Entomological Indicators of Malaria Transmission Prior to a Cluster Randomised Controlled Trial of a "Lethal House Lure" Intervention in Central Côte D'Ivoire

Rosine Zlanneu Wolie (✉ wolierosine@yahoo.fr)
Université Félix Houphouët-Boigny Unité de Formation et de Recherche en biosciences
https://orcid.org/0000-0002-3499-3114
Alphonsine A. Koffi
Institut National de Santé Publique: Institut National de Sante Publique
Lesley Ayuk-Taylor
Washington State Department of Health
Ludovic P. Ahoua Alou
Institut National de Santé Publique: Institut National de Sante Publique
Eleanore D. Sternberg
Liverpool School of Tropical Medicine
N'Nan Oulo Alla Yvonne
Université Félix Houphouët-Boigny: Universite Felix Houphouet-Boigny
Yao N'Guessan
Institut National de Santé Publique: Institut National de Sante Publique
Amal Dahounto
Institut National de Santé Publique: Institut National de Sante Publique
Welbeck A. Oumbouke
Institut National de Santé Publique: Institut National de Sante Publique
Innocent Z. Tia
Institut National de Santé Publique: Institut National de Sante Publique
Simon-Pierre A. Nguetta
Université Félix Houphouët-Boigny: Universite Felix Houphouet-Boigny
Jackie Cook
London School of Hygiene & Tropical Medicine
Matthew B. Thomas
New York University
Raphael N'Guessan
London School of Hygiene & Tropical Medicine

Research Article

Keywords: Malaria Transmission, Anopheles, Plasmodium, Insecticide resistance genes, Côte d'Ivoire.

Posted Date: December 21st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1146656/v1

Abstract

Background: A study was conducted prior to implementing a cluster randomised controlled trial (CRT) of a lethal house lure strategy in central Côte d'Ivoire and aimed to provide baseline information on malaria vectors in 40 village clusters.

Methods: Human landing catches (HLC) was performed between November-December 2016, capturing mosquitoes indoor and outdoor between 18.00-08.00. Mosquitoes were processed for entomological indicators of malaria transmission (human biting rates, parity rates, sporozoite infection rates and the entomological inoculation rates (EIR)). Species composition and allelic frequencies of *Kdr-w* and *Ace-1^R* mutations were also investigated within the *Anopheles gambiae* complex.

Results: Overall, 15,632 mosquitoes were captured. *Anopheles gambiae s.l.* and *Anopheles funestus* were the two malaria vectors found during the survey period, with predominance for *Anopheles gambiae* s.l. (66.2%) compared to *Anopheles funestus* (10.3%). The mean biting rate for *An. gambiae* s.l. was almost 5 times higher than that for *An. funestus* s.l.(19.8 bites per person per night for *An. gambiae* s. l. *vs* 4.3 bites per person per night for *An. funestus* s. l.) and this was evident indoor and outdoor. *An. funestus* was more competent to transmit malaria parasites in the study area, despite relatively lower number tested for sporozoite index (1.6% (1,373) for *An. gambiae* vs 4.7 % (722) for *An. funestus* s.l.).

There was no significant difference between the proportion infected outdoor and indoor for *An. gambiae* s.l. (1.6% *vs* 1.5%; OR=1.11[0.65-1.9]; P=0.676), but for *An. funestus*, more mosquitoes were infected outdoor (6.4%) than indoor (3.5%) (OR=1.86 [1.07-3.23]; P=0.0249). The majority of both infected vectors with malaria parasites harboured *P. falciparum* (90.6% for *An. gambiae* s. l. and 97, 8% for *An. funestus* s. l.). The EIR for both vectors (0.43 infected bites per night) were similar and there were no significant differences for transmission occurring outdoor and indoor for both species. Of the *An. gambiae* s.l. analysed, only *An. gambiae* (14.1%) and *An. coluzzii* (85.9%) were found. The allelic frequencies of *Kdr* and *Ace-1^R* were higher in *An. gambiae* (0.97 for *Kdr* and 0.19 for *Ace-1^R*) than in *An. coluzzii* (0.86 for *Kdr* and 0.10 for *Ace-1^R*) (P<0.001).

Conclusion: Despite universal coverage of long-lasting insecticidal nets (LLINs) in the area, there was an abundance of malaria vectors in the study in area in central Côte d'Ivoire, specifically highly resistant *An. gambiae* s.l. as well as *An. funestus* s.l.. The malaria sporozoite rate was higher in *An. funestus* s.l than *An. gambiae* s.l.. but EIR rates in these two species were similarly high, both indoor and outdoor. Novel tools or strategies are urgently needed to further reduce malaria transmission in this area.

Background

Malaria is caused by protozoan parasites belonging to the *Plasmodium* genus which are transmitted by the female *Anopheles* mosquito during blood feeding. Over the last 10 years, considerable efforts have been made to control malaria in many parts of the world, especially in Sub-Saharan Africa. This has led to the decline in malaria transmission in many parts of Africa[1, 2]. According to the last world health

organization (WHO) malaria report[3], the significant progress in malaria control can be attributed to a scale-up of vector control interventions, as well as improved diagnostic testing, rapid and efficient treatment of malaria patients. However, despite these considerable efforts to reduce transmission, malaria remains one of the major causes of morbidity and mortality in sub Saharan Africa[1, 3]. Vector control relies on a handful of insecticides used for indoor residual spraying (IRS) and treatment of long-lasting insecticidal nets (LLINs) and insecticide resistance has been widely detected in malaria vectors across the continent[4–7]. The situation is particularly worrying with an increase in intensity and mechanisms of insecticide resistance detected over time [7, 8]. Therefore there is a pressing need for effective, sustainable tools or strategies for malaria control.

