SURVIVORSHIP AND VECTORIAL CAPACITY OF *ANOPHELES GAMBIAE* IN BALI DISTRICT, TARABA STATE, NIGERIA

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ABSTRACT

The principal malaria vector in Africa south of the Sahara is Anopheles gambiae. This study aimed at determining the parity rates, survival rates and vector potentials of An. gambiae in Bali district Taraba State, Nigeria. Indoor mosquito collections were carried out between June and December 2018 using Pyrethrum Spray Catch in Daniya Bali, Fundalara and Gazabubarkeji communities. The number of people that slept overnight in the room was noted. Collected and freshly preserved mosquitoes were morphologically identified and female An. gambiae was counted. The ovaries of fed and unfed An. gambiae were extracted and quickly transferred to drop of distilled water on the slide and examined under high magnification for tracheal skein and classified as parous or nulliparous. The blood meal source of blood fed samples was determined using CSP-ELISA. A total number of 485 adult An. gambiae species were identified. The probabilities of daily survival of individuals of the mosquito, 0.75 and 0.688 during wet and dry seasons were not significantly different (p>0.05). The life expectancy of adult mosquitoes was 8.0 and 5.9 days during wet and dry seasons and was significantly different (p<0.05). During wet season the mosquitoes survive long enough to become infectious than during dry season. The vectorial capacities was higher significantly in wet season 1.98 than that of the dry season 0.09 (p<0.05). The An. gambiae population in Bali district was dominantly old ones due to high rates of daily survival. The longevity of the mosquito makes for high vectorial potential.

Keywords: *Anopheles gambiae, Plasmodium*, Life expectancy, Infection potentials, Guinea savannah

INTRODUCTION

Malaria is still the major cause of death in children in sub-Saharan Africa. The disease in this region takes the life of a child every 2 minutes (WHO, 2015). The targets of the Global Technical Strategy for Malaria 2016 – 2030 (GTS) is by 2030: to reduce malaria incidence and mortality rates globally by at least 90 % compared with 2015 levels; to eliminate malaria from at least 35 countries in which malaria was transmitted in 2015; and to prevent re-establishment of malaria in all countries that are malaria free (WHO, 2016). *Anopheles* is best known for its role in transmitting malaria worldwide, but in some areas it can also transmit filariasis (Awolola *et al.,* 2002; Service, 2004; WHO, 2013). Malaria is by far one of the greatest killer diseases in the world. Mosquito's density coupled with entomologic inoculation rate (EIR) is a major determinant of malaria transmission (WHO, 2013).

In Africa, the two main vectors *Anopheles gambiae sensu lato* and *Anopheles funestus* are complex species (Awolola *et al*., 2005; Temu *et al.,* 2007; Godfray, 2013).

People living in poor rural areas are confronted with a multitude of barriers when assessing malaria prevention especially on the knowledge of the biology and ecology of the vectors. This is because the species composition and other biological parameters of the mosquitoes are poorly known in different ecological zones of Nigeria and in most of the malaria endemic areas due to the difficulties in the morphological identification of certain species complex (Awolola *et al*., 2005).

Although a lot of work have been done on the above factors to understand the epidemiology of malaria in Africa, Nigeria inclusive (Awolola *et al.,* 2002; Awolola *et al*., 2005, Lamidi, 2009; Lamidi *et al.,* 2017a; Lamidi *et al*., 2017b; Lamidi *et al.,* 2018; Lamidi *et al*., 2019). Studies have also been conducted on parity and survival rates of *Anopheles* mosquitoes in Illorin and Calabar, Nigeria (Olayemi and Ande, 2008; Uttah *et al.,* 2013), but not much work have been done on influence of survivorship and density of mosquitoes on efficiency as vectors. Thus, this study aimed at determining the parity rates, survival rates and vector potentials of the principal malaria vector in Africa, *An. gambiae s. l.* in Bali district, Bali Local Government Area of Taraba State, Nigeria.

MATERIALS AND METHODS

Study Area: Taraba State is located between longitude 8.5^{o}– 11.6^{o}E and latitude 6.5^{o}– 9.5^{o}N (8^{o}00′N and 10^{o}30′E coordinates) in the north-eastern geopolitical zone of Nigeria with a size of 54,473 square kilometres representing 5.89% of the country landmass (Wikipedia, 2015). It has an estimated population of 2,688,944 based on 2006 census, giving a population density of 27 people per km^{2}, representing 1.90 % of the total population of Nigerians (Wikipedia, 2015).The study was carried out in three major riverine communities in Bali district, Bali Local Government Area of Taraba State (Fundalara, Gazabubarkeji and Daniya Bali).

The study communities were selected based on dense population, house types, presence of natural water bodies, both permanent slow running ones and stagnant prevailing pools of water that serve as breeding sites for mosquitoes.

