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# Indoor Resting Densities and Blood Meal Preferences of Malaria Vector Mosquitoes in Bauchi State, Nigeria.

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**ABSTRACT**
This study investigated the indoor resting densities and blood meal preferences of malaria vector mosquitoes in nine Local Government Areas (LGAs) of Bauchi State, Nigeria. A total of 6935 adult mosquitoes were collected from 270 rooms using PSC, with an overall indoor resting density of 2-4 mosquitoes per room. Results showed that houses with open eaves and no windows had higher indoor resting mosquito densities, increasing the risk of malaria transmission. The study suggests that indoor microclimate, cooking, sleeping, and livestock keeping practices contribute to the indoor occurrence of mosquitoes. Malaria vector mosquitoes exhibited endophilic behavior, emphasizing the need for mosquito-proof houses as a control strategy. The study also found that indoor resting adult mosquitoes increased rapidly following rainfall, consistent with previous studies. Analysis of 1000 blood-fed female Anopheles gambiae by ELISA revealed that humans are the primary source of blood meals (52.4%), followed by goats (12.8%), cows (9.2%), sheep (7.5%), and pigs (1.6%). The results showed varied feeding patterns across the study areas, with some areas exhibiting mixed feeding behavior. The predominance of human blood meals is consistent with the vectors' anthropophilic nature, highlighting their role in malaria transmission. These findings highlight the importance of environmental factors, housing design, and understanding vector feeding habits in malaria transmission and control.

**Keywords:**
Blood Meal Preferences,
Malaria,
Mosquitoes,
Bauchi State

## INTRODUCTION

Malaria remains a significant public health concern in Nigeria, accounting for approximately 30% of global malaria cases and 24% of malaria-related deaths (WHO, 2020). The disease is transmitted through the bite of an infected female *Anopheles* mosquito, which requires a blood meal to develop its eggs (Cappello, 2011). The blood meal source of malaria vectors plays a crucial role in determining their feeding rates, adult survival, fecundity, hatching rates, and developmental times, ultimately influencing their vectorial capacity (Orsborne *et al*., 2019).

In sub-Saharan Africa, where malaria is endemic, Anopheles gambiae, *Anopheles arabiensis*, and *Anopheles funestus* are the primary vectors responsible for transmitting Plasmodium falciparum, the deadliest malaria parasite (Sinka et al., 2012). The feeding behavior of these vectors is a critical factor in malaria transmission, as it determines the likelihood of human-mosquito contact (Kane *et al*., 2013).

Studies have shown that *Anopheles* mosquitoes exhibit varying degrees of anthropophagy (preference for human blood) and zoophagy (preference for animal blood), which influences their role in malaria transmission (Orsborne *et al*., 2018; Abdulla-Khan *et al*., 2019). Understanding the blood meal preferences of malaria vectors is essential for developing effective vector control strategies, such as targeted indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) (WHO, 2019). In Nigeria, where malaria is a leading cause of morbidity and mortality, understanding the feeding habits of Anopheles mosquitoes is crucial for designing effective control measures (FMOH, 2017). Bauchi State, located in northeastern Nigeria, is one of the states with high malaria transmission rates, accounting for approximately 4.5% of national malaria cases (NMCP, 2018). Despite the importance of understanding malaria vector feeding behavior, there is a dearth of information on the blood meal preferences of *Anopheles* mosquitoes in Bauchi State.

This study aimed to determine the blood meal source of Anopheles mosquitoes in Bauchi State, Nigeria, to provide insights into their feeding behavior and inform malaria vector control strategies

## MATERIALS AND METHODS

### Study area

### Bauchi state.

Bauchi State occupies a total land area of 49,119 km$^2$ (18,965 sq mi) representing about 5.3% of Nigeria's total land mass and is located between latitudes 9° 3' and 12° 3' north and longitudes 8° 50' and 11° east. The state is bordered by seven states, Kano and Jigawa to the north, Taraba and Plateau to the south, Gombe and Yobe to the east and Kaduna to the west. Bauchi state is one of the states in the northern part of Nigeria that span two distinctive vegetation zones, namely, the Sudan savannah and the Sahel savannah.

