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DISTRIBUTION OF *PLASMODIUM* SPOROZOITES IN ANOPHELINE MOSQUITO VECTORS IN SOME AREAS OF KATSINA STATE, NIGERIA

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ABSTRACT
Control of malaria is highly dependent on accurate identification of *Plasmodium* species infecting, because of their variability in terms of sensitivity to drugs and susceptibility to the immune system. Therefore a survey of *plasmodium* species present in Katsina State was carried out from 2009 to 2014 to determine the distribution of Plasmodium sporozoites in Anopheline mosquito vectors in some areas of Katsina State, Nigeria. *Anopheles* mosquitoes were caught indoors using the Pyrethrin spray sheet method and were subjected to VecTest, using Dipstick assay. Zone A (Southern Katsina) recorded more *P. falciparum* and *P. malariae* than Zone B (Central Katsina) which in turn recorded more than Zone C (Northern Katsina). *P. falcifarum* was the most preponderant in Zone A, having the highest of 163 at Dandume and its lowest was 40 at Daura. There was no significant difference between *P. falciparum* and *P. malariae* within the zones (p<0.05, F=6.79). No *P. vivax* 210 nor *P. vivax* 247 were encountered during the current study. There was a very high significant difference between *P. falciparum* and *P. malariae* isolated from *An. gambiae* and *An. arabiensis* within the zones (p <0.05, F=161.44). There is a very high significant difference between malaria sporozoites isolated from *An. gambiae s.s.* than in *An. arabiensis* or *An. funestus s.s. nor An. brucei* (p <0.05, F=161.44). *An. gambiae* s.s recorded the highest number of *P. falcifarum* (190) and *P. malariae* (289), while *An. quadrimaculatus* had the lowest for both species. The highest infections with *Plasmodium malariae* species in 2009 and 2010 were in the month of July with 82 and 78 respectively. The implications of the above findings is that both *An. gambiae* and *An. funestus* are transmitting *P. malariae* and *P. falciparum*. In conclusion, there were more *P. malariae* than *P. falcifarum* species throughout 2009, 2010 and 2011. The month of July was the peak of infection for *Plasmodium* species both in 2009 and 2010.

Keywords: Distribution, abundance, *plasmodium*, sporozoites, VecTest, Dipstick assay

INTRODUCTION

To control malaria effectively, the *Plasmodium* species present in a locality must be identified accuarately. Malaria is a burden both socially, economically, psychologically around the world. For instance, the cost of malaria treatment and prevention in Nigeria has been estimated to be over about US$37.55 per case (Ezenduka *et al*., 2017) and upto a $1 billion per annum (Odaibo, 2006; Olayemi and Ande, 2008). Malaria killed 401 people within the four weeks of September, 2011 (IGNtake, 2011) in Katsina State (according to local health officials)(IGNtake, 2011). And up to 50,000 malaria cases were recorded in the same year 2011 (WHO, 2011).

About 90% of global malaria mortalities occur in Africa (African Malaria Report, 2003). This high figure is a result of the efficient transmission of *Plasmodium falciparum* by *An. gambiae*, *An. arabiensis*, and *An. funestus* (Fanello *et al*., 2003). *An. gambiae* and *An. funestus* both feed on human blood and that facilitate their vectorial capacity (Brady *et al*., 2016). However diurnal differences exist between *An. gambiae* and *An. funestus*, therefore there should be more reliable identification of both species (Fanello *et al*., 2003) so as to facilitate control of mosquitoes and malaria. Factors that affect a mosquito's ability to transmit malaria include its innate susceptibility to *Plasmodium*, the choice of host and its life span (Fanello *et al*., 2003).

Currently there is no data to incriminate the specific malaria parasite responsible for malaria episodes seen in Katsina State (Umar and Nock, 2018). Does the malaria bouts currently reported originate from mosquitoes present in areas of Katsina State or are the malaria parasites imported from neighbouring states of Kano, Kaduna or even from Niger Republic. These can only be answered through the isolation and identification of *Plasmodium* parasites from locally available mosquitoes prevalent in Katsina State. And which data can be utilised to produce maps of areas of relative malaria risk as a result of the spatial heterogeneity of mosquito populations in Katsina State as was done earlier elsewhere by numerous authors like Fanello *et al*. (2003). Consequently, the current research's aim is to determine the Distribution of *Plasmodium* sporozoites in Anopheline Mosquito Vectors in some areas of Katsina State, Nigeria.

