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Determination of Sporozoite Infection Rate of Indoor Resting Female Anopheles Mosquitoes in Gombe South, Gombe State, Nigeria

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Abstract
Infected female Anopheles mosquitoes are the culprits of malaria. The abundance and distribution of malaria vectors in communities contribute significantly in malaria transmission. Understanding the magnitude and intensity of sporozoite infection rates is very sensitive and powerful indices for describing the epidemiology of malaria which will aid in strategizing malaria control programme. The present study determines the sporozoite infection rates of female Anopheles mosquitoes in the four Local Government Areas (LGA) of Gombe South, Gombe State, Northeastern Nigeria. Mosquitoes were collected using Pyrethrum Spray Catch (PSC) and were dissected to check the presence of sporozoites. Out of the 1,860 female Anopheles mosquitoes dissected, 665(35.8%) were infected with sporozoites. The highest infection rate was observed from Kaltungo LGA 145(41.7%), followed by Billiri LGA 182(36.3%), and 205(35.4%) was recorded from Balanaga. The lowest infectivity rate was observed from Shongom LGA 133(30.9%), p<0.005 which is highly significant statistically. With regards to monthly distribution of sporozoites, the highest infection rate was recorded in December (64.7%), January 60.0%, (64.5%), and August (60.7%) from Balanga, Billiri, Kaltungo and Shongom LGA respectively. Meanwhile the least was observed in July (2.8%), December (7.7%), April (20.0%), and March (11.6%) from Balanga, Billiri, Kaltungo and Shongom LGA respectively, p<0.005 which is highly significant statistically. However, (0.0%) sporozoite infection was obtained in September and March from Billiri, Kaltungo and Shongom LGA respectively. The present study therefore recommends

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malaria vector control through community based efforts by destruction of mosquitoes breeding sites, use of insect's repellents, environmental sanitation and application of oil in stagnant water in order to reduce vectors population.

Keywords: Female Anopheles, Sprozoites, Infection Rate, Gombe South.

INTRODUCTION

Mosquitoes are insects of medical importance that pose great threat to human health because of their ability to act as vectors of pathogens, causing diseases such as: malaria, dengue fever, zika virus, yellow fever, encephalitis and filariasis. Mosquitoes associated diseases lead to huge economic importance like disease burden, death, poverty and social debility worldwide, particularly in tropical countries. Among the diseases, malaria remains the most serious vector–borne disease [World Health Organization (WHO, 2015)]. *Plasmodium* transmission via mosquito-to-human occurs when sporozoites from the salivary gland of the infected female *Anopheles* mosquito are injected into human skin during blood-feeding. Parasites then pass to the liver where they replicate, each sporozoite yielding many thousands of merozoites which go on to cause patent infection (Kelly-Hope and McKenzie, 2009). *Anopheles* mosquitoes breed in water bodies, and each species has its own breeding preference. Transmission is more intense in places where mosquito lifespan is longer (parasite has time to complete its development inside the mosquito) and where anthropophilic mosquitoes prevail. Forty-one of the 512 Anopheles species have been defined by experts as Dominant Vector Species (DVS). DVS are the most important malarial vector worldwide affecting humans, characteristics of DVS are their propensity for humans feeding, longevity, abundance and elevate vector capacity (Takken and Scott, 2003).

Malaria is an acute febrile illness caused by *Plasmodium* parasites, which are spread to people through the bites of infected female *Anopheles* mosquitoes. There are 5 parasite species that cause malaria in humans, and 2 of these species *P. falciparum* and *P. vivax* pose the greatest threat (WHO, 2020). *P. falciparum* is the deadliest malaria parasite and the most prevalent on the African continent. *P. vivax* is the dominant malaria parasite in most countries outside of sub-Saharan Africa. The disease is preventable and curable. In 2021, there were an estimated 247 million cases of malaria, and 619,000 deaths worldwide. The WHO African Region carries a disproportionately high share of the global malaria burden. However, 95% of malaria cases and 96% of malaria deaths. Children under 5 accounted for about 80% of all malaria deaths in the Region (WHO, 2022). Although much progress has been made in the fight against malaria, the number of people that contract this disease due to the bite of an *Anopheles* mosquito remains unacceptably high. A better understanding of the relationship between the *Plasmodium* parasite and its vector is of extreme importance and may allow for the development of new tools to fight the disease (Kojin and Adelman, 2019).

