



# Entomological surveillance reveals transmission of malaria but not lymphatic filariasis in two communities in North-West Nigeria

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## Abstract

Malaria and lymphatic filariasis (LF) are two serious public health challenges in sub-Saharan Africa, and both diseases are transmitted by *Anopheles* mosquitoes. Successful control of both diseases requires detailed information on transmission dynamics; thus, this study investigated malaria and LF transmission indices in two (2) communities (Jidawa and Kargo) in North-West Nigeria. *Anopheles* mosquitoes were sampled from twenty-five (25) randomly selected houses from each of the two communities using pyrethrum spray collection (PSC). The samples were identified morphologically and molecularly characterised using polymerase chain reaction (PCR). Human biting rate (HBR), indoor resting density (IRD), sporozoite rate (SR) and entomological inoculation rate (EIR) were calculated using standard formulae. The thorax region of the collected samples were dissected and smeared; then, *Plasmodium* and *Wuchereria bancrofti* parasites were identified using microscopy. A total of 2417 *Anopheles* mosquitoes were collected, and all were identified morphologically as *An. gambiae* s.l. Further molecular identification of sibling species revealed that *An. gambiae* and *An. arabiensis* were the only sibling species present. A total of 818 *Anopheles* mosquitoes were screened for *Plasmodium* and *Wuchereria bancrofti* parasites. A total of 180 samples were positive for *Plasmodium* parasites (Jidawa = 151; Kargo = 29), and none was positive for *W. bancrofti* (0%). Result of entomological indices for malaria transmission showed that indoor resting density was higher in Jidawa (10 mosquitoes/room/night) while human biting rate (2.07 bites/person/night), sporozoite rate (29.3%) and entomological inoculation rate (0.61) were higher in Kargo. In total, 35.2% of the samples were blood-fed while 67.4% were parous. There is active transmission of malaria in the two communities but not LF, suggesting the effectiveness of mass drug administration for LF. Concerted efforts should be focused on malaria control as transmission of the disease persists.

**Keywords** *Anopheles* mosquitoes · Malaria · Lymphatic filariasis · Entomological indices · Entomological surveillance · Nigeria

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## Introduction

Malaria remains a principal public health issue in Nigeria (Omotayo et al. 2022) because the country shares a major portion of the malaria burden (27%) in the world (WHO 2022). The disease is transmitted by infective female *Anopheles* mosquitoes and *An. gambiae* s.l, and *Anopheles funestus* complexes are the principal vectors in Nigeria (PMI 2021). Of all the principal vectors, *An. gambiae* s.s and *An. coluzzii* belonging to the *An. gambiae* complex are the most efficient vectors of malaria in Nigeria (Amaechi et al. 2018). The continuous endemicity of malaria in sub-Saharan Africa has partly been attributed to the climate (majorly temperature, humidity and rainfall) that is highly favourable to the existence of the vectors (Githeko et al. 2000). *Anopheles*

mosquito is also the major vector for lymphatic filariasis (LF), a neglected tropical disease (NTD) common to sub-Saharan Africa and is caused by the filarial nematodes *Wuchereria bancrofti*.

Considering the fact that both *Plasmodium* and *W. bancrofti* are transmitted by *Anopheles* mosquitoes, co-transmission of both parasites has been a major concern in communities where both parasites are endemic (Eneanya et al. 2019). *Plasmodium* and *W. bancrofti* are majorly transmitted by indoor resting *Anopheles* species with a high affinity for human blood. This makes human-vector contact a prerequisite for transmission of both parasites; thus, many control efforts have been directed at reducing the human-vector contact majorly through long lasting insecticide nets (LLINs) and indoor residual spray (IRS).

These vector control strategies have largely been the focus of malaria control programmes; however, programmes aimed at LF have majorly employed mass drug administration (MDA) of ivermectin. In 2021, the World Health Organization reported a high prevalence of malaria in Nigeria (WHO 2022). Also, Nigeria was reported to be the 3rd most endemic nation for LF prior to 2015 (FMOH 2012); however, several reports on LF (Luka et al. 2021; Eneanya et al. 2019) suggest that continuous MDA has largely reduced the incidence of LF to specific few locations. In locations where LF transmission is still active, the challenge of co-infection with malaria is of utmost concern. Taking into consideration that both diseases have the same vector, vector control programmes for both diseases can be integrated.