MATERIALS AND METHODS

Study Area

Katsina State is 1,696 feet above sea level, it is located between 12°15' N - 7°30' E and 12° 25'N - 7.5° 0'E (Fig 1). It has an area of 24,194 km^2, with a population of 3,878,344 million and a population density of 160.3/km^2 (KSMLS, 2010). The climate of Katsina State is characterized by two well demarcated seasons, the rainy season, extending from May to September and the dry season from October to April. The dry season is characterized by harmattan dust between November and

February. The maximum amount of rainfall occurs between

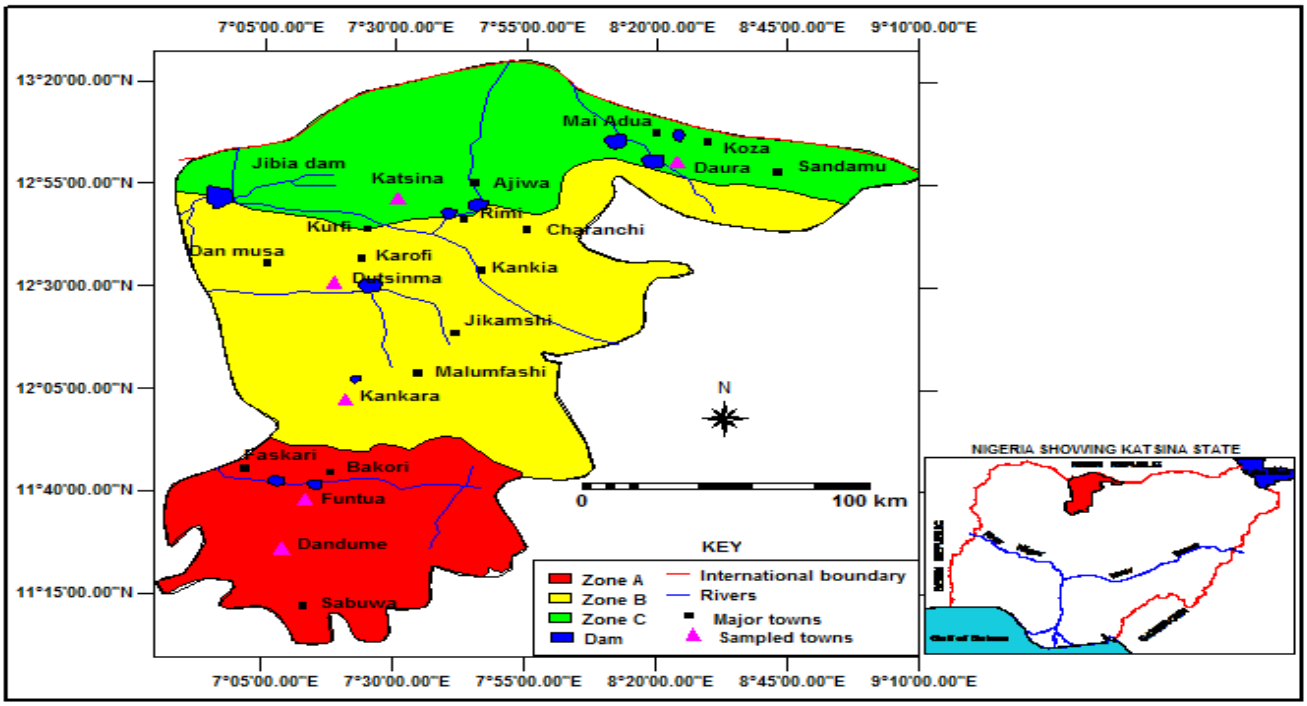

FIGURE 1 : KATSINA STATE SHOWING THE SAMPLED TOWNS AND VEGETATION ZONES

Source : Adapted and Modified from Katsina State Map, Ministry of Lands and Surveys

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green coloured area).

