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Vectorial Transmission of Malaria in Major Districts of Côte d'Ivoire

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Abstract

To better understand the influence of periodic mass distribution of Long-Lasting Insecticidal Nets (LLINs) on malaria transmission, a 1-yr entomological survey was conducted in three major districts of Côte d'Ivoire. Mosquitoes were sampled by Human Landing Catches (HLC) in urban and rural areas of San Pedro and Abidjan (coastal), and in Yamoussoukro (central). Mosquitoes were identified morphologically and by molecular methods. The *Plasmodium falciparum* circumsporozoite (CSP) indices were measured by ELISA, and the Entomological Inoculation Rates (EIR) were calculated for each species and area. *Anopheles gambiae s.l.* Giles (Diptera: Culicidae) and *Anopheles nili* Theobald (Diptera: Culicidae) were identified in coastal districts, while *An. gambiae s.l.* and *Anopheles funestus* Giles (Diptera: Culicidae) were reported in the central district. In urban areas, malaria vectors showed a low aggressiveness (<10 bites per person per night), except in Yamoussoukro, where up to 18.9 b/p/n were recorded. The annual EIR was higher in the central urban area (138.7 infected bites per person per year) than in coastal ones (10–62 ib/p/n). In rural areas, malaria vectors were highly aggressive with an average 13 b/p/n for *An. gambiae s.l*, 21.2 b/p/n for *An. nili* and 12 b/p/n for *An. funestus*. The annual EIR ranged between 94.9 and 193.4 infected bites per person per year. This work indicates that, despite repeated mass distribution of LLINs, malaria transmission remains high and heterogeneous across Côte d'Ivoire. Malaria transmission was lower in coastal urban areas than in the central one, and remains high rural areas where two additional *Anopheles* vectors are involved in malaria transmission.

Résumé

Pour mieux comprendre l'influence des distributions périodiques des moustiquaires imprégnées d'insecticide à longue durée d'action (MILDAs) sur la transmission du paludisme, une enquête entomologique d'un an a été menée dans trois grands districts de Côte d'Ivoire. Les moustiques ont été collectés par Capture sur Sujets Humains (CSH) en zones urbaines et rurales de San Pedro et Abidjan (zones côtières) et à Yamoussoukro (centre). Les moustiques ont été identifiés morphologiquement et par biologie moléculaire. Les paramètres entomologiques de la transmission du paludisme ont été calculés pour chaque espèce. *An. gambiae s.l.* et *An. nili* ont été identifiés en zone côtière, tandis que *An. gambiae s.l.* et *An. funestus* ont été capturés au centre du pays. En zones urbaines, les vecteurs du paludisme ont présenté une faible agressivité vis-à-vis de l'homme (<10 piqûres par personne et par nuit), sauf à Yamoussoukro, où jusqu'à 18,9 p/h/n ont été enregistrées. Le Taux d'Inoculation Entomologique (TIE) annuel était plus élevé au centre du pays (138,7 piqûres infectées par personne et par an) que dans les zones côtières (10 à 62 pi/h/a). En zones rurales, les vecteurs du paludisme étaient très agressifs, avec une moyenne de 13 p/h/n pour *An. gambiae s.l*, 21,2 p/h/n pour *An. nili* et 12 p/h/n pour *An. funestus*. Le TIE annuel variait entre 94,9 et 193,4 pi/h/a. Ce travail montre que, malgré la distribution massive et répétée de MILDAs, la transmission du paludisme reste élevée et hétérogène sur le territoire ivoirien. La transmission du paludisme est plus faible dans les zones urbaines côtières que celle du centre, et reste élevée dans les zones rurales où deux autres vecteurs sont impliqués dans la transmission.

Key Words: malaria, Anopheles, urban, rural, Côte d'Ivoire

Despite extensive malaria elimination efforts, there were an estimated 219 million cases and 435,000 related deaths in 2017, which primarily affected the sub-Saharan African population and children under 5 yr of age (Bouomrani and Regaïeg 2018). Control strategies include the distribution of Long-Lasting Insecticidal Nets (LLINs), improved diagnosis using malaria Rapid Diagnostic Tests (RDTs), and wider availability of Artemisinin-based Combination Therapy (ACT). Although these control strategies have been intensified during the past 10 yr in Côte d'Ivoire, *Plasmodium falciparum* malaria, which is transmitted by mosquitoes of the genus *Anopheles*, remains highly endemic. A mean prevalence of 54.1% has been reported by several studies (WHO 2018). Contrarily to Côte d'Ivoire, recent studies have shown that in countries such as Ethiopia, India, Pakistan, Rwanda, and Senegal, enhanced interventions have effectively reduced malaria transmission (Bouomrani and Regaïeg 2018). The heterogeneous results of malaria control programs highlight the complexity of malaria epidemiology and the necessity to adapt interventions to local epidemiological settings.

