

# High vectorial transmission of malaria in urban and rural settings in the northern, western and eastern regions of Côte d'Ivoire

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## ABSTRACT

**Background & objectives:** Malaria remains a public health problem in Côte d'Ivoire. To cope with this issue, the Ministry of Health established strategies through Long-Lasting Insecticidal Nets (LLINs) and artemisinin-based medicines. To better understand the influence of periodic mass distribution of LLINs on malaria transmission, this entomological survey was conducted in three regions of Côte d'Ivoire.

**Methods:** Mosquitoes were sampled by Human Landing Catches (HLC) in urban and rural settings of Korhogo, Man and Abengourou. Mosquitoes were identified morphologically and by molecular methods. *Plasmodium falciparum* infection was assessed by ELISA, and the Entomological Inoculation Rates (EIR) were calculated for each species and setting.

**Results:** Only *An. gambiae s.l.* was identified in Korhogo and in Abengourou while *An. gambiae s.l.* and *An. funestus s.l.* was reported in Man. *An. coluzzii* was the most abundant species of the *An. gambiae* siblings collected in Abengourou, and in Man while *An. gambiae* was most abundant in Korhogo. In urban settings, malaria vectors showed high aggressiveness (>11 bites per person per night) and the annual EIR was high (83.22-438.44 infectious bites per person per year). In rural settings, malaria vectors showed also high aggressiveness (19-52 b/p/n). The annual EIR is very high (>94 ib/p/yr). However, the weakest EIR was recorded in the northern region with 94.90 ib/p/yr.

**Interpretation & conclusion:** This work indicates that malaria transmission remains high and heterogeneous across Côte d'Ivoire, despite repeated mass distribution of LLINs. Also, in Man, malaria transmission is more intense with the involvement of two main vectors. Furthermore, in the village of Korhogo, the EIR remained relatively low.

**Key words** *Anopheles gambiae*; *Anopheles funestus*; urban; rural; Côte d'Ivoire

## INTRODUCTION

Malaria continues to have a devastating impact on public health and welfare in the African continent. This disease, transmitted by the bite of infected female *Anopheles* mosquitoes, is a major health burden in Côte d'Ivoire<sup>1-2</sup>. The dominant parasite in this country is *Plasmodium falciparum* and this disease remains the leading cause of consultation in health services<sup>2-3</sup>. In the country, four species of *Anopheles* are responsible of malaria transmission<sup>4-6</sup> (*Anopheles gambiae s.s.*, *Anopheles coluzzii*, *Anopheles funestus s.s.* and *Anopheles nili s.s.*).

Various factors contribute to differing malaria epidemiological profiles, including altitude, topography, hydrology and land use/land cover types<sup>7-8</sup>. Specifically, changes in environmental factors can impact malaria transmission by altering the microclimate of the immature stages and adult mosquitoes<sup>9</sup>, as it has been observed that shortening of *Plasmodium* sporogony and vector gonotrophic cycle leads to an increase of malaria transmission

risk across a highland environment in Kenya<sup>10</sup>. The level of malaria transmission is determined by the interactions between *Plasmodium* parasites, the *Anopheles* vectors and the human host. Understanding adult vector population dynamics by identifying the different species, their abundance, biting behaviour and entomological inoculation rates are important steps towards effective control of malaria, with vector abundance being a key determinant of malaria transmission force<sup>11</sup>. Potential reductions of the malaria burden in endemic and epidemic regions depend on the knowledge of malaria-transmitting mosquito species, populations and behavioral characteristics, and malaria exposure risks. In fact, in Côte d'Ivoire, entomological inoculation rates (EIR) of 158 and 339 infectious bites/person/year were respectively observed in Korhogo, in the North region<sup>12</sup> and in Bouaké in the Center<sup>13</sup>, mainly due to *An. gambiae s.l.* Similarly, in the mountainous West region, 289 and 303 infectious bites/person/night were recorded respectively in the localities of Gbatta and Gouin Houyé<sup>4</sup>. In the latter region, malaria transmission

