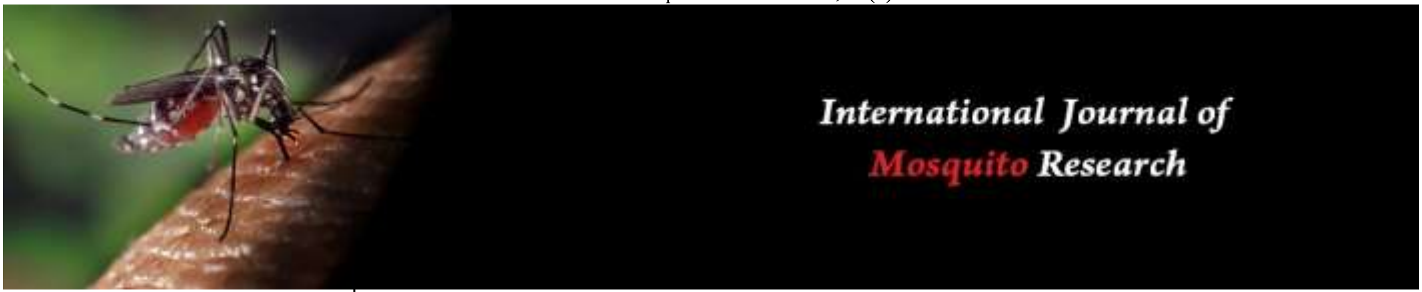

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Major vectors attitude in malaria transmission following a universal long-lasting insecticidal nets (LLINs) coverage in Bouaké, Côte d'Ivoire

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Abstract
To reduce malaria cases, several LLINs distribution campaigns were carried out in Côte d'Ivoire. This study aimed to investigate vector behavior and malaria vectorial transmission level in Bouaké area, following the 2017 large-scale LLINs distribution. Mosquito were collected in Belle-Ville neighborhood and Kpo-Kahankro village using Human Landing Catch and Pyrethrum Spray Catches. ELISA-CSP and ELISA-blood meal techniques determined *Plasmodium falciparum* infection and blood meals origin respectively. The Entomological Inoculation rate (EIR) recorded for *An. gambiae s.l.*, were 212 and 49 infectious bites/person/year (ib/p/year) and was endo-exophageous and exophageous in rural and urban setting respectively. *An. funestus* EIR in rural setting was 230 ib/p/year and was endophageous. *Plasmodium* transmission was higher in rural than in urban setting. *An. funestus* transmitted *plasmodium* at the same level as *An. gambiae s.l.* These vectors showed different behaviors in *Plasmodium* transmission and from one setting to another.

Keywords: Vectors transmission, LLINs, vectors behavior, Belle-Ville, Kpo-Kahankro, Côte d'Ivoire

1. Introduction

Malaria constitute a threat to human health worldwide. In 2022. WHO estimated at 249 million and 608,000, the number of malaria cases and deaths respectively, or an increase of 5 million cases and a reduction of 2.000 deaths compared to 2021 (WHO, 2023) [28]. African region represented in the world about 93.6% of malaria case and 95.4% of malaria death.

Côte d'Ivoire, like other countries of sub-Saharan Africa, isn't immune to malaria. To reduce malaria transmission in the country, the National Malaria Control Program (NMCP) of Côte d'Ivoire, like numerous African countries, has adopted a preventive approach based essentially on the LLINs use. Since 2014, several LLINs distribution campaigns have been carried out by NMCP (PSN, 2021-2025) [22]. This strategy has shown satisfactory results through a reduction in the malaria cases number from 4.7 million in 2013, before LLINs distribution to 3.6 million in 2018, after the 2017 campaigns (WHO 2018) [27], with over 15 million LLINs distributed and a coverage rate of 93% (MSHP, 2019) [19]. Also, malaria deaths number fell from 3.261 in 2013 to 1534 in 2022, or a 50% reduction in the mortality rate (WHO, 2018; NSP, 2021-2025) [27, 22]. However, after the large-scale distribution in 2021 with a coverage rate of 97% and over 19 million LLINs distributed (MSHP, 2022) [20], an increase in the malaria cases number to 8.026 million was observed in 2022 (NSP, 2021-2025) [22]. Despite the mortality reduction observed in 2022, malaria still remains a major public health problem. This can due to the importance of the vectorial transmission of *plasmodium* in some parts of the country. Malaria transmission in Côte d'Ivoire is perennial and ensured by *An. gambiae s.s.*, *An. coluzzii* (*An. gambiae s.l.* species). *An. funestus* and *An. nili* (Assouho *et al*., 2020; Adja *et al*., 2022) [5, 4].

