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ORIGINAL RESEARCH ARTICLE

# Resting and feeding behaviour of malaria vectors, in two areas of high long-lasting insecticidal nets coverage in Côte d'Ivoire

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## Abstract

The resting and feeding behaviour of the main malaria vectors was studied in rural areas of Côte d'Ivoire, after Long-Lasting Insecticidal Nets (LLINs) national coverage. Adult mosquitoes were sampled by Human Landing Catches (HLC) and Pyrethrum Spray Catches (PSC). The annual entomological inoculation rate (EIR) was calculated for each species. *An. coluzzii*, *An. gambiae s.s.* and *An. funestus* were the vector species identified in Toumbokro while *An. coluzzii*, *An. funestus* and *An. nili* were identified in Kassiapleu. These vectors feed preferentially on humans in the two villages. In Toumbokro, *An. gambiae s.l.* is exophagic while *An. funestus* is endophagic. In this village, the annual Entomological Inoculation Rate (EIR) average was high and estimated at 228.49 infectious bites per person per year (ib/p/y) with the contribution 80.81 ib/p/y either 35% for *An. gambiae s.l.* and 147.68 ib/p/y either 65% for *An. funestus*. *An. funestus* has become the main vector and provide its own more than half of this transmission. In Kassiapleu, this EIR was also high and estimated at 348.58 ib/p/y. *An. gambiae s.l.* and *An. funestus* have been responsible for 332.15 ib/p/y either 95% and 16.43 ib/p/y either 5%, respectively. *An. gambiae s.l.* is endophagic and has remained the main vector of malaria. This work shows that malaria transmission remains high and is assured by two main vectors (*An. funestus* and *An. gambiae s.l.*) in rural areas of Côte d'Ivoire. These vectors bite both inside and outside the house. Thus, effective malaria control requires combining indoor residual spraying and outdoor control tools at LLINs currently used against the vectors inside the home.

**Keywords** Resting behaviour · Feeding behaviour · *An. funestus* · *An. gambiae s.l.* · LLINs · Côte d'Ivoire

## Introduction

Malaria is a transmissible disease, potentially fatal (WHO 2017). Its transmission, closely linked to environmental conditions, makes the African continent the most malarial area with 95% of cases and 96% of mortality recorded worldwide (WHO 2022). In this continent, the parasites responsible for human malaria are mainly transmitted by primary vector species such as *Anopheles gambiae sensulato* (*s.l.*) Giles, *An. funestus s.l.* Giles and *An. nili s.l.* Theobald (Wiebe et al. 2017; Ossè et al. 2019). *An. gambiae s.l.* is a complex of seven morphologically similar sister species, identifiable by nucleotide replacements of rDNA (Scott et al. 1993; Riehl et al. 2011). Within this complex, *An. gambiae s.s*, *An. coluzzii and An. arabiensis* remain the most widespread vector species in sub-Saharan Africa. They are well adapted to various types of breeding sites such as puddles, shallow

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wells, footprints, rice paddies that are generally common in rural areas (Tia et al. 2016). Under natural conditions, these species are generally endophilic and anthropophilic with a longevity and density more abundant in the rainy season, causing a large number of malaria (Adja et al. 2011). Just like the members of the species complex of *An. gambiae*, *An. funestus s.l.* which is both endophilic and anthropophilic plays an increasingly important role in malaria transmission in several parts of Africa (Fontenille et al. 1990; Dabiré et al. 2007; Russell et al. 2011). Known as a major vector of malaria transmission in the dry season (Adja et al. 2011), *An. funestus* belongs to a group of ten (10) species that are morphologically similar, at least in adulthood (Cohuet et al. 2004; Coetzee et Fontenille 2004; Adja et al. 2011). Within this group, *An. funestus s.s.* is the most anthropophilic species and therefore considered the major vector of this group (Coetzee et Fontenille 2004; Dabiré et al. 2008).

In recent years, several control methods have been considered to reduce the global malaria morbidity and mortality. Vector control is the essential component of the overall control strategy. Indeed, grace to increased international funding, vector control based primarily on the use of Long-Lasting Insecticidal Nets (LLINs) and Indoor Residual Spraying (IRS) has been promoted in the WHO African Region (Ross 1911). These tools (LLINs, IRS), effective against the main vectors (*An. gambiae s.l.* and *An. funestus*) which prefer to feed and stay indoors, have made tremendous progress. They have reduced the number of cases and deaths due to this affection between the years 2010 and 2018 (WHO 2019). The Major promotion of LLINs has resulted in the average household possession and using rates of about 68% and 47% respectively in households (WHO 2022). However, this progress could be jeopardized by several factors including behavioural changes in vectors towards these control tools (Geissbühler et al. 2007; Protopopoff et al. 2008; Kleinschmidt et al. 2015).

In Côte d'Ivoire, malaria transmission is permanent and stable with an upsurge in the rainy season (Fofana et al. 2010; Raso et al. 2012; Edi et al. 2014). Several studies have shown that four vector species, *An. gambiae s.s*, *An. coluzzii*, *An. funestus* and *An. nili*, are responsible for malaria transmission (Diakité et al. 2015; Zogo et al. 2019; Zoh et al. 2020). *An. gambiae s.s* and *An. coluzzii* are the main vectors and are widespread in the country with a predominance of *An. gambiae s.s* in the north in savannah area and *An. coluzzii* in the rest of the country (Tia et al. 2017; Touré et al. 2018; Assouho et al. 2020). Unlike these two vectors, *An. funestus* and *An. nili* are more punctuated in the centre and southeast of the country respectively (Touré et al. 2018; Assouho et al. 2020; Yokoly et al. 2021).

