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Residual malaria transmission assessment: Quantification of the relative risk of malaria transmission at different night-time and location in Atacora region in Benin, West Africa

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Abstract

A significant reduction in malaria incidence has been observed in Africa, due to the extensive use of indoor residual spraying and insecticide-treated net. Even so, residual malaria transmission continues to occur. This study aims to quantify the potential risk of the different night-time and location in the context of high coverage of ITNs and IRS in Benin. Adult mosquito collections were carried out through Human Landing Catch. Vector species were identified using PCR. ELISA was used to determine sporozoite infections. The majority of biting occurred late at night with more infective bites observed outdoor. Considerable biting occurred early in the evening. The proportion of malaria transmission occurring before sleeping hours and outdoors is compromising the efforts towards malaria control. To curtail residual malaria transmission, additional interventions able to target vectors escaping conventional vector control interventions should be prioritized.

Keywords: Residual malaria, quantification

Introduction

Malaria is one of the most serious vector-borne diseases, affecting millions of people mainly in the tropics. It contributes heavily to the burden of disease in Benin, with 1.5 million cases reported annually among a national population of 11.1 million^[1]. In the past decade, a significant reduction in malaria incidence has been observed in Africa, due to the scale-up of interventions. Vector control has contributed to a significant decrease of malaria worldwide^[2-4]. Insecticide-treated nets (ITNs) and indoor residual spray (IRS), represent the two primary vector control interventions used for large-scale malaria prevention, and are an integral component of Benin's national strategic plan for malaria control^[1].

Despite the progress made in scaling-up of the interventions, malaria transmission continues to occur. The entomological indicators of malaria transmission such as density, sporozoite rate and entomological inoculation rate (EIR) are parameters used to assess the impact of the interventions and the intensity of malaria transmission. Several factors might be responsible for the persistence of malaria transmission, including vector behavioural change towards more exophagic (outdoor-feeding), exophilic (outdoor-resting) tendencies following the widespread use of ITNs and IRS^[5,6].

Vector control measures tackle only indoor and night biting vectors. The proportion of malaria transmission occurring before sleeping hours and outdoors can be an obstacle for malaria transmission control and elimination. Otherwise, a recent anthropological study from Ghana reported human night-time activities, including outdoor sleeping, that might increase exposure to malaria infection risk^[7]. Malaria transmission that can persist in the context of high levels of ITN or IRS coverage is known as residual transmission^[8,9] and represents a major challenge for malaria control. The main aim of this study was to assess the residual malaria transmission through the quantification of the potential risk of the different night-time and location in the context of high coverage of ITNs and IRS in Benin.

Methods**Study Area**

The study was carried out in Atacora region located in the Northwest of Benin in the district of

Kouandé (Fig. 1). This districts covered about 2,128 km² and had an estimated population of 108,956 in 2015. Atacora region has a sub-equatorial type climate with one dry season (December-May) and only one rainy season (June to November). The annual mean rainfall is 1,300 mm and the mean monthly temperature rangent between 22° and 33°C.

The major Economic activity is agriculture and it is characterized by the production of Cotton and millet Where Various classes of pesticides are used for Pest control. Since 2011, the Department has conducted a large scale Indoor Residual Spraying (IRS) and ITNs distribution.

