Scholars Academic Journal of Biosciences
Abbreviated Key Title: Sch Acad J Biosci
ISSN 2347-9515 (Print) | ISSN 2321-6883 (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

Biosciences

Effect of *Culex quinquefasciatus* Infection by *Wuchereria bancrofti* on the Ability to Resist Long-Lasting Insecticidal Nets

Yokoly N'dri Firmain[1,2*], Mamadou Traoré[3,4], Ouattara Allassane Foungoye[1,2,5], Behi Kouadio Fodjo[1,2], Alico Sylvestre Yao Armel[1,2], Koudou Guibehi Benjamin[1,2]

[1]Biology and Animal Cytology Laboratory, Natural Sciences, Nangui Abrogoua University, 02 BP 801 Abidjan 02, Côte d'Ivoire
[2]Vectors Control Unit, Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, 01 BP 1303 Abidjan 01, Côte d'Ivoire
[3]Centre d'Entomologie Médicale et Vétérinaire (CEMV), Alassane Ouattara University (UAO), Bouaké
[4]Unité Mixte de Recherche (UMR) Culicidés et Maladies Transmises (Paludisme, fièvre jaune etc.)
[5]Biostatistics Unit, Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, 01 BP 1303 Abidjan 01, Côte d'Ivoire

DOI: https://doi.org/10.36347/sajb.2025.v13i05.001 | **Received:** 11.03.2025 | **Accepted:** 16.04.2025 | **Published:** 01.05.2025

***Corresponding author:** Yokoly N'dri Firmain
Biology and Animal Cytology Laboratory, Natural Sciences, Nangui Abrogoua University, 02 BP 801 Abidjan 02, Côte d'Ivoire

Abstract **Original Research Article**

Limited information is available on the behavior of *Culex* mosquitoes in an insecticide-treated environment. We explored the effect of *Wuchereria bancrofti* infection on aggressiveness of *Culex quinquefasciatus* in long-lasting insecticide-treated mosquito nets (LLINs) presence. Adult mosquitoes were collected over four months in Tiassalékro experimental huts. The collected mosquitoes were pooled according to the hut compartments and treatment. Ten huts were selected, including five with LLINs and five with Untreated nets (UN). The mosquito DNA was extracted and analyzed using the polymerase chain reaction to detect *W. bancrofti* infection in different compartments. Overall, 5,273 mosquitoes were collected with 80.8% of *Anopheles* and 14.5% of *Culex*. *Culex quinquefasciatus* species (n= 620/763; 81.3%) was predominate among the *Culex* mosquitoes. The exophily rate and blood meal inhibition in LLINs presence was estimated to be 1.5 times than UN (P < 0.05). The *W. bancrofti* infection rate of *Culex* was 12 % in veranda-traps with LLINs and 18% indoor UN huts (p= 0.534). Infected *Culex* mosquitoes may be attracted to blood meals irrespective of the presence of an insecticide-treated mosquito net, even when the insecticide used is one to which they are known to be susceptible.
Keywords: *Culex Quinquefasciatus*, *Wuchereria Bancrofti*, Lymphatic Filariasis, Vector Competence, Long-Lasting Insecticidal Nets.

INTRODUCTION

Lymphatic filariasis (LF) is a non-fatal disease but is a leading cause of long-term disability worldwide, preventing the wellbeing of the sick people (Manimegalai and Sukanya, 2014; WHO, 2013). The main LF parasite in sub-Saharan Africa is *Wuchereria bancrofti* while *Brugia malayi* and *B. timori* are prevalent in Southeast Asia (WHO, 2018). Most infected individuals are asymptomatic, but infections can lead to disfigurement and disability, particularly in low-income and marginalised populations. The most common clinical symptom of LF is lymphoedema (swelling of the limb or limbs), after hydrocoele (scrotal swelling) (WHO, 2023). In 52 tropical countries, 80 million people are infected with around 850 million at risk (WHO, 2017), and more than one third are in sub-Saharan Africa (Hotez and Kamath, 2009). In the WHO African region up to 464 million people in 33 countries live in endemic areas(WHO, 2017).