In Côte d'Ivoire, the transmission of *P. falciparum* is ensured by three main vectors: *Anopheles gambiae* Giles (Diptera: Culicidae), *Anopheles funestus* Giles (Diptera: Culicidae), and *Anopheles nili* Theobald (Diptera: Culicidae). Vector transmission has been extensively studied in the past decade and malaria was described as hyperendemic and stable all year long, with seasonal upsurges (Dossou-Yovo et al. 1995, Koudou et al. 2007). The annual Entomological Inoculation Rate (EIR), which measures the exposure to *P. falciparum*-infected mosquitoes annually, was however very heterogeneous across the country, and ranged between 6 and 789 *P. falciparum*-infected bites per person per year (Dossou-Yovo et al. 1995, Henry et al. 2003, Koudou et al. 2007, Adja et al. 2008, Fofana et al. 2010, Adja et al. 2011). To fight against this disease, the National Malaria Control Program (NMCP) of Côte d'Ivoire has periodically organized, 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). To better understand the influence of these large-scale malaria control means on the level of vector transmission, an entomological survey was conducted from July 2015 to March 2016 in three districts of Côte d'Ivoire.

Material and Methods

Ethics Statement

An ethical clearance for this study was obtained from the National Ethics Review Committee of Côte d'Ivoire. This study also received an approval from health authorities of each district. In each study site, permission to work was granted by the chief of the district or village. Community members were informed in detail on the objectives, procedures, potential risks of harm, and benefits related to the study. Participation in mosquito collection was strictly voluntary, and all collectors were trained on the specific mosquito collection method prior starting the study. All collectors were placed on chemoprophylaxis in accordance with the recommendations of the NMCP of Côte d'Ivoire.

Study Area

Mosquitoes were sampled in Abidjan (5° 18′34″ N, 4° 00′45″ W) and San Pedro (4° 44′41″ N, 6° 38′23″W), in the southern forested area (coastal), and in Yamoussoukro (6° 49′13″N, 5° 16′ 36 ″W), in the pre-forested area (central). The coastal area is characterized by four seasons: a main rainy season (March–July), a minor rainy season (September–November), a main dry season (December–February), and a minor dry season (August), with a mean annual temperature of 28°C and more than 1,500 mm of rainfall per year. The central area is characterized by two seasons: one long rainy season from March to November followed by a short dry season from December to February. The mean annual temperature is 26°C with a mean annual rainfall of 801 mm. In each district, one urban and one rural site were selected. The cities of Djibi, Bardo, and Kokrenou were selected in Abidjan, San Pedro, and Yamoussoukro, respectively. Abidjan being a highly urbanized district, no rural site was selected for the mosquito sampling. The villages of Baba and Toumbokro were then selected as the rural sites in San Pedro and Yamoussoukro, respectively (Fig. 1).

Mosquito Collections and Field Processing

Adult mosquitoes were sampled from July 2015 to March 2016 using Human Landing Catches (HLC). A total of four surveys were conducted during this period: July 2015 (rainy season), October 2015 (rainy season), January 2016 (dry season), and March 2016 (rainy season). In each site, mosquito collections were carried out simultaneously indoors and outdoors at three randomly selected houses between 05:00 pm and 08:00 am. Collectors were organized in teams of two for each collection point and for two consecutive nights. The first team worked from 05:00 pm to midnight and the second one from midnight to 08:00 am. Mosquitoes were recorded by location and hours of capture and were then identified morphologically following the identification keys of Gillies & De Meillon and Gillies & Coetzee (Gillies and De Meillon 1968, Gillies and Coetzee 1987). The parity of each female anopheline mosquito was assessed by dissecting out ovaries and observing the degree of coiling of ovarian tracheoles (Detinova 1960). All collected anopheline females were stored individually in numbered vials with desiccant and preserved at −20°C until processing at the Transrisk laboratory at the Institute Pierre Richet of Bouaké.