To reduce malaria vector transmission, the National Malaria Control Program (NMCP) of Côte d'Ivoire, following the example of many African countries, adopted preventive control based essentially on the use of LLINs (WHO 2017; WHO 2018). Thus, through mass distribution campaigns, more than 29 million LLINs (14 million in 2014 and 15 million in 2017) were distributed throughout the national territory with a coverage rate of 93% (MSHP 2019). These actions have begun to have an influence positive on malaria mortality and morbidity rates. In fact, the number of deaths due to malaria fell from 3222 in 2017 to 1316 in 2020. e.i. a mortality rate down by around 50%. Also, the national incidence of malaria in children under the age of five fell by 26.06% (WHO/Régional Office for Africa 2022). However, this success could be hampered by changes in vector activity. Indeed, in recent years, several studies have shown that increasing LLIN coverage can lead to a change in vector biting behaviour and have a strong impact on the distribution, diversity of vectors and the level of malaria transmission (Riehle et al. 2011; Moiroux et al. 2012; Sougoufara et al. 2014). Such behavioural changes can have severe implications for the success of vector control programs. As Côte d'Ivoire is part of this context of malaria control based on LLINs using, we sought to know the possible behavioural changes that could take place in the main malaria vectors by setting the objective of assessing their biodynamics, five years after universal coverage in LLINs.

## Materials and methods

### Study area

The study was carried out from February to December 2019 in the Toumbokro and Kassiapleu villages, two rural areas of Yamoussoukro and Man districts respectively. These villages are part of the sentinel sites of the National Malaria Control Programe in Côte d'Ivoire. Yamoussoukro (6°15.7°35 N, 4°40 5°40 W) is located in the pre-forest zone in central Côte d'Ivoire, 240 km from Abidjan. This locality is characterized by a humid tropical climate with two seasons: a long rainy season (from March to October) and a dry season (from November to February). The region's coverage rate in LLINs is estimated at 96.9% in 2017. Precipitation in 2019 was 982.84 mm of water, with an average annual temperature of 26.57 °C (Data source: SODEXAM: Ivorian Society for Development and Exploitation of Meteorological Parameters). Toumbokro (6°54.711'N, 5°29.093'W), located at 27 km from the town of Yamoussoukro (Fig. 1), is characterized by the presence of a permanent watercourse constituting breeding sites for anopheles. Its population, estimated at 1,054 inhabitants, lives mainly from cereal and vegetable cultivation. The houses are made