In Bouaké, center of Côte d'Ivoire, several studies carried out about malaria vectorial transmission before LLINs implementation, showed the presence of two malaria vectors namely *An. gambiae s.l.*, and *An. funetus*. In rural setting, malaria transmission was ensured by both vectors, predominated by *An. gambiae s.l.* (Dossou-Yobo *et al*., 1995; Diakité *et al*., 2010; 2015) [13, 11, 12]. However in urban setting, malaria transmission was only ensured by *An. gambiae s.l.* (Dossou-Yovo *et al*., 1998) [14]. After LLINs implementation, studies led between 2014 and 2017 showed that transmission was ensured by *An. gambiae s.s.*; *An. coluzii* (*An. gambiae s.l.* species) and *An. funestus*. In rural setting, transmission was mainly ensured by *An. gambiae s.l.* (Zoh *et al*., 2020; Wolié *et al*., 2022) [32, 29]. *An. funestus* providing secondary transmission in some villages (Wolié *et al*., 2021) [29], even absent in others (Zoh *et al*., 2020) [32]. In urban setting, transmission was always ensured solely by *An. gambiae s.l.* (Adja *et al*., 2021) [3]. *An. gambiae s.l.* exhibited both endophagic and exo-endophagic behavior, while *An. funestus* was endophagic only.

Given the increase in the malaria cases number observed between 2017 and 2022, a better vector behavior understanding in *Plasmodium* transmission would be crucial for the success of current vector control methods. Thus, this study aimed to investigate vector behavior and malaria vectorial transmission level in Bouaké area, following the 2017 large-scale LLINs distribution campaigns.

2. Materials and Methods

2.1 Study Site

The study was carried out in the Bouaké area in the center of Côte d'Ivoire. at 7°44 north latitude and 5°41 south longitude, around 350 km from the economic capital Abidjan. It was conducted from February to December 2019, corresponding to four surveys. The climate is humid tropical, with two seasons: a rainy season from April to October, and a dry season from November to March, marked by the harmattan, a hot dry wind. Average annual rainfall is 1,227 mm and average temperature of 26.3 °C during the study (SODEXAM. 2019). The vegetation is the wooded savannah type.

This study is part of a PNLP project to assess the level of malaria vector transmission in different sentinel sites in Côte d'Ivoire, following the 2017 large-scale LLINs distribution campaign. Bouaké is one of these sites and the studt took place in an urban setting in the Belle-Ville neighborhood to the northeast, and in a rural setting in the village of Kpo-Kahankro to the south, 7 km from Bouaké city (Fig).