Laboratory Mosquito Processing

Heads and thoraces of anopheline females were tested by enzyme-linked immunosorbent assay (ELISA) for *P. falciparum* circumsporozoite protein (CSP) (Burkot et al. 1984, Wirtz et al. 1987). A sample was considered positive if its optical density (OD) value was twofold higher than the mean OD of four negative control wells (uninfected mosquitoes) on the ELISA plate. A random sample of 90 females belonging to the *An. gambiae* complex, together with all CSP-positive anopheline, were further classified by

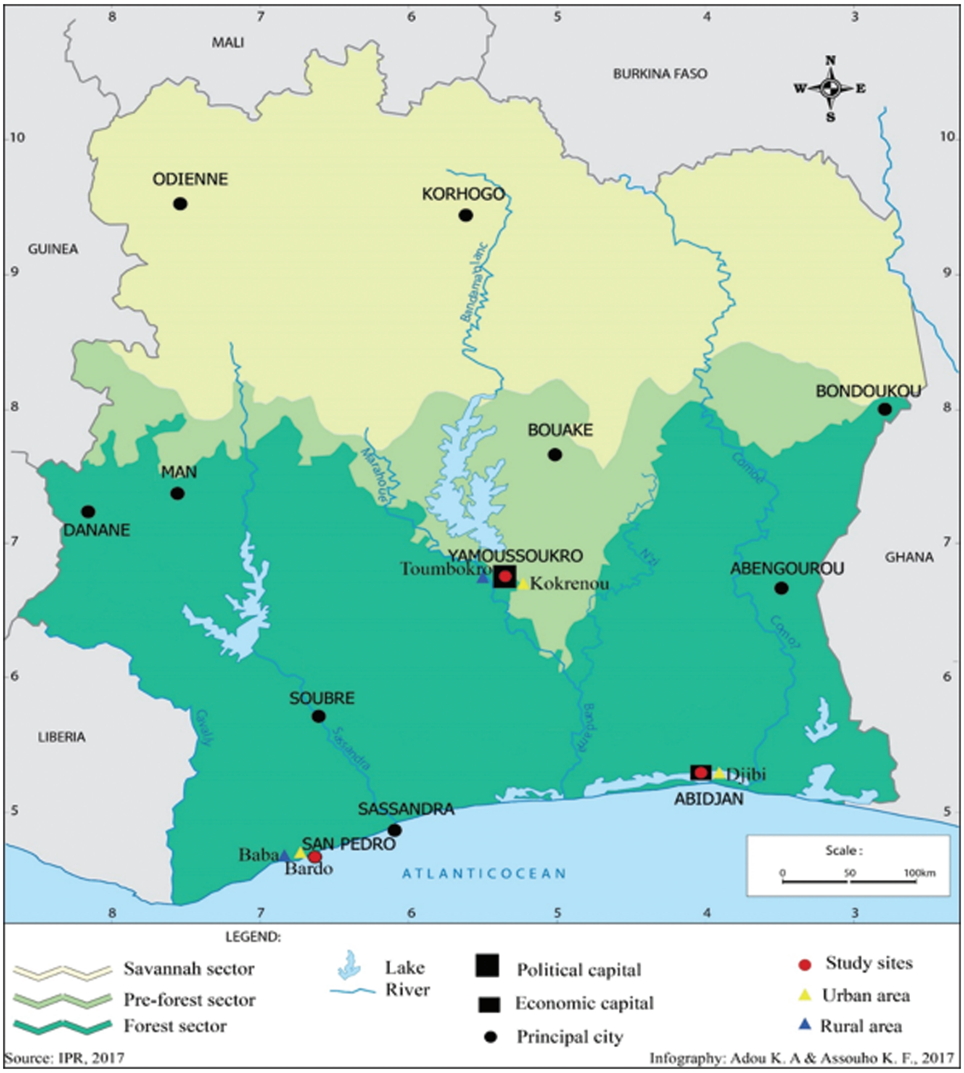

Fig. 1. Map of Cote d'Ivoire, showing the locations of the three study sites.

Table 1. Composition and abundance of mosquitoes in urban and rural settings of three major districts in Côte d'Ivoire

	Urban settings			Rural settings		
	Abidjan	San Pedro	Yamoussoukro	San Pedro	Yamoussoukro	
	Djibi *n* (%)	Bardo *n* (%)	Kokrenou *n* (%)	Baba *n* (%)	Toumbokro *n* (%)	Total *n* (%)
Anopheles spp.						
An. gambiae (s.l)	264 (9.15)	127 (4.03)	910 (40.27)	641 (21.60)	624 (21.57)	2,566 (18.13)
An. funestus	0	0	58 (2.57)	0	708 (24.47)	766 (5.41)
An. nili	0	0	0	1,020 (34.38)	0	1,020 (7.21)
Other *Anopheles* spp.	0	0	15 (0.66)	21 (0.71)	444 (15.35)	480 (3.39)
Anopheles	264 (9.15)	127 (4.03)	983 (43.50)	1,682 (56.69)	1,776 (61.39)	4,832 (34.14)
Culex spp.	604 (23.05)	3,006 (95.40)	1,212 (53.63)	944 (31.82)	201 (6.95)	5,967 (42.15)
Mansonia spp.	2,003 (76.45)	14 (0.44)	60 (2.65)	337 (11.36)	910 (31.46)	3,324 (23.48)
Aedes spp.	12 (0.46)	4 (0.13)	4 (0.18)	4 (0.13)	6 (0.21)	30 (0.21)
Coquilletidia spp.	1 (0.04)	0	1 (0.04)	0	0	2 (0.01)
Total	2,884 (100)	3,151 (100)	2,260 (100)	2,967 (100)	2,893 (100)	14,155 (100)

polymerase chain reaction (PCR) at the species and molecular form levels (Collins et al. 1987, Favia et al. 2001).