Collection and Assay of *Plasmodium* Sporozoites

Identification of *Plasmodium* Sporozoites

Plasmodium sporozoites were identified according to the procedure of Appawu *et al*. (2003) based on PCR techniques as follows: Only female Anopheline mosquitoes were used. Homogenized samples of mosquitoes were stored at - 20°C until processed.

Homoginization

One to 10 female Anopheline mosquitoes were placed into the conical grinding tube provided in the kit, so as to provide the adequate DNA required, and then placed in the tube stand provided.

Thirteen drops (250µl) of Grinding Solution from the dropper bottle was dispensed onto the mosquitoes in the tube. The mosquitoes were homogenized using a pestle provided in the kit. A test strip from the canister was then placed into the mosquito suspension in the grinding tube with the arrows pointing down. The desiccant cap was replaced on the canister to protect the remaining strips from moisture. Fifteen minutes was allowed for the test to be completed.

The test results were determined by removing The test strip was compared to the pictorial sample provided on the container. When the sample reaches the Control Zone, these unreacted conjugates binds to the antibody immobilized on the membrane and a signal is produced.

Before use, the Grinding Solution was mixed by gently inverting the bottle five times. The bottle was held in an upside-down, vertical position and the white portion of the dropper cap just above the tip was squeezed. Care was taken when dispensing drops of grinding solution into tubes by holding the bottle firmly and not at an angle, because the size of the drops may vary, and can cause the total amount of dispensed solution to be incorrect (Appawu *et al*., 2003).

The test results were rejected if the control line failed to develop and the tests were run again. The test was only interpreted as positive if two or more lines developed. When only one control line developed on the dipstick, the result was treated as negative. However the presence of two close lines indicated *P. falciparum*, two widely separated lines indicate *P. vivax* 210 however two far apart lines indicated *P. vivax* 247. Two closely packed lines indicated the presence of *P. falciparum.* Results were read within 30 minutes of performing the assay. Because thereafter, the dipsticks will developed a gray colour that can obscure accurate interpretation of results.

Anopheles mosquitoes caught indoors were subjected to VecTest, using Dipstick assay, during which aliquouts (150 ul) of *Anopheles* mosquito triturates made from squashed mosquito thoraces using grinding tubes were transferred into the wells of micro-titre plates (sero-wel; Bibby Sterilin Ltd, Stone Staffs, UK) for testing. VicTest dipstick strips (MAS TM; Camarillo, CA, USA), were placed separately in the test wells of the micro-titre plate and allowed to develop for 15 min at room temperature. Positive dipstick result were indicated by a horizontal reddish purple line in addition to a control line on each test strip in a distinct detection zone (Appawu *et al*., 2003).

Anopheles mosquitoes were caught indoors using the Pyrethrin Spray Sheet method of Service, (1993), once a month, for a period of 8 months during dry (4 months) and wet (4 months) seasons. All the windows and the door of a sampled room were closed and subsequently a sheet was spread to cover the entire floor of the room before a Pyrethrin insecticide was spread

round the room from top to bottom. Thereafter, the room was left closed for 15 minutes. Afterwich all the mosquitoes killed by the insecticide fell on the sheet and the sheet was carefully folded and the caught mosquitoes were transferred into a plastic container and labelled in terms of: Date of collection, name of collector and place of collection.

The monthly distribution data of *P. malariae* and *P. falciparum* during both dry and wet season was obtained from patient file records at Daura, Katsina and Funtua General Hospitals, after obtaining the consent of patients. Ethical clearance was strictly adhered to after screening by the ethical committee.

The seasonal distribution was evaluated by comparing the population of mosquitoes caught during rainy to those caught during dry season.

