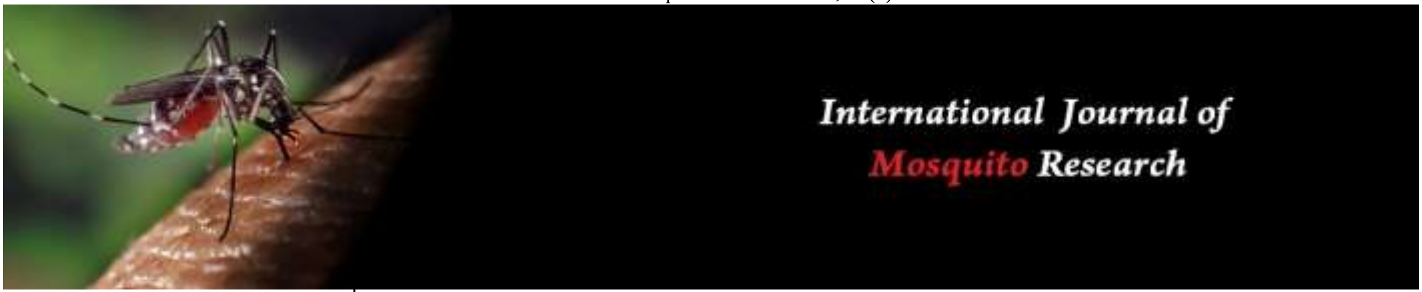

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Involvement of two major vectors of malaria, *Anopheles gambiae* Giles and *An nili* Theobald (Diptera: Culicidae), in *Plasmodium falciparum* transmission of two coastal villages of Côte d'Ivoire

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Abstract
Malaria is caused by parasites transmitted to people through the infected bites of female *Anopheles* mosquitoes. On the Ivorian coast, very few studies have been carried out on these vectors. The current study aimed to assess the involvement of malaria vectors in the transmission of malaria in Koukourandoumi and Baba, two rural areas of the Ivorian coast. Mosquitoes were collected by Human Landing Catches and Pyrethrum Spray Catches. *Plasmodium falciparum* species in these mosquitoes was identified by the ELISA-CSP technique. Behavioural data was also collected. The present study revelated the three malaria vector species in Côte d'Ivoire (*An. gambiae*, *An. nili* and *An. funestus*). *An. gambiae* was the most common *Anopheles* species in Koukourandoumi, whereas *An. nili* was the most abundant in Baba. The *An. gambiae* females feed indoor while *An. nili* feeds outdoor. Malaria vectors showed a low aggressiveness (<7 bites/person/night). The infection rates for *An. gambiae* were 1.73% and 4.40% in Baba and in Koukourandoumi respectively. For *An. nili*, these rates were 1.45% and 0% in Baba and in Koukourandoumi respectively. No *An. funestus* were infected. This study showed that *An. gambiae* is the only vector of malaria in Koukourandoumi. However, in Baba, *An. gambiae* and *An. nili* are responsible for malaria transmission, with *An. nili* accounting for most of the malaria transmission. This study shows the implication of *An. nili* in malaria transmission in these areas and the complexity of the malaria vectorial system that should be considered for any malaria control strategy.

Keywords: Infected bites, Ivorian coast, aggressiveness, malaria transmission, Baba, Koukourandoumi

Introduction

Malaria is a disease caused by parasites transmitted to people through the bites of infected Anopheles mosquitoes. In tropical Africa, the transmission of malaria parasites is ensured by primary vector species such as members of the *Anopheles gambiae* Giles complex and these of the *An. funestus* Giles and *An. nili* Theobald groups (Becker *et al*., 2010) [9]. The role of *An. gambiae s.l.* in the transmission of malarial parasites has been investigated several times in Côte d'Ivoire (Diakité *et al*., 2010; Koudou *et al*., 2010; Adja *et al*., 2011; Assouho *et al*., 2020) [17,21,3,6]. Another malaria vector, *An. funestus* has also described in the central zone of Côte d'Ivoire (Assouho *et al*., 2020) [6]. *Anopheles nili* s.l. is also regarded as one of the malaria vectors in Africa **(**Carnevale *et al*., 1992**;** Fontenille & Simard, 2004) [11,19]. It has been recognised as a vector in the northeast, in the village of Gansé, in the southwestern forest region, precisely in the Soubré area, along rivers (Adja *et al*., 2011) [3] and in the south-west of San Pedro (Assouho *et al*., 2020) [6]. The latter species proliferates in the vegetation of fast-flowing rivers (Carnevale *et al*., 2009) [13].