Entomological Parameters and Statistical Analysis

The following entomological parameters were 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) was calculated as the proportion of parous females; 3) the infection rate (IR) corresponding to the proportion of females infected by *P. falciparum*; and 4) the 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. The overall EIR for a given period was calculated as the sum of the EIR of each species. Data were analyzed using STATA Statistics 12.0. Chi-square tests were used to compare different proportions, and Kruskal–Wallis test was used to compare HBR between the three sites. All differences were considered significant at $P < 0.05$.

Results

Adult Mosquito Collection

A total of 14,155 female adult mosquitoes were caught by HLC in the three study districts. Overall, 4,832 *Anopheles* mosquitoes were morphologically identified, including 2,566 *An. gambiae s.l.*, 1,020 *An. nili*, and 766 *An. funestus* (Table 1). *An. gambiae s.l.* was the predominant *Anopheles* species in all urban sites, and the only species identified in urban Abidjan and San Pedro (coastal districts). In rural San Pedro (Baba) and Yamoussoukro (Toumbokro), *An. nili* and *An. funestus* were respectively the predominant species. No *An. funestus* was found in coastal districts (Abidjan and San Pedro), and no *An. nili* was found in the central district of Yamoussoukro.

Among the 90 *An. gambiae* s.l samples (3.5%) tested by PCR for the three districts, *An. coluzzii* represented about 97% and *An. gambiae* 3% (Fig. 2).

Biting Behaviors and Biting Rates

Among the 2,566 *An. gambiae s.l.* caught in the three districts, 1,399 (54.5%) were caught outdoors, indicating that this species was globally exophagic. This was confirmed in all study sites except in Bardo (urban San Pedro), where *An. gambiae s.l.* was endophagic (70/127, 55.12%). *An. funestus* caught in the district of Yamoussoukro was also exophagic in both urban (48/58, 82.7%) and rural areas (412/708, 58.2%). The same behavior was observed for *An. nili* in Baba (701/1020, 68.7%). Overall, malaria vectors were predominately exophagic in both urban and rural areas.

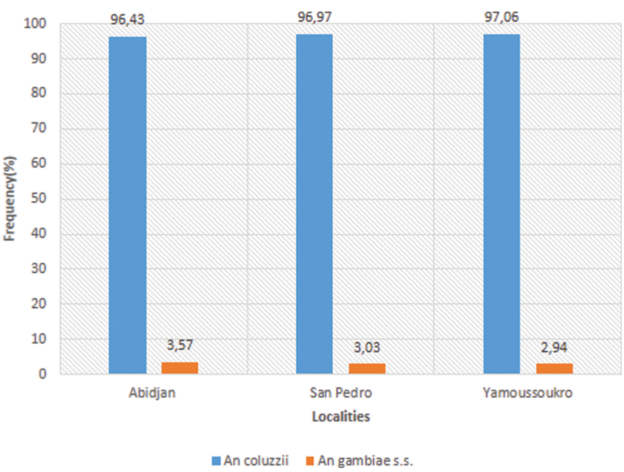

Fig. 2. Distribution of members of *Anopheles gambiae* complex in the three study localities.

The HBR of malaria vectors varied according to species and localities. Human landing catches gave an average biting rate of 9 *An. gambiae s.l.* bites per person per night in urban areas (2.6 b/p/n in Bardo, 5.5 b/p/n in Djibi and 18.9 b/p/n in Kokrenou) and 13.1 b/p/n in rural areas (12.9 b/p/n in Toumbokro and 13.3 b/p/n in Baba) (Table 2). In urban areas, Kokrenou had the highest *An. gambiae s.l.* HBR with 18.9 b/p/n, compared to Djibi (5.5 b/p/n) and Bardo (2.6 b/p/n) ($P < 0.0001$). No difference in *An. gambiae s.l.* biting rate was observed in rural sites.

Anopheles funestus, which was only caught in the district of Yamoussoukro, gave an average biting rate of 1.1 b/p/n in Kokrenou (urban area) and 12 b/p/n in Toumbokro (rural area) (Table 2).

Regarding *An. nili*, an average biting rate of 21.2 b/p/n was recorded in Baba, rural area of San Pedro.

