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Original Article

Survivorship Of *Anopheles gambiae* In Relation To Malaria Transmission In Ilorin, Nigeria

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Abstract:
For the first time in Africa, an entomological study went beyond the conventional practice of determining parity and survival rates of field-collected adult anopheline mosquitoes but also related these variables to duration of *Plasmodium* sporogony and estimated the expectation of infective life. Blood-seeking female mosquitoes were collected in Ilorin, Nigeria, from January 2005 to December 2006, and dissected for ovarian tracheations following WHO recommended techniques. The results indicated an annual mean parous rate of 70.92%, and significantly higher parous rates in the rainy than dry season, which also had very low densities. Mean probability of daily survival of the mosquitoes was 0.80, with annual mean life expectancy of 12.24 days. The probability of surviving the sporogonic cycle was low (< 0.4) but the expectation of infective life was long, especially in the rainy season (mean = 8.31 days). The epidemiological implications of these results were discussed. The *An. gambiae* population in Ilorin is dominated by older mosquitoes with high survival rates thus, suggesting a high vector potential for the species in the area. These information on the survival rates of *An. gambiae* in relation to malaria transmission would enhance the development of a more focused and informed vector control interventions.
Key Words: Infection, Life expectancy, Mosquitoes, Parity, *Plasmodium*, Sporogonic cycle

Introduction:

The burden of malaria remains enormous in Africa,[1] seven years after the launching of the World Health Organisation's Roll-Back-Malaria program in the year 2000. According to WHO statistics, approximately 40% of the world's population are at risk of malaria, with over 500 million people becoming critically ill with the disease annually.[2] In Nigeria, malaria is responsible for about 300,000 deaths every year and accounts for 40% public health expenditure.[3] The cost of malaria treatment and prevention in Nigeria has been estimated to be over $1 billion per annum.[4]

Anopheles gambiae is the principal vector of malaria in sub-Saharan Africa in general and Nigeria in particular.[5-6] For effective vector control interventions, as canvassed by the WHO's Roll-Back-Malaria initiative,[7] it is important that we know more about the local population biology of this mosquito, especially those factors that determine its epidemiological effectiveness. Among the more important of these factors are the total number of mosquitoes, the degree of their contact with man, their susceptibility to infection with Plasmodia and the proportion which survive to the infective age. Although most of these factors have been thoroughly studied in connection with the epidemiology of malaria in Africa,[8-10] not much has been done to understand the proportion of anopheline mosquitoes which survive till the end of *Plasmodium* sporogony. Published studies on anopheline survivorship in Nigeria are limited to those that merely provided estimates of parity rates and daily survival rates,[11-12] but did not relate these variables to the duration of sporogony in the mosquitoes. In order to fill this information gap and provide a good understanding of how survivorship influences the effectiveness of malaria vectors, this study was carried out to determine the age structure, survival rates and vector potentials of *Anopheles gambiae* mosquitoes in Ilorin, Nigeria.

Materials and Methods:

Study Area

Ilorin, the capital of Kwara state, Nigeria, is located within Longitudes 4° 30` and 4° 45`E and Latitudes 8° 25` and 8° 40`N, covering a land area of 75 Km^2 with an estimated population of 1.4 million people as at 2007.[13] The climate is tropical with mean annual temperature, relative humidity and rainfall of 27°C, 76% and 1800 mm, respectively. The climate presents two distinct seasons: a rainy season between May and Oct., with high rainfall during the months of Jun. and Aug., and a dry season (Dec. – Feb.) completely devoid of rains. The vegetation in Ilorin reflects that of the Guinea savanna zone, characterized by a predominance of tall grass, which are frequently removed by violent bush burning activities in the dry season.