As with several other known vectors, there is little information available on *An. nili* s.l. in Côte d'Ivoire, although there would appear to be many habitats that could support the breeding of these mosquitoes, especially in the southern forest zone (the larvae of *An. nili s.l.* are typically found in vegetation or dense shade along the edges of streams and large rivers) **(**Carnevale *et al*., 1992**;** Fontenille & Simard, 2004) [11, 19]. In addition, previous work has revealed the exophagous behaviour of this species in coastal areas (Adja *et al*., 2011; Assouho *et al*., 2020) [3, 6]. This behaviour, if it continues, will compromise the current methods of targeting internal vectors. The study aims therefore to assess the involvement of malaria vectors in the transmission of malaria in two coastal villages in the forest zone of southeastern and southwestern Côte d'Ivoire.

2. Materials and Methods

2.1 Study Site

This study was carried out from February to December 2019 in the villages of Koukourandoumi and Baba, located respectively in the south and the littoral of Côte d'Ivoire (Fig 1).

The village of Koukourandoumi (5° 31'N and 3° 10W) belongs to the locality of Aboisso. It is located in the southeast of Côte d'Ivoire at 116 km from Abidjan and 60 km from the border with Ghana. Located in the locality of San-Pedro, in the south-western of Côte d'Ivoire, the village of Baba (4 ° 75' N, 6 ° 73 W) is 348 km from Abidjan and 120 km from Liberia.

Koukourandoumi and Baba are part of the twelve sentinel sites of The National Malaria Control Program (NMCP), to assess the trend of the level of malaria transmission throughout the Ivorian territory. These two villages belonging to the same climate with four seasons are also located in the south of the country where there are several rivers. In fact, on the base on the meteorological data of the Société d' Exploitation et de Développement Aéroportuaire, Aéronautique et Météorologique (SODEXAM), the climate of Koukourandoumi and Baba is divided into four seasons. In Koukourandoumi, we find a long rainy season (February to June), a long dry season (July to August), a short rainy season (September to December) and a short dry season (January). The average annual rainfall was 1457.63 mm with an average temperature of 26.27 °C. In Baba, we have a long rainy season (March to July), a short dry season (August to September), a short rainy season (October to November), and a long dry season (December to February). The average annual rainfall was 74.39 mm with an average temperature of 26.19 °C. The-Bia-river crosses the village of Koukourandoumi and flows into the Aby lagoon in the south of Aboisso, whereas Baba is bordered by the Neron and the Digboué rivers.

2.2 Ethical consideration

This study received the approvals of the National Ethics and Research Committee of Côte d'Ivoire and of health authorities of each locality. Permission was obtained from heads of household to investigate their homes. In addition, community consent had been obtained beforehand in all the sites. The volunteer mosquito collectors gave their consent before participating in the study. They were also subjected to regular medical checkups with preventive malaria treatment in accordance with the recommendations of the NMCP of Côte d'Ivoire. They were all vaccinated against yellow fever.