is ensured by two major vectors: *An. gambiae s.l.* and *An. funestus s.l.* Moreover, in 2002, the entomological inoculation rate (EIR) of 789 and 249 infectious bites/ person/ year were respectively observed in Yamoussoukro and Dimbokro in the central region<sup>14</sup>, due to *An. gambiae s.l.* and *An. funestus s.l.* Finally, an entomological inoculation rate due to *An. gambiae s.l.* of 6.2 infectious bites/ person/ year was observed in Allaba in southern Côte d'Ivoire<sup>15</sup>. So, the annual Entomological Inoculation Rate, which measures the exposure to *P. falciparum*-infected mosquitoes annually, was very heterogeneous across the country. To fight this disease, the National Malaria Control Program (NMCP) of Côte d'Ivoire periodically organizes, since 2008, nationwide distributions of LLINs to achieve 100% coverage and 80% utilization combined with a wider availability of RDTs and ACTs for the diagnosis, and treatment of malaria cases. Between 2013 and 2014, e.g., more than 14 million LLINs were distributed to the population (unpublished data from the NMCP). The massive distribution of these control methods recommended by the NMCP should act on the malaria transmission, reducing host-vector contact and human biting rate. To better understand the influence of these large-scale malaria control means on the level of vector transmission, an entomological survey was conducted in urban and rural settings from July 2015 to March 2016 in three regions of Côte d'Ivoire. In fact, this work is a study requested by NMCP after the first distribution campaign, in order to have the status of the situation at the time of the study. These data were used as the basis to follow the trends of the situation.

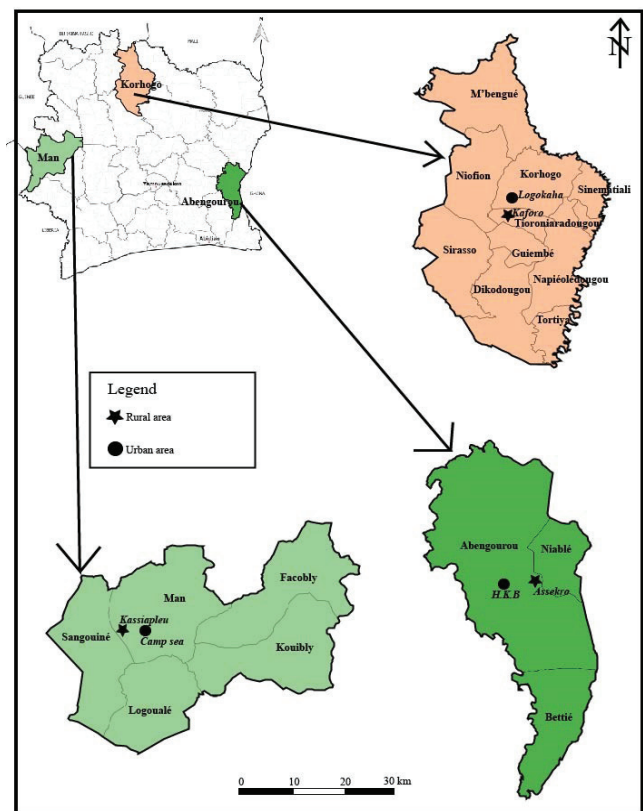
## MATERIAL & METHODS

### Study area

Mosquitoes were collected from three localities in urban area and rural settings: Korhogo (9° 27' 28" N, 5° 37' 46" W) in the northern savannah zone, Man (7° 24' 45" N, 7° 33' 13" W) in the western forest zone and Abengourou (6° 43' 46" N, 3° 29' 47" W) in the eastern forest zone. These localities were chosen because they are part of the sentinel sites of the NMCP and therefore included in the malaria epidemiological surveillance programme. In addition, they are tourist and trade towns and therefore places with high human traffic and activity. Moreover, according to the NMCP, the coverage rate in these localities is over 80%. In fact, in Abengourou, 89,298 households were counted, 73,305 households had at least one LLIN and 207,630 LLINs were distributed, with a coverage rate of 82.09%. In the mountainous zone of Man, 83,660 households were counted, 78,142 households had at least one LLIN and 234,904 LLINs were distributed, suggest-

ing a coverage rate of 93.45%. Finally, in the savannah zone of Korhogo, 198,679 households were counted and 163,302 LLINs were distributed with a coverage rate of 90%.

The northern zone is characterized by two seasons: one long rainy season (March–October) and a short dry season (November–February) with a mean annual temperature of 27°C and more than 1273 mm of water per year. The western and eastern zones are also characterized by two seasons: a long rainy season (March–November) and a short dry season (December–February). It has the mean temperature of 25°C and more than 1345 mm of water per year in these two sites. For a better appreciation of the realities of the field and a good interpretation of the results, two catch areas were chosen in each of the sentinel sites, one in an urban area and the other in a rural area. Thus, the neighborhoods of Logokaha (9° 27' 41" N, 5° 38' 19" W), Camp sea (7° 32' 48" N, 5° 32' 49" W) and H.K.B (6° 43' 46" N, 3° 29' 47" W) were chosen in urban area of Korhogo, Man and Abengourou, respectively. The villages of Kaforo, Kassiapleu (7° 21' 18" N, 7° 48' 47" W) and Assekro were chosen in rural areas of Korhogo, Man and Abengourou respectively (Fig. 1).



Source : Institut Pierre Richet, 2018 Infography : AZONGNIBO K.R.M. and ASSOHO K.F.