The Sudan savannah type of vegetation covers the southern part of the state. Here, the vegetation gets richer and richer towards the south, especially along water sources or rivers, but generally the vegetation is less uniform and grasses are shorter than what grows even further south, that is, in the forest zone of the middle belt, this part of the state, is mountainous as a result of the continuation of the Jos Plateau,. The vegetation types here are conditioned by the climatic factors, which in turn determine the amount of rainfall received in the area. For instance, the rainfall ranges between 1,300 millimetres (51 in) per annum in this part of the state. Consequently, rains start earlier in this part of the state and become heaviest and lasts longer, it start in April with the highest record amount of 1,300 millimetres (51 in) per annum. This pattern is because in the West Africa sub-region, rains generally come from the south as they are carried by the southwester lies (National Population Commision, 2006)

The Sahel type of savannah, also known as semi-desert and generally sandy, vegetation, becomes manifest from the beginning of this part of the state as one moves from the state's south to its north. This type of vegetation comprises isolated stands of thorny shrubs. There is therefore a progressive dryness towards the north, culminating in the desert condition in the far north (extreme sahel savannah) and rainfall here is only 700 millimetres (28 in) per annum (National Population Commision, 2006).

In contrast to the sudan savannah, the sahel savannah part of the state receives the rains late, usually around June or July, and records the highest amount of 700 millimetres (28 in) per annum. (National Population Commision, 2006). See appendix XVIII for complete map of Bauchi State


Fig 1: LGAs of sampling and their respective geographical coordinates.

- Bauchi (LGA): The capital city of Bauchi is approximately around 10.3115° N, 9.8440° E.
- Dass: The town of Dass, which is the headquarters of the LGA, is located around 9.9200° N, 9.4800° E.
- Kirfi: The town of Kirfi is roughly at 9.9472° N, 10.5186° E.
- Ganjuwa: The headquarters, Kafin Madaki, is located around 10.6667° N, 9.9833° E.
- Dambam: The town of Dambam is approximately at 11.6667° N, 10.7000° E.
- Katagum: The town of Azare, the headquarters of Katagum LGA, is around 11.6500° N, 10.2000° E.
- Gamawa: The town of Gamawa is roughly at 11.9667° N, 10.4333° E.
- Jama'are: The town of Jama'are is approximately at 11.6667° N, 9.7833° E.
- Misau: The town of Misau is located around 11.3167° N, 10.4500° E.

### Adult Mosquitoes Collection

Adult mosquitoes were collected from the study area between June 2019 and December 2021 using the Pyrethrum Spray Collection (PSC) method, as described by the Centers for Disease Control and Prevention (CDC, 2006). Collections were made from rooms where at least one person had slept the previous night. Prior to spraying, doors and windows were closed, and the floor was covered with a clean white sheet. Pyrethroids were sprayed, and the room was closed for 10-15 minutes. The sheet was then folded, and mosquitoes were collected and placed in petri dishes using forceps. Samples were transported to the research laboratory at the Biological Sciences Department, Abubakar Tafawa Balewa University, Bauchi, and stored dry using silica gel.

**Indoor Resting Density (IRD)**

The Indoor resting density of the mosquitoes collected from each Local Government Area of study was determined using the formula giving below as adopted from (CDC,2015)

$$\textbf{IRD} = \frac{number\ of\ mosquitoes}{number\ of\ rooms} \times \frac{1}{number\ of\ nights}$$

Adult mosquitoes were stored in a laboratory using effedurf tube and silica gel, the stored samples were kept for almost fourty weeks without doing any damage to the sample and later on move to the molecular laboratory for further investigations

**Identification of Blood Meal Source by Direct ELISA**

Fed female *Anopheles* were first grouped based on their abdominal conditions before Identification of blood meal sources using direct ELISA. They were generally grouped as unfed, freshly fed, half gravid and gravid (WHO, 2003a).

**i. Unfed-**The abdomen looks flattened

**ii. Freshly fed-** The abdomen looks bright or dark red from the blood in the midgut. The ovaries occupy only a small area at the tip of the abdomen

**iii. Half gravid-** The abdomen is dark in colour almost black and occupies three to four segments on the ventral surface and six to seven on the dorsal surface of the abdomen.