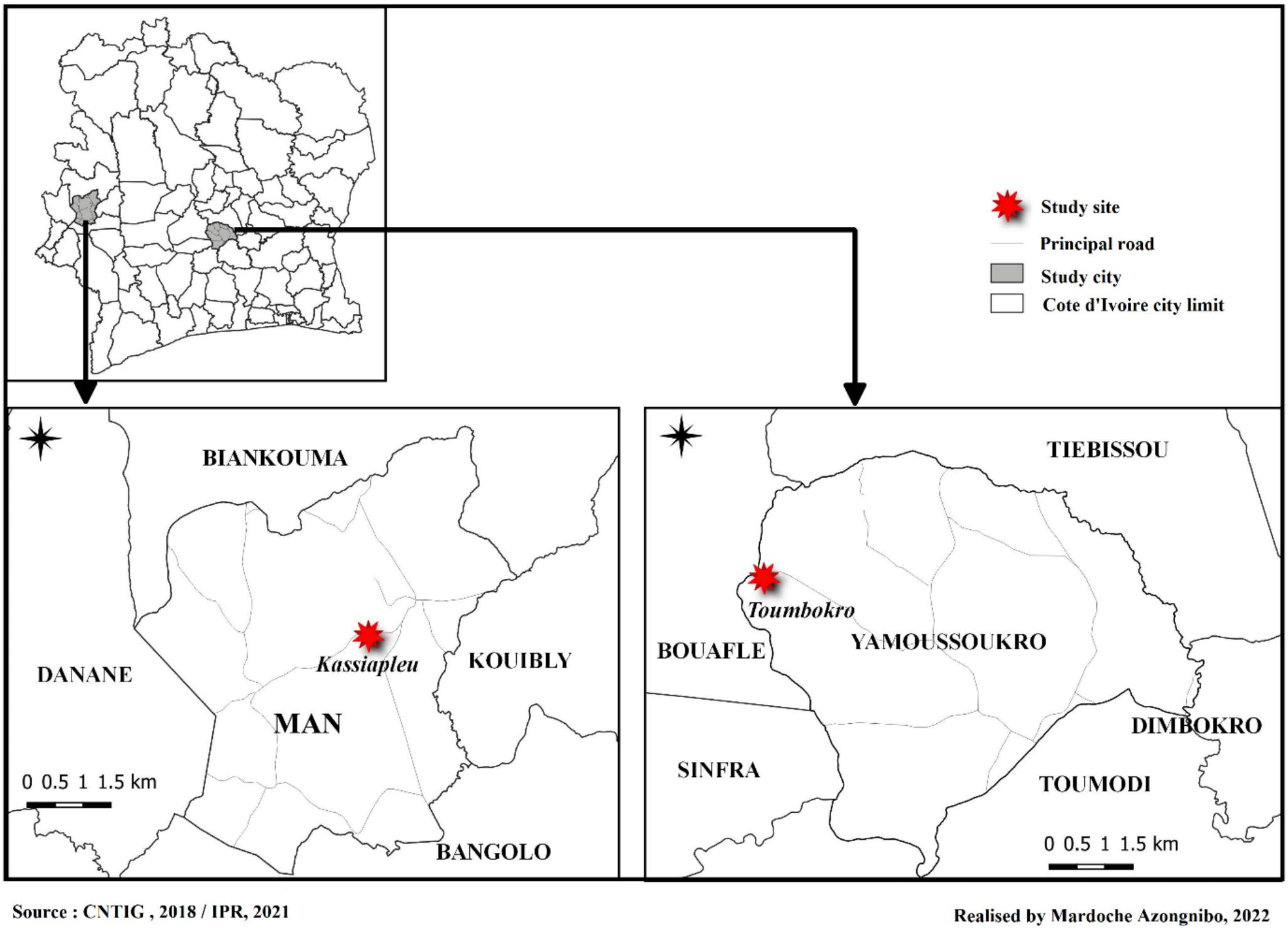


**Fig. 1** Map showing study sites

of earth or cement bricks and covered with a thatched roof or sheet metal.

Located about 580 km from Abidjan, Man district (7°24' N, 7°33' W) is a large city in the western part of Côte d'Ivoire. This city benefits from a humid tropical climate characterized by a long rainy season from February to October and a dry season from November to January. The regional coverage rate in LLINs is estimated at 99% in 2017. The annual rainfall average was 1,314.08 mm, with an annual temperature average of 24.63 °C (Data source: SODEXAM: Ivorian Society for Development and Exploitation of Meteorological Parameters). The village of Kassiapleu (7°21.500'N, 7°37.060'W), located at 9 km from the town of Man is characterized by the presence river and lowlands, the population was estimated at 1,078 inhabitants and lives mainly from the cultivation of coffee, tubers (cassava) and rice. The houses are also made of earth or cement bricks and covered with a thatched roof or sheet metal.

## Mosquito collection and processing

Adult mosquitoes were sampled during four surveys from February to December 2019. In each village, the sampling was conducted using two methods: the Human Landing Catches.

(HLC) from 06:00 p.m. to 08:00 a.m. and the Pyrethrum Spray Catches (PSC) from 6 a.m. to 8 a.m. For the HLC method, adult mosquitoes were sampled, simultaneously inside and outside three randomly selected houses in each site. In each collection point, collectors were organized into two teams. The first team worked from 06:00 p.m. to 01:00 a.m. and the second team from 01:00 a.m. to 08:00 a.m. for two consecutive nights every three month. Overall, this study required 96 man-nights of catches. The PSC was carried out at the same time as the HLC. At each site, a total of 10 houses were selected for each survey. In each house, the entire floor and furniture were covered with white sheets. The house was then sprayed on the walls with a 10% pyrethrin aerosol solution using the method described by Gimnig et al. (2003) and left closed for 10–15 min. The stunned mosquitoes and fell on the white sheet were then collected with tweezers and placed on moist filter paper in petri dishes labelled with the house number and name of the site. The number of people who had slept in the house the previous night was recorded. All the mosquitoes were identified separately on the basis of morphological criteria using the taxonomic determination keys of Mattingly (1971) for Culicidae in general and that of Gillies and De Meillon (1968) for Anophelian species in particular.

## Mosquito physiological status detection

Physiological status was determined only on anopheline vector females captured by SC. The status of female abdomens was examined according to the criteria of Hamon (1964) and the females were grouped according to their detected physiological status (fed, unfed, gravid, half-gravid).

## Mosquito parity determination

The ovaries of *Anopheles* vectors were dissected and observed to determine the degree of coiling of the ovarian tracheoles with the aim of determining their parity status based on the ovarian tracheation method of Detinova (1963). 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.

## Circumsporozoite protein (CSP) testing

The Enzyme-Linked Immunosorbent Assay (ELISA) was used to test the presence of circumsporozoite protein (CSP) of *Plasmodium falciparum.* 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) and Wirtz et al. (1987). 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.

## Analysis of blood meals

A direct enzyme-linked immunosorbent assay (ELISA) was used to identify the source of mosquito blood meals (Beier et al. 1988). In fact, the blood of engorged vector females was screened for potential host antigen reacting it with host-specific antibodies. The choice for antibodies tested was based on the presence of humans and animals in the study area. In our study, monoclonal antibodies from humans, beef, sheep and chicken were tested. These antibodies were all labelled with peroxide and stored at -20 °C. Dilutions of blood meals were carried out with human sera at 1/2000, beef at 1/1000, sheep at 1/500 and chicken at 1/2000.

## Molecular analysis

A DNA sample of 120 females (i.e. 60 females per site) belonging to the *An. gambiae* complex following the protocol of Cornel et al. (1996). Mosquitoes were placed one by one in 1.5 mL Eppendorf tubes. Each mosquito was then crushed in 200 µL of Cetyl trimethyl ammonium bromide (CTAB) 2%, then placed in a water bath. Subsequently, 200 µL of chloroform was added to each tube and centrifuged. The amplification solutions (premix) were prepared with 10 pmol of each primer: 0.2 mM dNTP, 2.5 mM MgCl2, 0.5 U of Taq polymerase, and 4 µl of DNA diluted 1/30 extracted from a single mosquito. This DNA solution is supplemented with water to a volume of 25 µl per reaction. The primers used are: SINE 200 F 5'- TCG CCT TAG ACC TTG CGT TA- 3' and SINE 200 R 5'- CGC TTC AAG AAT TCG AGA TAC – 3'. Electrophoretic migration of the amplified DNA was carried out on a 2% agarose gel, to which 2 drops of ethidium bromide (0.625 mg/mL) were added.