Fig. 1: Map of Cote d'Ivoire, showing the locations of the three study sites (the green and brown colors represent the forest and the savannah respectively).

### Study design and adult mosquito sampling

Repeated cross-sectional surveys were carried out to conduct this study every three months. Although data were collected during four months of the year, we considered the climatic seasons (rainy and dry). Two entomological surveys were carried out during the short and long seasons of each climate. However, there is no statistical variation in any of the season sections and these data are sufficient to translate the whole year, hence the extrapolation of the data from the four months of collection for the whole year. Mosquito collections were undertaken using Human Landing Catches (HLC) from July 2015 to March 2016. During two consecutive nights (from 17:00 h to 08:00 h), 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 h to midnight and the second from midnight to 8:00 h. 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). All mosquitoes collected were placed in holding cups labelled by hour until they were processed for morphological identification.

### Morphological identification of *anopheles* species and age-grading methods

After being differentiated at the level of genus as *Culex*, *Anopheles*, *Aedes* or *Mansonia*, *Anopheles*, mosquitoes were identified to species level using the taxonomy and identification key to female Afrotropical anophelines<sup>16–17</sup>. We dissected the ovaries of the females of *Anopheles* vectors (*An. gambiae s.l.* and *An. funestus s.l.*) and observed the degree of coiling of ovarian tracheoles to determine their parity status based on the ovary tracheation method of Dotinova<sup>18</sup>. All collected anopheline females were stored individually in Eppendorf tubes containing desiccant, labelled with 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.

### Determination of sporozoite rates in *An. gambiae s.l.* and molecular identification of *An. gambiae s.l.* complex members

The anopheline mosquitoes collected were tested for

the circumsporozoite (CSP) protein of *Plasmodium falciparum* using an Enzyme-Linked Immunosorbent Assay (ELISA). The head and thorax portion of each anopheles female were separated from the rest of the body. This portion was homogenized in blocking buffer (0.5% Casein, 0.1 N NaOH, 1 × PBS) and examined for the presence of *Plasmodium falciparum* CSP protein, using the method of Burkot *et al.*,<sup>19</sup> as modified by Wirtz *et al.*,<sup>20</sup> which is a reference technique for the detection of plasmodial species. All positive samples were extracted and disliked on new plates in subsequent tests for confirmation. A mosquito sample was considered positive if the optical density (OD) value was higher than twice the mean OD of 4 negative control wells (uninfected mosquitoes) on the ELISA plate. 90 specimens belonging to the *An. gambiae s.l.* siblings were further identified using molecular assays. DNA from the legs and wings of each individual specimen was extracted<sup>21</sup> and Polymerase Chain Reaction (PCR) was conducted to determine species<sup>22</sup>.

### Data processing and statistical analysis

Data were double entered into Microsoft Excel 2013 and transferred to STATA 14 (Stata Corp, College Station, TX, USA) for analysis. The human biting rate (HBR) (number of *Anopheles* per person and per night), Parous female percentages were calculated and the circumsporozoite protein (CSP) positive rate was calculated for each species in each setting. The CSP positive rate was calculated as the proportion of mosquitoes found to be positive for CSP. The EIR was defined as the *Anopheles* density by the CSP and estimated as the number of infectious bites per human per night. Data were analysed using STATA Statistics 14. Chi-square tests were used to compare different proportions and Kruskal-Wallis test was used to compare HBR. All tests were performed at the 5% significance level.

### Ethical statement

This study received the approval of the National Research Ethics Committee of Côte d'Ivoire. This study also received an approval from health authorities of each locality. Permission was sought from households to perform collections in their rooms. 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.

Table 1. Composition and abundance of mosquitoes in urban and rural settings of the three study sites from July 2015 to March 2016

Species	Urban settings			Rural settings			Total n (%)
	Logokaha n (%)	Camp sea n (%)	HKB n (%)	Kaforo n (%)	Kassiapleu n (%)	Assekro n (%)	
<i>Anopheles spp</i>							
<i>An. gambiae s.l.</i>	1876 (71.60)	1373 (76.83)	547 (46.59)	2483 (74.81)	1244 (76.79)	944 (97.32)	8467 (73.69)
<i>An. funestus s.l.</i>	0	21 (01.18)	0	0	317 (19.57)	0	338 (2.94)
Other <i>Anopheles spp</i>	31 (01.18)	2 (0.11)	1 (0.09)	64 (01.93)	18 (01.11)	1 (0.10)	117 (01.02)
Total 1	1907 (72.79)	1396 (78.12)	548 (46.68)	2547 (76.74)	1579 (97.47)	945 (97.42)	8922 (77.65)
Culicines							
<i>Culex spp</i>	506 (19.31)	376 (21.04)	529 (45.06)	233 (07.02)	12 (0.74)	2 (0.21)	1658 (14.43)
<i>Mansonia spp</i>	193 (07.37)	11 (0.62)	82 (06.98)	522 (15.73)	17 (1.05)	17 (01.75)	842 (07.33)
<i>Aedes spp</i>	14 (0.53)	4 (0.22)	15 (01.28)	16 (0.48)	12 (0.74)	6 (0.62)	67 (0.58)
<i>Uranotaenia spp</i>	0	0	0	1 (0.03)	0	0	1 (0.0001)
Total 2	713 (27.21)	391 (21.88)	626 (53.32)	772 (23.26)	41 (02.53)	25 (02.58)	2568 (22.35)
Grand Total	2620 (100)	1787 (100)	1174 (100)	3319 (100)	1620 (100)	970 (100)	11490 (100)