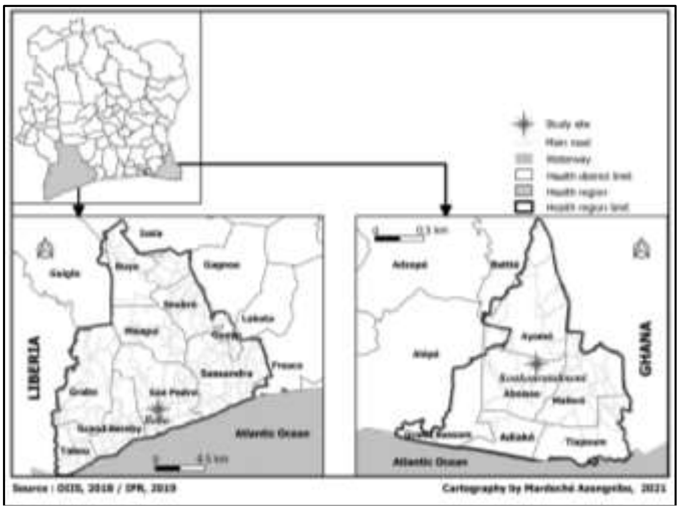

Fig 1: Location of the study sites in Côte d'Ivoire

2.3 Field Processing of Mosquitoes

Repeated cross-sectional surveys were carried out to conduct this study every three months. A total of four surveys was conducted from February to December 2019. Mosquito collections were undertaken using Human Landing Catches (HLC) method. During two consecutive nights (from 17:00 to 08:00 hrs), HLCs were performed in three houses per community with one collector indoor and one outdoor selected household. Householders were asked to assist designated mosquito collectors in each community for two days per study site. These catches were done by two teams of volunteers (local residents). The first team worked from 17: 00 hrs to midnight and the second from midnight to 8:00 hrs. The captured mosquitoes were grouped on an hourly basis per site and kept in separate sacks. Using this strategy, the Human Biting Rate (HBR) or Anopheles density per person per time unit (night) was estimated and used for the calculation of the entomological inoculation rates (EIR). Otherwise, resting females are collected by Pyrethrum Spray Catches (PSC) every three months in the morning after the second-night of human landing catching collections. The catching of *Anopheles* females in resting has been performed in 40 bedrooms of each study site. All collected mosquitoes were placed in holding cups labelled by hour until they were processed for morphological identification. After being differentiated at the level of the genus as *Culex*, *Anopheles*, *Aedes* or *Mansonia*, Anopheles mosquitoes were identified to species level using the taxonomy and identification keys to female Afrotropical anophelines (Coetzee, 2020) [14]. We dissected the ovaries of the females of Anopheles vectors and observed the degree of coiling of the ovarian tracheoles to determine their parity status based on the ovary tracheation method of (Detinova, 1968) [15]. All collected anopheline females were stored individually in Eppendorf tubes containing desiccant, labelled with the name of the study site, point and date of collection, and stored at -20 °C for further molecular analysis in the laboratory at the Institut Pierre Richet of Bouaké, Côte d'Ivoire.

2.4 Laboratory Processing of Mosquitoes

The Enzyme-Linked Immunosorbent Assay (ELISA) was used to test the presence of circumsporozoite protein (CSP) of *Plasmodium falciparum* (This parasite represents the predominant species and is the cause in more than 95% of cases). The head and the thorax of anopheles female were used for the test. These two portions were separated from the rest of the body and homogenized in blocking buffer (0.5% Casein, 0.1 N NaOH, 1 × PBS). The used procedures were those of Burkot *et al*. (1984) [10] and Wirtz *et al*. (1987) [24]. A mosquito sample is positive if the optical density (OD) value is higher than twice the mean OD of four negative control wells (uninfected mosquitoes) on the ELISA plate.

2.5 Entomological parameters and statistical analysis

Data were analysed using version 10.1 of the STATA software package (Stata Corporation, College Station, TX). The following entomological parameters are determined: 1) the human biting rate (HBR), expressed as the number of female anopheline bites per person per night (b/p/n); 2) the parity rate (PR) calculated as the proportion of gravid females; 3) the infection rate (IR) corresponding to the proportion of females infected by *P.* falciparum; 4) the entomological inoculation rates (EIR), expressed as the number of infective anopheline bites per person per year, was calculated as the product of the HBR and the IR of mosquitoes collected on humans; and the endophagous rate defined as the proportion of mosquitoes captured indoors among those caught indoors and outdoors from human landing catching. A P-value of ≤ 0.05 was considered indicative of a statistically significant difference. The χ^2 test was used to compare the parity and infection rates and the Kruskal–Wallis (KW)test for the human biting rates.

3. Results

3.1 Mosquito collections

Human Landing Catches allowed the collection of 1,913 mosquitoes in Koukourandoumi and 2,203 in Baba (Table 1). These mosquitoes were composed of 4 genera: *Anopheles*, *Aedes*, *Culex* and *Mansonia*. The *Culex* genu with 92.21% in Koukourandoumi and 48.75% in Baba, was predominant.

