section to ethical consideration

Comment [h21]: The authors should remove the highlighted section

Comment [h22]: The authors should mention the period the samples were collected

Comment [h23]: The authors should bevery clear concerning the under-mentioned issues:
-This is a hospital-based study not community.
-Do the patients attend the secondary health centers for suspected malaria or for others pathology, clarify.

ETHICAL CLEARANCE

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 attendees and guardian of children under 18 years gave written informed consent to participate in providingblood sample.A verbal explanation of the objectives,benefits and potential risks associated with participation in the study were given to all participants. Participant's personal information was kept confidential.

LABORATORY PROCEDURE

From each consented subject 5ml of venous blood was obtained. Thick (stained with Field stain A and B) and thin (stained with 3% Giemsa) blood films was 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.Molecular Plasmodium species identification was carried out at Nigerian Institute of Medical Research (NIMR) using 108 randomly collected samples (3 dry blood spot on each Whatman paper prepared).

Parasitaemia was calculated using the formular;

Parasite count × 8000/set range of WBC=parasite /µl (14).

DATA ANALYSIS

Chi-square test, analysis of variance and descriptive statistics were used for comparison of malaria parasiteamia in the study areas.

DNA EXTRACTION

Comment [h24]: The authors should note that the section is "ethical considerations" not "ethical clearance"

Comment [h25]: Please change the sub-title to "Ethical considerations"

Comment [h26]: Was informed consent obtained from parents or guardians of under 18 participants?

Comment [h27]: The authors should inform us the benefits of patients partaking in the study. This is part and parcel of "ethical consideration"

Comment [h28]: Were the samples positive for *Plasmodium specie* by microscopical examination or selected randomly

Comment [h29]: The authors should Specify the statistical package used to analyze the data. This section should be re-written

The spin column-based extraction of genomic DNA was carried out using DNA extraction kit (NIMR Biotech). Each dry blood spot was punctured 3 times into sterilizedeppendorf tube, 150µl of phosphate buffer saline (PBS) was added to soak the small cuts for 24 hours. The mixture was centrifuged at 10000rpm for 2 minutes after which 100µl of the upper layer was removed into a new sterile micro centrifuge tube and 300µl of lyses buffer was added. The mixture was then homogenized by vortexing and incubated at 50□C for 10 minutes to denature protein. To bring down the trapped liquid, the mixture was centrifuged at 10000rpm for 1 minute; absolute ethanol (200µl) was added to precipitate the DNA and transferred into the spin column; the spin column contains silica which attracts DNA in the presence of chaotropic salts and appropriate buffer. These was centrifuged at 10000rpm for 30 seconds (the centrifuge forced the solution through a silica membrane that is inside the spin column, under the right ionic conditions nucleic acid binds to the silica membrane as the rest of the solution passes through). The flow through was discarded and the collection tube blotted on tissue paper.

To the spin, 500µl of Wash Buffer (WB) 1 was added (WB 1 contains chaotropic agent and buffer solution with PH at or below the pKa of the surfacesilanol groups to enhance DNA absorption). These were centrifuged at 10000rpm for 30 seconds. The new flow through was discarded and the collection tube blotted on a tissue paper. To the spin, 500µl of Wash Buffer 2 was added (WB 2 contains buffer solution with PH at or below the pKa of the surface silanol group), then centrifuged at 10000rpm for 1 minute. The flow through was also discarded and the collection tube blotted on tissue paper. The spin column was centrifuged again at 12000-14000rpm for 3 minutes to remove all traces of ethanol. The spin column was placed into another micro centrifuge tube and 50µl of elution buffer was added to the column (low salt or aqueous liquid for eluting hydrophilic DNA). The eluent was incubated at room temperature for 1 to 2 minutes (for adequate incubation), then centrifuged at 10000rpm for 1 minute to elute the DNA. The DNA was stored at -20□C.

Comment [h30]: Provide this section as supplementary materials thus, it should be removed

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

MOLECULAR CHARACTERISATION OF PLASMODIUM SPECIES

Comment [h31]: Replace the sub-title by "Plasmodium speciation by species-specific nested PC "

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 (15).

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 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 electrophoresis.

