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Species Composition and *Plasmodium falciparum* Infection Rates of *Anopheles gambiae s.l.* Mosquitoes in Six Localities of Kwara State, North Central, Nigeria

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ABSTRACT: Entomological data gathering is essential for monitoring malaria vector disease risks and selection of appropriate interventions for the protection of exposed human populations. This study assessed the relative abundance, species composition, and sporozoite infection rates of indoor resting *An. gambiae* s.l. malaria vectors in six communities across 3 Local Government Areas in Kwara State, Nigeria. Total number of mosquitoes collected by Pyrethrum Spray Catch method over a period of eighth months were correlated with rainfall values in the area. *Plasmodium falciparum* sporozoite infection rates and sibling species identification of collected *An. gambiae* s.l. mosquito samples were determined by ELISA and PCR respectively. Results showed a positive correlation ($r = 0.639$, $p = 0.08$) between rainfall and numbers of *Anopheles* mosquitoes in the study areas. The overall composition of the *An. gambiae* s.l sibling species in the collected samples from all the six communities showed the predominance of *An. gambiae s.s* 298 (75.3%) compared to *An. coluzzii* 94(23.7%) and *An. arabiensis* 4(1.0%). However, the sporozoite infection rate of *An. coluzzii* (22.3%) was higher compared to *An. gambiae s.s* (12.8%) and *An. arabiensis* (0%)**.** Mean numbers of *An. gambiae s.l* mosquitoes were significantly higher in Ilorin west LGA compared to Asa ($F = 17.81$, $P < 0.001$) and Ilorin East LGAs ($F = 22.81$, $P < 0.001$)**.** Sporozoite rates of both *An. gambiae s.s* and *An. coluzzii* sibling species were higher in Ilorin West communities (Aiyede 21%, Ogundele 32%) compared to Asa (Idi Emi 11.1%, Lasoju 5.1%) and Ilorin East (Oke Oyi 2.4%, Ote-efan 0%) communities. Prevalence of sporozoite-infected *An. gambiae s.s* and *An. coluzzii* indoors highlight the need for effective insecticide treated bed-nets interventions to protect the residents from malaria risks. Higher numbers of *An. coluzzii* in the swampy rice marshed Ilorin West LGA communities require larval source management as an additional strategy for effective malaria vector control.

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The Nigeria National Malaria Strategic Plan (NMSP) 2014-2020 (FMoH, 2014) listed vector control as the first objective for reducing malaria burden. The NMSP aimed at ensuring that 80% of Nigerians utilize preventive measures by the year 2020. As such vector control remains the frontline means of preventing and reducing malaria transmission (WHO, 2018). Implementation of cost-effective vector control interventions rely on adequate knowledge of local vector species attained through sustained entomological monitoring and surveillance activities (WHO, 2019). Such surveillance activities provide information on the dynamics of vector species composition, relative abundance, and sporozoite infection rates (Mbogo *et al.,* 2003). Besides, these and other entomological indices such as vector behaviour and insecticide susceptibility status also serve as the basis for measuring the impact of vector control strategies on malaria transmission (WHO, 2019). Significant spatial heterogeneities have been observed in the abundance of indoor resting *Anopheles* mosquitoes, their species composition and sporozoite infection rates (Sinka *et al*., 2011). Characterization of entomological indices and mosquito behaviour in different settings, therefore, becomes necessary to guide the suitability of interventions such as Long-Lasting Insecticide Nets (LLINs) and Indoor Residual Spray (IRS) designed to target indoor biting or resting mosquitoes (Conn *et al.*, 2015). In Nigeria, the involvement of government and non – governmental partners in vector surveillance and insecticide resistance monitoring implementation has increased across the six geo-political zones (PMI-AIRS, 2018), thus providing comprehensive entomological data for the replacement of isolated entomological surveys of limited scope in Nigeria. Outcomes of these routine monitoring have provided data on malaria vector prevalence and infectivity rates in Nigeria.