The observation that host-seeking African malaria vectors predominantly enter human dwellings through open eaves motivated the development of the EaveTubes technology[9]. EaveTubes arean innovative delivery system where insecticide-treated inserts are placed in tubes installed in the eaves of houses. These inserts enable the transfer of a high dose of insecticide capable of killing even strongly insecticide-resistant *Anopheles* mosquitoes [10]. EaveTubes in combination with screening of windows and doors were found to reduce malaria transmission in a cluster randomized controlled trial (CRT) conducted in central Cote d'Ivoire between 2016 – 2019[11].

Baseline data on entomological parameters, including vector densities, malaria sporozoite rates, and insecticide resistant phenotypes, are valuable for interpreting the impact of an intervention like EaveTubes plus screening.

To collect these data, the current study was conducted prior to start of the CRT between November-December 2016 (the beginning of the dry season), across all study villages selected for the CRT in central Côte d'Ivoire..

Methods

Study Site

The study area has previously been described [12]. Briefly, it is an area of year around malaria transmission, with a major rainy season from April to July, a minor rainy season from August to November, and a dry season from December to March. Malaria transmission peaks after the minor rainy season in November[11].

For the CRT, forty village (clusters) were identified within a 60 kilometres radius around the city of Bouaké. The villages were selected to have 100-600 houses, of which at least 80% were suitable for installation of EaveTubes, and all of the villages were at least 2km apart from each other.

Mosquito Collection

A cross-sectional survey was conducted using human landing catches (HLC). Captures were done in each village over two consecutive nights, both indoor and outdoor, in five randomly selected households. For each capture point, one volunteer collected mosquitoes from 18:00 to 00:00 and a second volunteer took over from 00:00 to 08:00. Volunteers rotated from site to site to account for any possible differences in individual attractiveness to mosquitoes. The mosquitoes collected were kept in cool boxes and transported to the laboratory for processing the next morning.

Identification And Processing Of Mosquitoes

Mosquitoes were first identified using a morphological identification key[13]. Malaria vector species were then kept for additional processing. Due to the large numbers of *An. gambiae s.l.* and *An. funestus s.l.* captured during the HLC, only a subset of samples was analysed further.

For this subset, two to four female mosquitoes were randomly selected per collection site and their ovaries were dissected to determine parity status [14]. Of the parous female mosquitoes, up to 60 per village were randomly selected to be processed for sporozoite infection by quantitative polymerase chain reaction (qPCR) assay[15]. Up to 30 of these parous female mosquitoes were also tested for molecular identification of species[16] and to detect the Knockdown resistance gene L1014F (*Kdr-w*)[17] and the acetylcholinesterase gene G119S ($Ace\text{-}1^R$) mutations[18].

Data analysis

Indoor and outdoor human biting rates (HBR) measured were the mean number of vector bites received per person per night of collection (b/p/n). Parity rate was the proportion of parous mosquitoes over the total dissected. The *Plasmodium* sporozoite infection rate (SIR) in each vector species population was the number of mosquitoes infected with sporozoites, divided by the total number of mosquitoes tested. The nightly entomological inoculation rate (EIR) is the number of infectious bites per person per night. It is conventionally the product of the daily HBR and the SIR from the caught mosquitoes. For this study, nightly EIR was calculated using the following formula:

$$EIR = HBR * SIR \quad (1)$$

$$EIR = \left[\left(\frac{Totalvectorcaught}{Totalcapturenight} \right) * \left(\frac{Totalsporozoitepositive}{(Totaltested + totalnon-parous)} \right) \right] \quad (2)$$

In (2), the first term is HBR and the second is SIR. This approach was used because the SIR was estimated assuming that all non-parous mosquitoes were sporozoite negative.

HBR, parity, SIR, and EIR for indoor and outdoor catches were analysed using generalized linear mixed models (GLMMs) with binomial distribution for SIR and parity rates, and normal distribution using linear mixed model (lmer) for HBR and EIR, in R (version 3.6.3). Capture location was included in the model as

fixed effect and village as random effect. The sporozoite infection rate of identified local malaria vectors were compared using Pearson's chi-square test. The allelic frequencies of the two resistance genes (*Kdr* L1014F and *Ace-1*^{R} G119S) in *An. gambiae* s.l. sibling species were tested to Hardy–Weinberg equilibrium (HWE) conformity using the exact HW test and also compared.

Ethics Clearance

This study followed the ethics principles recommended by the Côte d'Ivoire Ministry of Health ethics committee (ref: 039/MSLS/CNER-dkn), the Pennsylvania State University's Human Research Protection Program under the Office for Research Protections (ref. : STUDY00003899 and STUDY00004815), and the London School of Hygiene and Tropical Medicine ethical review board (No. 11223).

Verbal and written informed consent from all participants were obtained in the local language prior to their enrolment in the study. Volunteer mosquito collectors were well trained on how to collect mosquitoes without being bitten. They received vaccination against yellow fever and the project offered treatment of confirmed malaria cases free of charge, according to the national malaria control programme policy.