The anopheline fauna was composed of two species in Koukourandoumi and six species in Baba. Three vector species *An. gambiae*, *An. funestus* and *An. nili* were identified in Baba while *An. gambiae* and *An. nili* were collected in Koukourandoumi (Table 1). In Koukourandoumi, *An. gambiae* was in the majority whereas in Baba, it is *An. nili*.

In Koukourandoumi and in Baba, 361 and 79 mosquitoes were sampled by Pyrethrum Spray Catches (Table 2). In the village of Koukourandoumi, these mosquitoes were composed of two genera, namely Culex and Anopheles. The Culex were in the majority (96.95%). The Anopheles represented only 3.05% of the total number of mosquitoes collected. In Baba village, the mosquitoes collected were composed of four genera, including Anopheles, Culex, Aedes and Mansonia. The Anopheles were predominantly represented (93.67%). Two malaria vectors, namely An. gambiae and *An. funestus* were identified in Baba with proportions of 70.89% and 22.78%. In Koukourandoumi, only An. gambiae was collected at 3.05%. No species of *An. nili* was encountered in the two villages using the PSC method despite their abundance observed through HLC.

3.2. Human biting rates

During the study period, 125 females of An. gambiae and 20 of *An. nili* were collected by the HLC in Koukourandoumi. In Baba, 199, 16 and 320 females of An. gambiae, *An. funestus* and *An. nili* and were collected respectively. The average aggressiveness rates of An. gambiae and *An. nili* recorded in Koukourandoumi were respectively 2.60 b/p/n [CI: 1.59-3.62] and 0.42 b/p/n [CI: 0.19-0.64] (Table 3). The aggressive density of An. gambiae was significantly higher than that of *An. nili* (KW = 20.640; p<0.0001). In Baba, the mean aggressive densities of An. gambiae, *An. nili* and *An. funestus* were respectively 4.15 [CI: 2.61-5.68], 6.67 [CI: 4.50-8.84] and 0.33 [CI: 0.15-0.52] b/p/n (Table 3). The aggressive density of *An. nili* and An. gambiae were comparable (KW = 3.26; p = 0.07). The aggressive densities of An. gambiae in Koukourandoumi and Baba were statistically identical (KW = 1.56; p = 0.21). On the other hand, those of *An. nili* were different between the two villages (KW = 61.79; p = 0.0001).

Table 1: Composition of mosquito fauna collected by Human Landing Catches in Koukourandoumi and in Baba

Genus	Species	Koukourandoumi			Baba		
		Int	Ext	Total (%)	Int	Ext	Total (%)
Anopheles	*An. gambiae*	67	58	125 (6.53)	119	80	199 (9.03)
	An. nili	9	11	20 (1.05)	151	169	320 (14.53)
	An. funestus	0	0	0	9	7	16 (0.73)
	An. pharoensis	0	0	0	1	3	4 (0.18)
	An. ziemanni	0	0	0	10	17	27 (1.22)
	An. brohieri	0	0	0	1	3	4 (0.18)
Total 1: *Anopheles*		76	69	145 (7.58)	291	279	570 (25.87)
Aedes	*Ae. Aegypti*	1	0	1 (0.05)	10	9	19 (0.86)
Total 2: *Aedes*		1	0	1 (0.05)	10	9	19 (0.86)
Culex	*Cx. Cinereus*	32	36	68 (3.55)	1	6	7 (0.32)
	Cx. Decens	103	131	234 (12.23)	0	0	0
	Cx. quinquefasciatus	773	667	1,440 (75.27)	113	107	220 (9.99)
	Culex. Sp	9	13	22 (1.15)	350	497	847 (38.44)
Total 3: *Culex*		917	847	1,764 (92.21)	464	610	1,074 (48.75)
Mansonia	*Man. Africana*	2	1	3 (0.16)	151	160	311 (14.12)
	Man. Uniformis	0	0	0	117	112	229 (10.39)
Total 4: *Mansonia*		2	1	3 (0.16)	268	272	540 (24.51)
Total Culicidae		996	917	1,913	1,033	1,170	2,203

Table 2: Composition of mosquito fauna collected by Pyrethrum Spray Cathes in Koukourandoumi and in Baba