Seasonal distribution of *P. malariae* and *P. falciparum* across sampled months based on Hospital Records

Determination of *Plasmodium* Sporozoites

Zone A recorded 581 *P. falciparum* and *P. malariae*, Zone B had 520 and Zone C, 253. The VecTest evaluation dealt solely with indoor-collected specimens, that is 926 of *An. gambiae* and 431 of *An. funestus* (Table 1). *P. malariae*was the most preponderant in Zone A, having the highest of 209 (35.9%) at Dandume and its lowest of 36 at Daura. And the highest distribution of *P. falciparum* was also in Zone B with 88 (16.9%) at Kankara and the least were in Zone C with 36 in Daura. There was no significant difference between *P. falciparum* and *P. malariae* within the zones (p<0.05, F=6.79).

RESULTS

Table 1: Zones, Locality and *Plasmodium* sporozoites identified by Vectest

Zone	Locality	*Plasmodium* sporozoites identified by Vectest				Total
		P. falcifarum	*P. malariae*	*P. vivax (210)*	*P. vivax* (247)	
Zone C	Katsina	54 (33.3%)	108 (66.7%)	0	0	162
"	Daura	36 (39.56%)	55 (60.44%)	0	0	91
Zone B	Dutsinma	78 (31.70%)	168 (68.29%)	0	0	246
"	Kankara	88 (32.11%)	186 (67.88%)	0	0	274
Zone A	Funtua	80 (27.97%)	206 (72.02%)	0	0	286
"	Dandume	86 (29.15%)	209 (70.84%)	0	0	295
	Total	422 (31.68%)	832 (62.42%)	0	0	1354

No *P. vivax* 210 nor *P. vivax* 247 were identified in any of the sampled Zones during the current study. There was a very high significant difference between *P. falciparum* and *P. malariae* isolated from *An. gambiae* and *An. arabiensis* within the zones (p <0.05, F=161.44). There was significant difference between *P. malariae* and *P. falciparum* sporozoites isolated from *An. gambiae s.s* (658); which was higher than those isolated from *An. arabiensis* (228*);* which was also higher than those isolated from *An. funestus s.s* (313); which was also higher than those of *An. brucei* (81) (p <0.05, F=161.44), across Zone A, Zone B and Zone C respectively. *An. gambiae* s.s. recorded the highest number of *P. falcifarum* with 277 and also the highest *for P. malariae* with 381, while *An. quadrimaculatus* had the lowest of 2 *P. falcifarum* and5 of *P. malariae* (Table 2).

Table: 2: Prevalence of *Plasmodium* sporozoites in the identified *Anopheles* spp.

Anopheles species	*Plasmodium* sporozoites identified by Vectest				Total
	P. fal.	*P. mal.*	*P. vivax (210)*strain	*P. vivax* (247) strain	
An. gambiae ss	277 (51.49%)	381 (47.98%)	0	0	658
An. arabiensis	95 (17.66%)	133 (16.75%)	0	0	228
An. funestus s.s	120	193 (24.31%)	0	0	313
An. brucei	(22.30%)	51 (6.42%)	0	0	81
An. rivulorum	30 (5.58%)	24 (3.02)	0	0	35
	11 (2.04%)				
An. maculipennis	3 (0.56%)	7 (1.18%)	0	0	10
An. quadrimaculatus	2 (0.37%)	5 (0.63%)	0	0	7
Total	538	794	0	0	1332

P. fal. = Plasmodium falciparum *P. mal. = P. malariae*

Distribution of Malaria Across Sampled Months Based on Hospital Records (Daura, Katsina and Funtua General Hospitals)

The highest malaria infections with *Plasmodium* species from 2009 to 2011 was in the month of July, 2011 with 110. Overall, there were more *P. malariae* (735) and less (389) *P. falcifarum* species recorded during 2009, 2010 and 2011 (Table 3, Fig 2, 3 & 4). However, more *P. falciparum* and less *P. malariae* were recorded across the three zones studied. One-Sample t-Test and Chi-Square indicated no significant difference between *P. falciparum* and *P. malariae* from June to May of 2009/2010, 2010/2011 and 2011/2012 seasons, but there was a significant Pearson correlation at 0.01 level. This implies that *P. falciparum* was responsible for most of the malaria episodes in the study area.