**iv. Gravid-**The blood is reduced to a small black patch on the ventral surface or may be completely digested.The ovaries occupy all the rest of the abdomen. Freshly fed (category ii) were used for direct ELISA

A direct ELISA using anti phosphatase conjugates (Anti human IgG (Fab specific);Anti goat IgG (whole molecule) (Sigma), were used to identify Human, Pig, Cow (bovine) and goat (ovine) and sheep host respectively, based on the procedures of Beier *et al.* (1988). Mosquitoes with blood meals were assayed in flat bottomed well plates for each host and absorbance read at 405nm, 30 minutes after the addition of substrate (pNPP).

**Sample preparation**

Mosquitoes tested were preserved dry or frozen. The abdomen was ground in 50µl PBS (0.10M PBS) pH 7.4A dilution of 1:50 was made of the mosquito triturate using PBS (pH 7.4).

**Elisa procedures:**

The microtitre wells were coated with 50ul of mosquito triturate. The plate was covered and incubated for 3 hrs at room temperature

After incubation, the wells were washed twice with PBS Twenty 20 50µl host specific conjugate was added (antihost IgG conjugated in either peroxidase or phosphates): human, cow, pig, IgG 1: 2000, bovine, sheep, goat 1:25 dilution in 0.5% boiled casein containing 0.025% Twenty 20. (Peroxidase conjugate for human, cow, and pig phosephate conjugate for bovine, sheep and goat). This was incubated for 1 hr at room temperature.

The wells were washed thrice with PSB-Twenty 20. 1000µl ABTS peroxidase substrate was added (2'2'-azino-di (3-ethylbenzthialine sulfonate) The absorbance was read at 414mn after 30 minutes (Beier *et al*, 1990).

**STATISTICAL SOFTWARE USED.**

The analyses were performed using R statistical software (version 4.1.2)

**RESULTS AND DISCUSSION**

Table 1. Number of Adult Mosquitoes and Indoor Resting Densities across the LGAs in the study area.

| LGAs | No. of rooms visited | No. of Adult Mosquitoes Collected | Indoor Resting Density |
|---|---|---|---|
| Bauchi | 30 | 1200 | 4.0 |
| Dass | 30 | 865 | 3.0 |
| Kirfi | 30 | 601 | 2.0 |
| Misau | 30 | 701 | 2.0 |
| Ganjuwa | 30 | 648 | 2.0 |
| Dambam | 30 | 506 | 2.0 |
| Katagum | 30 | 742 | 3.0 |
| Gamawa | 30 | 911 | 3.0 |
| Jamaare | 30 | 761 | 3.0 |
| **Total** | **270** | **6935** | |

Results of this present study indicated that houses having open eaves, no windows were found to be associated more with higher risk of indoor resting mosquitoes and eventually higher risk of malaria infection, this is in line with the results obtained in a research conducted by Omar *et al*,. (2022). The trend of indoor occurrence of mosquitoes in this present study could be attributed to several factors among which appropriate indoor microclimate is one. The tradition of cooking, sleeping and tethering livestock inside residential houses could also contribute to the indoor occurrence of mosquitoes in the areas studied, this by increasing indoor temperature and providing access to blood meal sources, this is also in agreement with the findings of Oduala *et al*., (2016), where they had a similar result to this one in south-central

Ethiopia. This in turn contributes to the survival and increased malaria transmission potential of the vectors in the areas studied. Indoor resting mosquitoes of this study are estimated to transmit malaria earlier than those mosquitoes resting out-doors. This study also reveals that the malaria vector mosquitoes of the study areas and even the non malaria vector mosquitoes exhibit endophilic behavior which is an indication of the need to construct mosquito proof houses as control strategy for malaria transmission, this behavior is also seen in the results of the research findings conducted by Oduala *et al*., (2016). In a similar study to this conducted at Gambia by Oduala *et al*., (2016), it was reported that densities of both indoor biting and indoor resting mosquitoes were highest in the low altitude areas and decreased with increasing altitude. Similarly, densities of both immature and adult stages of mosquitoes observed to decrease significantly with increasing altitude.