Genus	Species	Koukourandoumi		Baba	
		n	%	n	%
Anopheles	*An. gambiae*	11	3.05	56	70.89
	An. funestus	0	0	18	22.78
Total 1: *Anopheles*		11	3.05	74	93.67
Aedes	*Ae. aegypti*	0	0	1	1.27
Total 2: *Aedes*		0	0	1	1.27
Culex	*Cx. cinereus*	5	1.38	0	0.0
	Cx. decens	21	5.82	0	0.0
	Cx. quinquefasciatus	324	89.75	3	3.80
Total 3: *Culex*		350	96.95	3	3.80
Mansonia	*Man. uniformis*	0	0	1	1.27
Total 4: *Mansonia*		0	0	1	1.27
Total Culicidae		361	100	79	100

n: Species number, %: Species proportion

Table 3: Human biting rate, parity rate, infection rate and Entomological inoculation rate in Koukourandoumi and in Baba

Species	Koukourandoumi				Baba			
	HBR (b/p/n)	PR (%)	IR (%)	EIR (ib/p/n)	HBR (b/p/n)	PR (%)	IR (%)	EIR (ib/p/n)
An. gambiae	2.60	85.71	4.40	0.11	4.15	87.03	1.73	0.07
An. nili	0.42	80	0.00	0.00	6.67	94.42	1.45	0.10
An. funestus	-	-	-	-	0.33	81.25	0.00	0.00

HBR: human biting rate, PR: parity rate, IR: infection rate, EIR: entomological inoculation rate

3.3 Parity rates

In Koukourandoumi, examination of the ovaries of 119 *An. gambiae* females and 20 *An. nili* females revealed respective parity rates of 85.71 [CI: 79-92] and 80% [CI: 61-99] (Table 3). The proportion of parous females of *An. gambiae* was statistically identical to that of *An. nili* ($\chi^2 = 0.43$, p = 0.509). In Baba, the parity rates of 87.03 [CI: 82-92], 94.42 [CI: 92-97] and 81.25% [CI: 60-100] were respectively obtained in a total of 185, 287 and 16 females of *An. gambiae*, *An. nili* and *An. funestus*. However, *An. nili* species showed the highest rate of parity ($\chi^2 = 7.94$, p = 0.005). The proportion of parous females in the *An. nili* population in Baba was higher than in Koukourandoumi ($\chi^2 = 6.39$, p = 0.011). But for *An. gambiae*, no significant difference was observed between the proportions of parous females in the populations of the species at the two sites ($\chi^2 = 0.11$, p= 0.744).

3.4 Malaria vectors behaviour

The human landing catches of mosquitoes inside and outside the dwellings allows the identification of the biting behaviour of the anopheline malaria vectors. Estimates of the degree of endophagy and exophagy were obtained when relative proportions of vectors attempting to bite indoor and outdoor were compared. *An. gambiae* females were endophagous with proportions of 53.6% and 59.80% of mosquitoes caught inside houses in Koukourandoumi and in Baba (Table 4). On the other hand, *An. nili* females were exophagous with 55% and 52.81% of mosquitoes caught outside houses in Koukourandoumi and in Baba.

The catching of *Anopheles* females in resting has been performed in 40 bedrooms of each study site. In Koukourandoumi, with 11 *An. gambiae* females caught, the average resting density was 0.275 females per room per day (FBD). In the village of Baba, 56 females of *An. gambiae* and 18 females of *An. funestus* were collected. The mean resting densities were 1.4 FBD and 0.045 FBD for *An. gambiae* and *An. funestus* respectively. The resting density of *An. gambiae* collected at Baba was significantly higher than that obtained in Koukourandoumi (KW = 20; P= 10^{-4}). However, no *An. nili* has not caught at resting inside the bedrooms investigate despite their abundance observed through HLC.