LF is transmitted through the bites of mosquito vectors from various genera including *Anopheles*, *Culex*, *Mansonia* and *Aedes*, depending on the geographic area. *Coquillettidia* and *Ochlerotatus* genus have also been reported to be vectors of the LF parasites (de Souza *et al*., 2012; Simonsen and Mwakitalu, 2013). Furthermore, *Culex* genus is the most widespread mosquito worldwide (Bhattacharya and Basu, 2016) and is known to be highly opportunistic feeding on both humans and animals, which increases the potential to transmit arboviral diseases and LF (Goddard *et al*., 2002; Weissenböck *et al*., 2010). The main vectors of *W. bancrofti* in West Africa are *Anopheles gambiae* and *Culex quinquefasciatus* (Pi-Bansa *et al*., 2019). The cornerstone to achieve interruption of LF transmission in different epidemiological settings could be, among others, vector control (Bockarie *et al*., 2009). Long-lasting insecticidal nets (LLINs) continue to be an effective tool to reduce LF prevalence and transmission (Ashton *et al*., 2011). However, insecticide resistance (Butler, 2011) and vectors behaviour could undermine the progress made in vector control (Russell *et al*., 2011; Simonsen *et al*., 2010). Several studies have shown that *Cx. quinquefasciatus* exhibits resistance to the insecticides used in LLINs (Magesa *et al*., 1991).

In order to achieve the aims for LF elimination by 2030, Côte d'Ivoire has implemented a strategy for the control of LF (WHO, 2014), through the National Program for Neglected Tropical Diseases Control through Preventive Chemotherapy based on annual mass drug administration (MDA) using ivermectin and albendazole. However, the distribution of LLINs by the national malaria control program also impacts LF vectors. Thus, understanding how the behavior of mosquitoes in presence of insecticides affects LF epidemiology, emphasizing on mosquitoes that carry infectious stage of LF parasite is important. We hypothesize that *W. bancrofti* infection influence the behavior of *Cx. quinquefasciatus* in response to the insecticides. To investigate this, we assess changes in the behavior of infected mosquitoes when exposed to insecticides in experimental huts, aiming to enhance knowledge on the role of ITNs in LF control strategies.

METHODS

Ethical Considerations

This study was conducted in accordance with the protocol approved by the National Ethics Committee for Life Sciences and Health (CNESVS) of the Ministry of Health and Public Hygiene of the Republic of Côte d'Ivoire (025-18/MSHP/CNESVS-Kp). In addition, approval was received from the health authorities in each district. Community members were provided with comprehensive information regarding the study's objectives, procedures, potential risks, and benefits. Participation in mosquito collection was entirely voluntary, and all collectors were trained in the specific collection methods prior to the commencement of the study.

Study Area

The study was carried out at Tiassalé (5°54′ N. 4°50′ W), in southern Côte d'Ivoire, at the experimental station close to the rice irrigated field (Figure 1). The primary malaria vector in the region is *Anopheles coluzzii*, a member of the *An. gambiae* complex, which exhibits resistance to pyrethroids, organochlorines (DDT), carbamates, and organophosphates. Rice is cultivated year-round, with irrigation sourced from the Bandama River via a motor pump during the dry season. Rice fields are harvested asynchronously during a cycle. Consequently, paddy fields at different stages of rice development are created, corresponding to a suitable biotope for the development of culicid species because of the permanent presence of water. The climate is tropical and humid, and the average annual precipitation is 1,739 mm with an average annual temperature of 26.6 ° C. The average annual relative humidity is 90%.