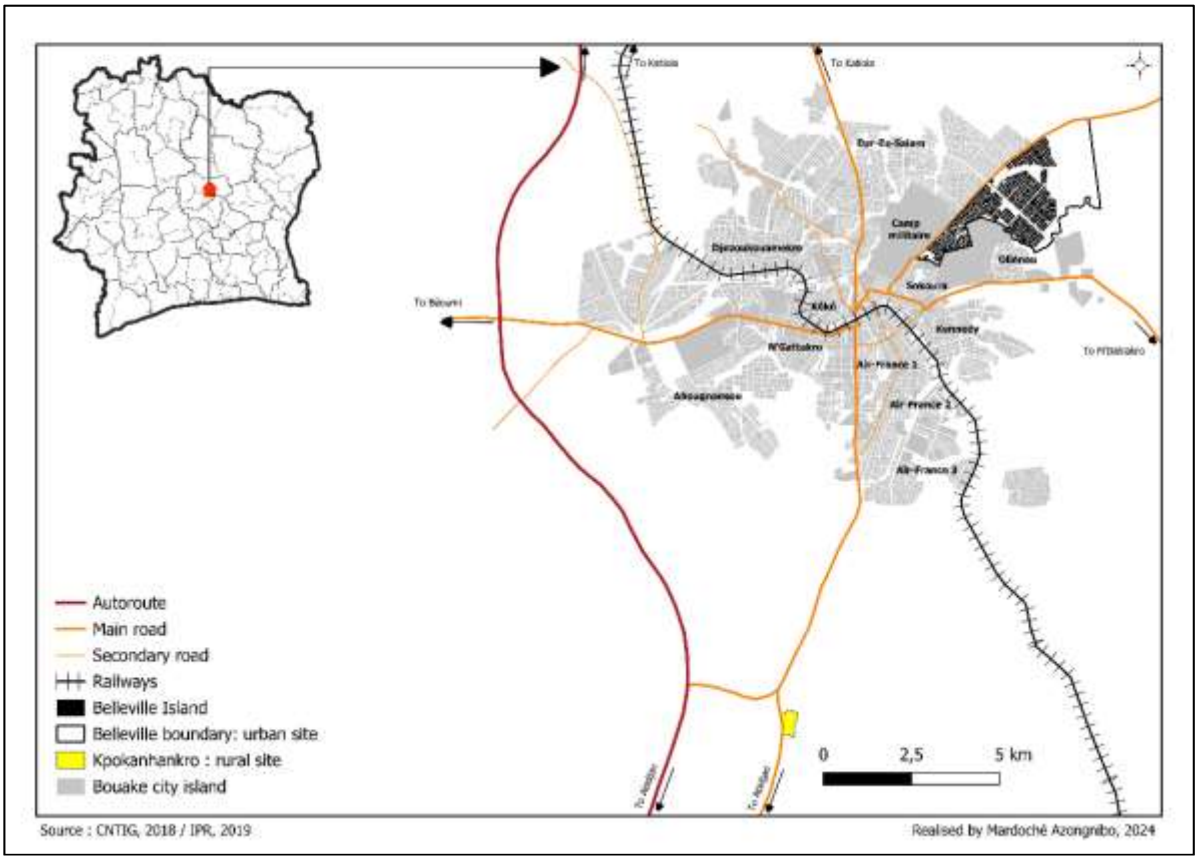

Fig 1: Location map of study sites in the Bouaké area

2.2 Ethical consideration

The study was approved by the Côte d'Ivoire National Ethics and Research Committee and the health authorities in each locality. Households heads have given authorization to conduct these survey in their homes. In addition, community consent was obtained in advance in all sites. Volunteer mosquito collectors gave their consent before participating in the study. They also underwent regular medical examinations with preventive treatment against malaria and yellow fever according to the recommendations of the Côte d'Ivoire NMCP.

2.3 Mosquito sampling

Mosquito collection was carried out in 2019 in the months of February, July, September and December using the Human Landing Catches (HLC) and pyrethrum spray catches (PSC) methods.

In each study site, mosquito sampling by HLC was carried out in three catching points, simultaneously inside and outside dwellings. Samplings began at 6 p.m. in each study site and ended at 8 a.m. next day. These catching were carried out in each study site during two consecutive nights in each survey month. The mosquitoes collected were identified morphologically using the Gillies and Coetzee (1987) [15] identification keys, and the malaria vectors females identified were kept for later analysis. Sampling by PSC involved spraying a 1% pyrethrin aerosol solution on the inside walls of bedrooms to collect resting mosquitoes. This was done early in the morning, between 6:30 and 7:30 a.m., on the next day after the last night of HLC method. These samplings was carried out in 10 bedrooms during each survey in each study site. The vectors were identified along with the same determination keys using for HLC method and the females which taken blood-meals were preserved to determine the blood meal origin.

2.4 Mosquito laboratory treatment

The mosquito sample treatment in laboratory has been done at Pierre Richet institute of Bouaké.

2.4.1 *Plasmodium falciparum*-infection research

The *Plasmodium falciparum* infection research in vectors was carried out using the ELISA-CSP technique according to the protocol of Burkot *et al*. (1984) [7], modified by Wirtz *et al*. (1987) [26]. This technique was performed using the head and thorax of female vectors collected by HLC.

2.4.2 Blood meal origin determination

Determination of blood meals origin taken by mosquitoes is carried out by ELISA-blood meal using the technique of Beier *et al*. (1988) [6]. This method is performed using the abdomens of the blood-fed females collected at rest in bedrooms by PSC.