## RESULTS

### Mosquitoes' composition and anopheline fauna diversity

In total 11,490 mosquitoes were collected during the survey period by HLC. Five genera were recorded based on the morphological identification of the specimens, including 77.65% *Anopheles spp.*, 14.43% *Culex spp.*, 7.33% *Mansonia spp.*, 0.58% *Aedes spp.* and 0.0001% *Uranotaenia spp* (Table 1). Overall, 8922 *Anopheles* mosquitoes were morphologically identified, including 8467 *An. gambiae s.l.* and 338 *An. funestus s.l.* The remaining 1.02% (117) of the anopheline species were: *An. pharoensis*, *An. coustani*, *An. ziemanni* and *An. wellcomei*.

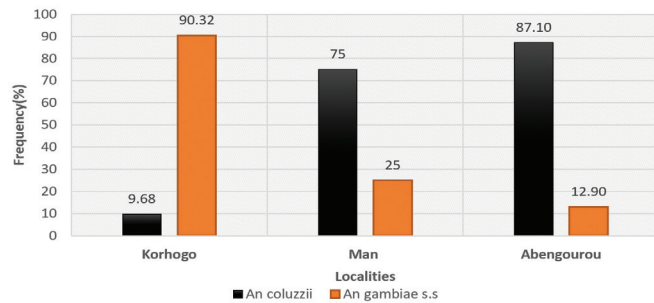


Fig. 2: Distribution of members of *Anopheles gambiae* complex in the three study localities.

*An. gambiae s.l.* was the predominant *Anopheles* species in all settings, both in urban and rural settings, and the only vector species identified in Korhogo (northern region) and in Abengourou (eastern region) while in rural Man (Kassiapleu), two malaria vectors were collected: *An. gambiae s.l.* and *An. funestus s.l.* analysis of *An. gambiae s.l.* siblings in each study sites revealed *An. gambiae s.s.* and *An. coluzzii*. In fact, *An. coluzzii* was predominant in the study sites except Korhogo, in the northern savannah where *An. gambiae s.s.* was most abundant. Thus, there were 90.32% *An. gambiae s.s.* and 9.68 *An. coluzzii* in Korhogo, 75% *An. coluzzii* and 25% *An. gambiae s.s.* in Man and finally 87.10% *An. coluzzii* and 12.90% *An. gambiae s.s.* in Abengourou (Fig. 2). Consequently *An. coluzzii* was the most abundant of the *An. gambiae* siblings collected by HLC in the study sites except Korhogo in the northern savannah where *An. gambiae s.s.* was abundant.

### Biting behaviours and biting rates of 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. The results showed that the biting behaviour of malaria vectors was variable (Table 2). In fact, in the urban settings,

Table 2. Biting behaviour of malaria vectors in urban and rural settings of the three study site from July 2015 to March 2016

Species	Setting	Females collected (%)		Total	Status
		Indoors	Outdoors		
<i>An. gambiae s.l.</i>	Logokaha	952 (50.75)	924 (49.25)	1876	Endo-exophagy
	Camp sea	523 (38.09)	850 (61.91)	1373	Exophagy
	HKB	239 (43.69)	308 (56.31)	547	Exophagy
	Kaforo	1250 (50.34)	1233 (49.66)	2483	Endo-exophagy
	Kassiapleu	632 (50.80)	612 (49.20)	1244	Endo-exophagy
	Assekro	453 (47.99)	491 (52.01)	944	Exophagy
<i>An. funestus s.l.</i>	Kassiapleu	183 (57.73)	134 (42.27)	317	Endophagy

Table 3. Trends of human biting rates with confidential interval in urban and rural sittings of *An. gambiae* in the study sites from July 2015 to March 2016