Comment [h32]: The authors should mention the content of the master mix

Comment [h33]: The authors should list the content of the 12.5µl PCR

Comment [h34]: The authors should note that no negative and positive control mentioned

Comment [h35]: The authors should specify the stain used for the agarose gel

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: MALARIA 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<0.05.

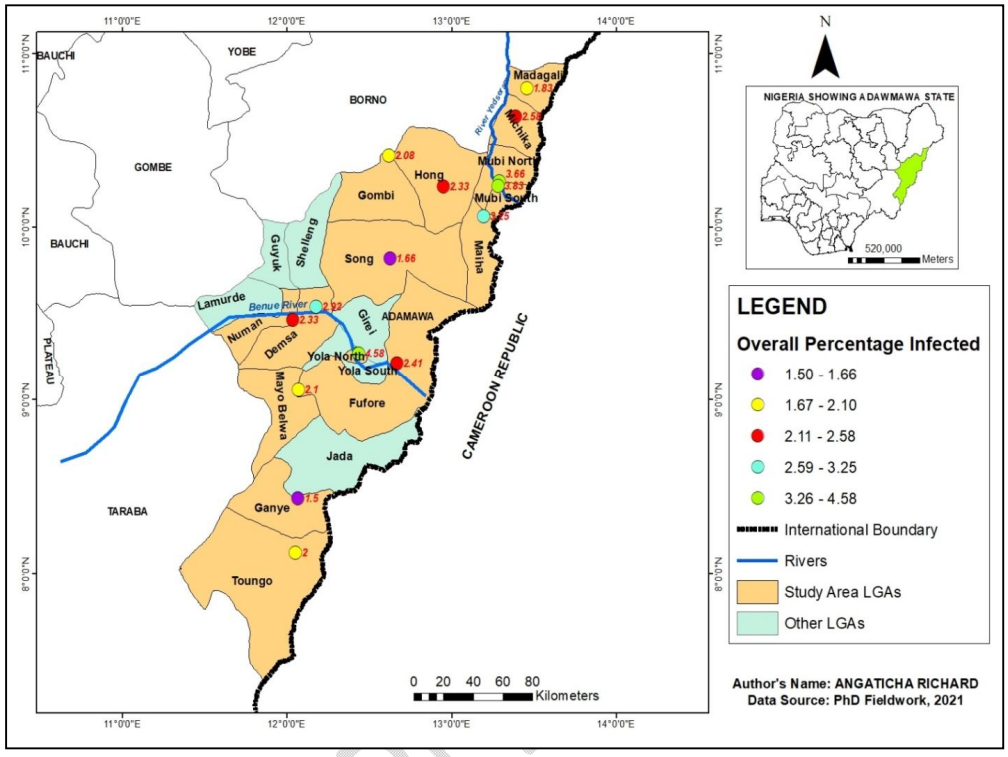

Figure 2: Map of Adamawa state showing statewide (overall %) infection in each location.

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<0.05.

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<0.05. 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, $\square^2$=14.204, P=0.001

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<0.05 Table 5. In relation to sex, female was higher with 17.9% infection and male had 13.5% P<0.05. 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.

