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THE INTERPLAY BETWEEN MALARIA VECTOR POPULATION, SEASONS, AND MALARIA PREVALENCE

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ABSTRACT
A retrospective scrutiny of dataset from a semi-longitudinal investigation reveals a dynamic interaction between seasons, vector population density and malaria prevalence. Indoor resting mosquitoes collected between the hours of 06:00 and 09:00 using Pyrethrum Spray Catch (PSC), were preserved dry over silica gel and used for morphological and molecular identification. Asymptomatic malaria prevalence was determined through Rapid Diagnostic Test (RDT), Microscopy test and PCR. A line list hospital attendance record was collated to explore the symptomatic malaria situation. Metrological data was collected from a weather station in the study community to see impact of some weather elements on the malaria situation. The four seasons under study shows a progressive increase in vector population density with increase in rainfall. A strong positive correlation exists between vector population and asymptomatic malaria prevalence [PCRtest-R^2=0.439(43.9%); RDT+MicroT -R^2=0.425(42.5%); RDT-R^2=0.342(34.2%)]. Symptomatic malaria peaked during the dry season (12.54%) with lower vector density while onset of rains with higher vector density recorded the least (4.7%). Established here is a dynamic interaction between malaria vector population, prevalence and the environmental landscape; hence the imperative for an ecological input in the face of fluctuating climatic elements.

Keywords: Malaria, Prevalence, Vector population, Climate, Seasons, Nigeria.

INTRODUCTION

Malaria is a foremost parasitic scourge ravaging man and is caused by protozoan parasites belonging to the family *Plasmodiidae* Sinden and Gilles (2002). These *Plasmodium* species are transmitted during the blood sucking activity of some members of the female *Anopheles* mosquitoes (Service & Townson 2002). Malaria continues to enjoy global priority position as a public health challenge. It is preventable, curable but can become complicated and fatal if inadequately contained. Concepts behind its control strategies are derivatives of acquired knowledge from the triad sustaining this formidable disease (the vector, the parasite and the human -either as a host or a victim).In the latest malaria report, the World Health Organization (WHO, 2023) declared climate change the single biggest health threat facing humanity, and with a major multiplier of other threats. Positing that, the direction of the effect of climate change on malaria transmission and burden will be non-linear and is likely to vary across different contexts.

It is estimated that there were 249 million cases of malaria in 2022 with the WHO African region recording the highest 93.6% of cases and 95.4% of deaths globally; 78.1% of all deaths in this region were among children aged under 5 years in 2022, compared with 90.7% in 2000 (WHO, 2023). Between 2019 and 2020, estimated malaria cases increased from 218 million to 230 million, and deaths from 552 000 to 604 000 in this region (WHO, 2023). In 2022, the 11 HBHI countries accounted for 67% of all cases and 73% of deaths globally. Nigeria accounted for the highest proportion of cases (27%) and deaths (31%) (WHO, 2023). About 97% of the 170 million people in Nigeria live in malaria endemic areas while only 3% live in malaria free highlands (NMCP, 2016). The fight against this advanced disease seems to have taken one step forward and is now taking ten steps backward. There have been a surge in last few years 2020-2022 global malaria burden (WHO, 2023). This is worrisome because malaria historically does make a comeback - and a vengeful one.

Intricately webbed complexities around the ecology of this ancient scourge has the capacity to impede malaria control reversing the progress made so far. Key gaps in our ecological knowledge base, insufficiency of presently employed malaria control tools (Ferguson, *et al*, 2010), increasing outdoor bites, resistance and other emerging challenges (Killeen, 2013); Cotter, *et al* 2013) are altogether capable of wasting the fragile gains towards malaria elimination. Several reports show association between malaria parasite, vector population distribution and sensitivity to climatic factors leading to seasonal variations of malaria infection and transmission indices (Patz *et al*, 2008; Peterson, 2009; Caminade, *et al* 2014*;* Hamad *et al* 2014). An expanded knowledge base is therefore critical for the development of novel tools complimentary to those currently engaged by the malaria control community. This paper reports findings surfaced from a retrospective scrutiny of a dataset from a semi-longitudinal investigation; revealing a dynamic interaction between seasons, vector population density and malaria prevalence.Thus, highlighting the importance of innovative vector surveillance as a core control tool in the face of fluctuating climatic elements. It involved a semi-longitudinal survey of the dominant vector species, a cross sectional survey of subjects in thirteen study communities over a two year (2015-2016) period and some weather elements. It is hoped that this vital information provides a basis for strategic seasonal and sub-national locale specific control intervention in the study communities and elsewhere as applicable.