Species	Months	HBR in urban settings (b/p/n)			HBR in rural settings(b/p/n)		
		Logokaha	Camp sea	H.K.B	Kaforo	Kassiapleu	Assekro
<i>An. gambiae s.l.</i>	July	24.33 (17.67-30.99)	13.25 (7.24-23.66)	10.67 (5.45-15.88)	10.08 (5.86-17.65)	54 (41.30-66.67)	10.75 (7.03-14.47)
	October	116.83 (86.21-147.45)	47.33 (32.54-61.58)	4.58 (1.72-7.44)	90.25 (79.54-116.45)	19.16 (8.64-29.70)	8 (4.28-11.71)
	January	4 (1.78-6.22)	4.16 (2.12-7.67)	3.33 (0.49-6.18)	0	1.33 (0.27-2.39)	1 (0.05-2.05)
	March	10.58 (4.78-16.38)	49.67 (35.56-63.76)	27 (20.08-33.91)	98.58 (84.96-111.89)	28.83 (22.04-35.62)	60.17 (35.24-85.09)
	Average	39.08 (23.82-54.06)	28.60 (18.62-38.59)	11.40 (7.92-14.88)	51.72 (37.18-66.28)	25.92 (19.00-32.67)	19.67 (11.07-28.88)

the anopheline populations feed both indoor and outdoor in Logokaha while they were exophagic in the two other neighbourhoods (Camp sea and HKB). In rural settings, the *An. gambiae s.l.* females in Kaforo feed both indoor and outdoor. The same behavior is observed in the village of Kassiapleu (Man). However, the *An. gambiae s.l.* populations remain exophagic in Assekro (Abengourou) as in urban setting. Otherwise, the *An. funestus s.l.* females, collected in the village of Kassiapleu (Man) were endophagic (57.73%). The HBR of malaria vectors was very high and varied according to species and localities (Table 3). HLC gave an average biting rate of 26.36 *An. gambiae s.l.* bites per person per night in urban settings (39.08 b/p/n in Logokaha, 28.60 b/p/n in Camp sea and 11.40 b/p/n in H.K.B) and 32.44 b/p/n in rural settings (51.72 b/p/n in Kaforo, 25.92 in Kassiapleu and 19.67 b/p/n in Assekro) (Table 3). In urban settings, Logokaha had the highest *An. gambiae s.l.* HBR with 39.08 b/p/n, compared to H.K.B (11.40 b/p/n, CI: 7.91-14.88,  $P < 0.0001$ ). However, this rate is comparable to that obtained from Camp sea, which was 28.60 b/p/n (CI: 18.62-38.59) ( $P=0.20$ ). In Camp sea (Man), the second vector (*An. funestus s.l.*) showed a low density which is 0.39 b/p/n (IC: 0.16-0.63). In rural settings, *An. gambiae s.l.* appeared to be the predominant anopheline species in Kaforo, with a peak biting rate, of 51.72 b/p/n (CI: 37.18-66.28). The HBR of this malaria vector in this village is comparable to that obtained in Kassiapleu which was 25.92 b/p/n (CI: 19-32.67) ( $P=0.0883$ ) and is higher than that observed in Assekro, which was 19.67 b/p/n (CI: 11.08-28.88,  $P=0.0024$ ). Regarding *An. funestus s.l.*, an average biting rate of 6.60 b/p/n (CI: 3.07-10.06) was recorded in Kassiapleu, rural setting of Man. The evolution of vector populations also varied according to seasons, with the highest biting rate recorded at the beginning of the rainy season (March) and in October for *An. gambiae s.l.* and *An. funestus s.l.*

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

The ovaries examination of anophelines females permitted to determine a parity rate of malaria vectors in each setting and overall this rate is high, suggesting that the malaria vectors are aged. In fact, in urban settings, the PR of *An. gambiae s.l.* was significantly higher in Logokaha (100%,  $n=1001$ ) than in Camp sea (99.16%,  $n=713$ , CI: 98.21-99.32) and HKB (89.91%,  $n=426$ , CI: 87.03-92.78) ( $P=0.004$ ). The PR of *An. funestus s.l.* was 100% in Camp sea. In rural settings, the PR in Kaforo was 96.16% ( $n=860$ , CI: 94.88-97.45) for *An. gambiae s.l.* This rate for *An. gambiae s.l.* was lower to that obtained in Kassiapleu which was 98.97% ( $n=678$ , CI: 98.20-99.73) ( $P < 0.001$ ) and higher to that obtained in Assekro which was 82.65% ( $n=461$ , CI: 79.18-86.12) ( $P < 0.001$ ). The females of the second vector (*An. funestus s.l.*) met in Kassiapleu were all aged and able to transmit malaria.