The HBR of malaria vectors also varied according to seasons, with the highest biting rate recorded at the beginning of the rainy season (March) for *An. gambiae s.l.* and *An. funestus* (Table 2). On the contrary, the highest biting rate for *An. nili* was recorded at the end of the rainy season (October).

Parity Rate

In urban settings, the PR of *An. gambiae s.l.* was significantly higher in Kokrenou (98.6%, CI: 97.6–99.6) than in Djibi (81.9%, CI: 77.3–86.5) and Bardo (63.7%, CI: 55.1–72.3) ($P < 0.001$). The PR of *An. funestus* was 100%.

In rural settings, the PR in Baba was 76.5% (CI: 73.1–79.9) for *An. gambiae* and 63.4% (CI: 59.6–67.2) for *An. nili.* The PRs of *An. gambiae s.l.* and *An. funestus* in Toumbokro were 99.7% (CI: 99–100) and 100%, respectively.

IR and EIR

The IR of malaria vectors ranged from 1 to 4% (Table 3). The *P. falciparum* IR for *An. gambiae s.l.* did not significantly vary between urban areas and was 3% (7/242, CI: 0.8–5) in Djibi, 1% (CI: –0.9 to 2.8) in Bardo, and 2% (CI: 0.9–3.7) in Kokrenou ($\chi^2 = 2.7191$; $P = 0.099$). The IR was 4% (CI: 1.2–4.7) for *An. funestus* in Kokrenou.

In rural areas, the *P. falciparum* IRs for *An. gambiae s.l.* and *An. nili* in Baba were 4% (CI: 2.6–6.3) and 1% (CI: –0.1 to 1.3), respectively. This IR was 3% for both *An. gambiae s.l.* (CI: 0.8–4.6) *An. funestus* (CI: 0.9–4.2) in Toumbokro.

The annual EIR for each malaria species and locality is presented in Table 4. In urban areas, the annual EIR for *An. gambiae s.l.* was 12-fold higher in Kokrenou (138.7 infected bites per person per year) than in Bardo (10.9 ib/p/y) and 2-fold higher in Kokrenou than in Djibi (62 ib/p/y). In rural areas, the annual EIR for *An. gambiae s.l.* and *An. nili* were 193.4 ib/p/y and 94.9 ib/p/y in Baba, and 142.3 ib/p/y and 131.1 ib/p/y for *An. gambiae s.l.* and *An. funestus* in Toumbokro. Overall, inhabitants in rural areas receive more infected bites per year than inhabitants in urban areas, except in urban Kokrenou (urban Yamoussoukro) where inhabitants are highly 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 districts of Abidjan, San Pedro and Yamoussoukro, even if *An. nili* and *An. funestus* were also involved in malaria transmission in Baba (San Pedro) and Toumbokro (Yamoussoukro), respectively (Table 4).

Table 2. Trends in human biting rates in urban and rural settings in major districts from July 2015 to March 2016

Species	Months	HBR in urban settings (b/p/n)			HBR in rural settings(b/p/n)	
		Djibi	Bardo	Kokrenou	Baba	Toumbokro
An. gambiae s.l.	July	3.5	0.91	13.7	18	3.1
	Oct.	0.7	0.8	18.4	9.8	10
	Jan.	7.9	2.7	7.6	1.7	1.3
	Mar.	9.8	6.2	36	23.8	37.4
	Average	**5.5**	**2.6**	**18.9**	**13.3**	**12.9**
An. funestus	July	0	0	0	0	2.9
	Oct.	0	0	0	0	17.4
	Jan.	0	0	0	0	5.9
	Mar.	0	0	4.8	0	22
	Average	**0**	**0**	**1.1**	**0**	**12.**
An. nili	July	0	0	0	13.2	0
	Oct.	0	0	0	46.9	0
	Jan.	0	0	0	24.8	0
	Mar.	0	0	0	0	0
	Average	**0**	**0**	**0**	**21.2**	**0**

Table 3. Infection rates in urban and rural settings in major districts from July 2015 to March 2016

		Abidjan		San Pedro		Yamoussoukro	
Settings	Species	IR (%)	CI (95%)	IR (%)	CI (95%)	IR (%)	CI (95%)
Urban	*An. gambiae*	3	0.8–5	1	0–2.8	2	0.9–3.7
	An. funestus	0		0		4	1.2–4.7
	An. nili	0		0		0	
Rural	*An. gambiae*	0		4	2.6–6.3	3	0.8–4.6
	An. funestus	0		0		3	0.9–4.2
	An. nili	0		1	0–1.3	0	