MATERIALS AND METHODS

Study communities

This study was conducted in thirteen communities located in Gboko (7° 19' 30" N, 9° 0' 18" E) and Otukpo (7° 19' 30" N, 9° 0' 18" E) local government areas of Benue State, North Central Nigeria. This region experiences two distinct seasons, the wet-rainy season which lasts from April to October and the dry season which, begins

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in November and ends in March. Temperature fluctuations generally falls between 21 – 37 ºC in the year. Much as most of these communities are located in the guinea savannah ecological zone, some of the rural communities in Otukpo local government area are located on the south western fringe- rainforest vegetation type. It can be described as a modified vegetation transition consisting of light deciduous forest and derived savannah laying between the guinea savannah and rainforest ecological zones. The rural settlers engaged in primary economic activity (Farming, hunting, fishing, grazing, lumbering) while the urban settlers by contrast engage in secondary and tertiary economic activities - industrial and commercial activities (Areola *et al,* 2009).

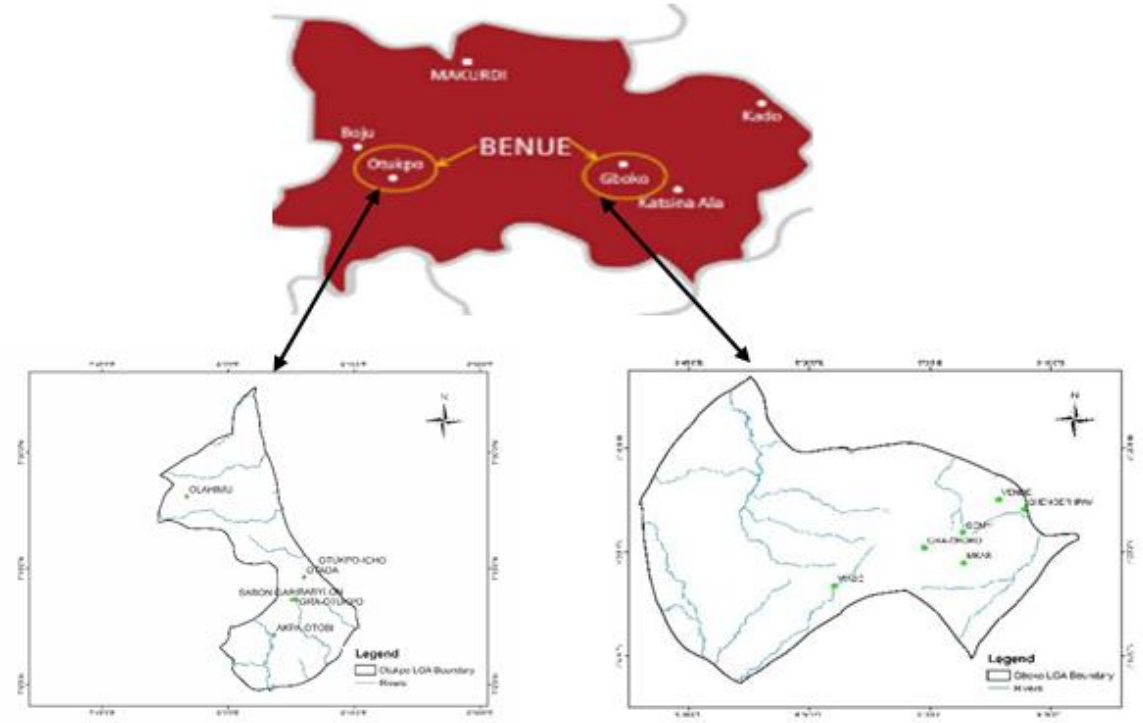

Figure 1. Map of Benue State Showing Study Sites.