Bali, Taraba State is in Guinea savannah (semi-arid) zone of the country. Rainy season is between May and early October and dry season between November and April. Daily temperature varies from 37 to 40 ^{o}C during the hottest months of March/April. It also varies from 32 to 37 ^{o}C during the coldest months of December/January. The relative humidity is about 23.00 % during the hot dry weather and can reach 80.00 % during the peak of wet season in July/August (Dammo *et al*., 2015).

Mosquito Sampling: Indoor mosquito collections were carried out every month of the study period (6 months), in each of the sites. Ten bedrooms were selected randomly from each station with at most three bedrooms from the same house. 180(6 months x 10 bedrooms x 3 sites) rooms were sprayed during the entire period of study.

Collection of mosquitoes by use of non-residual insecticide-pyrethrum (Spread Sheet Collection) was employed using World Health Organization standard procedure (WHO, 1995; WHO, 2003) and complemented with electronic mosquito hitting racket. All knocked down mosquitoes were preserved fresh and taken to laboratory.

Morphological Identification and Sorting out of Mosquitoes: Anopheline were separated from Culicine mosquitoes according to the morphological characteristics of their maxillary palps and identified mosquito genera were sexed based on the presence or absence of plumose (feathery) antennae. The morphological identification of female *An. gambiae s.l.* was done by studying the scales and colour of the palps at the head region, the patterns of spots on the wings, thorax, terminal abdominal segments, scales of the legs using dissecting microscope following the taxonomic keys of Gillett and Smith (1972) and Gillies and Coetzee (1987).

Determination of Parity and Estimation of Physiological Age: The identified female *An. gambiae* mosquitoes were kept in petri-dishes

with moist cotton wool or ice pack to prevent desiccations. Each was placed on a slide in a drop of physiological saline after removal of legs and wings. The ovaries were extracted and quickly transferred to drop of distilled water on the slide and examined under high magnification for tracheal skein and parous or nulliparous adopting the method of Beklemishev *et al.* (1959), WHO (2003) and Brogdon and Chan (2010). The proportion parous or parity rate was calculated using formula, n/total, where n represents the number of parous mosquito and total means all mosquitoes dissected.

Determination of Longevity/Survival Rate: The proportion parous (P) mosquito was used to determine the survival of the mosquitoes for one day (p) as explained by WHO (2003), using the formula, $p = \sqrt[x]{P}$; where x is the length of the gonotrophic cycle/interval of days between blood meals, which is usually presume to be 2 days. This formula for probability of surviving for one day (longevity) (p) assumes that the mosquito population has a stable size and age structure, and death rate is independent of age.

As p is the probability of surviving for one day, which is $\sqrt{P}$, p^n is the probability of surviving for n days. The expectation of life, the number of days survived and feed on human blood, for each species was calculated as: $1/ -\ln p$, where: n = duration of the sporogony cycle in the vector; p = probability of surviving one day.

Duration of sporogony/incubation period, n, was calculated by the formula $n = T/(t-t_{min})$, where: n= duration of sporogony; T = 111, 105 and 144 for *P. falciparum*, *P. vivax* and *P. malariae,* respectively; t = actual average temperature (^{O}C) and t_{min} = 16 for *P. falciparum* and *P. malariae* and 14.5 for *P. vivax* (WHO, 2003).

Determination of Vectorial Capacity: The capacity of the vector population to transmit malaria is given as $C = ma^2p^n/ -\ln p$, where: m = vector density in relation to man; a = Human Blood Index (x0.5); p^n = probability of surviving incubation period; $1/ -\ln p$ = longevity/life expectancy (WHO, 2003).

Source of Blood Meal/Host Preference ELISA: The blood meal sources were determined using CSP-ELISA as described by Okwa *et al.* (2007) and Brogdon and Chan (2010).

Human Blood Index (HBI) = Number of *Anopheles* mosquito with human blood/Total number of the mosquito species with blood.

Determination of Abundance: Abundance /Density was taken as total number of the *Anopheles* counted in the room where at least a human slept.

Mosquito Densities in Relation to Humans: The total *Anopheles* density / the total number of people that slept overnight in the sampled rooms.

Meteorological Data: Daily minimum and maximum atmospheric temperatures and other climatic data used in this study were obtained from the weather stations of College of Agriculture, Nigerian Meteorological Agency, Jalingo and Federal Polytechnic, Bali and used to calculate mean monthly/seasonal temperatures

Data Analysis: Generally data analyses were according to the World Health Organisation's technique (WHO, 2003). Mosquito density was determined as the total number of specimens collected during the study. Differences in survivorship parameters between seasons and vectorial capacities for the *Plasmodium* species were determined using chi-square test.