The presence of sporozoites in the salivary gland of mosquito thus defines mosquito infectiousness. These sporozoites are injected to human body through the bites of infected female *Anopheles* mosquito, thereby establish malaria parasites which is a major public problem in the tropics. Brugman *et al.* (2018) demonstrated that, *Anopheles* mosquitoes expel malaria sporozoites while feeding. The quantification of malaria sporozoites is of utmost importance, this would aid in predicting the forces of infection transmission and enhance field surveillance activities for a successful malaria elimination. The monthly distribution of sporozoites depict the seasonal dynamism of malaria in the study area. Understanding transmission biology of the malaria vectors is a key component of intervention strategies that target the spread of malaria parasites to humans (Meibalan and Marti, 2022).

MATERIALS AND METHODS

Study Area

The study was carried out in the southern part of Gombe State, northeastern Nigeria. Gombe south comprises of Balanga, Billiri, Kaltungo and Shongom Local Government Area (LGAs) as shown in Fig.1 below. Gombe State has a geographical coordinates of latitude 9°53N 11°26E and longitude 9.883°N 11.433°E on the Greenwich Meridian. Balanaga LGA has a population of 212,549 people, and a total land mass of 2140km², while Billiri LGA has a population of 202,144 people, with a total land mass of 655km² (3.9%), however, Kaltungo LGA has a population of 149,086, and a total land mass of 890km² (5.3%), and Shongom LGA has, a population of 151, 520 people, and a total land mass of 775km² (4.1%) [National Bureau of Statistics (NBS, 2007)]. The study area lies within the Sudan Savannah zone marked by dry and wet seasons. The rainfall season is from April to October with the rainfall figure ranging between 39-66mm. The relative humidity is between 44-68% while the temperature fluctuates between 22-29°C. The dry season is from November to March, and the vegetation of the zone consists of short grasses and medium shrubs that grow especially in July and September where there is high amount of rainfall [Nigerian Meteorological Agency (NMA, 2016)].

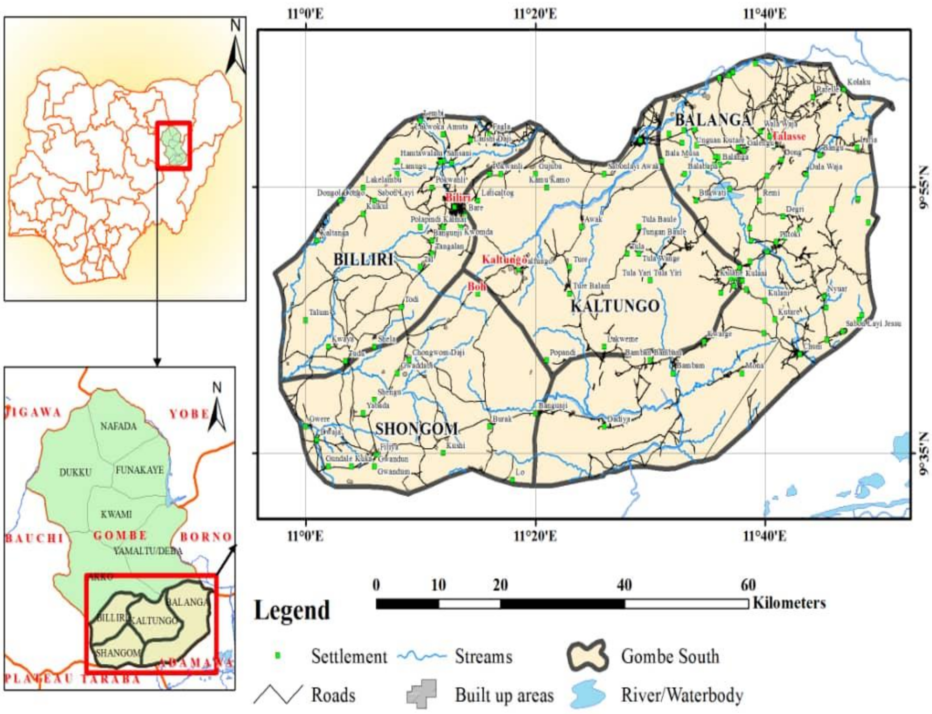

Figure1: Map of Gombe South, showing the study area (Mayomi *et al.,* 2018).

Ethical Consideration and Administration of Questionnaires

Ethical clearance was obtained from Gombe State Ministry of Health ethical committee with reference number: MOH/ADM/S/658/VOL.11/88. Before the commencement of the research work, the community people were gathered at the community gathering ground with the help of the gatekeepers, the purpose of the study was explained in English and Hausa language, then the dialect of the various people with the help of persons from the communities respectively.