Field collection, preservation and laboratory processing of mosquitoes

Field collection of mosquitoes

Field collection of indoor resting mosquitoes was carried out using Pyrethrum Spray Catch (PSC) between the hours 06:00 and 09:00 over a period of two years. Each individual sample was preserved dry over silica gel in well labelled Eppendorf tubes (1.5ml) prior to identification. The two distinct wet and dry seasons were broken down into sub-seasons and the mid months used for the collections. The sub divisions were derived from the twelve months of the year as follows: i) Late Dry Season (LDS-January, February, March); ii) Early Raining Season (EDS- April, May, June); iii) Late Raining Season (LRS-July, August, September) and iv) Early Dry Season (EDS-October, November, December).

Morphological identification of anthropophilic *Anopheles* species

Using a trinocular dissecting microscope (Amscope SZMT2/MU1000 10APTINA COLOR CMOS) morphological identification of anthropophilic *Anopheles* species was carried out with the help of standard keys (Gillies and Coetzee 1987; Gillies and De Meillon 1968; Gillett, 1972)

Molecular identification of anthropophilic *Anopheles* species

Molecular identification was done using the Deoxyribonucleic Acid Polymerase Chain Reaction (DNA-PCR), (Scott *et al*, 1993). Identification of the molecular forms of *Anopheles gambiae* s.s. was done using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP), (Favia *et al,* 1997; Fanello *et al,* 2002)

Metrological data

Data on annual rainfall amount (millimetres), rain days (days), minimum/maximum relative humidity (%) and mean monthly temperature (ºC) were gotten from a functional and reliable metrological station in study community. The Aperan Orshi College of Agriculture, Yandev, Agro- Metrological Station- Station Number 0709/20.

Asymptomatic malaria

Asymptomatic malaria prevalence was determined through a cross sectional survey of 272 apparently healthy symptomless children and adults (age 18months and above). Malaria parasites were detected by three test types: Rapid Diagnostic Tests (RDTs), conventional microscopic examination of blood stained films (thick and thin blood smears) and Polymerase Chain Reaction (PCR) based technique.

Blood collection: Using 5 ml capacity disposable syringes fitted with needles, venous blood was collected under sterile conditions

and dispensed into labelled tubes pre-lined with ethylene diamine tetra acetic acid (EDTA). Gentle mixing of the EDTA and blood was ensured and samples transferred to the General Hospital laboratory and used to prepare the various components for PCR test, RDT and microscopy.

Polymerase Chain Reaction (PCR): Using the method by Bereczky and co-authors (2005) DNA was extracted from Dry Blood Spot (DBS 60 µl each) soaked onto Whatman filter paper (GE Healthcare, UK, Grade 3 MM CHR CAT No: 3030–861). Each piece of the filter paper was carefully air dried and kept in individual sealable sachets of zip lock plastic bags together with desiccants before transferring them to the molecular laboratory at the Nigerian Institute for Medical Research, Yaba, Lagos. Same laboratory provided the standard 3D7 strain used as positive control. PCR was performed according to the method by Snounou (1996) nested protocol. Plasmo1: 5'GTT AAG GGA GTG AAG ACG ATC AGA-3' and Plasmo 2: 5' AAC CCA AAG ACT TTG ATT TCT CAT AA-3' were the Primers used. The PCR products were visualized with a transilluminator (UVP) after 1.5% agarose gel electrophoresis in 0.5 X Tris borate EDTA buffer and ethidium bromide (biohazard) staining.

Rapid diagnostic tests: with sixty microliters of blood RDT test was conducted, results recorded after about 15 to 20 minutes.
Microscopy: Blood smears (thick and thin) were prepared and stained with 3% solution of Giemsa (WHO, 2010) and examined under x100 oil immersion objective and paired x10 oculars. Slide validation was done by a staff of the University of Jos Teaching Hospital.

Symptomatic malaria
Two well-known and frequently visited health centres were selected for the study. Standard forms tagged annex 8 and 9 (WHO, 2016), were carefully explained and handed over to the prospective health workers. Data collection was monitored and picked up from source on a monthly basis.