A total of 3668 *Anopheles* mosquitoes (3502 *An. gambiae s.l.* and 166 *An. funestus s.l.*) was tested for *Plasmodium* infection using ELISA CSP and the IR of malaria vectors ranged from 0 to 7.7% (Table 4). The *P. falciparum* IR for *An. gambiae s.l.* did not significantly vary between urban areas and was 1.7% in Logokaha, 4.2% of Camp sea and 2% in H.K.B ( $P=0.72$ ). No infected *An. funestus s.l.* has been recorded in Camp sea. In rural areas, the *P. falciparum* IR for *An. gambiae s.l.* in Kaforo was 0.5%. This rate for *An. gambiae s.l.* is lower than those obtained in Kassiapleu and Assekro which were respectively 5.1% and 6.4% ( $P=0.325$ ). This IR of *An. gambiae s.l.* observed in Kassiapleu was 7.7%.

#### Contribution of malaria vectors to transmission expressed in terms of the EIR

The annual EIR for each malaria species and locality is presented in Table 5. According to this study, the biting and sporozoite rates recorded indicate that *An. gambiae*

Table 4. Number of mosquitoes tested and infection rates of malaria vectors in the three study sites from July 2015 to March 2016

Setting	Month	<i>An. gambiae s.l.</i>			<i>An. funestus s.l.</i>		
		Nb tested	IR (%)	CI (infected)	Nb tested	IR (%)	CI (infected)
Logokaha	July	248	(8)	3.2 (1.0-5.4)			
	October	303	(4)	1.3 (0.0-2.6)			
	January	41	(0)	0			
	March	105	(0)	0			
	Average IR	697	(12)	1.7 (0.8-2.7)	0	(0)	0
Camp sea	July	136	(7)	5.2 (1.4-8.9)	0	(0)	0
	October	151	(3)	2.0 (0.0-4.2)	7	(0)	0
	January	37	(4)	10.8 (0.3-21.3)	4	(0)	0
	March	223	(9)	4.0 (1.4-6.6)	0	(0)	0
	Average IR	547	(23)	4.2 (2.5-5.9)	11	(0)	0
H.K.B	July	129	(1)	0,8 (0,0-2,3)			
	October	46	(2)	4,4 (0,0-10,5)			
	January	35	(0)	0			
	March	236	(6)	2,5 (0,5-4,6)			
	Average IR	446	(9)	2,0 (0,7-3,3)	0	(0)	0
Kaforo	July	154	(0)	0			
	October	208	(1)	0.5 (0.0-1.4)			
	January	0	(0)	0			
	March	272	(2)	0.7 (0.0-1.8)			
	Average IR	632	(3)	0.5 (0.0-1.8)	0	(0)	0
Kassiapleu	July	353	(14)	4.0 (1.9-6.0)	0	(0)	0
	October	91	(9)	9.9 (3.6-16.1)	82	(5)	6.1 (0.8-11.4)
	January	15	(1)	6.7 (5.2-21.0)	0	(0)	0
	March	130	(6)	4.6 (1.0-8.3)	73	(7)	9.6 (2.7-16.5)
	Average IR	589	(30)	5.1 (3.3-6.9)	155	(12)	7.7 (3.5-12.0)
Assekro	July	115	(10)	8.7 (3.5-13.9)			
	October	98	(6)	6.1 (1.3-10.9)			
	January	13	(1)	7.7 (9.7-24.5)			
	March	365	(21)	5.8 (3.4-8.2)			
	Average IR	591	(38)	6.4 (4.5-8.2)	0	(0)	0

Table 5. Entomological inoculation rates in urban and rural settings in the three study sites from July 2015 to March 2016

Study areas	Species	No tested	Annual EIR (ib/p/yr)	RC (%)	
Urban settings	Logokaha	<i>An. gambiae s.l.</i>	697	242.49	100
	Camp sea	<i>An. gambiae s.l.</i>	547	438.44	100
		<i>An. funestus s.l.</i>	11	0	0
	H.K.B	<i>An. gambiae s.l.</i>	446	83.22	100
Rural settings	Kaforo	<i>An. gambiae s.l.</i>	634	94.90	100
	Kassiapleu	<i>An. gambiae s.l.</i>	589	482.50	72
		<i>An. funestus s.l.</i>	155	186.15	38
	Assekro	<i>An. gambiae s.l.</i>	591	459.42	100

No Tested: Number of mosquitoes tested, EIR: Entomological inoculation rate, RC: Relative contribution of each malaria vector in the infection.