Table 3: Monthly prevalence of *Plasmodium* species by hospital records

		Number of *Plasmodium* identified by Vectest								
		2009			**2010**			**2011**		
Month	Total	*P. fal.*	*P. mal*	*Total*	*P. fal.*	*P. mal*	*Total*	*P. fal.*	*P. mal*	
June	42	25	17	45	16	29	40	14	26	
July	48	29	19	40	15	25	110	22	40	
August	25	10	15	34	14	20	41	15	26	
September	22	7	18	31	13	18	53	18	35	
October	21	8	13	40	15	25	31	12	19	
November	19	6	13	62	25	37	62	35	75	
December	15	5	10	98	28	70	0	0	0	
January	0	0	0	63	19	44	0	0	0	
February	0	0	0	58	17	41	0	0	0	
March	0	0	0	62	22	40	0	0	0	
April	0	0	0	35	12	23	0	0	0	
May	0	0	0	27	6	21	0	0	0	
Total	192	90	102	595	183	412	337	116	221	

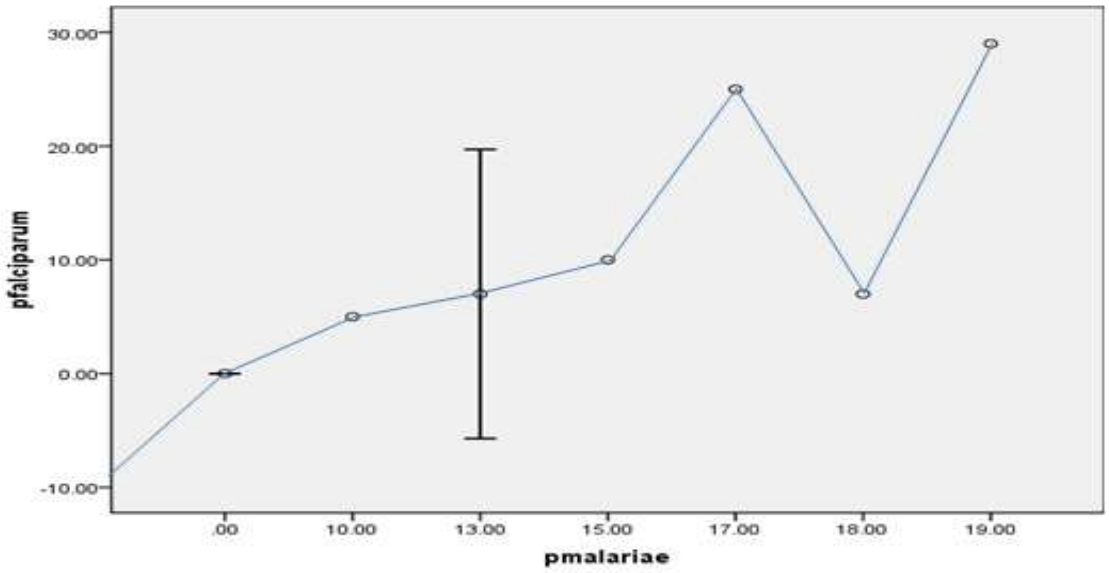

Fig 2: Correlation between the monthly distribution and abundance of *P. falciparum* (y-axis) and *P. malaria* (x-axis) between June 2009 to May 2010; during which a mono-peak abundance of the species occurred. There was a significance at 13.00