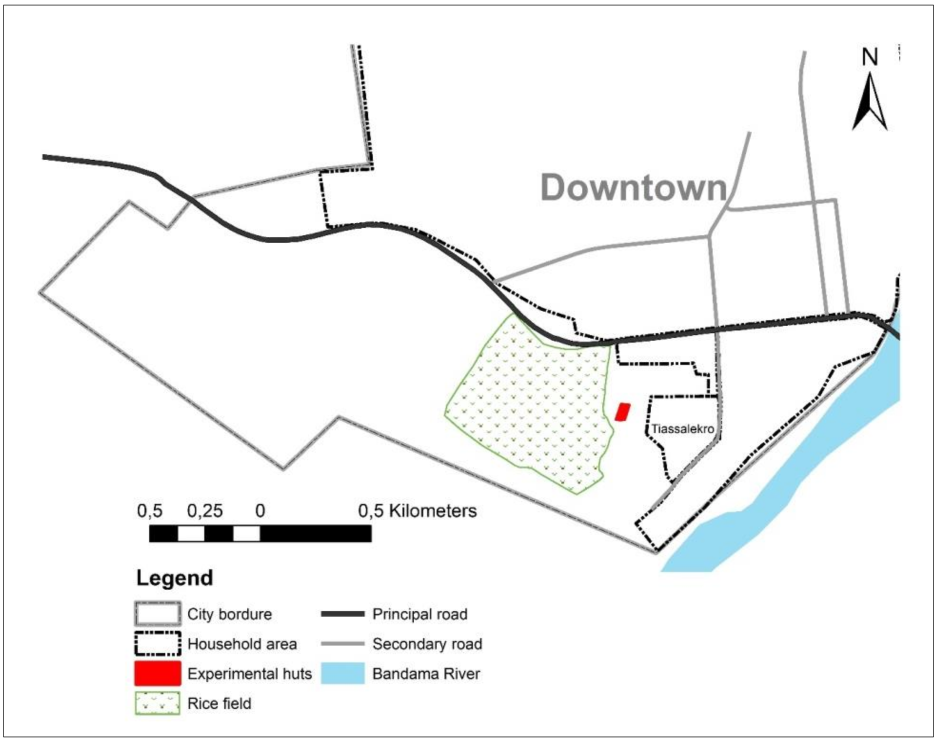

Fig. 1: Map showing the mosquito collection site in Tiassalé, Côte d'ivoire

Description of the Experimental Huts

The experimental station of Tiassalé is compound of 10 trap huts distributed into two rows of five trap huts about six meters apart. It is located close to a vast rice-growing perimeter. The trap huts measure 3.25 metres long, 1.75 metres wide and two metres high. They consist of a dwelling room with cement walls and floor, wooden frame and corrugated iron roof and the concrete floor surrounded by a water-filled moat (Smith and Webley, 1969). A plastic sheeting is stretched underneath the sheets to facilitate mosquito capture on the ceiling. The huts are equipped with 4 controlled openings or baffles that allow mosquitoes to enter the hut, but prevent them from exiting, and a rigid mosquito-netting verandah, the equivalent of an exit door for mosquitoes (Mosha *et al*., 2008; Oxborough *et al*., 2015).

Study Design

A total of 10 huts are used for this study. In five odd huts an untreated net is installed while in the other five even huts a long-lasting impregnated mosquito net is installed.

Mosquito Sampling and Species Identification

Collections of adult mosquito samples were performed monthly using hemolysis tubes for six consecutive days in each hut between 5 a.m. to 7 a.m. to catch resting mosquitoes inside huts. Mosquitoes were ranked into bags according to huts, transferred to the laboratory. Mosquitoes collected were and identified morphologically at species level using identification keys (Gillies and de Meilon, B., 1968; Mattingly, 1971). Mosquitos abdomen were inspected for meals intake (i.e unfed or bloodfed) and the alive or dead status recorded. *Culex quinquefasciatus* mosquitoes dead and alive were separately put in eppendorf tubes and freeze at -20°C for further analysis.