## Entomological parameters measured and data analysis

To assess the behaviour of key vectors and malaria transmission in these localities, the following entomological parameters were calculated: (i) Resting Density (RD) which expresses the number of female vectors anopheles per room per day (FRD); (ii) Human Blood Index (HBI) represents the proportion of blood meals taken from humans by females vectors; (iii) Human Biting Rate (HBR) expressed as the average number of bites of anopheles female vectors received per person per night (b/p/n); (iv) the Parity Rate (PR) which corresponds to the proportion of parous females; (v) the Infection Rate (IR) which corresponds to the proportion of female anopheles infected by *P. falciparum*, vi) the Entomological Inoculation Rate (EIR) corresponding to the number of infectious bites received per person per year. It is obtained by the product of the Human Biting Rate (HBR) and the Infection rate (IR) of mosquitoes collected to humans. Data was entered using Microsoft Office Excel 2016 spreadsheet and then transferred into the software STATA 8.0 (Stata Corporation, College Station, USA) for statistical analysis. The Chi2 ($\chi^2$) test was used to compare the proportions and Wilcoxon-Mann Whitney tests was used to compare the vector densities (human biting rate) between the two villages. The confidence interval was set at 95% and all differences were considered significant at $p < 0.05$.

### Ethics statement

Prior to the start of this study, ethical clearance N/Réf: 107 – 19/ MSHP/CNESVS-KP was obtained from the National Ethics and Research Committee (NERC) of Côte d'Ivoire. In each study site, permission to work was granted by health authorities and the village chief. The community members were informed about the objectives, procedures, potential risks of harm, and benefits of the study. The participation in the mosquito collection was strictly voluntary, and all the collectors were trained in the specific method of mosquito collection prior starting the study. Before starting the study, all volunteers were previously vaccinated against yellow fever and placed on chemoprophylaxis in accordance with the recommendations of the NMCP of Côte d'Ivoire.

## Results

### Mosquito species composition

A total of 6,429 adult female mosquitoes, divided into four genera (*Anopheles*, *Aedes*, *Culex* and *Mansonia*) was collected. Of this total, 5,834 (90.7%) were *Anopheles* identified with a predominance of *An. gambiae s.l.* (3,707/5,834 i.e. 63.54%) followed by *An. funestus* (2,010/5,834 i.e. 34.45%). The genus *Aedes*; *Culex* and *Mansonia* were collected in low proportions with respective values of 0.2%; 0.5% and 8.7% of the total fauna (Table 1).

In Toumbokro, there was a total catch of 3,810 adult mosquitoes including 3,267 (85.8%) Anopheline, 530 (13.9%) *Mansonia* spp, and 13 (0.3%) *Culex* spp which were collected by HLC. *An. gambiae s.l.* and *An. funestus* were the two malaria vectors identified in this village and accounted for 54.3% ($n = 1,773$) and 42.5% ($n = 1,389$) respectively of the anophelian fauna. These vectors were also collected inside the houses using PSC at respective percentages of 66.1 and 32.9. The other anophelian species identified were *An. ziemanne*, *An. paludis*, *An. coustani*, *An. wellcomei* and *An. pharoensis* (Table 1).

In the village of Kassiapleu, the human landing catching (HLC) has made it possible to obtain a total of 2,050 adult mosquitoes, of which 2,004 (97.8%) were anophelian females. In this village, three malaria vectors were identified: *An. gambiae s.l.* ($n = 1,558$) *An. funestus* ($n = 434$) and *An. nili* ($n = 7$) representing respectively 77.74%; 21.66% and 0.35% of the Anophelian fauna. Among these three vectors, only *An. gambiae s.l.* and *An. funestus* were collected using PSC respective percentages of 82.4 and 16.1 (Table 1). Apart from these vectors, two other species of Anopheles, *An. pharoensis* and *An. zemanni* have been identified (Table 1).

### Molecular identification of the species of *An. gambiae* complex

Molecular identification of 120 (i.e. 60 per site) specimens of the *An. gambiae* complex revealed the presence of two species (*An. coluzzii* and *An. gambiae* s.s) with a predominance of *An. coluzzii* in both villages. Indeed, in Toumbokro, *An. coluzzii* represented 60% of individuals tested by PCR against 40% for *An. gambiae s.s*. In the village of Kassiapleu, all the specimens tested by PCR were only *An. Coluzzii* (100%).

### Human biting behaviour

In Toumbokro, the vectors displayed different biting behaviour. *An. gambiae s.l.* was most collected outside the houses with a proportion of 57.47% (1,019/1,773), thus showing an exophagic behaviour. In contrast, *An. funestus* displayed

**Table 1** Composition and abundance of culcidian fauna collected in the villages of Toumbokro and Kassiapleu from February to December 2019