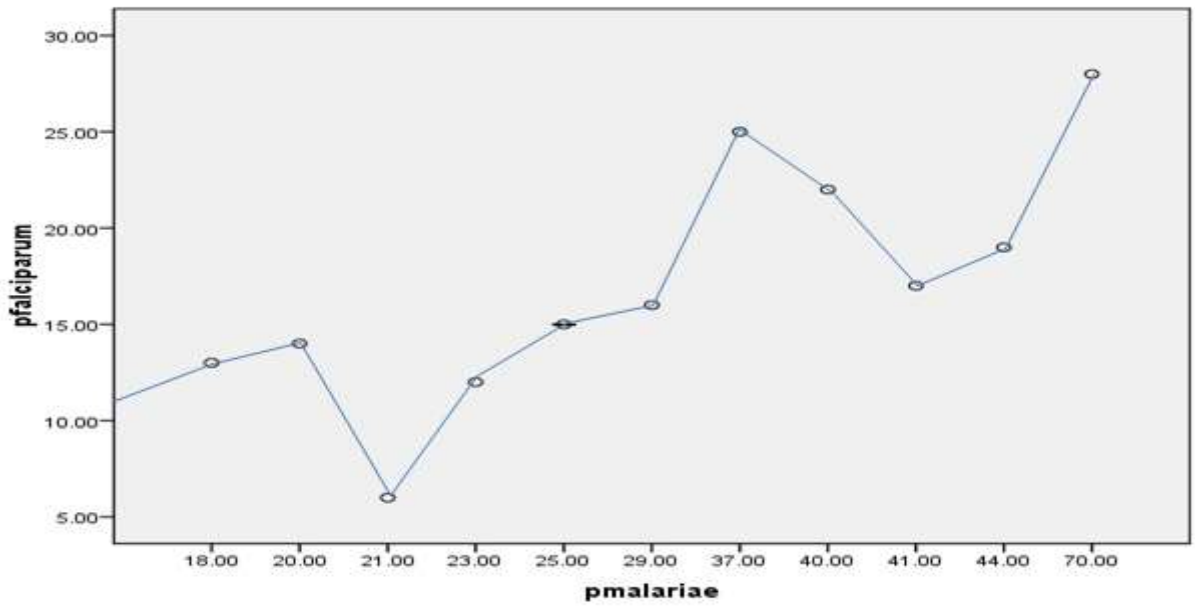

Fig 3: Correlation between the monthly distribution and abundance of *P. falciparum* (y-axis) and *P. malaria* (x-axis) between June 2010 to May 2011; during which a bi-peak abundance of these species was recorded. There was a significance at 25.00