In assessing the possibility and impacts of co-transmission of both malaria and LF, a thorough understanding of the diversity and bionomics of *Anopheles* mosquitoes is highly essential. It provides data about transmission indices of the diseases, which in turn will assist in the formulation and planning of suitable control programmes. Therefore, data on the diversity of *Anopheles* mosquitoes and their impacts in malaria and LF transmission especially in regions where both diseases remain a public health scourge becomes imminent. This informs this research that seeks to characterise the diversity of *Anopheles* mosquitoes in Kargo and Jidawa communities in Jigawa state, North-West Nigeria and assess entomological indices for both malaria and LF transmission in both communities.

## Materials and methods

### Ethical approval and community consent

Ethical approval (JHREC/2021/003) for the study was obtained from Jigawa State Health Research Ethics Committee. Community orientation and sensitization were

done with the help of health personnel from malaria and NTD unit of the Department of Public Health, Jigawa State Ministry of Health. Oral consent from household heads was obtained before house selection for mosquito sampling.

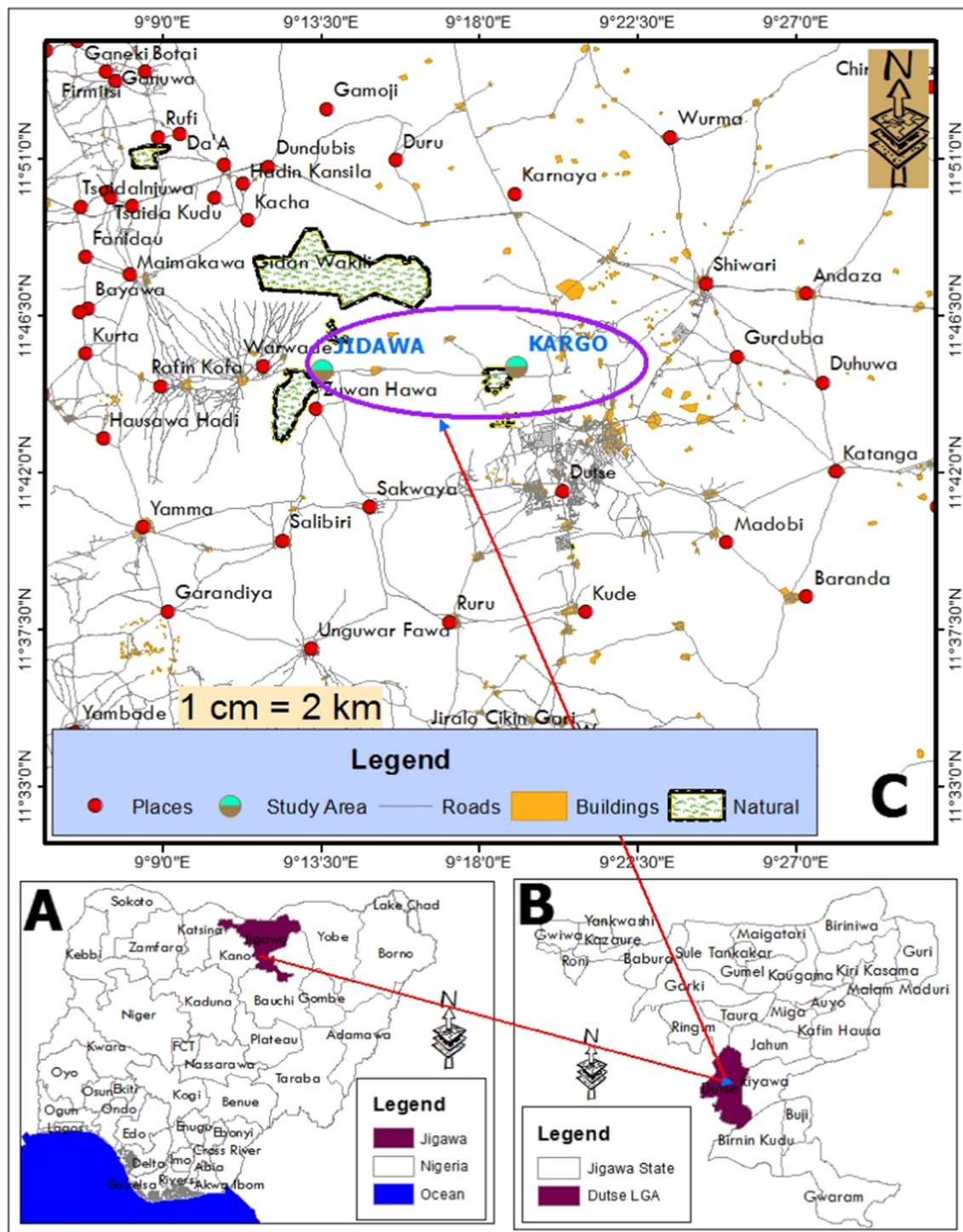
### Study area

Dutse Local Government Area is the State capital of Jigawa state located in North-West Nigeria (Fig. 1). Dutse has an estimated population of 251,135 (Federal Republic of Nigeria Official Gazette (2009)). Its vegetation falls within the Sudan savannah zone, with an annual rainfall of about 600–1000 mm in the month of July to September. Jidawa and Kargo communities lie between latitude 11.74391; longitude 9.187895 and latitude 11.72449; longitude 9.382033, respectively. Jidawa is about 30 km west of Dutse town while Kargo is about 4 km east of Dutse town. The communities are inhabited by Hausas (dominant), Kanuri and Fulani. The principal economic activities of the people include farming and animal husbandry. They also practice dry-season irrigation farming systems.