3.5 Circumsporozoite Protein Rate and Entomological Inoculation Rate

In Koukourandoumi, out of a total of 114 *An. gambiae* females tested by Elisa CSP, 5 were found to be infested with *Plasmodium falciparum*, i.e. an infestation rate of 4.40% [CI=

Table 4: Biting behaviour of malaria vectors in Koukourandoumi and in Baba

Espèces	Sites	Number of females collected			Status
		Indoor (%)	(%)	Total	
Anopheles gambiae	Koukourandoumi	67 (53.60)	58 (46.40)	125	Endophagy
	Baba	119 (59.80)	80 (40.20)	199	Endophagy
Anopheles nili	Koukourandoumi	9 (45.00)	11 (55.00)	20	Exophagy
	Baba	151 (47.19)	169 (52.81)	320	Exophagy

Table 5: Annual entomological inoculation rates relative contribution of malaria vectors in Koukourandoumi and in Baba

Study settings	Species	Annual EIR (ib/p/year)	RC (%)
Koukourandoumi	*An. gambiae*	41.76	100
	An. nili	0	0
Baba	*An. gambiae*	25.55	41.18
	An. nili	36.5	58.82
	An. funestus	0	0

EIR: Entomological inoculation rate, RC: Relative contribution of each malaria vector in the infection.

0.6- 8.2]. In Baba, the infestation rate was 1.73% [CI= 0.0-3.7] with 3 infested females out of 173 *An. gambiae* females tested. This rate is not different from that of Koukourandoumi (χ^2 = 1.1709; P = 0.279). For the *An. nili* species, only Baba recorded infections. Thus, out of 276 females tested, 4 were infested with a rate of 1.45% [CI= 0.2-3.4] (Table 3). In Koukourandoumi, the transmission of *P. falciparum* was ensured only by *An. gambiae* with an entomological inoculation rate of 0.11 infectious bites per person per night (ib/p/n) or 41.76 ib/p/year. In Baba, malaria transmission was ensured by *An. gambiae* and *An. nili*. These two species had entomological inoculation rates of 0.07 (ie 25.55 ib/p/year) and 0.10 ib/p/n (ie 36.5 ib/p/year) respectively (Table 5). Definitively, *An. gambiae* is the main vector of malaria in Koukourandoumi while in Baba, malaria transmission is mainly ensured by *An. nili*.

4. Discussions

In Côte d'Ivoire, malaria represents the first cause of morbidity, with 33% of the reasons for consultation. Mosquitos of the Anopheles genus are the vectors of this disease in the country. On the Ivorian coast, very few studies have been carried out on these vectors. The current study aimed to assess the involvement of the malaria vectors in the malaria transmission in Koukourandoumi and in Baba, two rural areas of the Ivorian coast. Mosquitoes sampling was done by Human Landing Catches (HLC) and Pyrethrum Spraying Catches (PSC) methods. The mosquitoes' catching showed a diversity of species dominated by those of the *Culex* genus. According to Carnevale *et al*. (1993) [12], the *Culex* are characteristic mosquitoes of urban areas. Their presence and predominance in Koukourandoumi and Baba could be due to the proximity of these villages to Aboisso and San Pedro cities, which gives them an urban-type landscape. Three anopheline vector species of malaria, namely *An. gambiae*, *An. funestus* and *An. nili*, have been collected in Baba. These species have been reported already by Diakité *et al.* (2015) [18], Touré *et al*. (2018) [23] and Assouho *et al*. (2020) [6], in central, northern and southern Côte d'Ivoire respectively. However, in Koukourandoumi, the absence of *An. funestus* could be due to the scarcity of breeding sites of the species. Indeed, *An. funestus* frequents more or less permanent water reservoirs overgrown with grass then these are almost absent in the environment of Koukourandoumi. *An. gambiae* was the most abundant vector species in Koukourandoumi. In Baba, it was *An. nili* that was the most abundant. Dia *et al*. (2003) [16] did the same observations in Senegal. The presence of *An. nili* in Koukourandoumi and Baba could be linked to the proximity of these sites to different rivers. This has also been observed in Longo and Gossonkaha, villages located near the Bandama River in Côte d'Ivoire (Touré *et al*., 2018) [23]. Some previous studies have also shown that the larvae of this species develop along the banks of streams and large rivers (Hamon & Coz, 1966; Akono *et al*., 2017) [20,4]. However, in Baba, the average annual density of *An. nili* was much higher than in Koukourandoumi. This fact could be due to the Nero and Digboué rivers located on either side of Baba. In fact, during the year, these rivers receive water from the rains and runoff, which allows them to maintain high water levels; this constitutes a significant factor in the proliferation of *An. nili* (Adja *et al*., 2011) [3].