Mosquito Collection, Processing and Identification

Adult mosquitoes were collected bi-weekly at four randomly selected sites using all night Human Landing Catches (HLC) from Jan. 2005 to Dec. 2006. There were two teams of two informed and consenting collectors per site. The human baits were rotated through the collection sites to compensate for differences in individual attraction or repulsion for mosquitoes. Captured mosquitoes were preserved in 4% formaldehyde solution, and identified using standard keys.[6,14]

Dissection of Mosquitoes and Examination of Ovaries for Parity:

The ovaries were dissected using WHO-recommended techniques.[15] Briefly, the legs and wings of the specimen were removed, and the mosquito was then placed on a slide in a drop of distilled water. While holding one dissecting needle on the thorax, under a dissecting microscope, the ovaries were removed by breaking the abdominal wall in the region of the 6th to 7th sclerite, and then pulling the tip of the abdomen away from the rest of the body with a second needle held in the right hand. The ovaries so revealed, were examined for ovarian tracheation under a compound microscope using the x10 objective, and when necessary, a confirmation was made using x40 objective. Those ovaries in which the terminal skeins of the tracheoles had become uncoiled were considered to be parous.

Meteorological Data:

Mean monthly temperature data, for the study period, were obtained from the weather station of the International Airport in Ilorin.

Data Analysis:

Generally, data analyses were according to WHO technique.[16] Mosquito density was determined as the total number of specimens collected per month. Parous Rate was determined as the proportion of dissected mosquitoes that were parous.

The probability of daily survival was estimated as the squared root of the proportion of parous mosquitoes.

Estimation of the duration of sporogony was done using the formula, $n = b/c$; where n = duration of sporogony, b = temperature degree–days, and c = the difference between mean temperature per time and the threshold temperature for extrinsic development of *Plasmodium* parasites, which was given as 16°C.[16] b was taken to be 111 degree-days. This is the temperature degree-days requirement for *Plasmodium falciparum* completion of sporogony. Over 90% of diagnosed malaria cases in Ilorin are due to *P. falciparum*.

The probability of surviving the sporogonic cycle was determined as P^n; where P = probability of daily survival and n = duration of sporogony.

Life Expectancy was estimated using the formula: $L = 1/-\log_e^P$; where L = Life Expectancy and P = probability of daily survival.

The Infective Life of the mosquitoes was calculated as the difference between the duration of sporogony and interval between adult emergence and blood meal put together, on one hand, and life expectancy on the other. For this study, the interval between adult emergence and blood meal was taking as two days.[16]

Differences in survivorship parameters between seasons and among months were determined using students' t-test and Chi-square test, respectively.

Results:

An annual average of 3,772 adult *An. gambiae* mosquitoes were caught (Table 1). Monthly densities of the mosquitoes varied significantly ($P < 0.05$) during the study period. The mosquitoes were least abundant in January/March, and the largest number of individuals was collected in September. Significantly ($P < 0.05$) higher numbers of mosquitoes were encountered in the rainy (May - October) than dry (December – March) season (Table 2). The results of the parous rates of the mosquitoes were similar to those of mosquito density (Table 1). However, the least and highest parous rates were recorded in February and July, respectively.

As shown in Table 1, the probability of daily survival of the individuals of this mosquito was quite high, with an annual mean probability of daily survival of 0.86. Mean monthly, as well as, mean seasonal probability of daily survival were not significantly different ($P > 0.05$) (Tables 1 & 2).

Table 1: Monthly variations in densities, survivorship and infection probabilities of *Anopheles gambiae* mosquitoes in Ilorin, between January 2005 and December 2006