2.5 Statistical data analysis

The human biting rate (HBR) corresponds to the average number of mosquito bites received per person and per unit of time (night. month or year). This index is used to monitor the vector population density variations in some area, based on mosquito catching on human per night. The *Plasmodium* infectivity rate (IR) corresponds to the female *anopheles* percentage carrying *Plasmodium* out of the total number of females examined. The entomological inoculation rate (EIR) corresponds to the number of *Plasmodium*-infected bites received per person and per unit of time (per night. per month or per year). It is the product of human biting rate and infectivity rate. It quantifies mosquito-to-man transmission. Anthropophilic rate (AR), corresponding to the vector females percentage which take their blood meals on humans. Data were analyzed using GraphPad Prism 5.1 software. Non-parametric Mann-Whitney tests were used to compare HBR average between study sites and between vectors in each site. Fisher tests were used to compare different proportions. All differences were considered significant at a p-value <0.05.

3. Results

3.1 Culicid fauna composition

3.1.1 Fauna collected by Human Landing Catching (HLC)

HLC method enabled to collect in Bouaké area a total of 12 culicid species divided into 4 genus.

In urban setting, 11 species belonging to 4 genus have been identified. *Cx. quinquefasciatus* predominated this fauna with a proportion of 76.65%, followed by *An. gambiae s.l.* with 19.74% of total fauna.

In rural setting, also 11 culicid species divided into 4 genus have been identified. However. *An. gambiae s.l.* was the predominate species with a proportion of 54.66%, followed by *An. funestus* with a proportion of 40.38% (Table 1).

Table 1: Culicid fauna collected on human in Bouaké urban and rural settings

Genus	Species	Belle-Ville			Kpo-Kahankro		
		In	out	Total (%)	In	Out	Total (%)
Anopheles	*An. gambiae*	186	278	464 (19.74)	810	813	1623 (54.66)
	An.funestus	1	0	1 (0.04)	862	337	1199 (40.38)
	An. pharoensis	0	1	1 (0.04)	0	7	7 (0.23)
	An. coustani	0	0	0	1	0	1 (0.03)
Total *Anopheles*		187	279	466 (19.82%)	1673	1157	2830(95.31%)
Aedes	*Ae. aegypti*	5	5	10 (0.43)	0	2	2 (0.06)
Total *Aedes*		5	5	10 (0.43)	0	2	2 (0.06)
Culex	*Cx. quinquefasciatus*	1108	741	1849 (76.65)	9	4	13 (0.44)
	Cx. annulioris	2	2	4 (0.18)	1	2	3 (0.10)
	Cx. nebulosus	1	0	1 (0.04)	1	2	3 (0.10)
	Cx. cinereus	2	3	5 (0.21)	0	1	1 (0.03)
	Cx. decens	4	8	12 (0.51)	0	0	0
Total *Culex*		1117	754	1871 (79.58)	11	9	20 (67.36%)
Mansonia	*Man. africana*	2	1	3 (0.13)	23	71	94 (3.17)
	Man. uniformis	1	0	1 (0.04)	7	16	23 (0.77)
Total *Mansonia*		3	1	4 (0.17%)	30	87	117 (0.12%)
Total culicid		1312	1039	2351	1714	1255	2969

In: Inside; Out: Outside; *An.*: *Anopheles*; *Cx.*: *Culex*; *Ae.*: *Aedes*; *Man.*: *Mansonia*

3.1.2 Endophilian fauna collected by Pyrethrum Spray Catches (PSC)

Endophilian fauna represented all mosquitoes resting inside households collected by PSC method.

In urban setting, 7 culicid species divided into 3 genus were identified. *Cx. quinquefasciatus* species was the most dominant of Endophilian fauna at 93.30%

However in rural setting, 3 species belonging to 2 genus were collected. *An. gambiae s.l.* was the predominant species at 68.75%, followed by *An. funestus* at 28.75% (Table 2).

Table 2: Endophilian fauna collected in Bouaké urban and rural settings

Genus	Species	Belle-ville	Kpo-Kahankro
		Total (%)	Total (%)
Anopheles	*An. gambiae*	6 (1.44)	55 (68.75)
	An.funestus	0	23 (28.75)
Total *Anopheles*		6 (1.44%)	78 (97.5%)
Aedes	*Ae. aegypti*	6 (1.44)	0
Total Aedes		6 (1.44%)	0
Culex	*Cx. quinquefasciatus*	390 (93.30)	0
	Cx. annulioris	5 (1.20)	0
	Cx. cinereus	3 (0.72)	2 (2.50)
	Cx. decens	8 (19.14)	0
Total *Culex*		406 (97.13%)	2 (2.50%)
Total Culicid		418	80

3.1.3 Vectorial fauna
In urban setting, 2 vectors namely *An. gambiae s.l.* (19.74%) and *An. funestus* (0.04%) have been collected by HLC method but with a very lower *An. funestus* proportion. However, PSC method enabled to collect *An. gambiae s.l.* (1.44%) alone.
In rural setting. *An. gambiae s.l.* (54.66% with HLC and 68.75% with PSC) and *An. funestus* (40.38% with HLC and 28.75% with PSC) were collected by both methods.