Ethics approval
Ethical approval (Project Number IRB/15/289) was obtained from the Institutional Review Board (IRB) of the Nigerian Institute for Medical Research (NIMR), Yaba, Lagos. The protocol and safety guidelines satisfy the conditions of NIMR-IRB policies regarding experiments that use human subjects. Community and family heads were duly consulted and verbal consent gotten prior to the commencement of the survey.

Data Analysis
ANOVA (analysis of variance) was used to test significant difference in vector population density and season between rural and urban communities. The strength and direction of relationship between Vector population and Malaria prevalence was determined using correlation analysis. Data obtained from the descriptive survey was analysed using Microsoft Excel 2013 and expressed in percentages. Using SPSS version, 20 and 23. Chi-square (χ^2) test was used to analyse all categorical variables. The P-value < 0.05 were considered statistically significant.

RESULTS

The four seasons under study shows a progressive increase in malaria vector population density (Table 1) form Late Dry Season (LDS-February), Early Raining Season (ERS-May) to Late Raining Season (LRS-August). There was a decrease only in Early Dry Season (EDS-November). Similarly, there was a significant difference (LDS-p-0.021; ERS- 0.004; LRS- 0.009) between rural and urban malaria vector species density and seasons with the exception of November (EDS-NS). The major malaria vector species identified were *Anopheles gambiae*, *Anopheles arabiensis* and *Anopheles coluzzii.*

Table 1: Vector Population Density and Season

Settlement	Late Dry (February)	Early Rain (May)	Late Rain (August)	Early Dry (November)
Rural	3.0±2.14	12.0±7.15	22.0±16.26	1.0±1.1
Urban	1.0±0.40	1.0±1.21	1.0±0.81	0.0±0.0
p-value	**0.021**	**0.004**	**0.009**	**NS**

NS=No Significant difference at P>0.05; Mean value±SD

No marked difference in metrological variables was observed in terms of mean monthly temperature during the period of study 2015(25.4-28.2 ^{o}C); 2016(25.3-28.5 ^{o}C). Minimum and maximum relative humidity ranged between 2015(16-95) and 2016(25-93). However, a marked difference was observed in number of rain days 2015(LDS=1, LRS=35); 2016(LDS=3, LRS=28). Details of these weather elements are shown in (Table 2). Total rainfall amount within the study period were 2015(LDS=10.5, LRS=629.2); 2016(LDS=16.9, LRS=537.8). Annual rainfall for 2015 was 1080.6 while that of 2016 was 1339.0 in millimetres.

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Table 2: Climate Data for Benue State (2015-2016)

Parameter	Unit	Year	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Annual Rainfall	mm	2015	10.5	0	0	39.6	177.6	83.8	225.9	209.9	193.4	123.8	26.8	0
		2016	0	0	16.9	35.6	79.9	269.5	76.6	311.9	149.3	103.5	284.6	11.2
Rain Days	Days	2015	1	0	0	3	11	9	12	9	14	11	1	0
		2016	0	0	3	5	8	12	8	7	13	12	4	2
Min. Relative Humidity	%	2015	28	27	31	50	61	64	72	71	72	64	36	16
		2016	26	25	33	53	62	65	70	73	72	66	50	26
Max. Relative Humidity	%	2015	66	65	67	75	81	85	89	92	95	87	95	85
		2016	64	64	68	74	80	86	88	90	93	86	88	78
Mean Monthly Temp.	°C	2015	26.9	26.5	28.2	27.5	28.1	26.4	25.4	25.6	25.5	27.3	27.6	25.5
		2016	26.6	26.8	27.5	28.2	28.5	26.1	26.6	25.8	25.3	26.9	28.0	25.7
Average Monthly Rainfall	mm	-	5.25	0	8.45	37.6	128.75	76.65	151.25	260.9	171.35	113.65	155.7	5.6

