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Abdominal Status and Blood Meal Preference of *Anopheles Gambiae* Complexes in Some Communities of Dutse Local Government Area, Northwestern Nigeria.

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Abstract

Mosquitoes are the greatest enemy of human beings as it transmits pathogen of medical and veterinary importance that results to morbidity and mortality globally. This study was aimed at identifying the most preferred host for malaria vector and classifying the female Anopheles based on their abdominal status. The study was conducted in three communities; Gurungu, Baranda and Warwade from April to October, 2022. Ten (10) houses were randomly selected in each of the three communities for the collection mosquitoes. Indoor resting adult mosquitoes were sampled using pyrethrum spray collection (PSC). Female Anopheles complexes blood meals were identified using polymerase chain reaction and abdominal status was classified using World Health Organization identification manual. Freshly fed female Anopheles were significantly highest (75.25%), followed by gravid (9.21%) and least were unfed

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(6.49%). Majority of female Anopheles tested fed on human blood (30%), followed by dog (10%) and those that were fed with both human and goat blood (10%) and from unknown source (50%).
The Findings of this study revealed diversity feeding habit of female Anopheles. It is therefore recommended that further investigation on the surveillance of the diseases transmitted by female Anopheles mosquitoes be carried out.

Keywords: Blood Meals, Abdominal Status, Host, *Anopheles,* Vector

INTRODUCTION

Mosquito vector transmits different pathogenic organisms of medical and veterinary importance, these pathogens including protozoans, viruses, bacteria and nematodes resulting to various kinds of diseases (Williann and Pinto, 2012). Despite decades of control efforts, mosquito borne diseases remain the major public health threat especially in sub-Saharan Africa, Nigeria inclusive. For instance, in 2020, WHO reported that there were an estimated 241 million malaria cases and 627,000 malaria deaths worldwide, representing about 14 milllion more cases in year 2020 compared to year 2019 (WHO, 2021). The infections caused my mosquitoes include malaria, dengue, yellow fever, lymphatic filariasis among others (Umar and Zakariyya, 2017; Jackman and Olson, 2012). The transmission of mosquito borne pathogens happens due to the feeding habit of mosquitoes. Each species of *Anopheles* has its own blood-feeding pattern and host preference (Becker *et al*., 2012). The correct identification of the preferred host for malaria vectors determines the major hosts in the support of the sustainability of vector population (Altahir *et al.,* 2022). Collection of adult mosquito is vital in entomological studies for monitoring vectors, especially in malaria endemic regions (Achee *et al*., 2015). Among these diseases, malaria account for the most disturbing and prevalence mosquitoe borne disease causing morbidity in both children, adults and pregnant women in dutse and its environ (Shuaibu *et al*., 2013; Balogun *et al*., 2021). Describing the interaction of mosquitoes with pathogens, host and community can help to understand region specific transmission dynamic, diseases spread and to design effort with a view to controlling (Sriwichai *et al.,* 2015). In the Bure district of Ethiopia, Adugna *et al*. (2021) observed that all *anopheles* mosquitoes tested for a bloodmeal source had mixed- rather than single-source bloodmeals. All *Anopheles* species, with the exception of *An. squamosus*, had frequencies for humans that were marginally higher than those for cattle. This indicated that, for each pair of vertebrate hosts, humans were a marginally preferred blood meal source over cattle. In Benue's north central region of Nigeria, human blood meal sources ranged from 97% to 100%, clearly indicating a high level of human-vector contact (Aju-Ameh *et al*., 2016). In instance, the state of Jigawa in northwest Nigeria lacks information on preferred blood meals by malaria vector. This study is intended to close this information gap, which is justified by the high level of animal domestication in the area. In order to categorize female *Anopheles* based on their abdominal state, the study was aimed at identifying the most preferred host for malaria vector and classifying the female *Anopheles* based on their abdominal status.