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Nevertheless, there is still a paucity of data in some states such as Kwara. This leaves a huge gap to be filled for proper planning, implementation and monitoring of the impact of interventions in reducing human-vector contact in different settings within Kwara and other states in the country where capacity for vector surveillance and insecticide resistance monitoring have not been built. This study provides information on the relative abundance, species composition and *Plasmodium falciparum* sporozoite infection rates of *Anopheles* mosquitoes in six communities across three selected Local Government Areas of Kwara State, North Central Nigeria.

MATERIALS AND METHODS

Description of Study area: The study was carried out in six rural communities: Lasoju (N 8°21.856' E 4°26.201'), Idi –Emi (N 8°21.889' E 4°26.491'), Oke - Oyi (N 8°35' 38.8' E 4°43' 30.0'), Ote-efan (N 8°34' 25.0' E 4°42' 18.8'), Ogundele (N 8° 29.384' E 4° 26.868') and Aiyede (N 8° 29.442' E 4° 27.374'). The communities are located in Asa (Lasoju and Idi –Emi), Ilorin East (Oke - Oyi and Ote-efan) and Ilorin West (Ogundele and Aiyede) LGAs in Kwara State, North-Central, Nigeria. Kwara state is within the transitional climate zone. The rainy season begins from the end of March and ends in October with two peak periods in June and September while the dry season starts in October and ends in February. The annual mean rainfall is about 1,352 mm (Alaaya *et al.,* 2013). Houses in all the study communities were built with mud and corrugated metal sheet, with no ceilings and window nets. The inhabitants are mostly farmers.

Mosquito sample and data collection: Indoor resting mosquitoes were collected in ten houses per community from September 2017 to April 2018 using Pyrethrum spray catch techniques (WHO, 2003). Houses with household heads willing to allow monthly long-term mosquito surveys in the sleeping rooms were chosen until ten houses were randomly selected. One room was surveyed in each house. The same rooms and houses were used for mosquito collections all through the period of the study. Rainfall data for the State was obtained from the Nigerian Meteorological Station at the Ilorin International Airport during the period of the study

Treatment and Analysis of Sample: Mosquitoes collected were preserved individually in Eppendorf tubes with desiccated silica gel. Morphological identification of collected *Anopheles* mosquitoes was conducted using standard keys (Gillies and De Meillon, 1968; Gillies and Coetzee, 1987). Mosquitoes identified as *Anopheles gambiae s.l* were subjected to PCR assays for sibling species identification using the method of Scott *et al.* (1993) and Favia *et al.* (1997). Presence of *Plasmodium falciparum* circumsporozoite protein (CSP) in the heads-thoraces of all the *Anopheles gambiae* s.l. mosquitoes collected was determined by the circumsporozoite ELISA as described by Wirtz *et al*. (1987). Positive controls and monoclonal antibodies used for the sporozoite ELISA were supplied by Centers for Disease Control and Prevention (CDC) in Atlanta USA. Non-blood-fed laboratory-reared *An. gambiae* Kisumu susceptible strain mosquitoes were used as negative controls. Unfed mosquitoes (4 in Oke-Oyi and 1 in Ote-efan) collected during the study were excluded from the ELISA analysis.

Statistical Evaluation of Data: Numbers of mosquitoes and rainfall values were transformed ($\sqrt{n+0.5}$) to accommodate zero values and attain normal distribution. Monthly numbers of mosquitoes collected from all the six communities were pooled and correlated (Pearson's correlation) with monthly rainfall for the state during the study period. Numbers of the *An. gambiae s.l* mosquitoes collected were compared across the six communities and 3 LGAs using ANOVA at $P < 0.05$ (SPSS statistical software version 20). Sporozoite rate was determined as numbers of mosquitoes that tested positive for *P. falciparum* sporozoites divided by the total numbers of a particular sibling species tested for sporozoites multiplied by 100.