### Collection, identification and sample preparation

Adult indoor resting mosquitoes were sampled monthly (April–September 2021) in Jidawa and Kargo communities, North-West Nigeria using pyrethrum spray collection (PSC) method. Twenty-five (25) houses were randomly selected and sampled each from Jidawa and Kargo communities. Samples were collected between 6:00 am and 8:00 am according to the method described in WHO (1995). Two (2) rooms in each house were sprayed using Baygon insecticide (containing pyrethroid, carbamate and organophosphorus insecticides), and after 15 min, mosquitoes knocked-down were collected and placed inside separate well-labelled petri dishes. The mosquitoes were transported to the insectary at Federal University Dutse for sample identification, dissection and preservation.

All collected mosquitoes were morphologically identified under dissecting microscope using the keys of Coetzee (2020). The method described in Yohanna et al. (2019) for the detection of sporozoites of *Plasmodium* in female *Anopheles* was employed to assess *Plasmodium* infection. For the detection of *Plasmodium* sporozoites, the lower part of the thorax was mashed to bring out the salivary gland. The salivary gland was placed on another slide, wet with a drop of normal saline and covered with a cover slip. Pressure was gently exerted to rupture the cells of the gland, and the content was observed for the presence of sporozoites. The method described by Muturi et al. (2006) was employed to detect the presence of filarial worms. Individual mosquito was placed on the stage of



**Fig. 1** A Map of Nigeria showing Jigawa State. B Map of Jigawa State showing Dutse Local Government Area. C Map of the study sites: Jidawa and Kargo communities in Dutse LGA. Source: Geo-

graphic Information System Laboratory, Department of Geography, Ahmadu Bello University, Zaria, Kaduna, Nigeria. Software: ArcGIS 10.3 Software

a stereomicroscope, and the appendages were removed. The contents of the dissected parts (the head, thorax and abdomen) were observed under a light microscope for the

presence of larvae of *Wuchereria bancrofti*. Also, the parity status of unfed mosquito samples was determined as described by Detinova (1962).