| Species | Human Landing Catches (HLC) | | | Pyrethrum Spray Catches (PSC) | | | |
|---|---|---|---|---|---|---|---|
| | Toumbokro *n* (%) | Kassiapleu *n* (%) | Total *n* (%) | Toumbokro *n* (%) | Kassiapleu *n* (%) | Total *n* (%) | Total *n* (%) |
| *Anopheles. sp* | | | | | | | |
| *An. gambiae s.l.* | 1,773 (46.5) | 1,558 (76) | 3,331 (56.8) | 151 (51) | 225 (82.4) | 376 (66.1) | 3,707 (57.7) |
| *An. funestus s.l.* | 1,389 (36.5) | 434 (21.2) | 1,823 (31.1) | 143 (48.3) | 44 (16.1) | 187 (32.9) | 2010 (31.3) |
| *An. nili s.l.* | 0 | 7 (0.3) | 7 (0.1) | 0 | 0 | 0 | 7 (0.1) |
| Other *Anopheles. sp* | 105 (2.8) | 5 (0.2) | 110 (1.9) | 0 | 0 | 0 | 110 (1.7) |
| Total 1 | 3,267 (85.7) | 2,004 (97.8) | 5,271 (89.9) | 294 (99.3) | 269 (98.5) | 563 (98.9) | 5,834 (90.7) |
| Culicines | | | | | | | |
| *Aedes. sp* | 0 | 10 (0.5) | 10 (0.2) | 0 | 0 | 0 | 10 (0.2) |
| *Culex. sp* | 13 (0.3) | 8 (0.4) | 21 (0.4) | 1 (0.3) | 4 (1.5) | 5 (0.9) | 26 (0.5) |
| *Mansonia. sp* | 530 (13.9) | 28 (1.4) | 558 (9.5) | 1 (0.3) | 0 | 1 (0.2) | 559 (8.7) |
| Total 2 | 543 | 46 | 589 | 2 | 4 | 6 | 595 (9.3) |
| Grand Total | 3,810 (100) | 2,050 (100) | 5,860 (100) | 296 (100) | 273 (100) | 569 (100) | 6,429 (100) |

**Table 2** Biting behaviour of malaria vectors in the villages of Toumbokro and Kassiapleu from February to December 2019

| Species | Setting | Females collected (%) | | Total | Status |
|---|---|---|---|---|---|
| | | Indoor | Out Door | | |
| *An. gambiaes.l.* | Toumbokro | 754 (42.53) | 1019 (57.47) | 1,773 | **Exophagy** |
| | Kassiapleu | 844 (54.17) | 714 (45.83) | 1,558 | **Endophagy** |
| *An. funestuss.l.* | Toumbokro | 733 (52.77) | 656 (47.23) | 1,389 | **Endophagy** |
| | Kassiapleu | 220 (50.11) | 219 (49.89) | 439 | **Endo-exophagy** |

**Table 3** Resting density expressed as number of females per room per day (FRD) and physiological status of *an. Gambiae s.l.* and *An. Funestus* females in Toumbokro and Kassiapleu to 2019

| Sites | Species | | physiological state of the female vectors | | | |
|---|---|---|---|---|---|---|
| | | | Fed | gravid | Other state | Total |
| Toumbokro | *An. gambiae s.l.* | number | 133 | 5 | 13 | 151 |
| | | FRD | 3.33 | 0.13 | 0.33 | 3.78 |
| | | % | 88.08 | 3.31 | 8.61 | |
| Kassiapleu | | number | 45 | 101 | 85 | 231 |
| | | FRD | 1.13 | 2.53 | 2.13 | 5.78 |
| | | % | 19.48 | 47.72 | 36.8 | |
| Toumbokro | *An. funestus* | number | 133 | 6 | 4 | 143 |
| | | FRD | 3.33 | 0.15 | 0.1 | 3.58 |
| | | % | 93.01 | 4.2 | 2.79 | |
| Kassiapleu | | number | 36 | 3 | 5 | 44 |
| | | FRD | 0.9 | 0.08 | 0.13 | 1.1 |
| | | % | 81.82 | 6.82 | 11.36 | |

FRD: number of females vectors per room per day

an endophage behaviour with 52.77% (733/1,389) of specimens collected inside the houses (Table 2).

Unlike this village, in Kassiapleu, *An. gambiae s.l* was endophagus with a proportion of 54.17% (844/1,558) collected inside the houses while *An. funestus* bite both inside (50.11%; 220/434) and outside (49.99; 219/434) the houses (Table 2).

## Mosquito resting behaviour

With regard to resting behavior, *An. gambiae s.l.* and *An. funestus* are endophilic. They were collected in the rooms in a gorged state in general, i.e. 88.08 and 93.01 respectively in Toumbokro and 19.48 and 81.82 respectively in Kassiapleu (Table 3).

## Human blood index

In these two villages *An. gambiae s.l.* and *An. funestus* feed preferentially on humans. They had a human blood index of 96.20% ($n=76$) and 92.71% ($n=89$) respectively in Toumbokro. In the village of Kassiapleu, these vectors are strictly anthropophilic (100%) with $n=38$ for *An. gambiae s.l.* and $n=18$ for *An. funestus*.

## Biting rate and peak biting time

The annual HBR varied from one village to another with a significantly higher value in Toumbokro (65.87 bites per human per night b/h/n, CI: 54.19–77.55) compared to Kassiapleu 41.66 b/h/n (CI: 23.89–59.43; U=42.44; df=1; $P<0.05$). In Toumbokro, the human biting rates of 36.95 b/h/n (CI: 27.2–46.6) and 28.95 b/h/n (CI: 24.2–33) were respectively recorded for *An. gambiae s.l.* and *An. funestus* (Fig. 2). These biting rates were statistically comparable between both species (U=0.05; df=1; $P=0.82$).

In Kassiapleu, the human biting rate average for *An. gambiae s.l.* was 32.5 b/h/n (CI: 27.2–46.6) andwas statistically superior to that of *An. funestus* which was 8.47 b/h/n (CI: 4.25–13.83; U=3.8; df=1; $p=0.05$) (Fig. 3). There was a low aggressive of *An. nili* females of 0.15 p/h/n (CI: -0.001-0.29). Also, there was statistical difference between the aggressiveness of *An. funestus* females recorded in Kassiapleu (8.47 b/h/n) and those of Toumbokro (28.95 b/h/n; U=41.91; df=1; $P<0.05$).