Results

Mosquito species composition, density and human biting pattern

A total of 15,632 female mosquitoes were captured using HLC, of which 66.2% (10,350) were *An. gambiae* s.l. and 1,615 (10.3%) were *An. funestus* s.l. (Table 1 and Fig. 1a). These two species were the only vectors of human malaria captured in the area. Both malaria vectors showed early biting activity (from 19.00 onward) to reach a peak around 02.00 (*An. gambiae* s.l.) or 03:00 (*An. funestus* s.l.) (Fig. 2). Biting then decreased steadily, and by dawn (06:00) it fell below 0.2 b/p/n. The mean biting rate for *An. gambiae* s.l. (19.8 b/p/n) was almost 5 times higher than that for *An. funestus* s.l. (4.3 b/p/n) and this was evident both indoor and outdoor (Table 2). Overall, the biting patterns indoor and outdoor were similar for gambiae s.l, however indoor biting was significantly higher than outdoor for *An. funestus* s.l.. (P=0.0088) (Supplementary Materials).

Table 1
Number of mosquitoes collected by human landing catch (HLC)

Mosquito species	Number of females collected (%)	Collection location	
		Number indoor (%)	Number outdoor (%)
An. gambiae s.l.	10,350 (66.2)	5,714 (55.2)	4,636 (44.8)
An. funestus s.l.	1,615 (10.3)	1,034 (64.0)	581 (36.0)
Other *Anopheles spp*	894 (5.7)	428 (47.9)	466 (52.1)
Mansonia sp.	1,990 (12.7)	1,074 (54.0)	916 (46.0)
Culex sp.	764 (4.9)	380 (49.7)	384 (50.3)
Aedes sp.	19 (0.1)	13 (68.4)	6 (31.6)
Total	15,632	8,643 (55.3)	6,989 (44.7)

Table 2
Mean values of entomological malaria parameters

	Capture location	*An gambiae* s.l.	*An funestus* s.l.	Both species combined
HBR (b/p/n) [95% CI]	Indoor	21.2 [10.6-31.8] [a]	5.8 [3.8-7.8] [a]	25.8 [15.2-36.2] [a]
	Outdoor	18.4 [7.8-29.0] [a]	2.8 [0.8-4.8] [b]	20.9 [10.4-31.4] [b]
	Total	19.8 [9.4-30.2]	4.3 [2.6-6.1]	23.2 [12.8-33.6]
Parity (%) [95% CI]	Indoor	95.0 [92.7-97.0] [a]	98.8 [97.4-99.7] [a]	96.9 [95.1-98.3] [a]
	Outdoor	94.3 [91.6-96.5] [a]	98.8 [97.0-99.7] [a]	96.3 [94.1-97.9] [a]
	Total	94.7 [92.3-96.7]	98.8 [97.6-99.6]	96.6 [94.7-98.1]
SIR (%) [95% CI]	Indoor	1.6 [0.8-2.9] [a]	3.5 [2.0-5.5][a]	1.8 [1.0-2.9] [a]
	Outdoor	1.5 [0.7-2.7] [a]	6.4 [3.8-9.9][b]	2.2 [1.3-3.5] [a]
	Total	1.6 [0.8-2.7]	4.7 [2.9-6.6]	2.0 [1.2-3.0]
EIR (ib/p/n) [95% CI]	Indoor	0.22 [0.15-0.38] [a]	0.26 [0.07-0.44] [a]	0.47 [0.27-0.66] [a]
	Outdoor	0.22 [0.11-0.33] [a]	0.25 (0.06-0.44] [a]	0.40 [0.21-0.59] [a]
	Total	0.24 [0.15-0.34]	0.25 [0.08-0.42]	0.43 [0.25-0.61]

SIR Sporozoite infection Rate; *EIR* Entomological Infection Rate; *CI* confidence interval

For each indicator, values in the same column sharing the same superscript letters do not differ significantly (95% confidence interval).

Parity Rate

Parity rate was high for both species caught indoor and outdoor; it averaged 94-95% for *An. gambiae* s.l. and 99% for *An. funestus* s.l., with no significant differences in the rates indoor and outdoor (P > 0.05) (Table 2 and Supplementary Materials).

Plasmodium **sporozoite infection rate**

Overall, infection rate for *An. funestus* s.l. (1.55%) was significantly higher (3-fold) than for *An. gambiae* s.l. (4.66%) (χ^2 = 17.91, P<0.0001). There was no significant difference between the proportion infected outdoor and indoor for *An. gambiae* s.l (1.64% *vs* 1.46%; OR=1.11[0.65-1.9]; P=0.676), but for *An. funestus* s.l., more individuals were infected outdoor (6.4%) than indoor (3.5%) (OR=1.86 [1.07-3.23]; P=0.0249) (Table 2 and Supplementary Materials).

The majority of *An. gambiae* s.l. infected with malaria parasites harboured *P. falciparum* (90.6%), and afew had *P. malariae* (9.4%) (Table 3). There was no *P. ovale* detected in any of the samples tested for *An. gambiae* s.l. Almost all *An. funestus* analysed were infected with *P. falciparum* (97%) and only one individual had *P. ovale* (3%), with no *An. funestus* s.l. testing positive for *P. malariae* (Table 3).

Table 3
Sporozoite infection rate (SIR) and malaria parasites.

Species	N_1 collected	N_2 tested	%SIR [95% CI]	Malaria parasite species			
				N	% P. falciparum (n)	% P. malariae(n)	% P. ovale (n)
An. gambiae s.l.	10,350	1,373	1.55[0.80-2.67]	63	90.6 (59)	9.4(4)	-
An. funestus s.l.	1,615	722	4.66[2.93-6.59]	59	97.0(58)	-	3.0(1)
Overall	11,965	2,095	1.96[1.17-3.02]	122	95.90(117)	3.28(4)	0.82(1)

N_1 number of mosquitoes; *N_2* number of mosquitoes tested, *N* total number of *Plasmodium* spp. *n* is the number positive for the given species.