RESULTS AND DISCUSSION

A total of 1,018 samples comprising *Anopheles* 864 (84.9%), *Aedes* 104 (10.2%) and *Culex* 50 (4.9%) mosquitoes were collected. In Oke – Oyi, higher proportions of *Aedes* spp. 69 (46.3%) mosquitoes were found indoors compared to all other sites. However, *Anopheles gambiae* s.l. was the predominant mosquito species (49.7% - 95.8%) across all the communities (Table 1). Though this is not the focus of this study, the close interaction of culicine mosquito vectors of filariasis and yellow fever among the residents of these three LGAs may imply increase risk of the residents to other mosquito-borne diseases apart from malaria. Sympatric occurrence of fewer numbers of indoor resting culicine mosquitoes relative to predominant *Anopheles* mosquitoes (85%) has been reported in rural communities located in both southwest and north-central regions of Nigeria (Oduola *et al*., 2013; 2016). The number of *Anopheles gambiae* s.l mosquitoes in October was higher compared to all other months (Table 2). Correlation between rainfall and pooled monthly numbers of *Anopheles* mosquitoes in all the communities was moderate and positive but not significant ($r = 0.639$, $p = 0.08$) (Table 2). The highest rainfall value (510.8mm) was recorded

in September. This could have been responsible for the higher numbers of *Anopheles gambiae* s.l mosquitoes collected during the following month of October compared to other months. Mean numbers of *An. gambiae* s.l. mosquitoes in the two communities within each LGA were not significantly different from each other; Idi-Emi and Lasoju in Asa LGA (F=0.75, P = 0.980), Oke-oyi and Otefan in Ilorin East LGA (F=0.25, P = 0.945), Aiyede and Ogundele in Ilorin West LGA (F=0.13; P = 0.993) (Table 3). These results showing no significant differences in the mean numbers of female *An. gambiae* s.l. mosquitoes between the two communities within the same LGA suggest that similar *Anopheles* mosquito resting behaviour existed within the communities in each LGA. Pooled mean number of *An. gambiae* s.l. mosquitoes was significantly higher in Ilorin West LGA (7.56±2.66) compared to Asa (4.64±2.6; F = 17.81, P < 0.001) and Ilorin East (3.00 ±3.16; F = 22.81, P < 0.001) LGAs (Table 4). Out of the 396 *An. gambiae* s.l. subjected to PCR analysis, three sibling species comprising *Anopheles gambiae s.s* 298 (75.3%), *Anopheles coluzzii* 94 (23.7%) and *Anopheles arabiensis* 4(1.0%) were identified (Table 5). *An. gambiae s.s* was predominant over *An. coluzzii* and *An. arabiensis* in all the communities and LGAs. Fewer numbers of *An. arabiensis* were found only in Idi Emi and Lasoju communities in Asa LGA (Table 5). Both *An. gambiae s.s* and *An. coluzzii* vector species carried the *P. falciparum* sporozoites. The infection rate in *An. gambiae s.s* and *An. coluzzii* across all the 6 communities ranged between 2.4% - 30% and 6.7% -36% respectively (Table 6.). The sporozoite infection rate of *An. gambiae s.s* (12.8%) across the communities was lower compared to that of *An. coluzzii* (22.3%). Overall, the sporozoite rate of *An. gambiae* s.l. was higher in Ilorin –West (Aiyede 21.1%, Ogundele, 32.0%) compared to Asa (Idi-Emi 11.1%; Lasoju, 5.5%) and Ilorin East (Oke Oyi, 2.4%; Ote-efan 0%) communities.