*s.l.* and *An. gambiae s.l.* were all vectors of *P. falciparum* in the study settings, although their relative importance varied with the setting. In urban settings, the annual EIR for

*An. gambiae s.l.* was 2-fold higher in Logokaha (242.49 infected bites per person per year) than in H.K.B (83.22 ib/p/y) and lower than that obtained in Camp sea (438.44

ib/p/y). In rural settings, the annual EIR for *An. gambiae s.l.* was 94.90 ib/p/y in Kaforo, 482.5 ib/p/y in Kassiapleu and 459.42 ib/p/y in Assekro. However, in Kassiapleu, the annual EIR for *An. funestus s.l.* was 186.15 ib/p/y. Overall, inhabitants receive more infected bites per year in the settings except in urban H.K.B (urban Abengourou) and in rural Kaforo (rural Korhogo) where the inhabitants are lowly exposed to *Plasmodium*-infected *Anopheles* bites. Taken together, these data indicate that *An. gambiae s.l.* was responsible for most of the transmission of *P. falciparum* parasites in the settings, even if *An. funestus s.l.* was also involved in malaria transmission in Kassiapleu (Man).

## DISCUSSION

The reduction of the malaria burden in endemic and epidemic regions mainly depends on the knowledge of malaria-transmitting mosquito species, populations and behavioural characteristics, as well as malaria exposure risks. The LLINs were scaled-up to prevent malaria transmission in Côte d'Ivoire. The present study carried out in urban and rural settings aimed to better understand the influence of mass distribution of LLINs on vector transmission of malaria in three regions of Côte d'Ivoire. In this study, we have highlighted the relative diversity and abundance of *Anopheles* mosquitoes in three regions of Côte d'Ivoire and assessed their implications in malaria transmission to local communities. Our results have shown that *An. gambiae s.l.* was most the abundant vector of malaria in the three regions<sup>6, 14, 23–24</sup>. *An. gambiae s.l.* and *An. funestus s.l.* were captured all year round and constituted more than 77% of the anopheline fauna. Their occurrence confirms an earlier study by Doucet *et al.*,<sup>25</sup> and those of some other authors<sup>14, 24, 26</sup>. Moreover, *An. gambiae s.l.* was the predominant species in all study sites. The high diversity and variation in the relative abundance of *Anopheles* mosquito species might result from a combination of ecological and climatic factors favouring the larval development of any species. Indeed, permanent water courses, puddles and small dams are probably abundant in these areas. In addition, we found that malaria vectors collected, exhibited high parity rates in all regions, thus suggesting that a significant proportion of the local vector population that have sufficient lifespan allowing for the completion of *Plasmodium* parasite lifecycle and transmission to humans. Similar findings have previously been reported in Côte d'Ivoire<sup>14</sup>. In this study, no *An. nili* species has been identified. This is explained by the rarity of the characteristic lodgings of this species. According to a recent study in Côte d'Ivoire, the preferential lodgings of this species

are the rivers<sup>4</sup>. The larval biology of this species is already described in the basic book of Gillies et De Meillon<sup>16</sup>.

Analysis by PCR of the members of *An. gambiae s.l.* complex has shown that *An. coluzzii* was the dominant species in forest area of Côte d'Ivoire, whilst *An. gambiae s.s.* was the dominant species in the savannah. The relative dominance of these two species may be associated with specific and characteristic breeding sites. The presence of *An. gambiae s.s.* and *An. coluzzii* has been previously reported in Côte d'Ivoire<sup>5–6</sup>. The abundance of *An. coluzzii* in our samples from forest areas could be related to the type of breeding sites and the climatic conditions in these study sites<sup>31–32</sup>. Several studies carried out in Côte d'Ivoire have shown a predominance of *An. coluzzii* in forest area<sup>6, 14</sup>. Therefore, *An. gambiae s.s.* was most abundant in the northern region. This result is similar to that obtained by several other authors<sup>27–28</sup> in the same region, suggesting that environmental conditions in savannah zones are unfavorable for the reproduction and the survival of *An. coluzzii*. This is in-line with observations made by other studies<sup>29–30</sup> which report that these molecular forms are cohesive and constitute exclusive taxonomic groups across their shared range. *An. coluzzii* is more adapted to urbanized and polluted environments<sup>31</sup> and environments having high salinity rates<sup>32</sup>, while *An. gambiae s.s.* seems predominant in semi-urban, rural and arid environments.

In our study settings, malaria vectors were frequently caught outdoors in Camp sea, H.K.B and Assekro, suggesting an important role of LLINs in this exophagic behavior. These observations were recently made by Assouho *et al.*,<sup>6</sup> in the major districts of Côte d'Ivoire. Indeed, several studies in rural eastern Sudan<sup>34</sup>, Benin<sup>35</sup> and Senegal<sup>36</sup> have reported an exophagic behavior of malaria vectors following the introduction of LLINs. In fact, a recent study in Benin also showed that one year after universal coverage, the exophagic rate increased from 45 to 68.1%<sup>37</sup>. The massive introduction of LLINs could, therefore, induce a modification of the biting behavior of malaria vectors. However, *An. funestus s.l.* species in rural Man (Kassiapleu), were exclusively endophagic with a high biting rate despite the presence of LLINs in houses. This could be due to their high resistance to pyrethroid insecticides used to treat LLINs but also raises questions about the current use of LLINs by the populations to avoid the bites of mosquito vectors and to prevent malaria. Nevertheless, in Logokaha, Kaforo and Assekro, malaria vectors feed both indoor and outdoor, thus reflecting the start of a change in vector behaviour.