Mosquito Collection

Ten (10) rooms were selected randomly from the communities in each LGA, the indoor resting mosquitoes were collected using Pyrethrum Spray Catch (PSC), within the periods of March, 2019 to February, 2020. A night before collection of the mosquitoes, all occupants of the various rooms were informed not to allow the window/doors open between the early hours of 6am to 9am. Furniture, food items, and other items that would either obstruct or be spoiled by the chemical were removed. White sheets were spread on the flow, windows were closed, then the insecticide was sprayed at all corners of the room, and the door was locked for at least 10 – 15 minutes. Knock-down/death mosquitoes were packed and transferred into labelled petri dishes and were brought to the laboratory for identification, using identification key before dissection (WHO, 2005).

Mosquito Dissection

The abdomen of each of the identified female mosquitoes was dissected to check for the presence of sporozoites, the head, wings, and legs of the mosquitoes was removed, then placed on a glass slide under dissecting microscope. Dissection was done between the 6th and 7th sclerite under. The ovaries were pulled out, and a little pressure was applied to burst the abdomen spring out the malpighian tubules and the stomach. This was then separated from other parts of the abdomen and transferred to a new slide. A drop of normal saline was added, then covered with a cover slip and examined under a compound microscope to check the presence of sporozoites (Williams and Pinto, 2012).

Determination of Sporozoite Infectivity Rate

Dissected mosquitoes were separated into two categories, the ones with sporozoites and those without sporozoite and was calculated as demonstrated below;

$$Infectivity\ Rate\ = \frac{Number\ of\ Mosquitoes\ Carrying\ Sporozoites}{Number\ of\ Mosquitoes\ Dissected}\ x\ 100$$

Statistical Analysis

Data obtained were analyzed using Statistical Package for Social Sciences (SPSS) version 26.0. Chi Square (χ^2) was used to analyze associations between variables. $p \leq 0.05$ were considered statistically significant.

RESULTS

A total of 1,860 mosquitoes were dissected out of which 665(35.8%) were infected with sporozoites, distribution by LGA showed: 145(41.7%) sporozoites was recorded from Kaltungo LGA which was the highest, then followed by Billiri 182(36.3%) infection rate, however, Balanaga LGA depicts 205(35.4%) infection rate, and the lowest infection rate was observed from Shongom LGA 133(30.9%) as presented in Table 1 below. Table 2 showed the monthly distribution of sporozoite infection rate, in Balanga LGA, the maximum infection was observed in December 33(64.7%), and the minimum in July 15(2.8%), meanwhile, Table 3 showcased the highest sporozoite infection rate was obtained in November 16(69.6%), and the lowest in December 3(7.7%), however, 0% sporozoite infection rate was recorded in September. Table 4 displayed the maximum sporozoite infection rate of 12(60.0%) in June, and the least in April 3(20.0%), meanwhile, 0% sporozoite was observed in March. Table 5 revealed the highest in July 25(64.1%) and the lowest in March 5(11.6%), 0.0% was recorded in September, $p<0.005$ in all the results obtained.

Table 1: Distribution of Sporozoite Infection Rate by Local Government Area

LGA (%)	Mosquitoes Dissected	Mosquitoes Infected	Sporozoite Infectivity Rates (%)	χ^2
Balanga	579	205	35.4	0.000
Billiri	502	182	36.3	
Kaltungo	348	145	41.7	
Shongom	431	133	30.9	
Total	1,860	665	35.8	

Table 2: Distribution of Sporozoite Infection Rate by Months in Balanga LGA

Month/Year	Mosquitoes Dissected	Mosquitoes Infected	Sporozoite Infectivity Rates (%)	χ^2
March, 2019	41	11	26.2	0.000
April, 2019	25	6	24.0	
May, 2019	53	22	41.5	
June, 2019	64	13	20.3	
July, 2019	72	15	2.8	
August, 2019	55	12	21.8	
September, 2019	49	16	32.6	
October, 2019	85	43	50.8	
November, 2019	42	10	23.8	
December, 2019	51	33	64.7	
January, 2020	18	10	55.5	
February, 2020	24	14	58.3	
Total	579	205(35.4)		