RESULTS

The survivorship parameters of *An. gambiae* during wet and dry seasons, June to December, 2018 (Table 1) indicated that a total number of 177(only unfed and freshly fed) were dissected for parity determination out of 485 *An. gambiae* collected and identified (Table 1). A number of the mosquitos were parous in both wet and dry seasons with an average proportion parous of 0.44 ± 0.35 (Figures 1 and 2).

Table 1: Seasonal variation in survivorship and longevity of *Anopheles gambiae* mosquitoes in Bali, June – December 2018

Seasons	Number dissected (N)	Number parous (P)	Proportion parous (P/N)	Probability of daily survival ($P = \sqrt{P/N}$)	Life expectancy (L=1/log *p*)
Wet	160	92	0.57	0.75	8.00
Dry	17	08	0.47	0.68	5.90
Total	177	100	1.04	1.43	13.90
Mean	88.5 ± 0.57	50 ± 0.59	0.44 ± 0.35	0.56 ± 0.13	3.97 ± 0.36

Figure 1: Ovary of newly emerged female *Anopheles* with terminal tracheoles showing characteristic tight skeins indicating that female is nulliparous (Source: Beklemishev *et al.*, 1959)

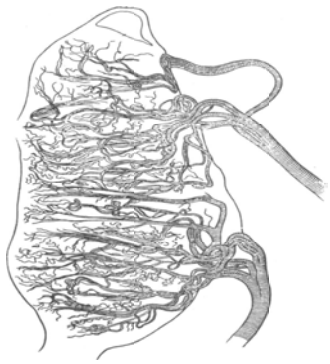

Figure 2: Ovary of female *Anopheles* after oviposition with terminal tracheoles showing net-like formation (Source: Beklemishev *et al.*, 1959)

The probability of daily survival of mosquito was high during both wet and dry seasons with seasonal mean probability of daily survival of 0.56 ± 0.13). Seasonal probabilities of daily survival were not significantly different (p>0.05).

The life expectancy (longevity) of adult mosquitoes was 8.0 and 5.90 days during wet and dry season respectively with a mean seasonal life expectancy of 3.97 ± 0.36 days. Life expectancy was significantly higher in wet season than dry season (p<0.05).

During the study period, atmospheric temperature was 27.0 ± 0.04 and 26.7 ± 0.29 ^{O}C during wet (July – September) and dry season (late October to December) with mean temperature of 26.9 ± 0.04 ^{o}C. No significant difference (p>0.05) in the temperatures were noticed (Table 2).

A total number of 485 adult *An. gambiae* mosquitoes were identified. The mosquitoes were higher in wet season 413(85.2 %) than dry season 72(14.8 %). Mosquito densities in relation to man were 4.13 and 0.81 during wet and dry seasons with mean density/man value of 3.11 ± 0.55. Seasonal densities of the mosquitoes were significantly different (p<0.05) (Table 2).

The extrinsic incubation period (sporogony development) of the *Plasmodium falciparum* lasted for 10 days in wet season and 10.4 days in dry season, the mean being 10.2 ± 0.01 days with no significant difference (p>0.05).

The probability that the *Anopheles* mosquitoes survived *Plasmodium* incubation period were very low. During wet season the mosquitoes were more likely to survive long enough to become infectious (0.06) than during dry season (0.02). The pattern of seasonal distribution of infective life of the mosquitoes was similar to the life expectancy. However, negative values were estimated for these parameters in the seasons of the study period (Tables 1 and 2).

Table 2: Seasonal variation in infection probability and vectorial capacity of *Anopheles gambiae* in Bali district, June – December, 2018

Season	Number collected (N)	Room occupant (O)	Density per man (N/O)	Atmospheric Temperature (°C)	Duration of sporogony (n)	Probability of surviving sporogony (P^n)	Infective life (L-n)	Vectorial capacity (C)
Wet	413	100	4.13	27.0	10.0	0.06	-2.0	0.99
Dry	72	88	0.81	26.7	10.4	0.02	-4.5	0.04
Total	485	188	4.94	53.7	20.4	0.08	-6.5	1.03
Mean	242 ± 0.50	94 ± 0.04	3.11 ± 0.55	26.85 ± 0.04	10.2 ± 0.013	0.04 ± 0.02	-6.2 ± 0.3	0.5 ± 0.4

Thus the vectorial capacities of the mosquitoes were low in both the seasons, although that of wet season (0.99) was significantly higher ($p<0.05$) than that of the dry season (0.04) with mean value of 0.02 ± 3.83.

The vectorial capacities of *An. gambiae* for *P. vivax* were estimated to be a double of that for *P. falciparum* under similar ecological conditions (Table 3).