## Parity rate (PR) and infection rate (IR)

The ovaries examination of vector anopheline females permitted to determine an average parity rate of malaria vectors which was 88.59% ($n=1069$; CI: 86.68–90.49) for *An. gambiae s.l.* and 84.18% ($n=924$; CI: 81.82–86.54) for *An. funestus*.

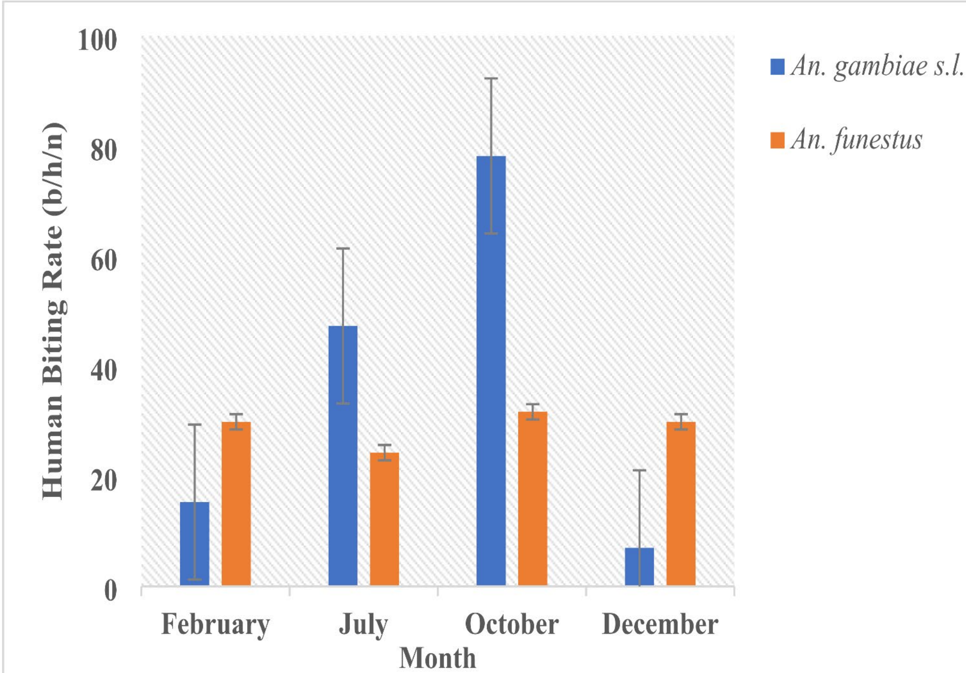


**Fig. 2** Variation of human biting rates of *An. gambiae s.l.*, *An. funestus* in Toumbokro from February to December 2019

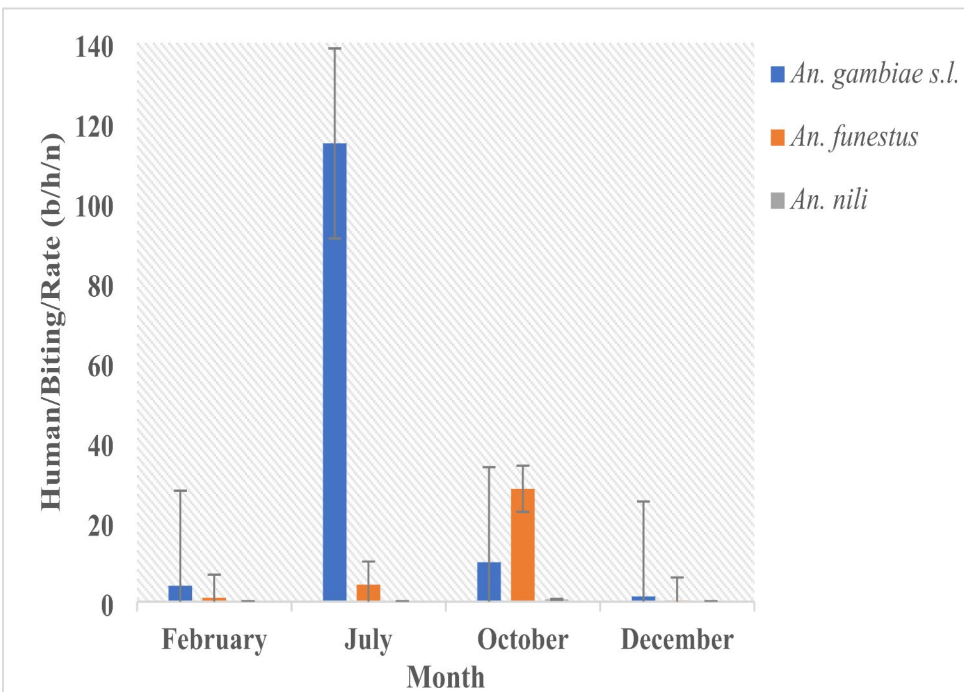


**Fig. 3** Variation of human biting rates of *An. gambiae s.l.*, *An. funestus* and *An. nili* in Kassiapleu from February to December 2019

In Toumbokro the parity rate average was 85.35% ($n=662$; CI: 82.65–88.05) for *An. gambiae s.l.* and 81.07% ($n=729$: CI: 78.22–83.92) for *An. funestus*. These values were statistically different ($\chi^2=4.52$; df = 1; $p<0.05$). In the village of Kassiapleu, the parity rate was 93.86% ($n=407$; CI: 91.51–96.20) for *An. gambiae s.l.*. This value was comparable ($\chi^2=1.02$; df = 1; $p=0.31$) to that of *An. funestus* which was 95.88% ($n=194$ CI: 93.05–96.20). The females of *An. nili* in this village were all parous (PR = 100%; $n=7$).