## Molecular identification of *An. gambiae* complex

Genomic DNA of the samples was extracted using the Nigerian Institute of Medical Research extraction kit following manufacturer's procedure. Molecular identification of sibling species of *An. gambiae* complex was conducted using polymerase chain reaction as described by Wilkins et al. (2006). One microlitre of extracted DNA was transferred into separate tubes each of 12.5 µl PCR-mix containing 1 × GeneAmp, PCR buffer I (New England Biolabs), 0.5 µl MgCl<sub>2</sub>, 1.25 µl dNTPs, 4.9 µl of water, 0.1 µl of *Taq* and 1.0 µl each of the primers (New England Biolabs).

The primers used in for PCR had the following sequences: IMP-UN 5'-GCTGCGAGTTGTAGAGATGCG-3', AR-3 T 5'-GTGTTAAGTGTCTTCTCCGTC-3', GA-3 T 5'-GCT TACTGGTTTGGTCGGCATGT-3', ME-3 T 5'-CAACCC ACTCCCTTGACGATG-3', QD-3 T 5'-GCATGTCCACCA ACGTAAATCC-3', IMP-S1 5'-CCAGACCAAGATGGT TCGCTG-3' and IMP-M1 5'-TAGCCAGCTCTTGTCCAC TAGTTTT-3' corresponding to universal primer, *An. arabiensis*, *An. gambiae*, *An. merus*, *An. quadriannulatus*, *An. gambiae* s.s and *An. coluzzii*, respectively. All primers share similar annealing temperatures and had no complementarity to each other to prevent formation of primer complexes. PCR was carried out with an initial denaturation at 94 °C for 5 min followed by 30 cycles of 95 °C for 30 s, 72 °C for 30 s, followed by one cycle of 72 °C for 5 min (Wilkins et al. 2006).

## Determination of transmission indices of malaria

In assessing malaria and LF transmission indices in the study communities, sporozoite rate, human biting rate, indoor resting density, entomological inoculation rate and parity rate were assessed as follows:

### Sporozoite rate (SR)

The sporozoite rate was determined using the formula described in Yohanna et al. (2019),

$$\text{Sporozoite rate} = \frac{\text{Number of mosquitoes carrying sporozoites} \times 100}{\text{Number of mosquitoes dissected}}$$

### Human biting rate (HBR)

The human biting rate is expressed as the number of bites a person receives from mosquito per night. The rate was determined according to the formula,

$$\text{Human biting rate (M)} = F/W$$

where:

- M* human biting rate
- F* Total number of freshly fed mosquitoes of a particular species
- W* Total number of human occupants in the houses used for collection

### Entomological inoculation rate (EIR)

Entomological inoculation rate is the product of human biting rate and sporozoite rate divided by 100,

$$\text{Entomological inoculation rate (EIR)} = \frac{\text{Human biting rates} \times \text{Sporozoite rate}(\%)}{100}$$

### Indoor resting density (IRD)

Indoor resting density is the number of female mosquito per house per night, and it was calculated using the formula outlined in Nsereko et al. (2020),

$$\text{IRD} = \frac{\text{No. of female Anopheles mosquitoes captured}}{\text{no. of houses} / \text{no. of nights}}$$

### Parity rate (PR)

The parity rate was determined using the formula below,

$$\text{Parity rate} = \frac{\text{Number of parous females Anopheles mosquitoes} \times 100}{\text{Number of female Anopheles mosquitoes}}$$

## Data analysis

Data collected were entered and processed using Microsoft Excel (Version 2016). Descriptive statistics was calculated, and standard formulae as outline above was used for calculating the entomological indices. *T* test was employed to compare IRD between the two locations, and level of significance was placed at < 0.05.