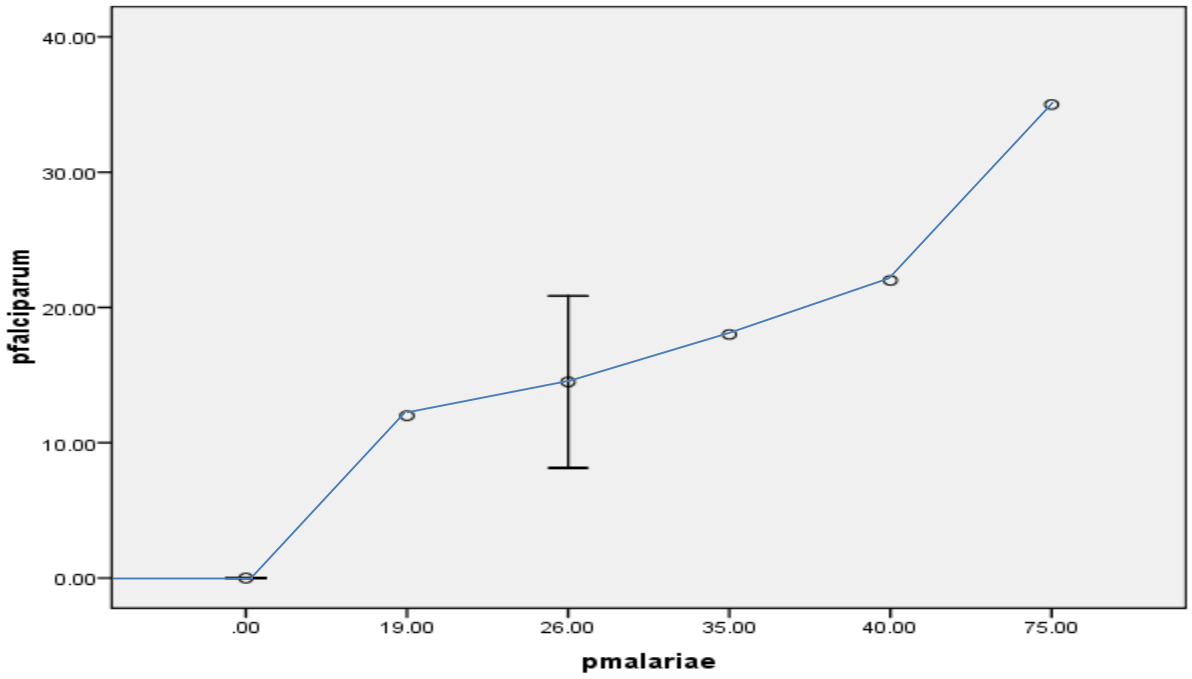

Fig 4: Correlation between the monthly distribution and abundance of *P. falciparum* (y-axis) and *P. malaria* (x-axis) between June 2011 to May 2012; during which a tri-peak abundance of these species occurred. There was a significance at 26.00

DISCUSSION

In this study, the high *Plasmodium* sporozoites recorded in *Anopheles* during the wet season actually coincides with the confirmed hospital cases of malaria observed based on retrospective malaria records in the sampled hospitals in the study areas as reported by Umar and Knock (2018). According to Kulwichit *et al.* (2000), *Plasmodium malariae* was successful in sustaining malaria because it persist in humans as an asymptomatic erythrocytic disease for many years if it is not treated conclusively, during which it causes problems in the liver (Kulwichit *et al.*, 2000). Therefore *Plasmodium malariae* plays a significant role in malaria infection and transmission within the studied zones.

During this study, it was determined that Zone A has more hospital recorded cases of malaria because malaria transmission is directly linked to mosquito proliferation (Umar and Knock, 2018). Eventhough, Umar and Knock (2018) have reported that hospital records of malaria transmission showed no significant difference in malaria cases in the three studied zones of Katsina State. This begs the question: is malaria prevalence related to other conditions apart from weather? The answer is yes, malaria transmission is promoted by the absence of proper drainage, inadequate sanitation, presence of adequate insecticide treated nets (ITNs) among others as also reported by Onyabe and Cohn (2001). The implication is that there is inadequate drainage, sanitation and ITNs in the study areas.

No *P. vivax* (210 strain) and *P. vivax* (247 strain) were identified during the study. A similar report was made by Gautret *et al*. (2001) and Culleton *et al*. (2008) who reported that *P. vivax* is absent from West and Central Africa due to the high prevalence of the Duffy negative phenotype in the indigenous populations. The Duffy binding proteins of *P. vivax* (PvDBP) are located on their merozoites (Fong *et al*., 2014). Consequently, *P. vivax* (210 strain) and *P. vivax* (247 strain) do not play any role in malaria transmission in the study area.

Hospital records of malaria in 2011 corroborated the high prevalence of malaria observed in the months of July and August during this study (Umar and Knock, 2018). This is consistent with IGNtake (2011) report that malaria killed 401 people in the last four weeks of September, in 2011 in Katsina State. Similarly, WHO (2011) reported 50,311 malaria cases in Katsina State in September, 2011. IGNtake (2011) attributed that to the heavy rainfall recorded in 2011 in Daura, Funtua, Ingawa, Kurfi, Dutsinma, Katsina and Bindawa towns. Similar fondings were reported by Legros *et al*. (2006) and WHO-CISD (2008).

Furthermore, Alaba and Alaba (2010) opined that the incidence of malaria varies with weather, and that affects the ability of *Anopheles* to survive or otherwise. In addition, tropical areas including Nigeria have the best combination of adequate rainfall, temperature and humidity which facilitate the breeding and survival of anopheline mosquitoes.

CONCLUSION

P. malariae is more preponderant than *P. falciparum* species in Zone A, which in turn recorded Katsina State and their vectors include *An. gambiae and An. funestus* species, which were more abundant in the month of July. No *P. Vivax* was identified during this study.

Zone A (Northern Katsina) recorded more *P. falciparum* and *P. malariae* than Zone B (Central Katsina) which in turn recorded more than Zone C (Southern Katsina). *P. falcifarum* was the most preponderant in Zone A

RECOMMENDATION

Plasmodium species are preponderant during the rains in Katsina, therefore people should take stringent measure against mosquitoes during the rains by staying indoors, using ITNs, seek prompt medical help when they observe fever symptoms, and ensure adequate environmental sanitation and drainage.

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