Higher numbers of female *An. gambiae* s.l. and increased *P. falciparum* sporozoite infection in the mosquitoes in the two communities in Ilorin West LGA, where there are available paddy rice farming areas for larval breeding, suggest increased exposure of humans to infected mosquitoes. Houses located around breeding sites have been reported to contribute significantly to increased abundance of indoor resting adult malaria vector species in Kenya (McCan *et al.,* 2017). In Ethiopia, Kibret *et al*. (2014) also reported significantly higher *Plasmodium falciparum* sporozoites rates and vector densities in *Anopheles arabiensis* and *Anopheles pharoensis* in irrigated villages compared to non-irrigated villages. Results found here are predictive indicators to ensuring increased access and use of LLIN among the residents in these communities. *An. gambiae s.s* was more abundant than *An. coluzzii* in the pooled samples from all the communities in Ilorin. This is in agreement with the overall composition from other surveillance sites in Nasarawa, Oyo and Plateau state sharing the same guinea savannah ecological zone with Kwara state (PMI-AIRS, 2018). The preponderance of *An. gambiae s.s* over *An. coluzzii* in Asa and Ilorin East LGAs is in contrast with Ilorin West LGA communities where a higher proportion of *An. coluzzii* mosquitoes were observed (Aiyede 43%; Ogundele 33%). This can be attributed to the presence of perennial paddy rice farms serving as the preferential mosquito larvae breeding sites in the Ilorin West LGA study communities. *An. coluzzii* larvae have previously been reported to utilize relatively permanent water bodies which are abundant in both urban and peri-urban Lagos in the forest ecological zone (Oduola *et al.,* 2012). Similar predominance of *An. coluzzii* have also been reported in Auyo (Kano State) and Rabah, Sentinel site (Sokoto State) (Ibrahim *et al.,* 2014; PMI, 2018) in the Sudan savannah ecological zone of Northern Nigeria. Also, *An. coluzzii* was reported predominantly in Ebonyi, located in forest ecological zone in South Eastern, Nigeria (PMI, 2018). All of these sites showing preponderance of *An. coluzzii* are associated with the historical practice of irrigation activities supporting vegetable and paddy rice farming systems. Occurrence of these favourable relatively permanent water bodies for *An. coluzzii* mosquito larval breeding may have influenced and supported the varying predominant patterns of *An. coluzzii* distribution observed along the various ecological zones. *Anopheles arabiensis* species were not encountered in most of the communities in this study probably because of the absence of suitable animal hosts to support its zoophilic tendencies and exophilic preferences (Massebo *et al.,* 2015). Higher proportion of *An. arabiensis* mosquito sibling species (65%) have been reported in Gaa – Bolorunduro, another community in Kwara state with heavy presence of Fulani Cattle herders and their animals (Oduola *et al.,* 2016). The negligible occurrence of *An. arabiensis* sibling species in the present study is in consonance with 0.6% *An. arabiensis* incidence recorded in two other non-cattle rearing communities in Kwara State (Obembe *et al.,* 2018). To our knowledge, this is a first report on the role of *Anopheles coluzzii* and *An. gambiae s.s* as vectors involved in malaria transmission in Kwara State, North Central Nigeria. Previous studies by Oduola *et al.,* (2016) only identified *An. gambiae s.s, An. coluzzii* and *An. arabiensis* in Ilorin East and Ilorin South LGAs but was unable to determine their infectivity rates.

Table 1: Number of female mosquito species collected from the rural Communities.

LGA	Community	Mosquito composition N (%)			Total
		Anopheles spp.	***Culex spp.***	***Aedes spp.***	
Asa					
	Idi-Emi	112(82.4)	8(5.9)	16(11.8)	136
	Lasoju	106 (89.0)	9 (7.6)	4 (3.4)	119
Ilorin East					
	Oke-Oyi	74(49.7)	6(4)	69(46.3)	149
	Ote-efan	69(77.5)	10(11.2)	10(11.2)	89
Ilorin-West					
	Aiyede	251 (95.8)	8 (3.1)	3 (1.1)	262
	Ogundele	252 (95.8)	9 (3.4)	2 (0.8)	263
Total		**864 (84.9)**	**50 (4.9)**	**104 (10.2)**	**1018**

N- Number of mosquitoes collected. (%) - Percentages of each mosquito species

Table 2: Correlation of total numbers of female *An. gambiae s.l.* mosquitoes with rainfall values