In both sites, Koukourandoumi and Baba, *An. gambiae* aggressiveness rates inside the houses were much higher than those obtained outside. These higher aggressiveness rates inside the house indicate high endophagic rates of *An. gambiae*, characteristic of malaria vectors in rural Africa (Adja *et al*., 2011; Adja *et al*. 2022) [3,1]. The endophagic behaviour of this vector could be explained by the fact that, in rural areas, a part of the population goes home earlier to sleep. In contrast to *An. gambiae*, *An. nili* was found to be exophagous in both sites. Thus, this vector can infect populations before taking shelter. Our results are contrary to those of Adja *et al.* (2011) [3], that showed endophagy of this species in rural areas in Gouin-Houyé. The behaviour of *An. gambiae* and *An. nili* in the villages of Baba and Koukourandoumi could be linked to their adaptation to the semi-rural and semi-urban habits of the population. Indeed, a part of this population returns to their homes early to go to bed while the other one stays out late at night before returning home or sleeps on terraces and verandas because of the heat and the village electricity.

In Koukourandoumi and in Baba, PSC allowed the collection of several females of *An. gambiae* inside houses, which confirms the endophilic behaviour of the species related in previous studies (Adja *et al*. 2015) [2]. The endophilic behaviour of *An. gambiae* females could be explained by the fact that these females prefer to stay indoors to digest after their blood meal. No specimens of *An. nili* was collected inside houses during the PSC throughout the study, which shows the exophilic behaviour of the species in Baba. This result is in line with those of Awono-Ambene *et al*., (2009) [8], Adja *et al*., (2011) [3] and Ossè *et al*., (2019) [22] in Cameroon, in Côte d'Ivoire and Benin respectively.

An. gambiae females were found infested with *plasmodium* in Koukourandoumi and in Baba villages, while females of *An. nili* were infested only in Baba, with high levels of *plasmodium* transmission. These *plasmodium* infestations observed in *An. gambiae* and *An. nili* could be explained by the large proportions of parous females in the populations of these two vectors, which are therefore epidemiologically dangerous. According to Anagonou *et al*. (2015) [5], the more parous females there are, the greater the probability that they would infest.

An. nili represented the principal malaria vector in Baba. However, it is not involved in malaria transmission in Koukourandoumi during the present study.

5. Conclusion

The current study carried out in Koukourandoumi and in Baba, two villages on the Ivorian coast, showed a diversity of species within the Culicidae fauna. The *Culex* genus was predominant and therefore represented the primary source of nuisance for the populations of these villages. Three malaria vectors, *An. gambiae*, *An. funestus* and *An. nili*, have been identified. All these vectors are present in Baba. In contrast, in Koukourandoumi, the species *An. funestus* was absent during the entire study period. In the both sites, *An. gambiae* showed a tendency to endophagy, and *An. nili* to exophagy. The Elisa CSP confirmed that *An. gambiae* is the principal malaria vector in Koukourandoumi. In Baba, this species became the secondary vector, and *An. nili* is the primary vector. The study also contributed to the knowledge of the involvement of *An. nili* in malaria transmission. The behaviour of this species, contrary to that of *An. gambiae* could undermine the effectiveness of the LLINs, the control method chosen by the

NMCP. Also, given the current context of environmental change, it would be interesting to carry out similar studies in the other sites of the NMCP where this vector is present to re-specify its importance in malaria transmission.

Authors' Contributions

Akré M. Adja, Emmanuel Tia and Négnorogo Guindo-Coulibaly conceptualized and designed the study. Négnorogo Guindo-Coulibaly, Emmanuel Tia, Akissi E. C. Anoh, Konan R. M. Azongnibo, Mintokapieu D. S. Kpan, Agnimou M. C. Sadia-Kacou and Moussa Koné conducted the field work. Konan F. Assouho, Nadro W. M. K. Djahouri and Dounin D. Zoh realized the laboratory work. Konan F. Assouho was responsible for the data management. Konan F. Assouho, Négnorogo Guindo-Coulibaly and Konan R. M. Azongnibo analysed the data. Konan F. Assouho, Négnorogo Guindo-Coulibaly and Akré M. Adja 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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