Entomological Inoculation Rate

The nightly EIR for *An. gambiae* s.l. and *An. funestus* s.l. were similar (0.24 – 0.25 ib/p/n) and there were no significant differences between outdoor and indoor EIR for both species (Table 2).

Frequencies of the Kdr 1014F **and** Ace-1^R 119S **alleles in** An. gambiae **s.l.**

Out of 1,374 *An. gambiae* s.l. mosquitoes analyzed by PCR, 1350 were successfully identified to species (<2% failure rate). Both *An. gambiae* s.s. (n =190; 14.1%) and *An. coluzzii* (n = 1160; 85.9%) were found within the *An. gambiae* complex analysed. For both *Kdr* and *Ace-1^R* genes, the allelic frequencies were higher in *An. gambiae* than in *An. coluzzii* (P<0.001) (Table 4).

Table 4

Kdr L1014F and *ace-1^R G119S* mutation frequencies in *Anopheles gambiae* s.l. populations.

Mutation	Species	N				Allelic frequency	p(HW)
			SS	RS	RR		
Kdr L1014F	*An. coluzzii*	1 145	43	233	869	0.861[a]	0.000
	An. gambiae	187	1	9	177	0.971[b]	0.140
ace-1 G119S	*An. coluzzii*	1 142	949	148	45	0.104[a]	0.000
	An. gambiae	185	121	55	9	0.197[b]	0.362

N number of mosquitoes genotyped; SS: susceptible; *RS* heterozygote; *RR* resistant; *p (HW)* exact Hardy-Weinberg test P-value for each mutation, allelic frequencies with different superscript letters differ significantly between species (G-test, P < 0.05)

For allelic frequency of each gene, values in the column sharing different superscript letters differ significantly (95% confidence interval).

Discussion

Here we have provided a descriptive analysis of the entomological indicators relevant to malaria transmission in central Cote d'Ivoire, prior to the start of a CRT evaluating a new malaria vector control intervention.

The human malaria vector species that we found in the study area at the time of sampling (November – December 2016) were *An. gambiae* s.l. and *An. funestus* s.l., with *An. gambiae* s.l. being more abundant. The predominance of *An. gambiae* s.l. could be explained by the presence of breeding sites favorable to *An. gambiae* s.l. (e.g. rice paddy fields, vegetable plots, marshes) throughout the study area[19–21]. This aligns with previous studies conducted in the same area, and elsewhere in Côte d'Ivoire, which reported the predominance of *An gambiae* s.l. among local malaria vectors [22, 23].

An. gambiae and *An. coluzzii* were the only members of *An. gambiae* s.l. identified in the study area. *An. coluzzii* found in high proportion (85.90%) was consistent with previous findings in the area of Bouaké[21, 24, 25] but contrasts with other studies in the northern savannah of the country, where*An. gambiae* was more prevalent[22, 26]. The difference observed is likely due to variations in mosquito larval habitats; *An. coluzzii* tends to exploit more permanent breeding sites, including those created by the type of irrigation for rice cultivation found in Bouaké and the surrounding area. Permanent availability of breeding sites, due to intensive and perennial agricultural practices could have led to the presence of *An. coluzzii*[27].

We found that increases in biting activity for both species coincided with the time when many people would be going to bed, with a peak in biting around 02:00 for *An. gambiae* s.l. and 03:00 for *An. funestus*

s.l.. This is similar to previous entomological studies conducted in same area around Bouaké[20] as well as the northern part of Cote d'Ivoire [22, 28] and elsewhere in Africa[29–31]. These biting profiles highlight the utility of insecticide treated bednets as a personal protective measure against host-seeking malaria vectors. However, the fact that outdoor biting *An. gambiae* s.l. mosquitoes were found in similar proportion to indoor biting mosquitoes is a sign that people are at risk of malaria transmission when they are outside in the evenings. It further highlights the need for novel strategies or tools to target outdoor malaria transmission[32, 33].

Mean parity rates and sporozoite infection rates were high in both species, especially in *An. funestus* s.l., indicating a high prevalence of older female mosquitoes who had already gone through several cycles of blood-feeding. Despite lower overall numbers, the sporozoite index rate for *An. funestus* s.l. was higher than *An. gambiae* s.l., indicating that it is still an important malaria vector in the area. These results are consistent with findings from previous studies in northern Côte d'Ivoire [22, 28], and show a need to better characterize the biology of *An. funestus* s.l. in this area[34], as well as careful monitoring of the epidemiological significance of *An. funestus* in malaria transmission; since in the current study, lots of clusters didn't have any *An. funestus* s.l. but they could be driving transmission in some clusters.

The mean nightly EIR in our study was 0.43 infected bites per person per night between November-December 2016. By extrapolation, it could correspond to 157 infected bites per person per year. Meta-analysis from a pool of studies conducted in various epidemiological settings across Africa reported EIRs ranging 1 to 1000 infected bites per person per year and that an annual EIR of 150 per person per year was consistently associated with malaria prevalence averaging 75%[29]. Similarity, in a baseline epidemiological study conducted at a similar time, in the same area, prevalence was reported to be 73.9% [11]. The area around Bouaké can therefore be considered as highly endemic for malaria. Moreover, EIR in the study area was equally high indoor and outdoor, possibly due to inconsistent LLIN use, despite universal coverage, or compromised LLIN efficacy due to intense insecticide resistance [8].