## Results

### Relative density and diversity of *Anopheles* species

The result of relative density and diversity of mosquito species collected in Jidawa and Kargo areas of Dutse is presented in Table 1. A total of two thousand four hundred and seventeen (2417) indoor resting adult *Anopheles* mosquitoes were collected within 6 months period from the two



**Table 1** Relative abundance of *An. gambiae* s.l mosquitoes

Species	Jidawa (%)	Kargo (%)	Overall (%)
<i>An. gambiae</i>			
Male	524	49	573
Female	1624	220	1844
Total	2148 (88.87%)	269 (11.13%)	2417 (100%)

communities. All the mosquitoes collected were identified morphologically as *An. gambiae* s.l. Of all the 2417 collected, 2148 (88.87%) were collected in Jidawa community, and 269 (11.13%) samples were collected in Kargo community. Also, 573 (23.70%) were male while 1844 (76.30%) were female. The density of mosquitoes was highest in June in both communities and lowest in September and July in Jidawa and Kargo communities, respectively (Fig. 2).

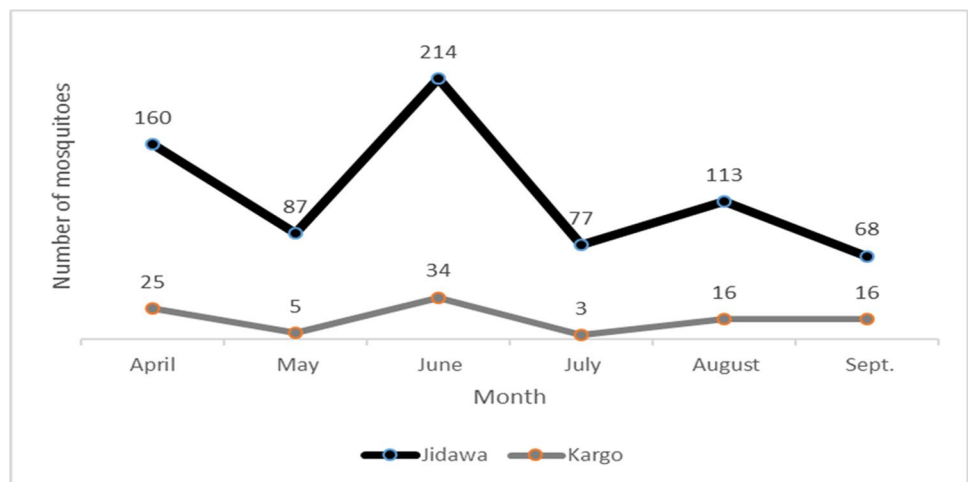
Result of molecular identification of sibling species of *An. gambiae* s.l collected showed that *An. gambiae* and *An. arabiensis* are the two sibling species present in the two communities. *An. gambiae* was dominant in Jidawa (78%)

while there was equal number of *An. gambiae* and *An. arabiensis* in Kargo.

### Infection rate of *Plasmodium* and *W. bancrofti* parasites

The result of infection rate of *Plasmodium* parasite and *W. bancrofti* is presented in Table 2. Out of the 2417 *An. gambiae* s.l collected throughout the study period, 818 *An. gambiae* s.l. were screened for *Plasmodium* and *W. bancrofti*. None (0%) of the dissected mosquitoes from the study sites was positive for *W. bancrofti* parasite. Of the 818 *An. gambiae* s.l. (Jidawa = 719, Kargo = 99), 180 were positive for *Plasmodium* parasite (Jidawa = 151, Kargo = 29). The sporozoite rate in Jidawa was highest in the month of September (36.8%), followed by May (31.0%) while the lowest infection rate was recorded in April (3.8%). In Kargo community, the sporozoite rate was highest in the month of June (44.1%), followed by May (40.0%) while the lowest was observed in July (0%) (Fig. 3).