3.2 Vectors behavior
3.2.1 Vectors biting behavior
Mosquito collected by HLC, carried out simultaneously inside and outside dwellings, enabled to determine the vectors biting behavior.
In urban setting, *An. gambiae s.l.* females feed mainly outside and are therefore exophagous. However, in rural setting, *An. gambiae s.l.* females showed a tendency towards endo-exophagy, i.e. these females feed both inside and outside households, whereas *An. funestus* females more feed inside, and therefore are tendency towards endophagy (Table 3).

Table 3: *An. gambiae* and *An. funestus* biting rate in Bouaké urban and rural settings

Species	Setting	Female collected			Status
		Inside (%)	Outside (%)	Total	
An. gambiae	Urban	186 (40.09)	278 (59.91)	464	Exophageous
	Rural	810 (49.91)	813 (50.09)	1623	Endo-exophageous
An. funestus	Rural	862 (71.89)	337 (28.11)	1199	Endophageous

3.2.2 Vector resting density
PSC method was used to determine the resting density of vector fauna after taking a blood meal. A total of 47 blood-fed *An. gambiae s.l.* females were collected in 80 bedrooms, giving an average resting density of 0.59 females per room per day (FRD).
In Belle-Ville neighborhood, in urban setting, this density was 0.15 (6/40) FRD, while in Kpo-Kahankro, in rural setting; it was 1.03 (41/40) FRD. As for *An. funestus*. 11 blood-fed females were collected in 40 bedroom in Kpo-Kahankro alone, giving a resting density of 0.23 FRD.

3.2.3 Anthropophilic rate
Vectors blood meal origin was analyzed only for *An. gambiae s.l.* in rural setting, due to the low number of *An. gambiae s.l.* blood-fed females collected in urban setting (n = 6) and *An. funestus s.l.* collected in rural setting (n = 9). This analysis has been done with abdomens of the blood-fed females collected by PSC method.
In rural setting. 41 out of 44 *An. gambiaes.l.* females analyzed had taken their blood meal on humans. They are therefore strict anthropophilic, with an anthropophilic rate of 93.18%.

3.3 *Plasmodium falciparum* transmission
3.3.1 Human Biting rate (HBR)
A total of 2.087 *An. gambiae s.l.* were collected from 96 men in all study sites. The overall average of biting rate has been estimated at 21.74 bites/person/night (b/p/n), or 7935 b/p/year.
In urban area, the average biting rate recorded for *An. gambiae s.l.* in the neighborhood was 9.67 (*CI = 5.96-13.38*) b/p/n. or 3530 b/p/year. Biting rates inside and outside the households were comparable (P = 0.16).
In rural area, the average biting rate for *An. gambiaes.l.* recorded in the village was 33.81 (*CI = 23.08-44.55*) b/p/n or 12341 b/p/year. This rate was significantly higher (P = 0.0007) than that recorded in urban area. However, as observed in the neighborhood, there was no difference (*p*>0.05) between inside and outside households.
For *An. funestus* biting rate recorded was 24.98 (*CI = 15.99-33.97*) b/p/n or 9118 b/p/year. This rate was comparable to that recorded for *An. gambiae* (*p*>0.05). *An. funestus* bite number received inside was more than twice as much (P = 0.04) that received outside (Table 4).