Month	Number collected and Dissected	Number parous	Parous Rate (%)	Probability of daily survival	Life Expectancy (Days)	Atmospheric Temperature (°C)	Duration of Sporogony (Days)	Probability of Surviving Sporogony	Expectation of infective Life (Days)
January	128^{a}	74^{a}	57.77^{a}	0.76^{a}	8.40^{a}	33.40^{b}	6.38^{a}	0.17^{a}	2.02^{b}
February	198^{b}	110^{b}	55.64^{a}	0.75^{a}	7.87^{a}	34.60^{b}	5.97^{a}	0.17^{a}	1.90^{b}
March	124^{a}	74^{a}	59.62^{a}	0.77^{a}	8.93^{a}	35.00^{b}	5.84^{a}	0.22^{a}	3.09^{b}
April	243^{b}	152^{c}	62.37^{a}	0.79^{a}	9.80^{a}	35.60^{b}	5.66^{a}	0.26^{a}	4.14^{b}
May	475^{d}	305^{e}	64.13^{b}	0.80^{a}	10.00^{a}	30.00^{a}	7.93^{a}	0.17^{a}	2.07^{b}
June	534^{d}	415^{f}	77.80^{c}	0.88^{a}	18.34^{c}	29.80^{a}	8.04^{a}	0.36^{a}	10.30^{d}
July	246^{b}	196^{d}	79.64^{d}	0.89^{a}	20.16^{c}	28.30^{a}	9.02^{b}	0.36^{a}	11.14^{d}
August	538^{d}	407^{f}	75.71^{c}	0.87^{a}	16.67^{c}	27.20^{a}	9.91^{b}	0.25^{a}	6.76^{c}
September	551^{e}	429^{f}	77.94^{d}	0.88^{a}	18.52^{c}	28.50^{a}	8.88^{b}	0.33^{a}	9.64^{d}
October	394^{c}	308^{e}	78.29^{d}	0.89^{a}	18.87^{c}	28.40^{a}	8.95^{b}	0.34^{a}	9.92^{d}
November	193^{b}	119^{b}	61.50^{a}	0.78^{a}	9.52^{a}	30.10^{a}	7.87^{a}	0.15^{a}	1.65^{b}
December	148^{a}	86^{a}	58.43^{a}	0.76^{a}	8.55^{a}	28.00^{a}	9.25^{b}	0.08^{a}	- 0.70^{a}
Mean	3772^{f}	2675^{g}	70.92^{c}	0.86^{a}	12.24^{b}	30.70^{a}	7.55^{a}	0.24^{a}	4.29^{b}

Values followed by same superscript alphabet in a column are not significantly different at $P = 0.05$.

Table 1 shows that the life expectancy of the adult mosquitoes ranged from 8.4 days in January to 20.16 days in July, with a mean annual life expectancy of 12.24 days. Life expectancy was significantly ($P < 0.05$) higher in the rainy than dry season (Table 2). During the study period, atmospheric temperature ranged between 27.20°C in August and 35.60°C in April, with an annual mean temperature of 30.70°C (Tab. 1). Significantly ($P < 0.05$) higher temperatures were recorded in the dry than rainy season (Table 2). The sporogonic development of the *Plasmodium* parasites lasted for approximately 6 days in the months of January to April (Table 1). Starting from May, there was an appreciable increase in the duration of sporogony until a peak of 9.91 days was attained in August. Though the duration of sporogony was longer in the rainy than dry season, the difference was however not significant (P > 0.05) (Table 2).

Table 2: Seasonal variations in densities, survivorship and infection probabilities of *Anopheles gambiae* mosquitoes in Ilorin, between January 2005 and December 2006

Parameter	Dry Season	Rainy Season
Mosquito Density	149.50^{a}	456.33^{b}
Parous Rate (%)	57.87^{a}	75.59^{b}
Probability of Daily Survival	0.76^{a}	0.87^{a}
Life Expectancy (Days)	8.44^{a}	17.09^{b}
Atmospheric Temperature (°C)	32.75^{b}	28.70^{a}
Duration of Sporogony (Days)	6.86^{a}	8.79^{a}
Probability of Surviving Sporogony	0.16^{a}	0.30^{a}
Expectation of Infective Life (Days)	1.67^{a}	8.31^{b}

Values followed by same superscript alphabet in a row are not significantly different at *P* = 0.05.