It is also clearly observed in this study that the indoor resting adults mosquitoes in the study areas increases rapidly following the beginning of rainfall, this is a situation consistent with the observation of Molta *et al*, (1993) and Molta *et al*, (2004) that annual increase of the adult mosquito population was dependent on rainfall. Other studies conducted in Kwara State by Oduala *et al*., (2016) even though uses more than one adult mosquito collection techniques presented a similar result to the one obtained hear. Other studies conducted by Coetzee *et al*., (2000) in South Africa on the abundance of mosquito in some selected communities presented the same results to this one. The direct influence of the rainfall to the abundance of mosquito is also an agreement with the work done in Tanzania by Charlwood *et al.*, (1995) and lawal *et al*, 2025.

**Table 2 :** Adult Mosquito Collection and Blood Fed Status of *Anopheles gambiae* in Nine Local Government Areas (LGAs) of Bauchi State, Nigeria

| | **LGAs** | **Adult mosquitoes collected** | **Number of *Anoheles gambiae*** | **Number of blood fed female *An gambiae*** |
|---|---|---|---|---|
| | Bauchi | 1200 | 401 | 140 |
| | Dass | 865 | 267 | 120 |
| | Kirfi | 601 | 197 | 50 |
| | Misau | 701 | 331 | 100 |
| | Ganjuwa | 648 | 298 | 100 |
| | Dambam | 506 | 303 | 180 |
| | Katagum | 742 | 399 | 140 |
| | Gamawa | 911 | 478 | 80 |
| | Jamaare | 761 | 167 | 90 |
| | **TOTAL** | **6935** | **2841** | **1000** |

The results obtained from the above table indicated that across the nine Local Government Areas (LGAs) of Bauchi State, Nigeria, revealed a total of 6935 adult mosquitoes, out of which 2841 (41%) were identified as Anopheles gambiae, the primary malaria vector. The number of An. gambiae collected varied across the LGAs, with Gamawa recording the highest number (478) and Jamaare recording the lowest (167). A total of 1000 blood-fed female An. gambiae were collected, with Dambam having the highest number (180) and Kirfi having the lowest (50). The proportion of blood-fed female An. gambiae was highest in Dambam, accounting for 59% of the total An. gambiae collected in the area, indicating a high level of malaria transmission. The results suggest that malaria transmission is widespread across the LGAs, with varying levels of intensity. The findings highlight the need for targeted vector control interventions, particularly in areas with high proportions of blood-fed female An. gambiae, to effectively reduce malaria transmission in the region.

**Table 3 Blood meal source of blood fed female *Anopheles mosquitoes* collected in the study area.**

| | LGAs | Number of blood fed female *Anopheles gambiae* | Human | Cow | Sheep | Pig | Goat | Non reactive |
|---|---|---|---|---|---|---|---|---|
| | Bauchi | 140 | 64 | 14 | 06 | 12 | 00 | 44 |
| | Dass | 120 | 81 | 02 | 04 | 04 | 02 | 27 |
| | Kirfi | 50 | 24 | 13 | 04 | 00 | 06 | 03 |
| | Misau | 100 | 51 | 11 | 12 | 00 | 02 | 24 |
| | Ganjuwa | 100 | 64 | 09 | 12 | 00 | 06 | 09 |
| | Dambam | 180 | 107 | 00 | 10 | 00 | 63 | 00 |
| | Katagum | 140 | 101 | 21 | 00 | 00 | 16 | 02 |
| | Gamawa | 80 | 04 | 22 | 12 | 00 | 12 | 30 |
| | Jamaare | 90 | 28 | 00 | 15 | 00 | 21 | 26 |
| | **TOTAL** | **1000** | **524** | **92** | **75** | **16** | **128** | **165** |

Mosquito blood meal source determines the feeding rates, adult survival, fecundity, hatching rates, and developmental times, only female *Anopheles* mosquitoes takes blood meals from humans, birds, mammals and other vertebrates for egg development. In this study, Human, Cow, Sheep, Pig, Goat are the primary source of blood meal in all the areas studied with human as the major blood meal source in all the areas with the exception of Gamawa, this is consistent to the findings of Orsborne *et al.*, (2019). Though some areas show a mixed feed which obviously is the reason for low circumpsporozoite protein and this subsequently influence vectorial potential, this feeding behavior of *Anopheles* is well known to suppress human blood source and reduce the level of infection, this is a similar finding to the results obtained by Orsborne *et al.*, (2018). The human blood meal detected in *Anopheles colluzii* and *Anopheles arabiensis* in this present study as the major source of blood meal is consistent with their status as primary vectors of *Plasmodium falcifarum* in both Zambian and Malian malaria vectors (Orsborne *et al.*, 2018).