MATERIALS AND METHODS

Study Area

The study was conducted in some selected communities of Dutse Local Government Area (LGA). According to Mansur, (2014) Dutse is situated in central part of the Jigawa state ($11^{0}42'05''$N and $9^{0}20'31''$E) with total area of 738 km^{2}. The city is characterized as tropical with both rainy and dry seasons with an average yearly temperature of 26^{o}C (Zangina, 2015). The relative humidity in Dutse ranges from 17% in January to the highest of 95% in August,

followed by September with 68% and July with 56%, respectively (Weather Online, 2022). The annual rainfall is between 600 to 1000 mm. The yearly rainfall usually is between the months of April and October. The type of vegetation in Dutse L.G.A. is Sudan savannah which ranges from dense woodland and shrub to barely degraded farm, with few scattered trees in dry season (Mansur, 2014). During the rainy season it appears green and densely vegetated. Majority of the population of the study sites are farmers who grow rice, millet, beans and groundnut. The rest of the populations are merchants and works in nongovernmental organizations. Animals such as cattle, sheep, goats and hens were rearing by the residents in the study villages. Most of the inhabitants in the study sites were resides in mud houses. This study was conducted in three villages of Dutse LGA., namely; Baranda, Warwade and Gurungo. Warwade and Baranda were purposively selected because they have dam and river respectively, while Gurungu was randomly selected among the villages without Water in Dutse LGA.

Collection of Mosquitoes

Thirty houses were used in this study. Each house was selected randomly. Firstly, houses were selected by giving an identification number. The number of the houses were written on papers, folded and put in a container, then shuffled among which ten folded papers were picked randomly. The houses whose numbers were picked were selected for the study. A rapport and consent of the respondents were sought prior to the study. Pyrethrum spray catches method was used for the collection of indoor resting adult mosquitoes for seven months (April to October 2022) in the morning (6:30 to 8:00 am). Prior to the collection of mosquitoes, food, water and domesticated animals were moved out of the room, door and window were also closed. A sheet of large white cloth was spread on the floor in a room, followed by spray of pyrethrum, baygon (0.05% Imiprothrin, 0.05% Prallethrin and 0.015% Cyfluthrin). After 15 minutes, the white sheet was taken outside, the mosquitoes that fell on the sheet were collected with forceps and stored in a petri dish. Separate petri dishes from each house of sampling sites were used and all the petridis were labeled with time, date, and location (Umar *et al.,* 2012).

Morphological Identification of Mosquitoes

Microscope was used for detailed observations and identification of the mosquitoes with particular reference to the palps, proboscis, and antenna according to Gillet (1972) and Coetzee (2000).

Determination of Blood Meal Source using Polymerase Chain Reaction

DNA extraction

The DNA of female *Anopheles* was extracted following the ethanol precipitation protocol described by LIVAK (Livak, 1984). The buffer was made by dissolving 5.48 g sucrose, 1.57 g Tris, 1.6 ml of 5M sodium chloride in 10.16 ml of 0.5M EDTA. This was then followed by a 2.5 ml 20% SDS and the volume was finally made up to 100 ml in a volumetric flask. The buffer solution was then filtered and sterilized. 5 ml aliquots were stored at -20^{o}C which was heated in a water bath and whirled to re-dissolve precipitate before use.

Mosquitoes were homogenized individually using a battery-operated mortar and pestle (SIGMA) in 50µl pre-heated grind buffer in 1.5 ml Eppendorf. The pestle was rinsed with a further 50µlof the buffer to make a total of 100 µl. Homogenate was incubated at 65^{oC} for 30 minutes. The condensation was collected after the mixture was microfuged and 14 µl of 8M of potassium acetate was added, then mixed and incubated for 30 minutes on ice. Tubes were

centrifuged for 20minutes at 4^{oC} after which the supernatant was transferred carefully to a 1.5 ml eppendorf. At this point, 200 µl of 100% ethanol was added and mixture spun for 15 minutes at 4^{o}C. Pellets was rinsed in approximately 100 µl ice cold 70% ethanol, air-dried for two hours and then re-suspended in 100µl of distilled water. Tubes were finally incubated at 65^{o}C for 10 minutes.