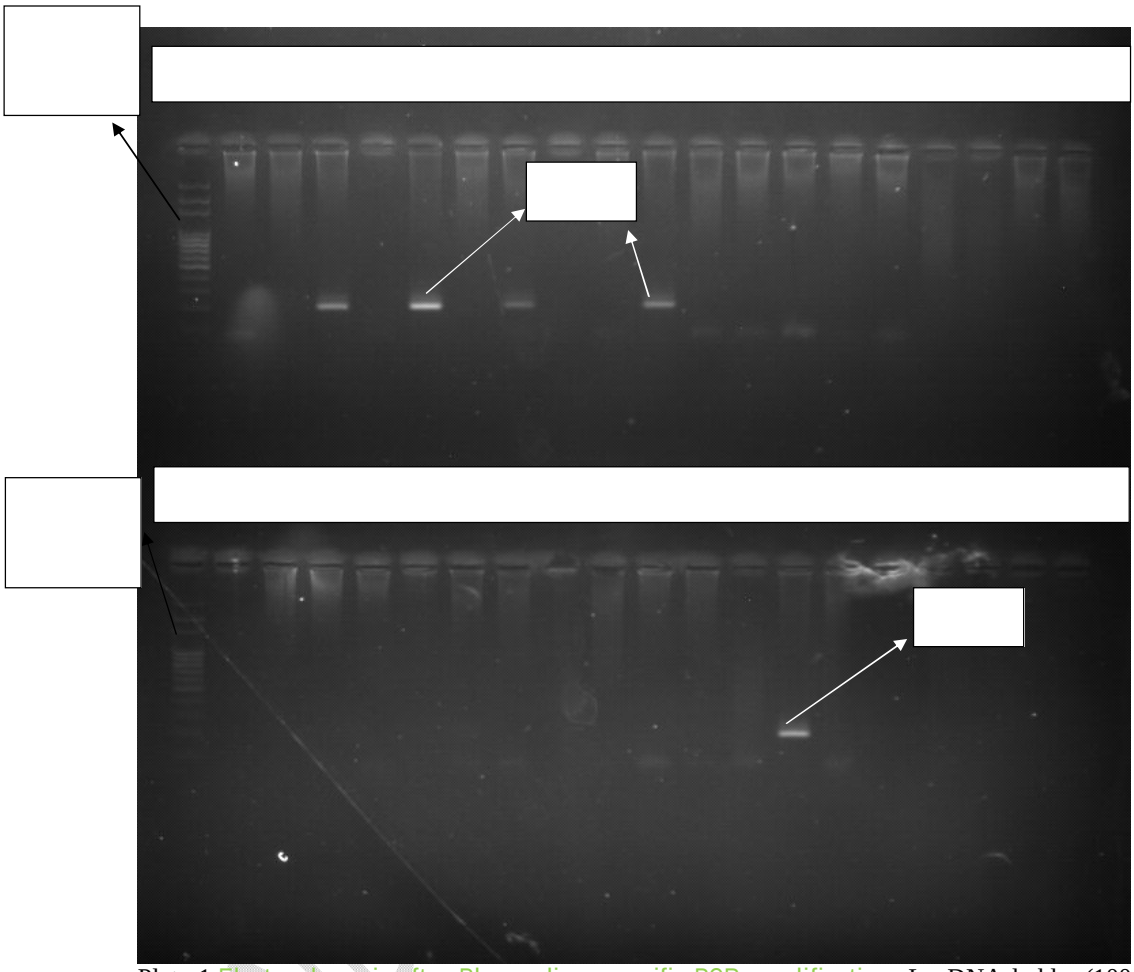

Plate 1:Electrophoresis after Plasmodium-specific PCR amplification: L= DNA ladder (100 base pair), Lanes 65, 8, 6, 2, and 58 are positive.

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% $P<0.05$. 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<0.05 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<0.05. 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<0.005. 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

Malaria remains a significant global burden despite being a preventable and treatable infection. As reported in 2020, Nigeria and Democratic Republic of Congo leads in global malaria cases

with 27% and 12% respectively (7). 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 with visual presentation to provide information on the epidemiology of the infection for data driven interventions. 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 (16). It also concurs with findings in Rivers state, Nigeria (17) and Kano, Nigeria (18). 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 (7).

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 (19). 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 could serve as contact sites with mosquitoes amidst other factors like clustered city setting leading to high rate of infection in Mubi town. Difference in number of infection by location in Adamawa state was not statistically significant as $P>0.05$ whereas parasite density was significant as $P<0.05$. 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 (11) 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 (20). The difference in percentage infection by zone was significant as $P<0.05$.

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 (7 & 21). 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 (22). 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 (19) and in Emohua, Rivers state, Nigeria (17).

According to socioeconomic status, the middle class recorded highest infection 43% followed by higher class 38.6% $P<0.05$. 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) 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), hence ownership is not enough (25). 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 $P<0.05$.

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. Free malaria communities could lead to improved socioeconomic status as the infection is associated with economic difficulty.