Consistent with recent studies carried out in the area of Bouaké [6, 8, 24, 35], there was a high frequency of both *Kdr* and *Ace1*^{R} genes in *An. gambiae* and *An. coluzzii*, with a higher frequency for *An. gambiae.* Resistant individuals seem to have better adaptive response in *An. gambiae* than *An. coluzzii* as evidenced elsewhere in Côte d'Ivoire[7, 36] and other parts in sub-Saharan Africa[22, 37, 38].

Resolving the problem posed by outdoor transmission of malaria has become critical[39, 40] LLINs and IRS are effective strategies controlling malaria but unfortunately they can only operate indoor [41, 42]. Once again the high outdoor transmission of malaria in our study triggers the urgent search for innovative tools or strategies to overcome outdoor transmission of malaria.

Conclusion

Densities of *An. gambiae* s.l. and *An. funestus* s.l. were high in central Côte d'Ivoire prior to the start of a CRT evaluating a new method of malaria vector control. Densities of *An. gambiae* s.l. were higher than

An. funestus s.l., however *An. funestus* s.l. had higher rates of infection with *Plasmodium* parasites (sporozoite index). Consequently, EIR for these two species were similarly high, indicating that both species are probably important malaria vectors in the study area. Moreover, EIR was high both indoors and outdoors, despite universal coverage of LLINs in the area, which reinforces the urgent need for novel tools or strategies to address outdoor malaria transmission.

Abbreviations

WHO
World Health Organization
LLINs
Long Lasting Insecticidal Nets
IRS
Indoor Residual Spraying. L1014F *Kdr*:West knockdown resistance
Ace-1^{R}
Acetylcholinesterase-1 resistance
VCPEC
Vector Control Evaluation Centre
IPR
Institut Pierre Richet
Ace-1^{R} G119S
G119S mutation in *Ace-1*^{R}
OR
odds ratio
R
Resistant
S
Susceptible

Declarations

Acknowledgements

We would like to thank the technical staff at the Institut Pierre Richet, Bouaké, Côte d'Ivoire for their valued support during mosquito collection surveys and laboratory analysis. The authors are very grateful to colleagues from various disciplines from Côte d'Ivoire, especially from the Unité de Recherche et de Pédagogie de Génétique, UFR Biosciences, Université Félix Houphouët-Boigny, Abidjan, for their useful contribution. We also thank the volunteer mosquito collectors in villages for their participation towards the study.

Authors' contributions

RZW, AAK and RN designed the study. RZW, LAT, YN, IZT, WAO and AAPL conducted the field and laboratory. RZW and AD analysed the data. RZW wrote the manuscript. AAK, AAPL, ONA, EDS, JC TBM and RN supervised the study and revised the manuscript. All authors reviewed and approved the final manuscript.

Funding

This study is supported by a grant to the Pennsylvania State University from the Bill & Melinda Gates Foundation (OPP1131603), for evaluating the impact of an intervention composed of household screening plus a novel insecticide delivery system called In2Care EaveTubes.

Availability of data and materials

The datasets supporting the conclusions of this manuscript are included within the manuscript and its additional files, and are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Ethical clearance and consent information are included within the manuscript.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Université Félix Houphouët-Boigny, UFR Biosciences, Unité de Recherche et de Pédagogie de Génétique, Abidjan, Côte d'Ivoire. [2]Vector Control Product Evaluation Centre, Institut Pierre Richet (VCPEC-IPR), Bouaké, Côte d'Ivoire. [3]Institut Pierre Richet (IPR) / Institut National de santé Publique (INSP), Bouaké, Côte d'Ivoire.[4]Washington DC Department of Health, USA, [5]Department of Entomology, Center for Infectious Disease Dynamics, The Pennsylvania State University; University Park, PA, USA. . [6]Department of Vector Biology, Liverpool School of Tropical Medicine, Liverpool L3 5QA, UK, [7]Department of Disease Control, London School of Hygiene and Tropical Medicine, London, UK., [8]Université Alassane Ouattara, Bouaké, Côte d'Ivoire. [9]Innovative Vector Control Consortium, IVCC, Liverpool, UK, [10]London School of Hygiene and Tropical Medicine; International Statistics and Epidemiology Group, Department of Infectious Disease Epidemiology [11]York Environmental Sustainability Institute, University of new York, USA.