The PCR reaction was also carried out using KAPATaq DNA polymerase. The total reaction volume was 15 µl comprises of 1 µl of each of the genomic DNA, 1.5 µl of 10 buffer, 0.5 µl of dNTP, 0.75 µl of $MgCL_2$, 0.5 µl Forward primers of Pig573F, Human741F, Goat894F, Dog368F, and UNRev1025, 0.2 µl of Kapatez all in 7.05µl of ddH_2O.Amplification was carried out using the following conditions: initial denaturation of 3 minutes at 95℃, followed by 35 cycles each of 60 s at 94 ℃ (denaturation), 60 s at 58 ℃ (primer annealing) and 60 seconds at 72 ℃ (extension). This was followed with 10minutes final extension at 72 ℃.PCR products were separated in a 1.5 % agarose gel stained with ethidium bromide. Primers sequences and base pair of each of the hosts are presented in Table 1.

Table 1: Primers and Base pairs of the Hosts

Primers	5′-3′ sequences	Product size with UNREV1025(BP)
Pig 573F	CCTCGCAGCCGTACATCTC	453
Human741f	GGCTTACTTCTCTTCATTCTCTCCT	334
Goat894F	CCTAATCTTAGTACTTGTACCCTTCCTC	132
Dog368F	GGAATTGTACTATTATTCGCAACCAT	680
Cow121F	CATCGGCACAAATTTAGTCG	561
UNREV1025	GGTTGTCCTCCAATTCATGTTA	_

Sources: Kent and Norris (2005)

Determination of Abdominal Status of Female *Anopheles*

The abdominal condition of *Anopheles* was determined based on blood digestion and the ovarian development by the use of standard keys as unfed, freshly fed and half gravid and gravid (WHO,2013). Both freshly and half gravid was taken for bloodmeal preference/source (Figure 1).

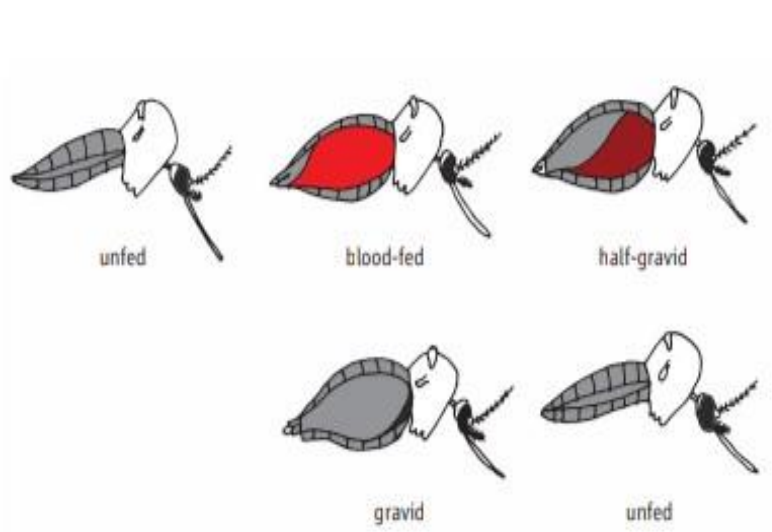

Figure 1: Abdominal Condition of Female Anophelines
Source: (WHO, 2013)

Data Analysis

Abundance of female *Anopheles* based on abdominal status was subjected to one-way ANOVA, using SPSS (version 22) statistical package. P value was considered significant when the $P < 0.05$

RESULTS

Monthly Abdominal Status of female *Anopheles* in sampling sites

The results in Table 2 show the monthly abdominal status of female anopheline in Dutse L.G.A. From the results the highest freshly fed anophelines were observed, in April (95.44%), the highest half gravid (25.05%) and gravid (18.0%) were recorded in the month of May and July respectively while the highest unfed anophelines were recorded in October (34.78%). More so, October recorded the least abdominal status for freshly fed (65.22%), half gravid (0.00%) and gravid (0.00%) while the least unfed anopheline were recorded in the month of April (1.44%). Freshly fed anophelines were the significantly ($P<0.05$) highest (75.25%) and unfed were the least (6.49%) out of the total female anophelines collected.