Table 4. EIR in major districts of Côte d'Ivoire from July 2015 to March 2016

Study areas		Species	No Tested	Annual EIR (ib/p/y)	RC (%)
Urban settings	Djibi	*An. gambiae*	242	62.0	100
	Bardo	*An. gambiae*	206	10.9	100
	Kokrenou	*An. gambiae*	470	138.7	88.4
		An. funestus	55	18.2	11.6
Rural settings	Baba	*An. gambiae*	476	193.4	67.1
		An. nili	495	94.9	32.9
	Toumbokro	*An. gambiae*	297	142.3	52
		An. funestus	353	131.4	48

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

Discussion

Misunderstanding vectors that are involved in malaria transmission could lead to a failure of implemented vector control strategies (Molineaux and Gramiccia 1980). In Côte d'Ivoire, LLINs were scaled-up to prevent malaria transmission. The present study aimed to better understand the influence of mass distribution of LLINs on vector transmission of malaria in three districts of Côte d'Ivoire. The longitudinal entomological follow-up carried out in both urban and rural areas from July 2015 to April 2016 allow the identification of 7 *Anopheles* species, among which three were already incriminated in malaria transmission in Côte d'Ivoire (Doucet et al. 1960, Adja et al. 2006). This diversity of species could be explained by a combination of various ecological and climatic factors favorable to the larval development of each species: permanent watercourses, puddles, small dams. Malaria vectors captured in these environments are *An. coluzzii*, *An. gambiae s.s*, *An. nili,* and *An. funestus,* with *An. coluzzii* being the dominant species. This report confirms the existence of *An. gambiae s.l*, *An. nili*, and *An. funestus* in Côte d'Ivoire (Doucet et al. 1960). The dominance of *An. coluzzii* in these localities was previously reported (Akono et al. 2017a, Tia et al. 2017) owing to the permanent nature of this species' breeding sites (Akono et al. 2017b, Ntonga et al. 2017). The distribution and predominance of *An. gambiae s.l* are consistent with previous studies (Coetzee et al. 2000, Wanji et al. 2003, Antonio-Nkondjio et al. 2006). The adaptability of this species to different topographic settings has also been widely reported (Tchuinkam et al. 2010, Tene Fossog et al. 2015).

In our study sites, all malaria vectors were frequently caught outdoors in both urban and rural areas, suggesting an important role of LLINs in this exophagic behavior. Indeed, several studies in rural eastern Sudan (Hamad et al. 2002), Benin (Corbel and N'Guessan 2013), and Senegal (Ndiath et al. 2014) have reported an exophagic behavior of malaria vectors following the introduction of LLINs. A recent study in Benin also showed that 1 yr after universal coverage, the exophagic rate increased from 45 to 68.1% (Moiroux et al. 2012). The massive introduction of LLINs could, therefore, induce a modification of the biting behavior of malaria vectors. However, *An. gambiae s.l.* species in urban San Pedro (Barbo), were exclusively endophagic 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 bed nets.

Anopheles gambiae s.l, *An. funestus,* and *An. nili* are efficient vectors of *P. falciparum* both in urban and rural areas of Côte d'Ivoire. Although infection rates were comparable between study sites, the intensity of transmission was very heterogeneous. In urban areas, malaria transmission was higher in Kokrenou (central) than in Djibi and particularly in Bardo (coastal). It is clear that LLINs have beneficial effect on the population of coastal neighborhoods by reducing the density of vector populations and the level of 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 coastal and central districts. This high transmission of malaria could be due to the high infection rate of malaria vectors. In addition, the presence of several vectors in the same area (Yamoussoukro and San Pedro) could significantly increase the risk of malaria transmission. Malaria control interventions should be strengthened in rural areas where populations have limited access to healthcare to go towards malaria elimination.

Conclusion

This study showed that, despite repeated mass distribution of LLINs, malaria transmission remains high and heterogeneous across Côte d'Ivoire with the presence of several vectors: *An. gambiae s.l.*, *An. nili* and *An. funestus*, with *An. gambiae s.l.* being the dominant species. Malaria vectors were mainly exophagic both in urban and rural areas, which could be a consequence of mass distribution of bed nets by the National Malaria Control Program. Malaria transmission remains high in major districts, but is less intensive in the urban areas of coastal cities (Abidjan and San Pedro) than in the central city of Yamoussoukro. In addition, malaria transmission was more intensive in the rural areas where two additional vectors are involved (*An. funestus* and *An. nili*). The NMCP should take into account the exophagic behavior of malaria vectors in the development of control strategies in both rural and urban areas to effectively control this disease.

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References Cited

Adja, A. M., K. E. N'Goran, P. Kengne, G. B. Koudou, M. Toure, A. A. Koffi, E. Tia, D. Fontenille, and F. Chandre. 2006. [Vectorial transmission of malaria in shrubby Savannah area at Ganse, Ivory Coast]. Med. Trop. (Mars). 66: 449–455.