Table 3: Distribution of Sporozoite Infection Rate by Months in Billiri LGA

Month/Year	Mosquitoes Dissected	Mosquitoes Infected	Sporozoite Infectivity Rates (%)	χ^2
March, 2019	33	7	21.2	0.000
April, 2019	57	17	29.8	
May, 2019	38	11	28.9	
June, 2019	73	28	38.4	
July, 2019	88	41	46.6	
August, 2019	32	10	31.3	
September, 2019	21	-	-	
October, 2019	48	23	47.9	
November, 2019	23	16	69.6	
December, 2019	39	3	7.7	
January, 2020	20	12	60.0	
February, 2020	33	14	42.4	
Total	502	182(36.3)		

Table 4: Distribution of Sporozoite Infection Rate by Months in Kaltungo LGA

Month/Year	Mosquitoes Dissected	Mosquitoes Infected	Sporozoite Infectivity Rates (%)	$\chi 2$
March, 2019	13	-	-	0.000
April, 2019	15	3	20.0	
May, 2019	18	7	38.8	
June, 2019	20	12	60.0	
July, 2019	24	13	54.2	
August, 2019	45	22	48.8	
September, 2019	36	10	27.7	
October, 2019	51	18	35.2	
November, 2019	14	6	42.9	
December, 2019	40	21	52.5	
January, 2020	31	20	64.5	
February, 2020	41	13	31.7	
Total	348	145(41.7)		

Table 5: Distribution of Sporozoite Infection Rate by Months in Shongom LGA

Month/Year	Mosquitoes Dissected	Mosquitoes Infected	Sporozoite Infectivity Rates (%)	$\chi 2$
March, 2019	43	5	11.6	0.000
April, 2019	30	7	23.3	
May, 2019	24	7	29.2	
June, 2019	53	10	18.9	
July, 2019	39	25	64.1	
August, 2019	28	17	60.7	
September, 2019	49	-	-	
October, 2019	33	5	15.2	
November, 2019	20	6	30.0	
December, 2019	51	27	52.9	
January, 2020	31(7.2)	11	35.5	
February, 2020	30	13	43.3	
Total	431	133		

DISCUSSION

Understanding the magnitude and intensity of sporozotes infection rate is a very sensitive and powerful parameter for describing the epidemiology of malaria which will aid in strategizing control programme. This research work investigated the sporozoites infection rate of infected female *Anopheles* mosquitoes in Gombe south LGAs, Gombe State, northeastern Nigeria. A total of 1,860 female *Anopheles* were dissected, out of which 665(35.8%) sporozoites infection rate was observed as the overall in the four LGAs, the outcome of this research concur with the findings of (Salum *et al*., 2021; Oduola *et al*., 2021; Manyi *et al*., 2014; Najila *et al*., 2019) where in separate studies form Kilombero velleys, southeastern Tanzania, Kwara state and Markurdi in north-central Nigeria respectively, but differs with the reports of (Lamogo *et al*., 2019; Maia *et al*., 2019; Obembe and Awopetu, 2014). from some selected communities in Shongom LGA, Gombe state, and Sri Lanka, the difference in the results could be as a result of geographical factors variation such as: the amount of rainfall received in the site, temperature, and availability of potential breeding sites of the vectors, the size of communities covered and other factors like conduciveness of environments for the *Anopheles* mosquito to live comfortably.

The prevalence of sporozoite obtained from Balanga, Billiri, Kaltungo, and Shongom LGA depicts: 35.4%, 36.3%, 41.7%, and 30.9% respectively, the slight variation amongst the for LGAs could be affiliated to the similarities in geographical factors like the rainfall, temperature, and humidity experienced in the zone in which the four LGAs are in cluster. This disagreed with the result of (Okorie *et al*., 2014) where low sprozoite infection rate was reported from Ibadan southern Nigeria respectively, but conform with the findings of (Manyi *et al*., 2014) as compared in the study. The highest prevalence of sporozoite by month showed, (60.0%) in June, and (64.1%) in July, this corresponds with the work of (Manyi *et al*., 2014) where the maximum sprozoite infection rate was observed in May and June from Wurkun and Makurdi localities in Benue State Nigeria, both May, June, and July are in the rainy period. In Balanga and Billiri LGA, the highest sprozoite infection rate was observed in October (64.7%), and November (69.6%) while this is in contrast with (Manyi *et al*., 2014) result from North-Bank locality in Makurdi, Benue State where (16.7%) sprozoite infection rate was observed.

CONCLUSION

It is therefore concluded that the prevalence of sporozoite infection rate is high in the study site, but Kaltungo LGA recorded the highest percentage while the other three LGAs have similar sporozoite prevalence rate.

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