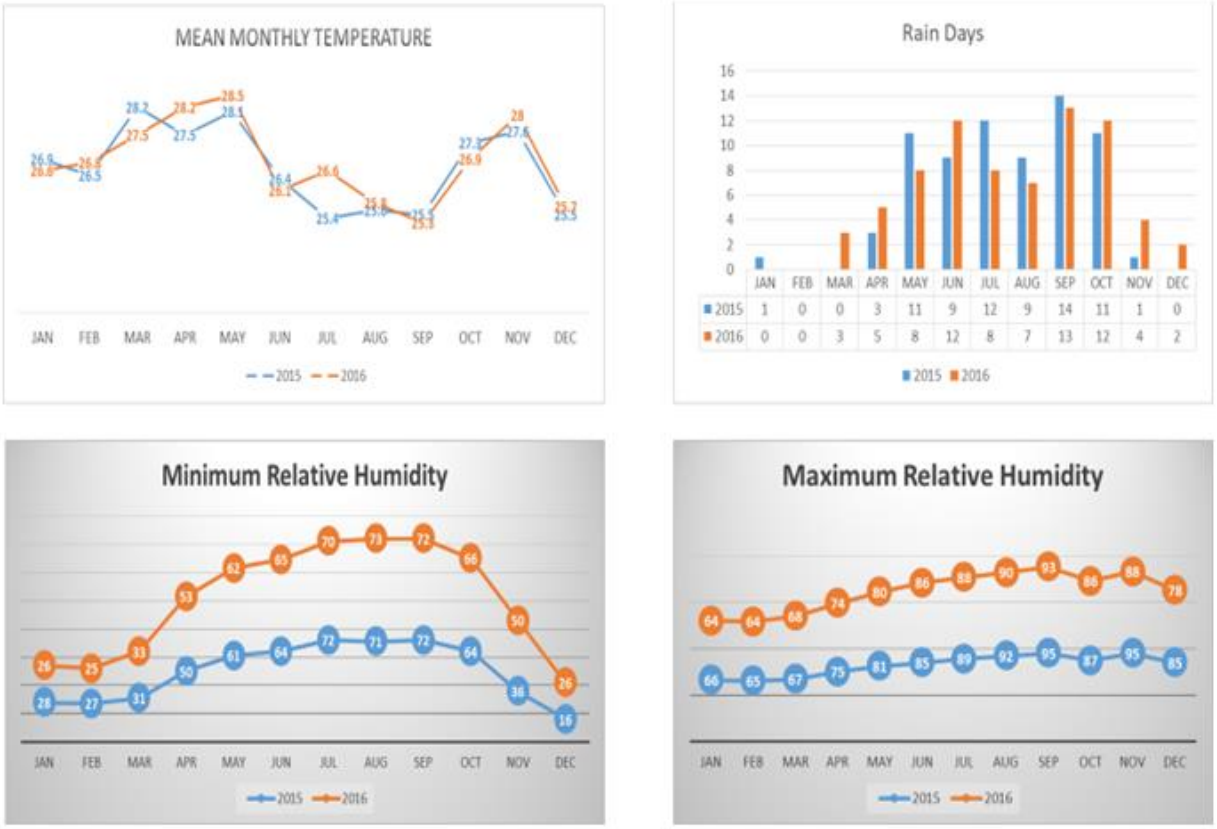

Figure 2: A composite of selected prevailing climatic elements in the study location

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The overall comparison of *Anopheles* malaria vector species abundance in 2015 and 2016 revealed that 2015 had the higher abundance of 275(83.33%) compared to year 2016 55(16.67%). The data set showed a significant difference (χ^2=91.561, df=10, p = 0.001) in abundance between 2015 and 2016 at ($p<0.05$). Asymptomatic malaria prevalence in relation to vector population density by Polymerase Chain Reaction (PCR), Rapid Diagnostic Test (RDT) and Microscopy Test (Micro T) showed that when vector population increases malaria prevalence increases (Fig 3). A strong positive correlation exists between vector population and PCR test- R^2=0.439(43.9%); RDT+MicroT -R^2=0.425(42.5%); RDT- R^2=0.342(34.2%) while a weak/low positive correlation exist between vector population and Micro T- R^2=0.178(17.8%) (Figs 3-6).