Adja, A., S. Assi, A. Somian, A. Koffi, A. Alou, J. Djaman, and M. Kone. 2008. Exploitation des bas-fonds et transmission du paludisme en milieu urbain, Adzopé, Côte d'Ivoire. Revue d'Épidémiologie et de Santé Publique 56: 305.

Adja, A. M., E. K. N'goran, B. G. Koudou, I. Dia, P. Kengne, D. Fontenille, and F. Chandre. 2011. Contribution of *Anopheles funestus*, *An. gambiae* and *An. nili* (Diptera: Culicidae) to the perennial malaria transmission in the southern and western forest areas of Côte d'Ivoire. Ann. Trop. Med. Parasitol. 105: 13–24.

Akono, P. N., A. M. Mbida, C. Tonga, A. Y. Kayoum, L. E. Youmbi, and L. Lehman. 2017a. Données préliminaires sur le paludisme humain en zones rurale et sémi-urbaine du département du Nkam (Littoral-Cameroun). J. Appl. Biosci. 115: 11441–11452.

Akono Ntonga, P., C. Tonga, J. A. Mbida Mbida, C. Ndo, P. Awono Ambene, L. G. Lehman, R. Ngaha, O. E. Ngo Hondt, M. F. Peka Nsangou, and N. Tedongmo Lopedji. 2017b. Malaria transmission and the sensitivity of aggressive mosquitoes to insecticides in a poorly urbanized area of the Deido health district in Douala (Cameroon). Med. Sante Trop. 27: 82–89.

Antonio-Nkondjio, C., C. H. Kerah, F. Simard, P. Awono-Ambene, M. Chouaibou, T. Tchuinkam, and D. Fontenille. 2006. Complexity of the malaria vectorial system in Cameroon: contribution of secondary vectors to malaria transmission. J. Med. Entomol. 43: 1215–1221.

Bouomrani, S., and N. Regaïeg. 2018. Épidémiologie du paludisme dans le monde et la Tunisie. Pan African Medical Journal 7: 35.

Burkot, T. R., J. L. Williams, and I. Schneider. 1984. Identification of *Plasmodium falciparum*-infected mosquitoes by a double antibody enzyme-linked immunosorbent assay. Am. J. Trop. Med. Hyg. 33: 783–788.

Coetzee, M., M. Craig, and D. le Sueur. 2000. Distribution of African malaria mosquitoes belonging to the *Anopheles gambiae* complex. Parasitol. Today. 16: 74–77.

Collins, F. H., M. A. Mendez, M. O. Rasmussen, P. C. Mehaffey, N. J. Besansky, and V. Finnerty. 1987. A ribosomal RNA gene probe differentiates member species of the *Anopheles gambiae* complex. Am. J. Trop. Med. Hyg. 37: 37–41.

Coluzzi, M., A. Sabatini, V. Petrarca, and M. Di Deco. 1979. Chromosomal differentiation and adaptation to human environments in the *Anopheles gambiae* complex. Trans. Royal Soc. Trop. Med. Hyg. 73: 483–497.

Corbel, V., and R. N'Guessan. 2013. Distribution, mechanisms, impact and management of insecticide resistance in malaria vectors. 19: 580–633.

Dossou-yovo, J., J. M. Doannio, F. Rivière, and G. Chauvancy. 1995. Malaria in Côte d'Ivoire wet savannah region: the entomological input. Trop. Med. Parasitol. 46: 263–269.

Detinova T. S. 1963. Méthodes à appliquer pour classer par groupes d'âges les diptères présentant une importance médicale. Notamment certains vecteurs du paludisme. WHO Monog Ser. 47:220.

Doucet, J., J. P. Adam, and G. Binson. 1960. Mosquitoes of the Ivory Coast. Annales de Parasitologie Humaine et Comparee 35: 391–408.

Favia, G., A. Lanfrancotti, L. Spanos, I. Sidén-Kiamos, and C. Louis. 2001. Molecular characterization of ribosomal DNA polymorphisms discriminating among chromosomal forms of *Anopheles gambiae s.s.* Insect Mol. Biol. 10: 19–23.

Fofana, D., K. L. Konan, V. Djohan, Y. L. Konan, A. B. Koné, J. M. Doannio, and K. E. N'goran. 2010. [Specific diversity and culicidian nuisance in the villages of N'gatty and Allaba in laguna area of Ivory Coast]. Bull. Soc. Pathol. Exot. 103: 333–339.

Fontenille, D., A. Cohuet, P. H. Awono-Ambene, C. Antonio-Nkondjio, C. Wondji, P. Kengne, I. Dia, D. Boccolini, J. B. Duchemin, V. Rajaonarivelo, et al. 2003. [Systematics and biology of Anopheles vectors of Plasmodium in Africa, recent data]. Med. Trop. (Mars). 63: 247–253.