REFERENCES

1. https://stanfordhealthcare.org/medical-conditions/primary-care/malaria/types.html

2. Enock K Pukuma SM Augustine LM, Gundiri L.B., Zamdayu NM and Daniel JL. Prevalence and associated risk factors of malaria infection among pregnant women attending antenatal clinic in Yola North, Adamawa state. *The zoologist*2002; 18:19-25. https://dx.doi.org/10.4314/tzool.v18i1.4
3. Wahedi JA., Ande AT.,Oduola AO, Obembe A,Tola M, Oyeniyi TA and Awolola TS. Dynamics of malaria vector indices in two vegetation zones within North Eastern Adamawa state, Nigeria. Tropical Biomedicine 2020; 37(3): 637-649.
4. Warral E, Suprotik B, and Hara H. (2003). The relationship between socioeconomic status and malaria: a review of the literature. https://assets.publishing.service.gov.uk/media/57a08c12e5274a27b2000f71/WP01_03.pdf.
5. Somi MF, Kachur, SP, Butler J, Njau JD, Vahid, F, & Abdulla S. Is there evidence for dual causation between malaria and socioeconomic status? Findings from rural Tanzania.*The American journal of tropical medicine and hygiene*, 2007; 77 6, 1020-7.
6. World Health Organisation. Malaria keyfacts 2023
7. World malaria report 2022. World Health Organisation 2022. Licence: CC BY-NC-SA3.0 IGO
8. World Health Organisation. Malaria fact sheet, 2018.
9. Nigeria Malaria Fact Sheet.United State Embassy in Nigeria, 2011.
10. https://www.citypopulation.de/en/nigeria/admin/NGA002_adamawa/
11. Wahedi JA., Ande AT.,Oduola AO, Obembe A,Tola M, Oyeniyi TA and Awolola TS. Dynamics of malaria vector indices in two vegetation zones within North Eastern Adamawa state, Nigeria. Tropical Biomedicine 2020; 37(3): 637-649.
12. Glenn DI. Determining sample size. Programme evaluation development, Florida PEOD-6 2003; Pp 4.
13. www.mobile-CDR.com
14. World Health Organisation. Malaria parasite counting. Malaria microscopy standard operating procedure-MM-SOP-09. 2016.
15. Snounou G, Viriyakboso S, Zhu XP, Jarra W, Pinheiro L, doRosario VE, Thaithong S, Brown KN. High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. Mol Biochem Parasitol 1993; 61:315-320.
16. World Health Organisation (2019). Malaria factsheet.
17. Richard A., Nduka F.O., Awi-waadu G.D.B., and Wogu M.N. Malaria parasitaemia among selected residents of Emohua Local Government Area and Port Harcourt metropolis, Rivers state, Nigeria. *Nigerian Journal of Parasitology*2019; 4:1.32-36.
18. Salwa D, Hesham M, Al-Mekhlafi, II, Jamaiah I, Wahid MA, Awatif MA *etal*.Is Nigeria winning the battle against malaria? Prevalence risk factors and KAP assessment among

Hausa communities in Kano state, Malaria journal 2016; 15. Doi:10.1186/s12936-016-1394-3.
19. Kunihya I.Z., Samaila AB, Nassai I, Sarki A, and Haruna MY. Prevalence of malaria infection among children attending specialist hospital Yola, Adamawa state, Nigeria. *JournalofMedical and Biological Science Research.*2016; 1:8: 132-142.
20. Kumur JH, Elishama LJ, Joshua DZ, John AG and Adamu BM. Regional trends of malaria cases in Adamawa state. *GlobalScientificJournals*2019; 8:732-737.
21. Abossie A, Yohanes T, Nedu A, Tafesse W and Damitie M. Prevalence of Malaria and Associated Risk factors Among Febrile children under five years: A cross-sectional study in Arba Minch Zuria District, South Ethiopia. Infection and drug resistance, 2022; 13,363-372. https://doi.org/10.z147/IDR.S223873
22. Naphtali RS, Babylon P, Musa ZJ, and Atinga A. Prevalence of Malaria Infection in Yola-South L.G.A. of Adamawa state. IORS *Journalof Pharmacy and Biological sciences*2017; 12:5:58-61.
23. Health think team (2017). Malaria in Nigeria: Where are we now?
24. Bertozzi-Villa A, Bever CA, Koenker H, Weiss DJ, Vargas-Ruiz C, Nandi AK, *etal*. Maps and metrics of insecticide-treated net access, use and nets-per-capita in Africa from 2000-2020. *Nature communications*2021; 12:3589.
25. Orish VN, Maalman RSE, Donkor OY, Cemantis BYM, Osei E., Amu H, *etal*. Assessing health-seeking behavior and malaria prevention practices among communities in four districts of the Volta region of Ghana. *Malariajournal*2021; 20:450.