Table 3: Vectorial capacity of *Anopheles gambiae* for *Plasmodium* species in Bali district, June – December 2018

Season	Vectorial capacity of *Anopheles gambiae* for two *Plasmodium* species in the tropics	
	Plasmodium falciparum	***Plasmodium vivax***
Wet	0.99	1.4
Dry	0.04	0.09
Total	1.03	1.49
Mean	0.02 ± 3.89	0.04 ± 1.08

DISCUSSION

The seasonal variations in the mosquito density observed in this study was similar to the findings of Oyewole *et al*. (2005) in tropical rain forest, Lamidi (2009) in Sahel savannah and Lamidi *et al*. (2017a) in Guinea savannah. Higher numbers of mosquitoes were collected in wet season than dry (cold) season. This was attributed to lack of rain in dry season and temporary water holding places were drying up. The fact that the mosquito abundance coincides to a great extent with period of rainfall is an indication that rainfall plays significant role in mosquito population dynamics.

This implies that in all the study areas, abundant rainfall and duration, temperature and relative humidity are factors that determine abundance of mosquito due to availability of breeding sites throughout the year (Lamidi *et al*., 2017b).

The parous rates were high because none of the season was less than 50 %. This is an indication that a large number of *An. gambiae* in the study area were anthropophagic with HBI of 1, and had laid eggs at least once. Such high parous rate had been attributed to poor adult mosquito control measures and high distribution of larval habitats (WHO, 2001). Higher parous rate observed in wet season in this study was in line with the report of Olayemi and Ande (2008) in Ilorin, a similar ecological zone to Bali in Nigeria but in contrast to the findings of Uttah *et al.* (2013) who observed higher parous rate in dry season in Calabar, Cross River State, a rainforest/swamp forest zone of Nigeria. The variation might be due to the fact that rainforest/swamp forest zone experience rainfall in almost all months of the year and the variations in the values of other climatic parameters like temperature and humidity might also be more favourable to mosquito survival in dry season.

The probability of daily survival of the mosquitoes was very high throughout the seasons, indicating that *An. gambiae* is well adapted to the ecological conditions in Bali. This mosquito is the principal vector of malaria in tropical Africa, and has thus adapted itself to the prevailing conditions in the study area and geographical region (Lamidi *et al*., 2017b). The results of this study about life expectancy of *An. gambiae* imply that the mosquitoes are long-

lived because the values for both wet and dry seasons (8.00 and 5.9 days respectively) fall within the already discovered mean life expectancy of the *Anopheles* species which is 6 to 9 days (Brogdon and Chan, 2010), although the life expectancy recorded in this study is lower than that of findings of Olayemi and Ande (2008). A long live female mosquito allows for increase opportunity for vector-man contact and malaria transmission.

The seasonal mean temperature recorded for the study area favours mosquito survival and *Plasmodium* sporogony development. Temperatures of 20 to 30 ^{o}C are optimal for *Anopheles* to survive and be effective vector (Paaijman *et al*., 2011). The duration of sporogony (incubation period of *Plasmodium*) was slightly longer in dry season than wet season in this study unlike the findings of Olayemi and Ande (2008). This observation may be due to slightly lower temperatures recorded in dry season (being a cold/harmattan period). This is because a lower temperature is expected to bring about increase in *Plasmodium* incubation period in *Anopheles* mosquitoes (WHO, 2003).

The likelihood that the mosquitoes will survive incubation period in this study was very low, the expectation of infective life was negative and the mean vectorial capacity was low. This result was in agreement with the previous studies which noted that small fraction of *Anopheles* mosquitoes live long enough for the completion of extrinsic incubation period of malaria parasites in the gut of the mosquitoes notwithstanding the unfavourable climatic factors (Awolola *et al*., 2002; Afrane *et al*., 2008). This might be due to mortality factors that become more limiting with increasing age and influence of previous diet (Beklemishev *et al*., 1959; Okech *et al*., 2004) and effects of mosquito genes on *Plasmodium* development and external environmental factors (Osta *et al*., 2004; Paaijman *et al*., 2011). This is a reason why results on infection rate or sporozoite rates of *Anopheles* have been very low in reported research works (Awolola *et al*., 2002; Oyewole *et al*., 2005; Lamidi *et al*., 2019).

The difference recorded for the vectorial capacities of *Anopheles* mosquitoes for the *P. falciparum* and *P. vivax* is due to difference in incubation period of these *Plasmodium* species depending on ambient temperatures (WHO, 2003; Afrane, 2008; Bi *et al.,* 2013).

Conclusion: This kind of study with ultimate objective of determining the potential rate of contact between infectious principal malaria vectors and susceptible hosts is very rare. The *An. gambiae* population in Bali district is dominated by old mosquitoes due to high rates of daily survival. The longevity of this mosquito in this area with the favourable atmospheric temperature for *Plasmodium* development, make for high vectorial potential to the transmission of malaria. This information on the survivorship and vectorial capacity of *An. gambiae* can pave way for more informed vector control measures.

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