The probability that the mosquitoes survived sporogony were rather low (Table 1). The months of June to October were the period when the mosquitoes were most likely to survive long enough to become infectious. The pattern of the monthly distribution of infective life of the mosquitoes was similar to that observed for life expectancy (Table 1). However, a negative value (-0.70 day) was estimated for this parameter in December. The annual mean expectation of infective life indicated that a mosquito stays infective for about 4 days in Ilorin. The infective life of the mosquitoes was significantly (P < 0.05) longer in the rainy (mean = 8.31 days) than dry (mean = 1.67 days) season (Table 2)

Discussion:

The monthly density variations of the mosquitoes observed in this study is similar to those reported elsewhere in Nigeria.[17,18] Significantly higher densities of mosquitoes were collected in the rainy than dry season. A study in Kenya opined that the rainy season presents favourable environmental conditions that enhance mosquito breeding and survival, through the proliferation of larval habitats and improved humidity, respectively.[19] The mean monthly parous rates were high, as none was less than 50%. This observation indicates that a large proportion of the female *An. gambiae* mosquitoes in Ilorin had already practiced haematophagy. In the past, such high parous rates of anopheline mosquitoes has been related to the non-application of control measures and closeness of mosquito collection sites to larval habitats.[16] Comparable data on anopheline parity rates in Nigeria are rare, however, results similar those of this study were obtained in Makurdi.[12]

The probability of daily survival of the mosquitoes remained very high throughout the year, suggesting that *An. gambiae* is well adapted to the environmental conditions in Ilorin. This mosquito is the principal vector of malaria in sub-Saharan Africa, and thus has adapted itself fully to the prevailing tropical conditions in the region.[5] The mean life expectancy of anopheline mosquitoes in nature ranges from 6 to 9 days.[20] The results of this study indicate that the *An. gambiae* mosquitoes in Ilorin are long-lived. The mean annual life expectancy was 10 days, with the adult mosquitoes surviving for about 20 days in July. This observation is of considerable importance with respect to vector efficiency of *An. gambiae* for malaria parasites in Ilorin. A long-lived adult female mosquito allows for increased opportunities to encounter an infected human host, the malaria parasites to multiply and reach the salivary glands after an infective blood meal, and transmission of parasites in later blood meals to previously uninfected hosts.

The annual mean monthly temperature recorded for the study area favours mosquito and Plasmodia development. While, temperatures of 20°C to 30°C are optimal for *Anopheles* to survive long enough to acquire and transmit *Plasmodium* parasites,[21] the extrinsic incubation period of the parasite is shortest at temperatures of about 30°C.[22] The incubation period of the *Plasmodium* parasites was longer in the rainy than dry season. This observation may be due to the lower temperatures recorded in the rainy season; a drop in temperature is expected to increase the incubation period of *Plasmodium* parasites in *Anopheles* mosquitoes.[23]

The likelihood of surviving the sporogonic cycle was low. This result agrees with those of previous studies which noted that only a relatively small fraction of anopheline populations live long enough for the *Plasmodium* sporogonic cycle to be completed, due to mortality factors that become more limiting with increasing age.[20] The expectation of infective life of the mosquitoes was slightly more than 8 days in the rainy season. The gonotrophic cycle of *An. gambiae* has been estimated at 2 days and is, of course, affected by ambient temperature.[16] Thus, an infective *An. gambiae* mosquito in Ilorin could take up to four blood meals, with considerable potential for malaria parasite transmission.

Conclusions:

This study achieved, for the first time in Africa, a definite objective of determining what proportion of the principal vector of malaria in the continent survives to become a threat to human health. The *An. gambiae* population in Ilorin is dominated by older mosquitoes due to the high rates of daily survival. The long life expectancy of this mosquito in the area coupled with optimal temperatures, for parasite development, make for high vector potential for the transmission of malaria. These information on the survival rates of *An. gambiae* in relation to malaria transmission would enhance the development of a more focused and informed vector control interventions.

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