References

1. Bhatt S, Weiss DJ, Mappin B, Dalrymple U, Cameron E, Bisanzio D, et al. Coverage and system efficiencies of insecticide-treated nets in Africa from 2000 to 2017. eLife. 2015; 4:e09672
2. Ranson H, N'Guessan R, Lines J, Moiroux N, Nkuni Z, Corbel V. Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? Trends Parasitol. 2011; 27:91–8.
3. World malaria report 2020: 20 years of global progress and challenges.: 299.
4. Riveron JM, Irving H, Ndula M, Barnes KG, Ibrahim SS, Paine MJI, et al. Directionally selected cytochrome P450 alleles are driving the spread of pyrethroid resistance in the major malaria vector *Anopheles funestus*. Proc Natl Acad Sci USA. 2013; 110:252–7.
5. Mulamba C, Riveron JM, Ibrahim SS, Irving H, Barnes KG, Mukwaya LG, et al. Widespread Pyrethroid and DDT Resistance in the Major Malaria Vector *Anopheles funestus* in East Africa Is Driven by Metabolic Resistance Mechanisms. PLoS ONE. 2014; 9:e110058.
6. Koffi AA, Ahoua Alou LP, Adja MA, Chandre F, Pennetier C. Insecticide resistance status of *Anopheles gambiae* s.s population from M'Bé: a WHOPES-labelled experimental hut station, 10 years after the political crisis in Côte d'Ivoire. Malaria J. 2013;12:151.
7. Kouassi BL, Edi C, Tia E, Konan LY, Akré MA, Koffi AA, et al. Susceptibility of Anopheles gambiae from Côte d'Ivoire to insecticides used on insecticide-treated nets: evaluating the additional entomological impact of piperonyl butoxide and chlorfenapyr. Malar J. 2020;19:454.
8. Oumbouke WA, Pignatelli P, Barreaux AMG, Tia IZ, Koffi AA, Ahoua Alou LP, et al. Fine scale spatial investigation of multiple insecticide resistance and underlying target-site and metabolic mechanisms in Anopheles gambiae in central Côte d'Ivoire. Sci Rep. 2020; 10: 15066.
9. Knols BGJ, Farenhorst M, Andriessen R, Snetselaar J, Suer RA, Osinga AJ, et al. Eave tubes for malaria control in Africa: an introduction. Malaria J. 2016; 15:404.
10. Andriessen R, Snetselaar J, Suer RA, Osinga AJ, Deschietere J, Lyimo IN, et al. Electrostatic coating enhances bioavailability of insecticides and breaks pyrethroid resistance in mosquitoes. Proceedings of the National Academy of Sciences. 2015; 112:12081–6.
11. Sternberg ED, Cook J, Alou LPA, Assi SB, Koffi AA, Doudou DT, et al. Impact and cost-effectiveness of a lethal house lure against malaria transmission in central Côte d'Ivoire: a two-arm, cluster-randomised controlled trial. The Lancet. 2021; 397:805–15.
12. Sternberg ED, Cook J, Ahoua Alou LP, Aoura CJ, Assi SB, Doudou DT, et al. Evaluating the impact of screening plus eave tubes on malaria transmission compared to current best practice in central Côte d'Ivoire: a two armed cluster randomized controlled trial. BMC Public Health. 2018; 18: 894.
13. Gillies MT, Meillon B de. The Anophelinae of Africa south of the Sahara. Suppl: Afrotropical region. Johannesburg; 1987; 55.
14. Detinova TS. Age-grouping methods in diptera of medical importance: 1962; 213.
15. Mangold KA, Manson RU, Koay ESC, Stephens L, Regner M, Thomson RB, et al. Real-Time PCR for Detection and Identification of *Plasmodium spp*. J Clin Microbiol. 2005; 43:2435–40.

16. Favia G, Lanfrancotti A, Spanos L, Sidén-Kiamos I, Louis C. Molecular characterization of ribosomal DNA polymorphisms discriminating among chromosomal forms of *Anopheles gambiae* s.s.: *An. gambiae* s.s. rDNA polymorphisms. Insect Mol Biol. 2001; 10:19–23.
17. Bass C, Nikou D, Donnelly MJ, Williamson MS, Ranson H, Ball A, et al. Detection of knockdown resistance (kdr) mutations in *Anopheles gambiae*: a comparison of two new high-throughput assays with existing methods. Malar J. 2007; 6:111.
18. Bass C, Nikou D, Vontas J, Williamson MS, Field LM. Development of high-throughput real-time PCR assays for the identification of insensitive acetylcholinesterase (ace-1R) in *Anopheles gambiae*. Pestic Biochem Physio. 2010; 96:80–5.
19. Dossou-yovo J, Doannio J, Rivière F, Duval J. Rice cultivation and malaria transmission in Bouaké city (Côte d'Ivoire). Acta Trop. 1994; 57:91–4.
20. Dossou-Yovo J, Doannio JMC, Diarrassouba S, Chauvancy G. The impact of rice fields on the transmission of malaria in Bouake, Cote d'Ivoire. Bulletin de la société de pathologie exotique. 1998;91:327–33.
21. Zoh DD, Yapi A, Adja MA, Guindo-Coulibaly N, Kpan DMS, Sagna AB, et al. Role of Anopheles gambiae s.s. and Anopheles coluzzii (Diptera: Culicidae) in Human Malaria Transmission in Rural Areas of Bouaké, in Côte d'Ivoire. Hamer G, editor. J Med Entomol. 2020; 57:1254–61.
22. Zogo B, Soma DD, Tchiekoi BN, Somé A, Ahoua Alou LP, Koffi AA, et al. *Anopheles* bionomics, insecticide resistance mechanisms, and malaria transmission in the Korhogo area, northern Côte d'Ivoire: a pre-intervention study. Parasite. 2019; 26:40.
23. Yokoly FN, Zahouli JBZ, Small G, Ouattara AF, Opoku M, de Souza DK, et al. Assessing *Anopheles* vector species diversity and transmission of malaria in four health districts along the borders of Côte d'Ivoire. Malar J. 2021;20:409.
24. Camara S, Koffi AA, Ahoua Alou LP, Koffi K, Kabran J-PK, Koné A, et al. Mapping insecticide resistance in *Anopheles gambiae* (s.l.) from Côte d'Ivoire. Parasit Vectors. 2018;11:19.
25. Koffi AA, Ahoua Alou LP, Djenontin A, Kabran J-PK, Dosso Y, Kone A, et al. Efficacy of Olyset ® Duo, a permethrin and pyriproxyfen mixture net against wild pyrethroid-resistant *Anopheles gambiae s.s.* from Côte d'Ivoire: an experimental hut trial. Parasite. 2015;22:28.
26. Zogo B, Koffi AA, Alou LPA, Fournet F, Dahounto A, Dabiré RK, et al. Identification and characterization of Anopheles spp. breeding habitats in the Korhogo area in northern Côte d'Ivoire: a study prior to a Bti-based larviciding intervention. Parasites Vectors. 2019; 12:146.
27. Gimonneau G, Pombi M, Choisy M, Morand S, Dabiré RK, Simard F. Larval habitat segregation between the molecular forms of the mosquito Anopheles gambiae in a rice field area of Burkina Faso, West Africa. Med Vet Entomol. 2012; 26:9–17.
28. Adja AM, N'Goran KE, Kengne P, Koudou GB, Toure M, Koffi AA, et al. Vectorial transmission of malaria in shrubby Savannah area at Ganse, Ivory Coast. Med. Trop. 2006; 66:449–55.
29. Killeen GF, Githure JI, Beier JC. Short report: entomologic inoculation rates and *Plasmodium falciparum* malaria prevalence in Africa. Am J Trop Med Hyg. 1999; 61:109–13.