Laboratory Analysis for *Wuchereria Bancrofti* Detection in Mosquitoes

All *Cx. quinquefasciatus* mosquitoes in good condition were pooled into batches of 10 considering the collection compartment. A total of 62 pools (25 pools for LLINs huts and 37 pools for untreated huts) were analyzed using the polymerase chain reaction (PCR) (Ramzy *et al*., 1997). The pools were mixed with a set of solution then incubated and centrifuged for 5 min. The supernatant was mixed with an alcoholic solution and then centrifuged for 15 min to concentrate the DNA at the bottom of the tube. Then, the DNA is purified and reconstituted in 20 µL DNase-free water (Sigma-Aldrich, United Kingdom).

Two oligonucleotide primers, NV-1 (5'- CGT GAT GGC ATC AAA GTA GCG – 3') and NV-2 (5' – CCC TCA CTT ACC ATA AGA CAA C – 3') were used for PCR assay to identification of *W. bancrofti* DNA in *Cx. quinquefasciatus* mosquitoes. PCR was run at an initial denaturation of 94 °C for 3 minutes, 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 1 minute and extension at 68 °C for 1 minute, and final extension at 68 °C for 5 minutes. An electrophoresis with a UV transluminator allowed to visualize bands.

Data Analysis

Data were entered in Microsoft Excel and transferred into R version 3.5.1 (The R foundation for statistical computing) for analysis. A poison generalized mixed linear model with huts and months fixed as a random effect was used to compare mosquito densities between LLINs and untreated net. The generalized mixed negative binomial model to compare exophily rate. Chi-square test ($\chi 2$) was used for infection rate comparison. The significant level was set at 0.05.

RESULTS

Mosquito Composition

Table 1 indicates the species composition of the mosquitoes collected in the huts. A total of 5,273 mosquitoes of four genera *Anopheles* (n=4,259; 80.8%), *Culex* (n=763; 14.5%), Aedes (n=8; 0.2%), *Mansonia* (n=243; 4.6%) were collected. *Culex quinquefasciatus* (n= 620; 81.3%) was the most abundant compared to *Cx. nebulosus* (n= 102; 13.4%), *Cx. cinerus* (10; 1.3%), *Cx. poicilipes* (n=13; 1.7%), *Cx. tigripes* (n= 14; 1.8%) and *Cx. descens* (n= 4; 0.5%) among *Culex* genera ($p<0.001$).

Table 1: Species composition of mosquitoes collected in huts.

Espèces	**LLINs**		**NINs**		**Total**	
	n	**(%)**	**n**	**(%)**	**n**	**(%)**
Anopheles s.l.	1,755	80.8	2,504	80.7	4,259	80.8
Aedes sp	2	0.1	6	0.2	8	0.2
Culex quiquefasciatus	252	11.6	368	11.9	620	11.8
Cx. nebulosus	41	1.9	61	2.0	102	1.9
Cx. cinerus	6	0.3	4	0.1	10	0.2
Cx. poicilipes	8	0.4	5	0.2	13	0.2
Cx. tigripes	7	0.3	7	0.2	14	0.3
Cx. decens	3	0.1	1	0.0	4	0.1
Mansonia sp	98	4.5	145	4.7	243	4.6
Total	2 ,172	100	3,101	100	5,273	100
n : number, LLINs: long-lasting insecticidal nets , NIN: no-impregnated nets						

Deterrence

Culex density in untreated nets ranged from 1 to 4 females/hut/day (f/h/d) during the four collection months. Regarding mosquitoes collected in LLINs huts, density was highest (from 4 to 7 f/h/d) (Figure 2). *Culex* density from LLINs huts was 1.44 (95% CI: 0.89-2.32) times higher than *Culex* density from untreated nets (p=0.130).