The Table 4 presents the *P. falciparum* infection rates of vector species in the two villages. Only *An. gambiae s.l.* and *An. funestus* were found infected with *P. falciparum* while no *An. nili* specimens tested were found to be infected.

In Toumbokro out of 1,204 female vectors tested by ELISA CSP, 12 were found infected with *P. falciparum* corresponding to an infestation rate average of 1% (CI: 0.4–1.6). This rate was 0.6% ($n=634$; CI: 0.0-1.2) for *An. gambiaes.l.* and 1.4% ($n=570$; CI: 0.4–2.4) for *An.*

**Table 4** Infection rate of *an. Gambiae s.l.* and *An. Funestus* in the villages of Toumbokro and Kassiapleu in 2019

| Sites | Month | An. gambiae s.l. | | | | An. funestus | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | No tested | No positive | IR (%) | IC (95%) | No tested | No positive | IR (%) | IC (95%) |
| Toumbokro | February | 132 | 2 | 1.5 | 0.0-3.6 | 111 | 2 | 1.8 | 0.0-4.3 |
| | July | 196 | 2 | 1 | 0.0-2.4 | 163 | 3 | 1.8 | 0.0-3.9 |
| | October | 240 | 0 | 0 | | 127 | 2 | 1.6 | 0.0-3.8 |
| | December | 66 | 0 | 0 | | 169 | 1 | 0.6 | 0.0-1.8 |
| | **average** | **634** | **4** | **0.6** | **0.0-1.4** | **570** | **8** | **1.4** | **0.4–2.4** |
| Kassiapleu | February | 53 | 2 | 3.8 | 0.0-9.1 | 0 | 0 | 0 | |
| | July | 241 | 4 | 1.7 | 0.0-3.3 | 23 | 0 | 0 | |
| | October | 59 | 3 | 5.1 | 0.0-10.9 | 143 | 0 | 0 | |
| | December | 7 | 1 | 14.3 | 0.0-49.2 | 28 | 1 | 3.6 | 0.0-10.9 |
| | **average** | **360** | **10** | **2.8** | **1.1–4.5** | **194** | **1** | **0.5** | **0.0-1.5** |

IR: Infection Rate; No: Number of mosquitoes

**Table 5** Entomological inoculation rate and relative contribution of *an. Gambiae s.l. et an. Funestus* in the villages of Toumbokro and Kassiapleu in 2019

| Toumbokro | | | | Kassiapleu | | | |
|---|---|---|---|---|---|---|---|
| Species | No tested | Annual EIR (ib/p/y) | RC (%) | Species | No tested | Annual EIR (ib/p/y) | RC (%) |
| *An. gambiae s.l.* | 634 | 80.81 | 35 | *An. gambiae s.l.* | 360 | 332.15 | 95 |
| *An. funestus* | 570 | 147.68 | 65 | *An. funestus* | 194 | 16.43 | 5 |

No: Number of mosquitoes; RC: Relative contribution of each malaria vector in the transmission; EIR: Entomological Inoculation Rate

*funestus*. Which is more than twice that of *An. gambiae s.l.* No significant differences were observed between the two values ($\chi^2$ = 1.82; df = 1; $P$ = 0.18). Females of *An. funestus* were found to be infected throughout the study period with a rate of 1.7% in the rainy season that is statistically comparable to that observed during the dry season (1.1%; $\chi^2$ = 0.43; df = 1; $P$ = 0.51).

In Kassiapleu, the average of the malaria vector infection rate was 2% ($n$ = 554; CI: 0.8–3.2). This rate was 2.8% ($n$ = 360; CI: 1.1–4.5) for *An. gambiae s.l.* and 0.5% ($n$ = 194; CI: -0.5-1.5) for *An. funestus*. These values are statistically comparable ($\chi^2$ = 3.32; df = 1; $P$ = 0.07). Unlike in the village of Toumbokro, infections of *An. funestus* were observed only in December with a rate of 3.6% (CI: 0.0-10.9) in Kassiapleu (Table 4**).**

## Entomological inoculation rate

During this study, the transmission of *Plasmodium falciparum* was almost permanent and was transmitted by *An. gambiae s.l.* and *An. funestus* in the two villages. In the village of Toumbokro, the annual EIR average was 228,49 infectious bites per person per year (ib/p/y). The contribution of females of *An. gambiae* and *An. funestus* were 80.81 ib/p/y or 35% and 147.68 ib/p/y or 65% respectively. These vectors have together transmitted *P. falciparum* but with a larger share of *An. funestus* which provided more than half of this transmission. *An. funestus* seems to play a major role in malaria transmission in this village (Table 5).

In Kassiapleu, the annual EIR average of *P. falciparum* was estimated at 348.58 ib/p/y. *An gambiae s.l.* and *An. funestus* females are responsible for 332.15 ib/p/y and 16.43 ib/p/y respectively. *An. gambiae s.l.* remains the main vector of malaria in Kassiapleu and provides 95% of the transmission. The remaining 5% was provided by *An. funestus* (Table 5).