30. Kilama M, Smith DL, Hutchinson R, Kigozi R, Yeka A, Lavoy G, et al. Estimating the annual entomological inoculation rate for *Plasmodium falciparum* transmitted by *Anopheles gambiae* s.l. using three sampling methods in three sites in Uganda. Malaria J. 2014; 13: 111.
31. Conn JE, Norris DE, Donnelly MJ, Beebe NW, Burkot TR, Coulibaly MB, et al. Entomological Monitoring and Evaluation: Diverse Transmission Settings of ICEMR Projects Will Require Local and Regional Malaria Elimination Strategies. Am J Trop Med Hyg. 2015; 93:28–41.
32. Russell, T.L., Govella, N.J., Azizi, S. et al. Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. Malar J. 2011; 10: 80.
33. Musiime, A.K., Smith, D.L., Kilama, M. et al. Impact of vector control interventions on malaria transmission intensity, outdoor vector biting rates and *Anopheles* mosquito species
34. Byrne I, Chan K, Manrique E, Lines J, Wolie RZ, Trujillano F, et al. Technical Workflow Development for Integrating Drone Surveys and Entomological Sampling to Characterise Aquatic Larval Habitats of *Anopheles funestus* in Agricultural Landscapes in Côte d'Ivoire. J. Environ. Public Health. 2021; 2021:1–14.
35. Wolie RZ, Koffi AA, Ahoua Alou LP, Sternberg ED, N'Nan-Alla O, Dahounto A, et al. Evaluation of the interaction between insecticide resistance-associated genes and malaria transmission in *Anopheles gambiae* sensu lato in central Côte d'Ivoire. Parasit Vectors. 2021; 14:581.
36. Alou LPA, Koffi AA, Adja MA, Assi SB, Kouassi PK, N'Guessan R. Status of pyrethroid resistance in *Anopheles gambiae* s. s. M form prior to the scaling up of Long Lasting Insecticidal Nets (LLINs) in Adzope, Eastern Cote d'Ivoire. Parasit Vectors. 2012;5.
37. Koukpo CZ, Fassinou AJYH, Ossè RA, Agossa FR, Sovi A, Sewadé WT, et al. The current distribution and characterization of the L1014F resistance allele of the kdr gene in three malaria vectors (*Anopheles gambiae*, *Anopheles coluzzii*, *Anopheles arabiensis*) in Benin (West Africa) Malaria J. 2019; 18:175.
38. Soma DD, Zogo BM, Somé A, Tchiekoi BN, Hien DFS, Pooda HS, Coulibaly S, Gnambani JE, Ouari A, Mouline K, Dahounto A, Ouédraogo GA, Fournet F, Koffi AA, Pennetier C, Moiroux N, Dabiré RK. Anopheles bionomics, insecticide resistance and malaria transmission in southwest Burkina Faso: A pre-intervention study. PLoS One. 2020; 3;15(8):e0236920.
39. Moshi IR, Manderson L, Ngowo HS, Mlacha YP, Okumu FO, Mnyone LL. Outdoor malaria transmission risks and social life: a qualitative study in South-Eastern Tanzania. Malaria J. 2018; 17, 397.
40. Russell TL, Govella NJ, Azizi S, Drakeley CJ, Kachur SP, Killeen GF. Increased proportions of outdoor feeding among residual malaria vector populations following increased use of insecticide-treated nets in rural Tanzania. Malar J. 2011;10:80.
41. Dossou-Yovo J, Guillet P, Rogier C, Chandre F, Carnevale P, Assi S-B, et al. Protective efficacy of lambda-cyhalothrin treated nets in *Anopheles gambiae* pyrethroid resistance areas of Côte d'Ivoire. Am J Trop Med Hyg. 2005; 73:859–64.

42. Bhatt S, Weiss DJ, Cameron E, Bisanzio D, Mappin B, Dalrymple U, et al. The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. Nature. 2015;526:207–11.