Table 2: Monthly Abdominal Status of female Anophelines in Dutse Local Government Area

Months	Freshly fed (%)	Half gravid (%)	Gravid (%)	Unfed (%)	Total (%)
April	398 (95.44)	11 (2.64)	2 (0.48)	6 (1.44)	417 (100)
May	130 (58.82)	62 (28.05)	5 (2.26)	2 (10.86)	221 (100)
June	95 (64.19)	26 (17.57)	12 (8.11)	15 (10.14)	148 (100)
July	182 (55.49)	51 (15.55)	61 (18.60)	34 (10.37)	328 (100)
August	553 (71.17)	74 (9.52)	88 (11.33)	62 (7.98)	777 (100)
September	631 (84.58)	19 (2.55)	79 (10.59)	17 (2.28)	746 (100)
October	30 (65.22)	0 (0.00)	0 (0.00)	16 (34.78)	46 (100)
Total	2019 (75.25)	243 (9.06)	247 (9.21)	174 (6.49)	2683 (100)
P-value	0.005	0.28	0.019	0.11	

Blood Meal Source of *Anopheles gambiae* Complexes

Blood meal source of *Anopheles gambiae* complexes is presented in Table 3, and figure 2 and 3. Out of total mosquitoes tested for blood meal source, 6 samples tested positive for human Blood, 2 samples tested positive for goat blood and 1 sample was also tested positive for dog. More so, 2 were found to be positive for both human and goat.

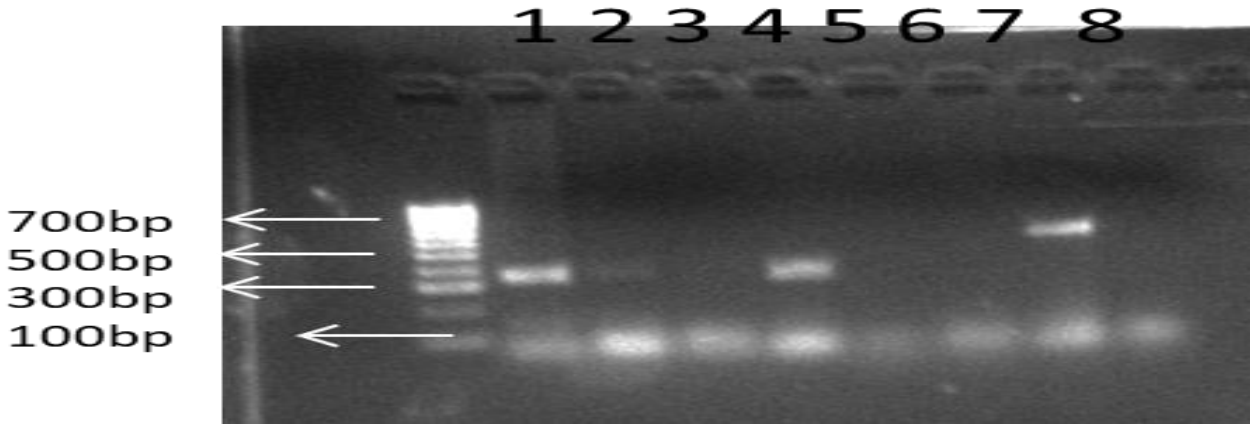

Figure 2 : Ethidium Bromide-stained Gel Agarose showing cytochrome b Polymerase Chain Reaction products amplified from Female *Anopheles* Mosquitoes: Lane 1, 2 and 4 (human, 334bp) and lane 8 (dog, 680bp)