Month	Asa		Ilorin East		Ilorin West		ATM-M	ATM-R	Correlation
	IE	La	OO	OF	Ai	Og			
Sept	21	23	5	2	26	27	104(10.22)	510.8(22.6)	r = 0.639
Oct	42	54	51	63	82	57	349(18.69)	367.9(19.2)	
Nov	10	8	3	2	52	19	94(9.72)	26.9(5.23)	P= 0.087
Dec	6	5	5	0	6	11	33(5.79)	10(3.24)	
Jan	1	0	0	1	1	11	14(3.81)	1.9(1.55)	
Feb	10	1	2	0	31	38	82(9.08)	1.33(1.35)	
Mar	5	3	1	0	19	40	68(8.28)	1.24(1.32)	
Apr	17	12	3	0	34	49	115(10.75)	2.8(1.82)	

Where ATM-M = Actual (Transformed) Monthly total Numbers of mosquitoes*; ATM-R* = Actual (Transformed) Monthly total Rainfall values (mm); IE = idi-Emi; La = Lasoju; OO = Oyeke-Oyi; OF = Ote-Fan; Ai = Aiyede; Og = Oygyundele; *P and r values are from correlations of transformed monthly total numbers of mosquitoes and transformed rainfall values*

Table 3: Comparisons of mean numbers of *An. gambiae s.l* collected from the six communities.

Month	Asa			Ilorin East			Ilorin West		
	IE ATN	La ATN	P value	OO ATMN	OF ATMN	P value	Ai ATMN	Og ATMN	P value
Sept	21(4.64)	23(4.85)		5(2.35)	2(1.58)		26(5.15)	27(5.24)	
Oct	42(6.52)	54(7.38)		51(7.18)	63(7.97)		82(9.08)	57(7.58)	
Nov	10(3.24)	8(2.92)		3(1.87)	2(1.58)		52(7.25)	19(4.42)	
Dec	6(2.55)	5(2.35)	0.980	5(2.35)	0(0.71)	0.945	6(2.55)	11(3.39)	0.993
Jan	1(1.22)	0(0.71)		0(0.71)	1(1.22)		1(1.22)	11(3.39)	
Feb	10(3.24)	1(1.22)		2(1.58)	0(0.71)		31(5.61)	38(6.20)	
Mar	5(2.35)	3(1.87)		1(1.22)	0(0.71)		19(4.42)	40(6.36)	
Apr	17(4.18)	12(3.54)		3(1.87)	0(0.71)		34(5.87)	49(7.04)	
Mean number ±S.D	3.49 ± 1.62^{ab}	3.11 ± 2.17^{a}		2.39 ± 2.01^{a}	1.90 ± 2.48^{a}		5.14 ± 2.49^{bc}	5.45 ± 1.61^{c}	

ATN = Actual (Transformed) no *Mean values having the same letter superscript along the same row are not significantly different at P < 0.05.; ATMN* = Actual (Transformed) no

Table 4: Comparisons of mean numbers of female Anopheles mosquitoes across the three LGAs

Month	Asa			Ilorin East			Ilorin West		
	IE A	**La A**	**TAT**	**OO A**	**OF A**	**TAT**	**Ai A**	**Og A**	**TAT Med**
Sept	21	23	44(6.67)	5	2	7(2.74)	26	27	53(7.31)
Oct	42	54	96(9.82)	51	63	114(10.70)	82	57	139(11.81)
Nov	10	8	18(4.30)	3	2	5(2.35)	52	19	71(8.46)
Dec	6	5	11(3.39)	5	0	5(2.35)	6	11	17(4.18)
Jan	1	0	1(1.22)	0	1	1(1.22)	1	11	12(3.54)
Feb	10	1	11(3.39)	2	0	2(1.58)	31	38	69(8.34)
Mar	5	3	8(2.92)	1	0	1(1.22)	19	40	59(7.71)
Apr	17	12	29(5.43)	3	0	3(1.87)	34	49	83(9.14)
Mean number ±S.D			4.64 ± 2.66^{a}			3.00 ± 3.16^{a}			7.56 ± 2.66^{b}

Mean values having the same letter superscript along the same row are not significantly different at P < 0.05. IEA= Idi-Emi Actual no; La A= Lasoju Actual no; TAT = Total Actual (Transformed) no; OO A= Oke-oyi; OF A = Ote-fan Actual no; Actual no; TAT = Total Actual (Transformed) no; Ai A = Aiyede Actual no; Og A = Ogundele Actual no; TAT med = Total Actual (Transformed) no

Table 5: Sibling species composition of *An. gambiae* s.l. collected from the communities.