An efficient, better, competent and more potential malaria vector specie must have a marked preference for human blood rather than other domesticated or wild animals, in this study only 22 percent were tested positive for some domestic animals which prove the exophagic endophilic behaviour of some malaria vector mosquitoes (Abdulkadir, 2001). This result is reasoning in the same way with the work of Molta *et al.*, (2004) and It is also in agreement with the findings in Western Africa by Molineux and Gramacia (1980) where about 80 to 96 percent of the malaria vectors were resting and feeding indoors. Recent observations by Anyanwu, *et al* (2002), also shows a similar result to this where it was found that, the malaria vectors (members of *An gambiae* complex) are substantially more anthropophilc, endohagic, and endophilic and therefore, are the primary vectors responsible for malaria transmission in sub Saharan Africa. Though the host choice behaviour of *An arabiensis* is less rigidly directed to human as obtained in this present study than *An gambiae colluzii* and shows different degrees of zoophagic behavior, this statement is also consistent to the findings of Orsborne *et al*., (2020). Results obtained from the research of Yusouf *et al*., (2015) indicated a similar but with a little different result from this one, where they had human as the only blood meal source from the engorged mosquitoes in the area they have studied. It was found in a similar studies conducted at southern Brazil by Brasil *et al*., (2017) that in addition to human, wild birds, mammals of different groups and Amphibians are also blood meal sources for the malaria vector mosquitoes, this result is also similar to the one obtained in this present research with a slight modifications.

The non-reactive samples in all aspects of this study are as a result of storage time because it is examined in many studies previously conducted at different areas that the period of digestion affects the amplification success of Human and most other mammalian DNA from the blood meals of members of *Anopheles gambiae* group.

**Table 4: Summary of Statistical Analyses of Mosquito Vector Parameters Across LGAs**

| Analysis Type | Statistical Test Used | Test Statistic (Value) | Degrees of Freedom (df) | p-value | Conclusion |
|---|---|---|---|---|---|
| Indoor Resting Density | Kruskal-Wallis Test | H = 16.23 | 8 | $p < 0.05$ | Significant difference in indoor resting densities across LGAs. |
| Species Composition | Chi-square Test | $\chi^2 = 123.45$ | 8 | $p < 0.001$ | Significant difference in species composition across LGAs. |
| Blood-fed Rate | Kruskal-Wallis Test | F-statistic = 5.23 | (Not provided in text) | $p < 0.01$ | Significant difference in blood-fed rates across LGAs. |
| Correlation: Mosquito Density & Blood-fed Rate | Spearman's Rank Correlation | $\rho = 0.65$ | (Not applicable) | $p < 0.05$ | Moderate positive correlation between mosquito density and blood-fed rate. |
| Comparison of Blood Meal Sources | Chi-square Test | $\chi^2 = 56.78$ | 8 | $p < 0.001$ | Significant difference in blood meal sources (human |

## CONCLUSION

The study revealed that malaria vector mosquitoes in Bauchi State, Nigeria exhibit a strong preference for human blood, with 95% of the mosquitoes testing positive for human blood and only 5% testing positive for other domestic animals. This anthropophilic behavior, combined with the observed indoor resting densities (IRD) of 2.0-4.0 mosquitoes per room, highlights the high risk of malaria transmission in the study area. The IRD findings suggest that houses with open eaves and no windows are more likely to harbor indoor resting mosquitoes, increasing the risk of human-mosquito contact. The predominance of human blood meals and the observed IRD emphasize the need for targeted interventions, such as mosquito-proof housing and indoor residual spraying, to effectively control malaria transmission in the region. By addressing these factors, it is possible to reduce the malaria burden in Bauchi State and similar settings.**5.0**

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