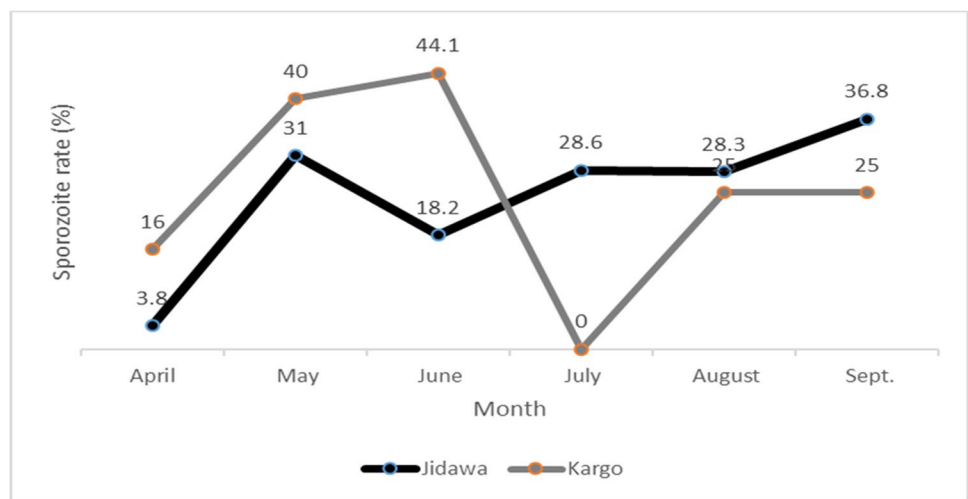
**Fig. 2** Monthly density of *Anopheles* mosquitoes collected in Jidawa and Kargo communities

**Table 2** *Plasmodium* parasites and *W. bancrofti* co-infection in *An. gambiae* s.l

Month	Jidawa					Kargo				
	Number dissected	Pp	SPR (%)	W.b	% Co-infection	Number dissected	Pp	SPR (%)	W.b	% Co-infection
April	160	6	3.8	0	0	25	4	16	0	0
May	87	27	31	0	0	5	2	40	0	0
June	214	39	18.2	0	0	34	15	44.1	0	0
July	77	22	28.6	0	0	3	0	0	0	0
August	113	32	28.3	0	0	16	4	25	0	0
Sept	68	25	36.8	0	0	16	4	25	0	0
Total	719	151	21	0	0	99	29	29.3	0	0

Pp *Plasmodium* parasite, SPR Sporozoite rate in percentage, W.b *Wuchereria bancrofti*

**Fig. 3** Monthly *Plasmodium* sporozoite rate of *Anopheles* mosquitoes collected in Jidawa and Kargo communities



**Table 3** Entomological indices for *Plasmodium* transmission in Jidawa and Kargo communities

Study area	Number dissected	HBR	SPR (%)	EIR	IRD
Jidawa	719	1.26	21	0.27	10.0
Kargo	99	2.07	29.3	0.61	1.8
Total	818	1.06	22	0.23	6.5

SPR sporozoite rate, HBR human biting rate, EIR entomological inoculation rate, IRD indoor resting density

**Entomological indices for Plasmodium transmission**

The result of entomological indices for malaria transmission is shown in Table 3. In Jidawa, sporozoite rates and human biting rates (HBR) for *An. gambiae* s.l. (n = 719) were 21% and 1.26 bites/person, respectively, while the sporozoite rate and HBR for *An. gambiae* s.l. in Kargo (n = 99) were 29.3% and 2.07 bites/person, respectively. The overall sporozoite rate and HBR for *An. gambiae* (n = 818) over the 6-month period were 22% and 1.06 bites/person, respectively. Entomological inoculation rates (EIR) in Jidawa and Kargo were 0.27 and 0.61, respectively, while the overall EIR was found to be 0.23. None of the examined *An. gambiae* s.l. was infected with *W. bancrofti*; hence, SPR for filaria infection

was 0% and EIR was 0%. The result for indoor resting density (IRD) is shown in Table 3. Indoor resting density of *Anopheles* mosquitoes was significantly higher (t = 4.06; P = 0.008) in Jidawa (10.0 mosquitoes/house/night) compared to Kargo (1.8 mosquitoes/house/night).

**Physiological and parity status of *An. gambiae* s.l.**

The results of physiological status of the female *An. gambiae* s.l. are shown in Table 4. The result revealed that 35.0% and 36.4% of *An. gambiae* collected in Jidawa and Kargo, respectively, were blood-fed. Only 13.4% of the total samples were unfed. The proportion of unfed to fed *An. gambiae* was higher in Kargo, while Jidawa had higher proportions of half-gravid/gravid *An. gambiae*. The parity rate of the mosquitoes dissected showed that majority of the *An. gambiae* s.l. collected in Jidawa (65.9%) and Kargo (75%) were parous.