Table 4: Vectors biting rate in Bouaké urban and rural settings

Setting	Species	Biting rate (b/p/n)		
		In (CI)	Out (CI)	Total (CI)
Urban	*An. gambiae s.l.*	7.75 (3.04-12.46)	11.58 (5.58-17.58)	9.57 (5.96-13.38)
Rural	*An. gambiae s.l.*	33.75 (17.82-49.68)	33.88 (18.25-49.50)	33.81 (23.08-44.55)
	An. funestus	35.92 (19.89-51.94)	14.04 (7.11-20.97)	24.98 (15.99-33.97)

3.3.2 *Plasmodium falciparum* Infectivity Rate
From a total of 999 *An. gambiae* females analyzed. 16 females were infected to *Plasmodium falciparum*, or an annual infectivity rate of 1.6%.
In urban area, out of 362 *An. gambiae s.l.* females. 5 were infected to *Plasmodium falciparum* with an annual infectivity rate of 1.38%. Averages rates recorded estimated at 1.23% (CI = 0-2.94) and 1.51% (CI = 0-3.22) respectively. Infectivity rates inside and outside were statistically similar (P = 1.0000).
In rural area, out of 639 *An. gambiae s.l.* tested. 11 were infected to *Plasmodium falciparum*. Infectivity rate recorded of 1.73% (IC = 0.71-2.75) was similar (P = 0.80) to neighborhood. Inside and outside infectivity rate were also similar (P = 1.0000).
Concerning *An funestus*, 12 out of 476 females tested were infected. The infectivity rate for this vector was 2.52% (CI = 1.11-3.93) and was similar (P = 0.40) to that recorded for An. gambiae s.l.. The rates recorded inside and outside were comparable (P = 0.75) (Table 5).

Table 5: Vectors infectivity rate in Bouaké urban and rural settings

Site	Species	Infectivity rate (%)		
		In (CI)	Out (CI)	Total (CI)
Urban setting	*An. gambiae s.l.*	1.23 (0-2.94)	1.51 (0-3.22)	1.38 (0.17-2.59)
Rural setting	*An. gambiae s.l.*	1.86 (0.38-3.35)	1.59 (0.20-2.98)	1.73 (0.71-2.75)
	An. funestus	2.85 (1-4.69)	1.87 (0-4)	2.52 (1.11-3.93)

3.3.3 Entomological Inoculation Rate (EIR)

Global annual Entomological Inoculation Rate of *An. gambiae s.l.* recorded in this study was estimated at 128 infectious bites/person/year (ib/p/year).

At Belle-Ville, in urban area the EIR was 0.13 ib/p/n. or one *An. gambiae s.l.* infectious bite received every 10 days in this neighborhood. Annuel EIR was estimated at 49 ib/p/year. EIRs recorded inside and outside households have been estimated at 37 and 62 ib/p/year. Thus, one person living in this neighborhood received every 10 days about twice as much infectious bites outside than inside.

At Kpo-Kahankro in rural area. EIR recorded for *An. gambiae s.l.* was 0.58 ib/p/n and estimated at 212 ib/p/year or one infectious bite every 2 days corresponding at less 5 times more *An. gambiae s.l.* infectious bite received every 10 days in this village than in the neighborhood. EIRs have been 230 ib/p/year inside and 197 ib/p/year outside corresponding to one infectious bite as well inside as outside every 2 days

EIR recorded for *An. funestus* has been 0.63 ib/p/n or an annual EIR of 230pi/p/year. One person in the village also received one *An. funestus* infectious bite every 2 days just like *An. gambiae s.l.*. EIR recorded inside of 372 ib/p/year was twice higher than that recorded outside with 95 ib/p/year (Table 6).

Table 6: Entomological Inoculation Rate of malaria vectors in urban and rural setting of Bouaké

Setting	Species	Entomological Inoculation Rate (ib/p/n)		
		Inside	Outside	Total
Urban	*An. gambiae s.l.*	0.1	0.17	0.13
Rural	*An. gambiae s.l.*	0.63	0.54	0.58
	An. funestus	1.02	0.26	0.63

Discussion

Human Landing Catches (HLC) and Pyrethrum Spray Catches (PSC) methods carried out during this study, revealed the involvement of *An. gambiae s.l.* and *An. funestus*, in malaria transmission in Bouaké area. Previous studies had already shown the role of these vectors in malaria transmission in this area (Dossou-Yovo *et al*., 1998; Diakité *et al*., 2015; Wolié *et al*., 2022) [14, 12, 29].

An. gambiae s.l. and *An. funestus* presented different behaviors in *Plasmodium* transmission.