# Discussion

A total of 8 species of *Anopheles* genus were identified in both villages, including three malaria vectors that are *An. gambiae s.l.*, *An. funestus* and *An. nili*. The three vectors species reported here were already reported for their involvement in malaria transmission in Côte d'Ivoire (Zogo et al. 2019; Assouho et al. 2020; Adja et al. 2022). The species diversity could be explained by the presence of different ecological factors such as permanent rivers, puddles, small dams, rice-growing areas and vegetable areas. Indeed, these biotopes offer specific breeding sites for the development of larvae (Betsi et al. 2012; Etang et al. 2016; Assouho et al. 2020). *An. gambiae s.l.* and *An. funestus* were the two malaria vectors identified all year round in Toumbokro while in Kassiapleu, in addition to these two vectors, *An. nili* has been identified. The occurrence of these vectors confirms earlier studies by Assouho et al. (2020) and those of other authors (Akono et al. 2017; Tia et al. 2017). Our results showed the abundance of *An. gambiae s.l*, which represented 63% of the anopheline fauna, but also that of

*An. funestus*, vector of malaria, which constituted more than 34.5% of the anopheline fauna. The relative abundance of this Anopheles species might result from a combination of ecological factors as permanent water courses and puddles, favoring the larval development of each species. Our results confirm an earlier study by Doannio et al. (2006) and those some other authors (Koudou et al. 2007; Adja et al. 2011, 2022; Moiroux et al. 2012; Zoh et al. 2020). The presence of *An. nili* in Kassiapleu could be explained by the presence of fast-flowing streams as the rivers which are favorable breeding sites for this species. Indeed, the larval biology of this species has been already described in the basic book of Gillies et De Meillon, 1968 and according to a recent study in Côte d'Ivoire, the preferential lodgings of *An. nili* are the rivers (Adja et al. 2006).

Molecular identification of species of *An. gambiae* complex revealed the presence of two species: *An. coluzzii* and *An. gambiae s.s.*, with a predominance of *An. coluzzii.* The predominance of this vector in both villages is linked to the permanent nature of its breeding sites (sunny stagnant waters; shallow abandoned wells). Our results are similar to the results obtained by other authors in the forest area particularly in the west and south-east of Côte d'Ivoire (Alou et al. 2012; Koffi et al. 2013). That has shown the its ability of *An. coluzzii* to adapt. *An. gambiae s.l.* showed an exophagic biting behaviour in Toumbokro. Which suggests an adaptation in the biting behaviour of this vector due to the introduction of LLINs. Our results confirm the results of the study by Adja et al. 2022 and those of other authors (Sougoufara et al. 2014; Assouho et al. 2020), that have reported an exophagic behaviour of malaria vectors following the introduction of LLINs. Contrary to *An. gambiae s.l.*, *An. funestus* species collected in this village, were exclusively endophagus with a high biting rate despite the presence of LLINs in houses. This could be from by the development of resistance to insecticides used for the treatment of LLINs. Indeed, these last years resistance to different insecticide classes (pyrethroids, carbamates and DDT) was increasingly proven in *An. funestus* in several African countries (Mzilahowa et al. 2016; Riveron 2016). In Kassiapleu, females of *An. funestus* feed both indoor and outdoor in homes. The ability of this vector to bite inside and outside homes in this village could be translated in to the start of a change in its biting behaviour. Similar results have already been reported in southern Benin, in Tanzania and recently in Côte d'Ivoire mainly in Logokaha, Kaforo and Assekro (Russell et al. 2011; Corbel et al. 2012; Adja et al. 2022).

Infection testing of vector mosquitoes for *Plasmodium falciparum* showed that *An. gambiae s.l.* and *An. funestus* were the primary vectors for malaria transmission in both villages. In Toumbokro, these vectors together transmitted *P. falciparum* but with a larger share of *An. funestus*. This vector become pre-empting and ensured 2/3 of malaria transmission. Unlike many studies conducted in Côte d'Ivoire, indicating the involvement of *An. funestus* in malaria transmission in the dry season (Adja et al. 2011; Touré et al. 2018), this study showed its involvement throughout all year with high infestation rate. *An. funestus* has probably gained in importance in recent years and now plays a major role in malaria transmission in rural areas of Côte d'Ivoire. The similar findings were reported in some countries such as Sénégal (Dia et al. 2000) and Benin (Djouaka 2016). In Kassiapleu, *An. gambiae* remained the main vector of malaria and responsible of the majority of transmission e.i.95%. The remaining 5% is provided by *An. funestus* which was involved in the transmission during the dry season, playing thus a secondary vector role in this village. These results were consistent with recently of Adja et al. (2022) and with previous studies made in the country (Adja et al. 2011; Touré et al. 2018).

## Conclusion

The present study shows that malaria vector transmission remains relatively high and ensured by two vectors (*An. gambiae s.l.* and *An. funestus*) in rural areas of Côte d'Ivoire, after repeated mass distribution of LLINs. In Toumbokro, the exophagic biting behaviour of *An. gambiae s.l.* females are well pronounced, which could undermine the current control measures applied in the houses. Also in this village, *An. funestus* is the main vector and ensures the vast majority of malaria transmission. Moreover, this vector has become endophagous even though the LLINs are in the houses. *An. funestus* has certainly begun to have an ability to tolerate insecticides contained in LLINs. In Kassiapleu, *An. gambiae s.l.* is still the main malaria vector and also presents endophagous. Thus, face the high level of vector transmission of malaria and the varied behaviours of malaria vector populations that feed both indoors and outdoors in rural areas, other complementary control strategies such as indoor residual spraying and outdoor control tools (e.g., repellents, automatic insecticide dispensers, fumigation) should be associated with the LLINs currently used against vectors inside the house.

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## Declarations

**Ethical approval** The study protocol was approved by the National Committee for Ethics and Research of Côte d'Ivoire. The informed consent, a letter of agreement and insurance of the participants has been obtained.

**Conflict of interest** The authors declare that they have no conflict of interests.

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