Gillies, M., and M. Coetzee. 1987. A supplement to the Anophelinae of Africa South of the Sahara. Publ S Afr Inst Med Res 55: 1–143.

Gillies, M. T., and B. De Meillon. 1968. The Anophelinae of Africa south of the Sahara (Ethiopian zoogeographical region), 2nd ed. The South African Institute for Medical Research, Johannesburg. 54: 343.

Hamad, A. A., A. e. l. H. Nugud, D. E. Arnot, H. A. Giha, A. M. Abdel-Muhsin, G. M. Satti, T. G. Theander, A. M. Creasey, H. A. Babiker, and D. E. Elnaiem. 2002. A marked seasonality of malaria transmission in two rural sites in eastern Sudan. Acta Trop. 83: 71–82.

Henry, M. C., C. Rogier, I. Nzeyimana, S. B. Assi, J. Dossou-Yovo, M. Audibert, J. Mathonnat, A. Keundjian, E. Akodo, T. Teuscher, et al. 2003. Inland valley rice production systems and malaria infection and disease in the savannah of Côte d'Ivoire. Trop. Med. Int. Health. 8: 449–458.

Koudou, B. G., A. M. Adja, B. Matthys, M. Doumbia, G. Cissé, M. Koné, M. Tanner, and J. Utzinger. 2007. [Agricultural activities and malaria transmission in two eco-epidemiological settings in central Côte d'Ivoire]. Bull. Soc. Pathol. Exot. 100: 124–126.

Moiroux, N., M. B. Gomez, C. Pennetier, E. Elanga, A. Djènontin, F. Chandre, I. Djègbé, H. Guis, and V. Corbel. 2012. Changes in *Anopheles funestus* biting behavior following universal coverage of long-lasting insecticidal nets in Benin. J. Infect. Dis. 206: 1622–1629.

Molineaux, L., and **G. Gramiccia. 1980**. Le projet Garki. Recherches sur l'épidémiologie du paludisme et la lutte antipaludique dans la savane soudanienne de l'Afrique occidentale.Geneve, Organisation mondiale de la sante. p. 354.

Ndiath, M. O., C. Mazenot, C. Sokhna, and J. F. Trape. 2014. How the malaria vector *Anopheles gambiae* adapts to the use of insecticide-treated nets by African populations. Plos One. 9: e97700.

Ntonga Akono, P., A. Mbouangoro, A. Mbida Mbida, C. Ndo, M. F. Peka Nsangou, and S. Kekeunou. 2017. [The *Anopheles gambiae* species complex and Kdr resistance gene at the periphery of Douala, Cameroon]. Bull. Soc. Pathol. Exot. 110: 122–129.

Tchuinkam, T., F. Simard, E. Lélé-Defo, B. Téné-Fossog, A. Tateng-Ngouateu, C. Antonio-Nkondjio, M. Mpoame, J. C. Toto, T. Njiné, D. Fontenille, et al. 2010. Bionomics of Anopheline species and malaria transmission dynamics along an altitudinal transect in Western Cameroon. BMC Infect. Dis. 10: 119.

Tene Fossog, B., D. Ayala, P. Acevedo, P. Kengne, I. Ngomo Abeso Mebuy, B. Makanga, J. Magnus, P. Awono-Ambene, F. Njiokou, M. Pombi, et al. 2015. Habitat segregation and ecological character displacement in cryptic African malaria mosquitoes. Evol. Appl. 8: 326–345.

Tia, E., M. Chouaibou, C. N. G. Gbalégba, A. M. O. Boby, M. Koné, and A. K. Kadjo. 2017. [Distribution of species and kdr gene frequency among *Anopheles gambiae* s.s. and *Anopheles coluzzii* populations in five agricultural sites in Côte d'Ivoire]. Bull. Soc. Pathol. Exot. 110: 130–134.

Wanji, S., T. Tanke, S. N. Atanga, C. Ajonina, T. Nicholas, and D. Fontenille. 2003. Anopheles species of the mount Cameroon region: biting habits, feeding behaviour and entomological inoculation rates. Trop. Med. Int. Health. 8: 643–649.

WHO. 2018. World malaria report 2018. World Health Organisation, Geneva.http://apps.who.int/iris/bitst ream/handl e/10665 /27586 7/97892 41565 653-eng.pdf?ua. Accessed 24 February 2019.

Wirtz, R. A., T. R. Burkot, P. M. Graves, and R. G. Andre. 1987. Field evaluation of enzyme-linked immunosorbent assays for *Plasmodium falciparum* and *Plasmodium vivax* sporozoites in mosquitoes (Diptera: Culicidae) from Papua New Guinea. J. Med. Entomol. 24: 433–437.