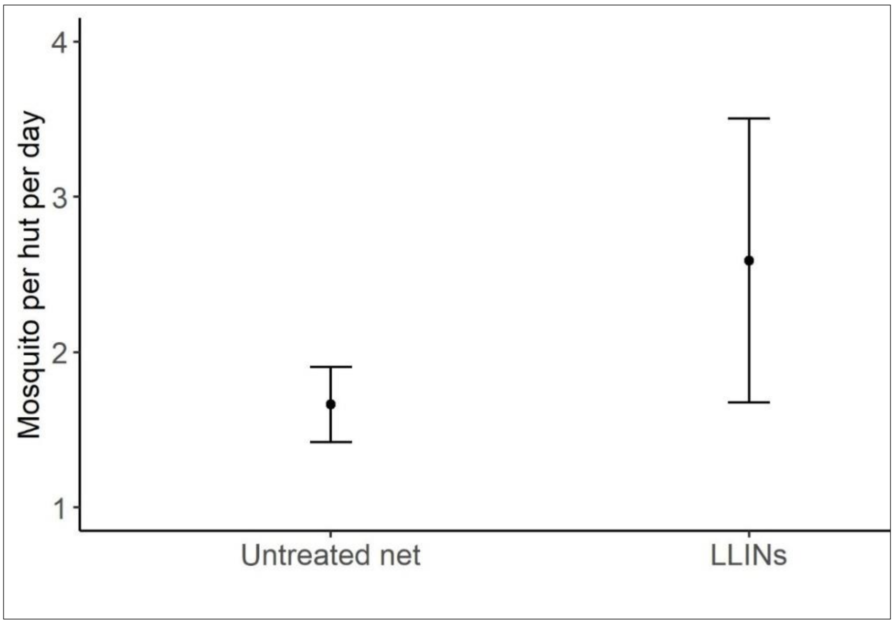

Figure 2: Endophilic rate

Exophilic Rate

Exophily rate in untreated net huts was 49.0% while the rate in LLINs was 66.0% (Figure 3). Exophily rates in LLINs was 1.38 [IRR= 1.38 (95% CI: 1.06-1.80)] higher than untreated nets (p=0.019).

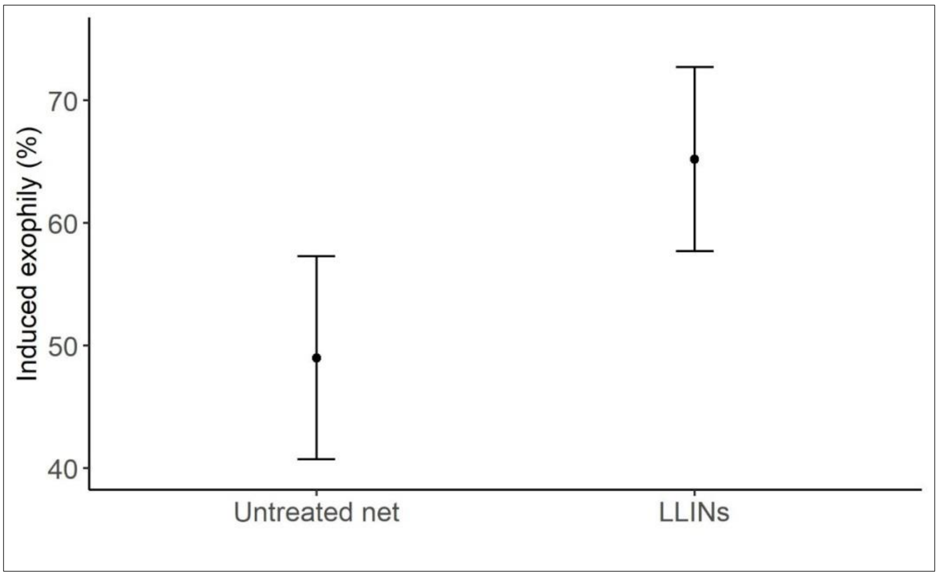

Figure 3: Induced exophily

Bloodmeal Inhibition Rate

The bloodmeal inhibition rate from untreated nets was 43.9%, while the rate from LLINs huts was 64.4% (Figure 4). Bloodmeal inhibition rate from LLINs huts was significantly higher compared to untreated net huts [IRR=1.32 (95% CI: 1.04-1.70)] (p=0.015).