**Discussion**

While malaria remains the major vector-borne disease in Nigeria, LF is one of the major neglected tropical diseases that have continued to attract the attention of public health experts in sub-Saharan Africa. Before 2015, Nigeria was

**Table 4** Physiological status and parity rate of adult female *An. gambiae* s.l. in Jidawa and Kargo communities

Study site	Physiological status					Parity status		
	No. collected	Unfed (%)	Blood fed (%)	Half gravid (%)	Gravid (%)	No. dissected	Nulliparous (%)	Parous (%)
Jidawa	1624	197 (12.1)	569 (35)	413 (25.4)	445 (27.4)	455	155 (34.1)	300 (65.9)
Kargo	220	51 (23.2)	80 (36.4)	35 (15.9)	54 (24.5)	88	22 (25)	66 (75)
Total	1844	248 (13.4)	649 (35.2)	448 (24.3)	499 (27.1)	543	177 (32.6)	366 (67.4)

All numbers in parenthesis are in percentages

considered to be the third most endemic country globally for LF. Data from LF mapping activities revealed that Jigawa State is one of the few States with all its LGAs mapped as endemic to LF (FMOH 2012). Parasites of both malaria and LF are efficiently transmitted by *Anopheles* mosquitoes in sub-Saharan Africa. The region has a favourable climate for the continued existence of the vector, and this has made co-transmission of both malaria and LF a major public health concern in the region. Thus, this study investigated entomological indices and co-transmission of malaria and LF in two communities in North-West Nigeria.

In the present study, a total of 2417 *Anopheles* mosquitoes were collected throughout the sampling period. All the *Anopheles* mosquitoes were morphologically identified as *An. gambiae* s.l. *An. gambiae* s.l. has been documented as the major *Anopheles* species in sub-Saharan Africa (Awolola et al. 2002; Omotayo et al. 2022), and this has been attributed to the favourable climatic condition in the region (Githeko et al. 2000). This is also in agreement with earlier findings from close communities in the rural villages of Mare and Gunduwa, where *An. gambiae* s.l. was also found to be the dominant *Anopheles* species (Dogara et al. 2012). *An. gambiae* s.l. is the major vector of both malaria and LF in Nigeria, and the abundance of the vector in Northern Nigeria has the potential to impact the transmission of the two diseases.

Molecular characterisation of sibling species of *An. gambiae* complex revealed the presence of two sibling species: *An. gambiae* and *An. arabiensis* in both study areas. Awolola et al. (2002) had earlier identified *An. gambiae* and *An. arabiensis* as the major vectors of malaria in Nigeria, and they are extensively distributed throughout most of the tropical African countries (Oduola et al. 2016). It is worthy of note that *An. coluzzii* was not found among the samples collected in this study; however, several other studies had reported *An. coluzzii* from different parts of Nigeria (Oduola et al. 2012; Omotayo et al. 2022). Likewise, Habibu et al. (2017) also reported *An. coluzzii* as the dominant sibling species of *An. gambiae* complex in Auyo LGA, a close location to the present study sites in the same Jigawa State, Nigeria. While *An. gambiae* and *An. arabiensis* were found in both study sites, *An. gambiae* was highly preponderant in Jidawa community. *An. gambiae* has been reported to mostly rest indoor and feed on human unlike *An. arabiensis* that are mostly exophilic and exhibit zoophilism (Eikenberry and Gumel 2018); however, both vectors have been incriminated in the transmission of malaria, lymphatic filariasis and other mosquito-borne diseases (Lagare et al. 2019).