The exophagic tendency of *An. gambiae s.l.* observed in Bouaké urban setting corroborates Dahoui *et al.* (2023) [10] observations done after LLINs distribution campaigns of 2017 in two Bouaké neighborhoods. These results demonstrate LLINs effectiveness inside households, which led these vectors to feed outside. Indeed, studies carried out in other localities in Côte d'Ivoire before LLINs implementation of 2014 had shown an endophagic tendency of *An. gambiae s.l.* (Adja *et al*., 2006; 2011) [1, 2]. This change behavior from endophagic to exophagic after LLINs implementation in household has been reported in Equatorial Guinea (Reddy *et al*., 2011) [23] and Tanzania (Russell *et al*., 2011; Kreppel *et al*., 2020) [25, 17]. However, *Plasmodium falciparum* transmission by *An. gambiae s.l.*, higher outside households also represent a threat to the effectiveness of current vector control strategies because LLINs were designed to specifically target vectors feeding inside households.

Endo-exophagic tendency was also observed in same vector in Bouaké rural setting, would indicate an *An. gambiae s.l.* adaptation beginning to LLINs used in this setting. These observations have also been done in other localities in Côte d'Ivoire (Adja *et al*., 2022) [4] and also in other African countries (Russell *et al*., 2011; Corbel *et al*., 2012) [25, 9]. Furthermore, the indoor transmission persistence with indoor control tool could be explained by the resistance development of *An. gambiae s.l.* to pyrethroids, an insecticide recommended by WHO for impregnating mosquito nets, due to their rapid action and their safety for humans (Zaim *et al*., 2000) [30]. Several studies have shown resistance of this vector to this insecticide throughout the country (Koffi *et al*., 2013; Zoh *et al*., 2018; Camara *et al*., 2018) [16, 31, 8].

Anopheles funestus endophagy behavior in Bouaké rural setting confirms Wolié *et al*., (2022) [29]; Kroko *et al*., (2024) [18] works results. This behavior could be beneficial for the current vector control, because *An. funestus* will have a high likelihood to encounter LLINs inside households. However, this behavior also could constitute a concern source, especially as *Plasmodium falciparum* transmission by this vector is higher inside households. This could herald an *An. funestus* resistance to insecticides used in the vector control tools impregnation, although it is still little studied in Côte d'Ivoire, compared with that of *An. gambiae s.l.*. Furthermore, resistance to different classes of insecticides has been increasingly demonstrate in *An. funestus* in several African countries (Mzilahowa *et al*., 2016; Riveron *et al*., 2016) [21, 24].

This present study also showed that *An. gambiae s.l* and *An. funestus* had the same level of *P. falciparum* transmission in rural setting. Yet, *An. funestus* had been shown by several previous studies as a secondary vector after *An. gambiae s.l.* (Dossou-Yovo *et al*., 1995; Diakité *et al*., 2010; 2015; Wolié *et al*.; 2022) [13, 11, 12, 29]. In view of these results, a control strategy that takes into account each malaria vectors behavior is needed to reduce the malaria number cases. A better understanding of the *An. funestus* resistance to insecticides used in vector control, as well as a good assurance of the possession and effective use of LLINs by the population, could help to improve these control strategies in the Bouaké area.

Conclusion

The aim of this study that was to investigate vector behavior and malaria vectorial transmission level, was showed that *An gambiae s.l.* and *An. funestus* are major malaria vectors in Bouaké area. In urban setting, malaria transmission is ensured by *An gambiae s.l.* and in rural setting, transmission is ensured by *An gambiae s.l.* and *An. funestus* at the same level. These vectors showed different behaviors in *Plasmodium* transmission and from one site to another. In urban setting, *An. gambiae s.l.* transmitted *Plasmodium* more outside and showed an exophagic behavior, whereas in rural setting, this same vector transmitted as well outside as inside and showed an endo-exophagic behavior. However, *An. funestus* remained endophageous in rural area and transmitted *Plasmodium* more inside households. More than 90% of *An. gambiae s.l.* are strictly anthropophilic behavior.

Authors' Contributions

Akré M. conceptualized and designed the study. Dounin D. Zoh. Konan F. Assouho and Konan R. M. Azongnibo. conducted the field work. Dounin D. Zoh. Konan F. Assouho and Dipomin F. Traore realized the laboratory work. Dounin D. Zoh analysed the data and wrote the manuscript. All authors read and approved the final version of the article.

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Conflict of Interest

The authors declare no competing interests.

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