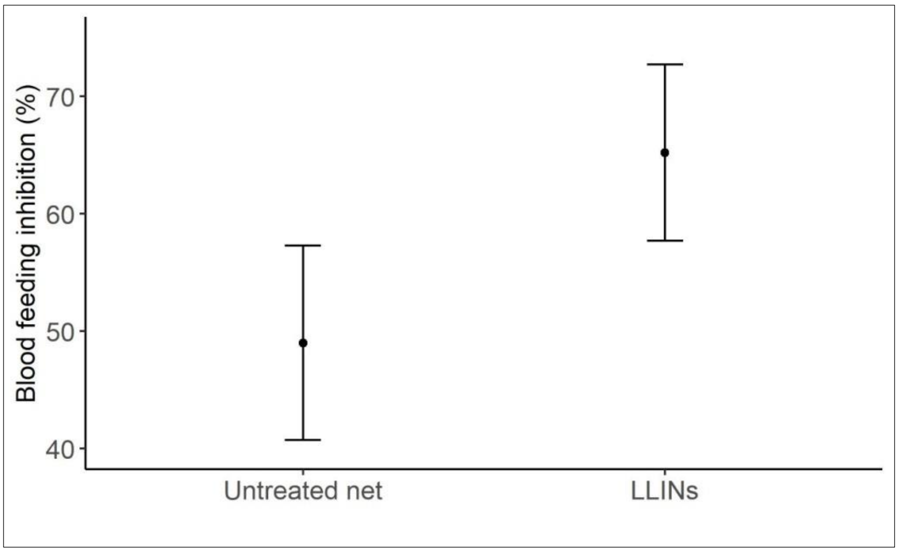

Figure 4: Bloodmeal inhibition rate in *Culex* mosquitoes in the huts

Mortality Rate

The mortality rate was 48.7% in untreated nets and 89.8% in LLINs huts (Figure 5). Significant difference was observed between the two treatments [IRR=1.06 (95% CI: 0.306-3.661)] (p=0.009).

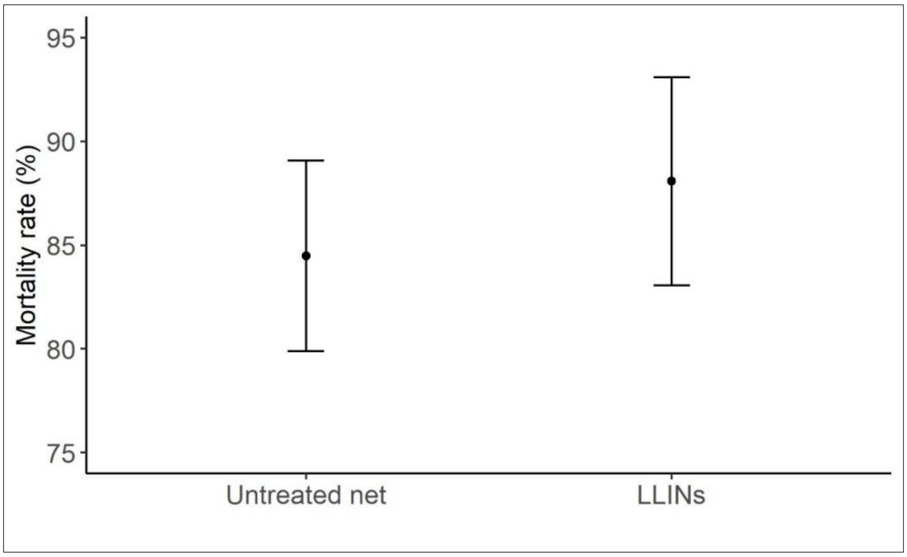

Figure 5: Mortality rate in *Culex* mosquitoes in the huts

Rate of Mosquito Infected by *Wuchereria Bancrofti*

Three pools from LLINs huts verandah were found to be infected by *W. bancrofti*. The infection rate was estimated at 0.12 (95%IC: 0.025-0.312). Regarding the untreated net huts, seven pools were found to be infected with the infection rate of 0.19 (95%IC: 0.079-0.352). No significative difference was observed between the infection rate from LLINs and untreated nets huts (p= 0.534).