Figures

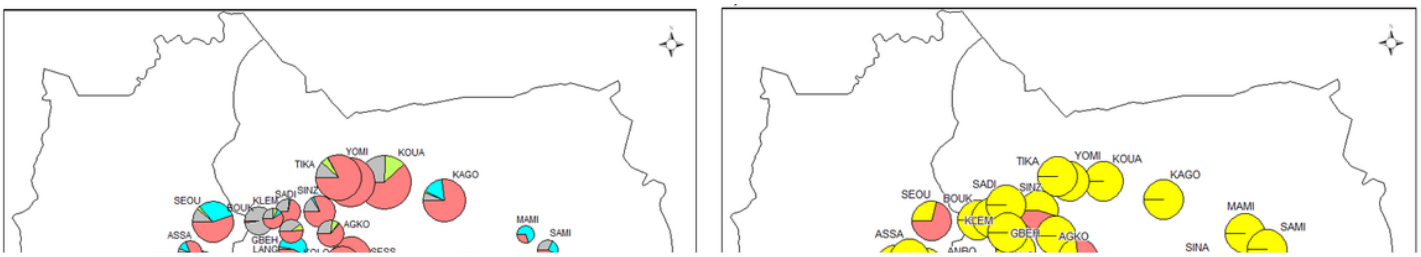

Figure 1

Map of mosquito densities and composition in the 40 village-clusters of the study area. A) Overall mosquito density; B) *An. gambiae* s.l. species complex distribution in the forty (40) villages

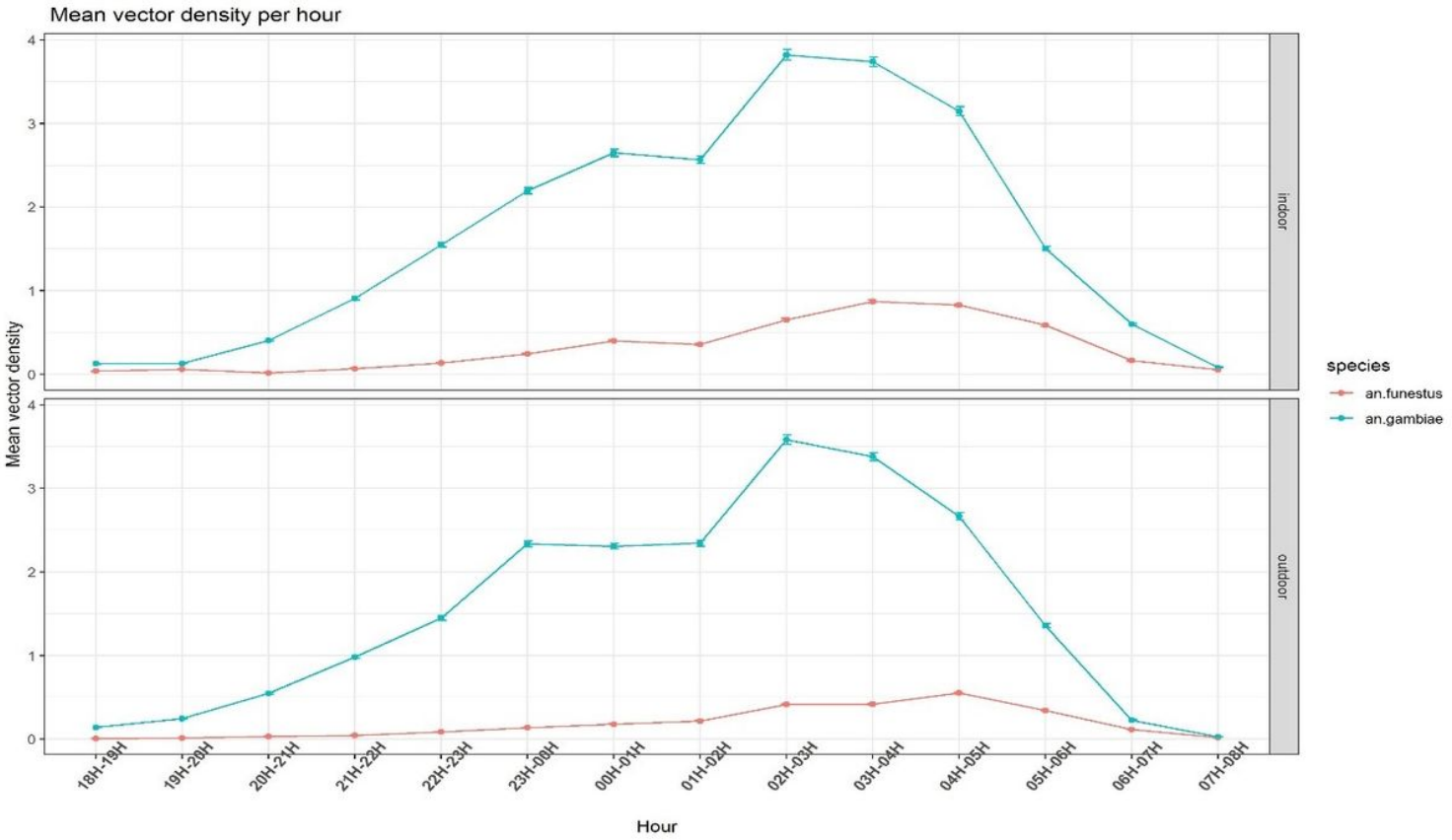

Figure 2

Hourly outdoor and indoor biting profiles of *An. gambiae* s.l. and *An. funestus* across all the study villages. Points show mean and bars indicate hourly change in number of mosquito bites

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterialsRosineZ.Wolie.docx