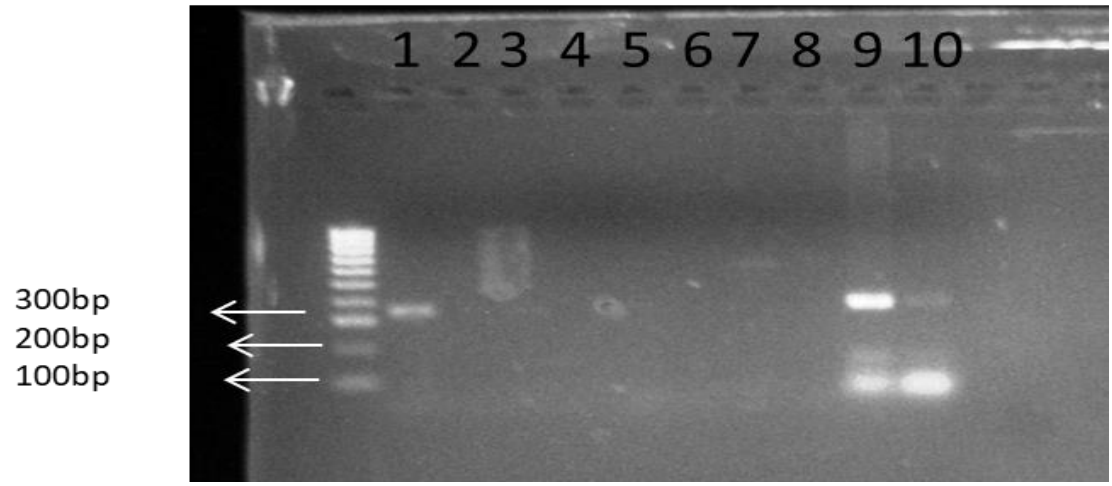

Figure 3: Ethidium Bromide-stained Gel Agarose showing cytochrome b Polymerase Chain Reaction products amplified from Female *Anopheles* Mosquitoes: Lane 1, 9 and 10 (human, 334bp) , Lane 9 and 10 (human and goat, 132bp) and lane 7 (dog,680bp)

Table 3: Blood Meal Source of Female *Anopheles* Mosquito

No. of Female *Anopheles* tested (%)	Human	Dog	Human and Goat	Unknown
20 (100)	6 (30)	2 (10)	2 (10)	10 (50)

DISCUSSION

Mosquitoes are the greatest enemy of human beings, because it transmits pathogens of medical and veterinary importance that result to morbidity and mortality globally. Result obtained from this study shows the highest abdominal status of collected *Anopheles* mosquitoes to be freshly fed. This may likely be due to the endophagy and zoophilic nature of the female *Anopheles* mosquitoes. The peak of freshly female *Anopheles were* recorded in the month of September. This also indicates the chances of malaria transmission during raining season. This is contrary to the findings of Adugna *et al.* (2021) in northwestern Ethiopia, of which 69.7% of the female *Anopheles* mosquitoes were unfed, followed by fed (24.5%), gravid (3.9%), and half-gravid (1.9%), and also contrary to the finding of Altahir *et al.* (2022) who reported blood meal profile among different malaria vectors in three (3) states in Sudan. 67.1% of the sampled mosquitoes were gravid, 17.6% of the sampled mosquitoes were blood fed and 15.3% of the sampled mosquitoes were unfed (Altahir *et al.,* 2022). The findings of this study also collaborate the outcome of Getachew *et al.* (2019) and Animut *et al.* (2013) who reported freshly fed and *Plasmodium* infection rates of *Anopheles* mosquitoes. The highest abundance of freshly fed malaria vectors may also be related to the lack of awareness on the proper use of insecticide.

For blood meal preference, blood meals of humans, dogs, and goats were identified (Agudna *et al.,* 2021; Finny *et al.,* 2021; Getachew *et al.,* 2019). The majority of the host fed on humans followed by dogs and the remaining malaria vector fed on both Humans and goat. This indicates that human are more attracted to the vector and more prone to malaria and other diseases transmitted by mosquito. The preference of blood meal by mosquitoe in the study area contradict the finding of Finny *et al.* (2021), who reported outdoor mosquitoes having high frequency of human blood meal, while the indoor mosquitoes fed more on livestock blood. Altahir *et al.* (2022) reported that the blood meals of cattle was higher, followed by human, dogs and goat. The present studies also support the previous finding and suggestion that malaria vectors have little shifted from feeding on human to animals (Agudna *et al.,* 2021;

Finny *et al.,* 2021; Getachew *et al.,* 2019). The presence of domesticated animals in the same house with human could play a role in blood feeding habit of mosquitoes from both humans and animals (Ndenga *et al.,* 2016). More so, presence of alternative host like goats and dogs can significantly affect mosquitoes feeding preference (Mayagaya *et al.,*2015; Ogola *et al.,*2017).

CONCLUSION

The results obtained in this study have indicated diversity in the feeding habit of female *Anopheles*. Female mosquitoes can feed broadly on human beings, goats and dogs

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