LGA	Villages	N	*An. gambiae* n (%)	*An. coluzzii* n (%)	*An. arabiensis* n (%)
Asa	Idi-Emi	63	46(73.0)	15(23.8)	2(3.2)
	Lasoju	55	51(92.8)	2(3.6)	2(3.6)
	Total	**118**	**97(82.2)**	**17(14.4)**	**4(3.4)**
Ilorin East	Oke-Oyi	42	41(97.6)	1(2.4)	0.0
	Ote-efan	47	45(95.7)	2(4.3)	0.0
	Total	**89**	**86(96.6)**	**3(3.4)**	**0.0**
Ilorin West	Aiyede	114	65(57.0)	49(43.0)	0.0
	Ogundele	75	50(66.7)	25(33.3)	0.0
	Total	**189**	**115 (60.8)**	**74 (39.2)**	**0.0**
Grand total		**396**	**298(75.3)**	**94(23.7)**	**4(1.0)**

N= Number of mosquitoes analyzed, n = numbers identified by PCR

Table 6. *Plasmodium falciparum* infection rates of *An. gambiae* sibling species in the communities

			An. gambiae		*An. coluzzii*		*An. arabiensis*		
LGA	**SC**	**NBF**	**PCR identified**	**No +ve for CSP N (SR)**	**PCR identified**	**No +ve for CSP N (SR)**	**PCR identified**	**No +ve for CSP N (SR)**	**Overall**
Asa	Idi-Emi	112	46	6(13.0)	15	1(6.7)	2	0(0)	11.1
	Lasoju	106	51	3(5.9)	2	0(0)	2	0(0)	5.5
Ilorin	Oke-Oyi	70	41	1(2.4)	1	0(0)	0	0(0)	2.4
East	Ote-efan	68	45	0(0)	2	0(0)	0	0(0)	0.0
Ilorin	Aiyede	251	65	13(20)	49	11(22.4)	0	0(0)	21.1
West	Ogundele	252	50	15(30)	25	9(36)	0	0(0)	32.0
	Total	**859**	**298**	**38(12.8)**	**94**	**21(22.3)**	**4**	**0(0)**	**15.1**

N = Number positive for Circumsporozoite protein out of the numbers identified by PCR, SR = sporozoite rate; SC = Study communities; NBF = No of blood-fed and half-gravid An. gambiae s.l; Overall = Overall An. gambiae s.l Sporozoite infection rates

Significant association was previously observed between the occurrence of *An. gambiae s.s* and *An. coluzzii* and a high incidence of reported malaria cases in Ilorin – East LGA (Oduola *et al.,* 2016). Strong indoor presence of two confirmed vectors of malaria validates the need for increased access of communities in Kwara state to protective interventions such as indoor residual spray or insecticide-treated nets. This will protect residents in the communities from host-seeking *Plasmodium falciparum*-infected mosquitoes. Likewise, selective larviciding will be appropriate to reduce the density of *Anopheles gambiae* s.l. mosquitoes observed in the Ilorin- West LGA communities.

Conclusion: This study presents a first report on the role of *An. coluzzii* and *An. gambiae s.s* mosquito sibling species as vectors involved in malaria transmission in Kwara State, North Central Nigeria. Endophilic nature of the highly infected *An. coluzzii* and *An. gambiae s.s* mosquito collected indoors in the study areas suggests the need for effective use of indoor malaria vector control interventions such as insecticide treated bed-nets for the prevention of malaria morbidity and mortality in the localities.

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