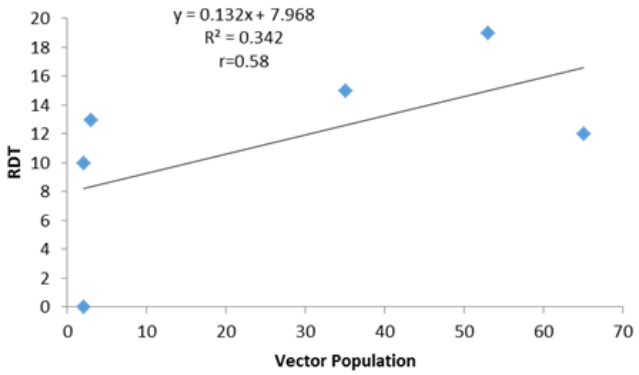

Figure 3: Strength and direction of Relationship between vector population and malaria prevalence in respect to RDT

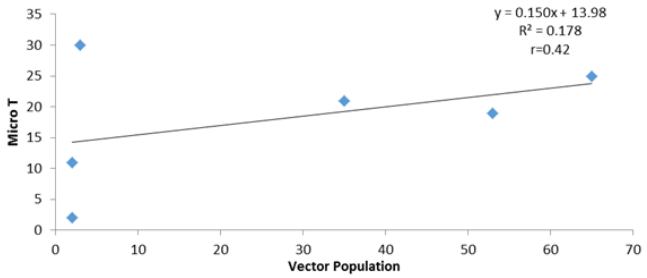

Figure 4: Relationship between vector population and malaria prevalence in respect to Microscopy Test

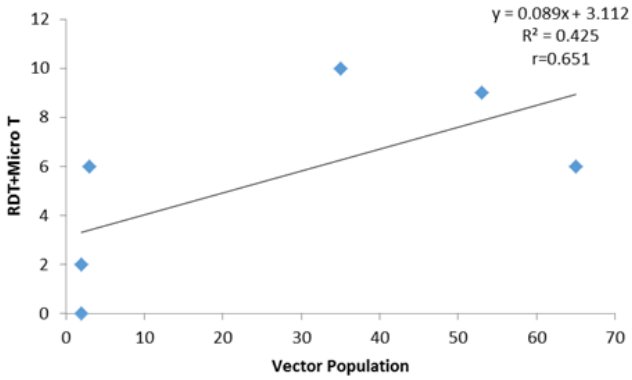

Figure 5: Relationship between vector population and malaria prevalence in respect to RDT+ Microscopy (+ve) Test

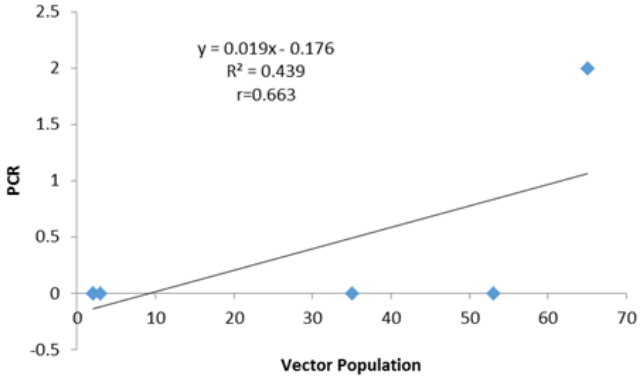

Figure 6: Relationship between vector population and malaria prevalence in respect to PCR.

Symptomatic malaria from line list record of hospital attendance showed no zero malaria month with a dry season peak, for more details (Fig 3). Late dry season (LDS-January-12.5%; February-11.3%) had the highest malaria admission -recording one death while the early raining season (ERS-April-4.7% and May- 4.7%) had the least (Fig 7)

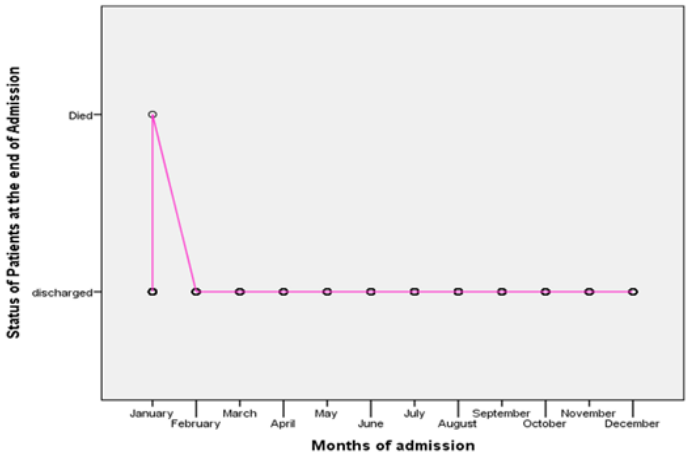

Figure 7- Malaria Hospital Ward Admission Record in Gboko Local Government Area of Benue State.