The ability of mosquitoes to rest indoor confers high parasite transmission potential in communities where most of the populations stay indoor especially at night. Resident of both communities in this study spend a good percentage of their time indoor in houses typical of rural communities

in Northern Nigeria where openings and eaves in houses are not well screened. The indoor resting density (IRD) in Jidawa was approximately 6 times higher than what was recorded in Kargo community. The high IRD (10 mosquitoes/room) recorded in Jidawa community suggests high exposure of human to the vectors indoor, and this portends high-risk transmission potential for both malaria and LF. Most of the samples collected in the present study were also blood-fed and parous. The high parity rate of the *Anopheles* populations is an indication that most of the *Anopheles* population in the communities have lived enough to be able to transmit the parasites.

Result of entomological indices reveal that the human biting rate in Kargo was almost twice of what was recorded in Jidawa. The HBR in Kargo was more than 2 bites/person thereby revealing the possibility of easy transmission of *Plasmodium* or *W. bancrofti* since every human in the community receives an average of 2 bites/day. Considering the homogeneity of the two communities in terms of climate and demography of inhabitants, the difference in HBR calls for further assessment. However, difference in the abundance of breeding habitat in the two communities might be responsible for more abundance of mosquito population in Kargo community. Likewise, the study documented 29.3% and 21% prevalence of *Plasmodium* sporozoites in Kargo and Jidawa, respectively, with no *W. bancrofti* larvae found in all of the mosquitoes examined. While the indoor resting density is higher in Jidawa community, the human biting rate and *Plasmodium* sporozoite rate in Kargo suggest that human population in Kargo are at higher risk of being infected with malaria but not LF. The malaria transmission capacity of the *An. gambiae* s.l. in the study areas is typical of many regions in sub-Saharan Africa. The total absence of *W. bancrofti* in the two communities could be attributed to the effectiveness of the intervention of World Health Organization (WHO) (Macrofilaricidal drugs aimed at LF elimination have been distributed through MDA Programme) and other agencies in Jigawa state since 2011.

The climatic condition of sub-Saharan Africa is a conducive climate for reproduction and development of *Anopheles* mosquitoes, and this has continued to influence the continuous transmission of malaria and lymphatic filariasis parasites. Despite huge efforts aimed at control of the vector of both diseases, transmission of malaria keeps escalating, whereas the incidence of lymphatic filariasis has been mitigated throughout Nigeria. The decline in the incidence of LF has been mainly attributed to intervention programmes focusing on mass drug administration of ivermectin, and this has been highly instrumental in reducing the challenges that would have surfaced with co-infection of malaria and LF. Additionally, there is a need for constant and thorough surveillance for co-endemicity of both malaria and LF

especially in regions where LF was formerly endemic, and this surveillance should employ more advanced molecular techniques to assess the incidence of LF.

## Conclusion

This study provides information on the status of malaria and filariasis transmission in Jidawa and Kargo communities in North-West Nigeria. The absence of LF parasites in *Anopheles* populations from the two communities reveals the success of intervention programmes sustained over the years in Northern Nigeria. Conversely, the high transmission indices of malaria show the endemicity of malaria in the two communities and in effect a call for more efforts to be geared towards reducing the incidence of malaria.

**Abbreviations** *LF*: Lymphatic filariasis; *SPR*: Sporozoite rate; *HB*: Human biting rate; *EIR*: Entomological inoculation rate; *IRD*: Indoor resting density; *PCR*: Polymerase chain reaction; *MDA*: Mass drug administration

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**Author contribution** H.A.A., D.M.M., B.J.B. and O.A.I. conceived and designed the study. H.A.A., H.A.A.<sup>2</sup> and A.S.S. collected field samples. H.A.A., B.J.B. and A.K.A. conducted microscopy. O.A.I. and A.K.A. conducted molecular analysis. H.A.A. and O.A.I. drafted the manuscript. All authors read and approval the final draft of the manuscript. H.A.A and O.A.I are joint first authors.

**Data availability** All data generated or analysed during this study are included in this published article.

## Declarations

**Ethics approval and consent to participate** Ethical approval (JHREC/2021/003) for the study was obtained from Jigawa State Health Research Ethics Committee. Consents of household heads were obtained before sampling of households for adult mosquitoes.

**Competing interests** The authors declare no competing interests.

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