DISCUSSION

The aim of this study was to assess the impact of the presence of *W. bancrofti* in *Cx. quinquefasciatus* on the ability to resist nets coated with deltamethrin. The findings showed a high and significant exophylic rate (66%), blood inhibition rate (64%) and mortality rate (90%) in LLIN huts compared to untreated nets where deterrence was not statistically significant. Moreover, the proportion of mosquitoes feeding in LLIN huts and untreated nets was not significantly different.

Indeed, resistance to pyrethrinoids in *Cx. quinquefasciatus* has been reported (Konan *et al*., 2003), along with high levels of resistance organophosphates in Côte d'Ivoire (Chandre *et al*., 1997). However, LLINs have an irritant effect on mosquitoes, which helps prevent blood-feeding. These findings are consistent with previous studies which have shown that the excito-repellent effect of insecticides whether sprayed in experimental huts or used for net impregnation is consistently high compared to control conditions (Koudou *et al*., 2011). The mortality rate of *Cx.*

quinquefasciatus was higher in huts with LLINs compared to those with untreated nets. This high mortality rate is due to the action of the insecticides used in LLINs. Similar mortality rates have already been reported in studies conducted in experimental huts during insecticide evaluation trials in the M'bé Valley (Côte d'Ivoire) (Darriet *et al*., 2002) and Tanzania (Malima *et al*., 2017).

Although some species belonging to *Anopheles*, *Aedes*, *Culex* and *Mansonia* mosquito genera have been identified as carriers of *W. bancrofti* DNA or parasites, their geographic distribution plays a key role in determining their vectorial capacity (WHO, 2013). In West Africa, *An. gambiae* is recognized as the primary vector for lymphatic filariasis (Anosike *et al*., 2005; Pi-Bansa *et al*., 2019). Conversely, the vectorial capacity of *Cx. quinquefasciatus* appears significantly reduced compared with the same species in east Africa (Curtis *et al*., 1981; Jones *et al*., 2018).

In this study, we observed that mosquitoes entered huts regardless of the presence of mosquito nets. This consistent with previous findings indicating that this species possesses pyrethroid resistance genes (Kodindo *et al*., 2018). However, infected mosquitoes were found in all huts. In huts equipped with LLINs, infected mosquitoes were collected from the verandah, whereas in untreated huts, they were found within the nets. A plausible explanation for this observation could be the presence of *W. bancrofti* infection or eggs in the abdomen, although this aspect was not evaluated in the study. Nevertheless, despite the insecticide resistance of *Culex* mosquitoes, LLINs still exert a repellent effect on mosquitoes infected with *W. bancrofti* DNA. This study highlights the behavior of *Cx. quinquefasciatus* in the epidemiology of LF in Côte d'Ivoire. However, the lack of data on LF prevalence assessed through microscopy or rapid diagnostic tests in the populations of the study area represents a significant limitation. The study could be further improved by increasing the number of surveys and expanding to additional sites.

CONCLUSION

This study identified the presence of potential African vectors of *W. bancrofti* in Tiassalé, namely *An. gambiae* and *Culex* species, and highlighted the behavior of *Cx. quinquefasciatus* infected with *W. bancrofti* DNA in relation to LLINs. The findings indicate that, despite insecticide resistance, LLINs exert a significant repellent effect on infected mosquitoes. This information is critical for the design of more effective and cost-efficient vector control strategies, particularly those targeting high-transmission urban areas. We recommend that annual mass drug administration (MDA) for LF control in urban settings be complemented by the widespread distribution of LLINs. Additionally, further studies are needed to assess the long-term impact of these combined interventions on LF prevalence.

Acknowledgements

The authors are grateful to health authorities, local authorities, and the mosquito collection teams.

Author Contributions

YNF drafted the manuscript, BKF and TM revising the manuscript critically for important intellectual content; OAF involved in data acquisition, analyse and interpret the data; ASYA acquire the data; KBG designed the study; all authors approved the final version submitted.

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