Our improved understanding of malaria transmission at the local level is essential for the development and implementation of effective vector control strategies.

Thus, to identify potential vector species of malaria in our various study sites, individual females of *An. gambiae s.l.* and *An. funestus s.l.* were tested using the ELISA CSP method for the presence of *P. falciparum* sporozoites. The finding showed that the two species were involved in malaria transmission in Man, while *An. gambiae s.l.* being the main malaria vector in all regions. Although infection rates were almost similar between study sites, the intensity of transmission was very heterogeneous and this study revealed relatively high parity rates of female *Anopheles* ( $\geq 82\%$ ) in the study areas<sup>33</sup>. These variations underline the great variability of parity rates observed sometimes between study sites, and within the same site depending on the species density dynamics. The heterogeneity of malaria transmission observed in the present study is consistent with a recent study<sup>6</sup>. This may also be the effect of differences in intervention strategies coordinated by the National Malaria Control Program (NMCP), especially free distribution of LLINs to vulnerable populations and the use of ACTs for the early treatment of malaria cases. *An. gambiae s.l.* and *An. funestus s.l.* are efficient vectors of *P. falciparum* both in urban and rural settings of Côte d'Ivoire. Although, the intensity of transmission was very heterogeneous. In urban settings malaria transmission was higher in Logokaha and in Camp sea than in H.K.B. It is clear that LLINs have a beneficial effect on the population in the H.K.B neighborhood by reducing the level of malaria transmission. However, this reduction was not sufficient to eliminate the risk of infection of the city dwellers. In rural areas, malaria transmission remains relatively high in both western and eastern regions, but it remains relatively low in northern region. This result is consistent with the findings of Fofana in two villages of the southern region of Côte d'Ivoire<sup>38</sup> and in Benin<sup>39</sup>. This high transmission of malaria could be due to the high infection rate of malaria vectors and could be the result of relatively high parity rates recorded during this study<sup>33</sup>, therefore malaria control interventions should be strengthened to reduce transmission and for the future elimination of malaria. Moreover, high sporozoite infection and parity rates were recorded in all three regions and highlighted the high transmission of malaria within local populations. These findings suggest that either the distributed LLINs are not being used properly by the populations or the local vector populations are developing resistance to the insecticide use in the LLINs.

Between the two species recorded, *An. gambiae s.l.* was responsible for most malaria transmission with *An. funestus* playing a secondary vector role. A similar observation was reported in a savannah area in the central regions of the Côte d'Ivoire<sup>14</sup>. By contrast, in a village of

northern region (Kaforo), the infection rate was low. This result is consistent with the findings of Fofana *et al.*,<sup>38</sup> in two villages of the southern region of Côte d'Ivoire and in Benin<sup>39</sup>.

## CONCLUSION

The present study showed that malaria transmission remains high and heterogeneous across Côte d'Ivoire, despite repeated mass distribution of LLINs. It is also found that in Man (Western region), two vectors are involved while only one is involved in Korhogo (Northern region) and in Abengourou (Eastern region) in the transmission. Malaria transmission is very high in rural settings except Kaforo (Korhogo) where the EIR remained low. In urban settings, malaria transmission is high. In addition, malaria vectors variously observed biting behaviour, which could be a consequence of mass distribution of LLINs by the National Malaria Control Program. The results underscore that these behaviours should be considered in the development of complementary control strategies in both rural and urban settings to effectively control this disease. Indeed, taking into account these results, which show that malaria vector populations feed both indoors and outdoors in urban and rural areas, it would be interesting to combine indoor residual spraying with current control. In addition, outdoor control tools (automatic insecticide dispenser, fumigation) need to be tested as current tools only to target indoor vectors.

Given the high level of transmission in the sampled localities, continued and intensified sensitisation is necessary to increase the use of LLINs. Other means such as indoor residual spraying should also be combined with the currently used LLINs.

*Conflict of interest:* None

## ACKNOWLEDGEMENTS

The authors would like to thank the Coordination of the National Malaria Control Program of Côte d'Ivoire, global fund to fight AIDS, tuberculosis and malaria, study site populations and health authorities for assistance in field surveys.

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Received: 22 November 2020

Accepted in revised form: 12 July 2022