Vegetation was observed to be very dense with breeding sites at favourable peak during the late raining season, while the converse obtains during the late dry seasons. Captured images of seasonal expression are available on request.

DISCUSSION

Vector-borne diseases are a part of an intricately linked larger ecosystem. Interactions between the malaria vector, parasite and humans are complex, dynamic and constantly evolving (Oyewole *et al,* 2007; Dery *et al,*2010). Increase in vector population density and increase in rain fall indicates availability of favourable breeding sites and resting shades from the dense vegetation (Peterson, 2009; Okwa *et al* 2009; Govoetcha *et al*, 2014). Correlation analysis shows increase in asymptomatic malaria prevalence as vector population increases. Unexplainably the hospital attendance record shows a much higher symptomatic malaria prevalence in

January- peak of the late dry season- recoding one death. Late dry season hardly have any visible breeding sites and has less malaria vector species. Data confirms no zero malaria month. Dry season and perennial malaria have been reported in Benin a neighbouring country. Yet to be unmasked may be a critical link between perennial malaria, the inadvertent human host (asymptomatic malaria parasite carriers) and compromised malaria vector; even when the vector population reduces in and out of historically acclaimed non-malaria seasons. There are accompanying questions:

i) Could *anopheles* be breeding in unsuspecting places? Where would those dry season refugia be in these communities?

ii) Could there be any other yet to be incriminated mosquito species responsible for P*lasmodium* transmission to humans now especially outdoors?

iii) Or is it a change of baton over biting dominance between already established vector species (indoor vs outdoor species)?

Of the three weather elements only rainfall data showed a marked difference compared to temperature and relative humidity. A much longer time span may reveal influential variations. Snow *et al* in 2017 opined ''Although short-term seasonal cycles are fundamental to malaria epidemiology, longer-term climate anomalies and shifting environmental and intervention landscapes also alter the likelihood of contact between mosquitoes and humans or the duration of host infection' 'The present contribution lends a voice to the unequivocal suggestions of other researchers (Killeen *et al,* 2017) for a broader scope of malaria research and the call for development of expanded tool box based on an increased knowledge spectrum. The reduction in the number of *Anopheles* malaria vector species in the second year (2016) may be attributable to the residual effect of the insecticide spray used indoors for mosquito capture in the previous year (2015). Control intervention during the course of study and vector behaviour shift (Wamae *et al,* 2015; Gilles, 2002) are the likely reasons for the unprecedented decrease in vector population in the second year. In gleaning from the past, it will not be out of place to re-visit the experiences outlined by Gilles on milestones in the history of malaria and its control (Aju-Ameh *et al,* 2018). Such success stories of malaria/mosquito elimination as adjudged applicable in multi-layered strategies, could be selected and leveraged upon for countries lagging behind in attaining both national and international elimination targets. The success of malaria control and elimination effort is predicted on known facts behind the several intricately webbed phenomena that sustains this formidable, ancient and advanced disease. That is to say the more accurate our knowledge is, the better equipped we are in combating this debilitating scourge.

Conclusion

With increase in malaria burden, in the face of extreme and erratic weather events such as prolong or shortened raining/dry seasons, floods, temperature fluctuations and other weather elements, an ecological impute becomes imperative in unearthing links embedded within the complex ecosystem that sustains malaria. An all year round climate and vector surveillance is recommended.

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Availability of data and materials
Data supporting the conclusions of this article are included within the article and its supporting files. The datasets used and/or analyzed during the study are available from the corresponding author upon reasonable request.

Competing interests
The authors declare no competing interest.

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

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