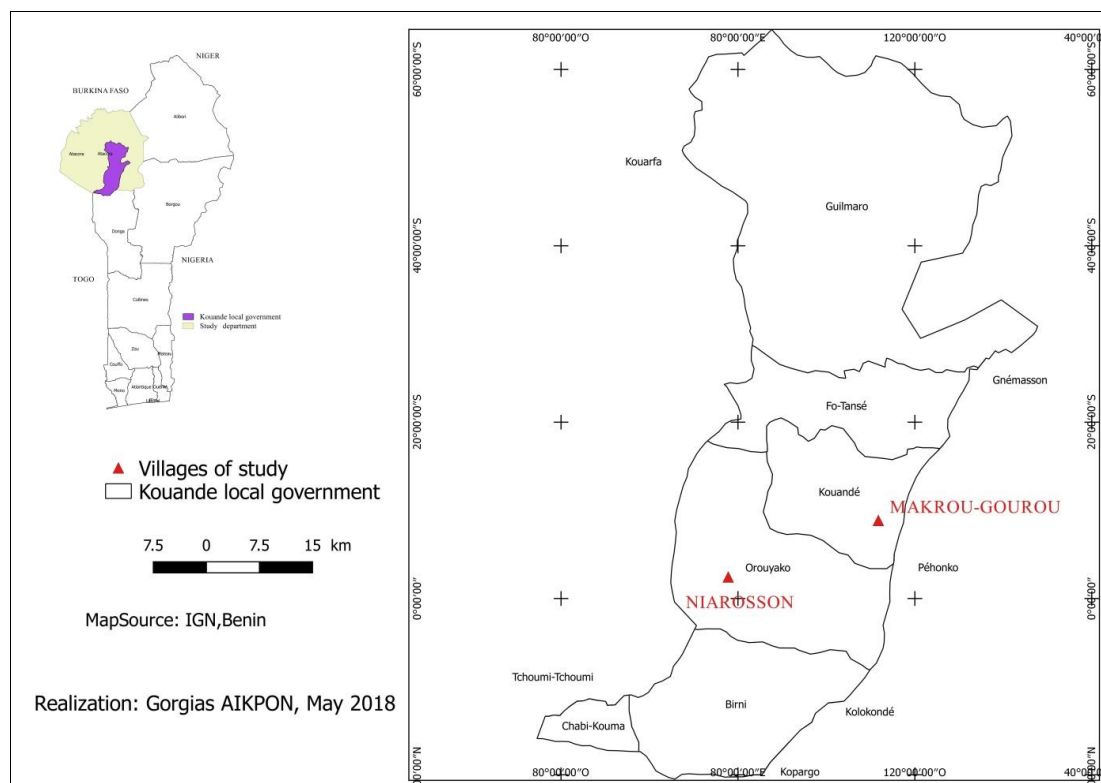


Fig 1: Map of study area

Mosquito Sampling

Adult mosquito collections were carried through Human Landing Catch (HLC). Two villages were selected, and two houses were chosen per village for mosquito collection to monitor malaria transmission. Monthly, mosquito collections were carried out from 6 p.m. to 6 a.m. inside and outside houses using a mouth aspirator, by human volunteers who had previously given consent. Two nights of mosquito collections per month were carried out from January to December 2016. A total of twenty four catches were performed. In each village, two houses and four collectors were selected for the mosquitoes collection. The recorded data were used to assess the night-time aggressiveness (HBR), sporozoite index and entomological inoculation rate vectors (EIR).

Identification of vector species complexes

Members of *An. Gambiae s.l.* and *An. Funestus s.l.* groups were identified to species by polymerase chain reaction (PCR), following the protocols developed by Scott *et al.* For *An. Gambiae s.l.* [10] and Koekemoer *et al.* For *An. Funestus s.l.* [11].

Sporozoite ELISA

Dried head and thorax of the preserved *Anopheles* mosquito specimens were carefully separated from the abdomen and tested for *P. falciparum* circumsporozoite proteins (CSPs) using sandwich ELISA method [12, 13].

Data analysis

The sporozoite rate was the proportion of *P. falciparum* CSP

positive mosquitoes divided by the total number of mosquitoes caught. The human-biting rate (HBR) was expressed as the number of bites per person per night (b/p/n) determined by dividing the number of mosquitoes collected by the number of volunteers per night. The entomological inoculation rate (EIR) was estimated by multiplying the sporozoite rate by the human-biting rate [14]. Vector densities by HLC were weighed according to the proportion of individuals sleeping both indoors and outdoors. Chi-square test was employed to test the difference in vector species composition between indoor and outdoor.

Results

Composition and abundance of mosquito fauna

A total of 528 mosquitoes belonging to 14 different species were caught in the lading catches during the study period. Most (57.95%) of these were anopheline, and most the anopheline mosquitoes collected were members of the *An. gambiae* complex (75.89%) and *An. funestus* group (13.04%) (Table 1).

Among the 231 female *An. Gambiae s.l.* identified in species level in PCR-based assay, *An. Gambiae*, *An. Coluzzii* and *An. Arabiensis* were found in sympatry in the study area. However, *An. Gambiae* was predominant, representing 66.23% (n=153) followed by *An. Coluzzii* (30.30%; n=70) and *An. Arbiensis* (3.46%; n=8). Besides, all of the females of the *An. Funestus* group investigated were identified as *An. Funestus s.s.*

Table 1: Numbers of adult female mosquitoes collected on human bait in study area from January to December 2016.

Species	Number	Percentage (%)
<i>Anopheles gambiae s.l</i>	231	43.75
<i>Anopheles funestus s.l</i>	40	7.58
<i>Anopheles pharoensis</i>	8	1.52
<i>Anopheles ziemanni</i>	27	5.11
Total <i>Anopheles</i>	306	57.95
<i>Aedes aegypti</i>	11	2.08
<i>Aedes vittatus</i>	17	3.22
<i>Aedes longipalpis</i>	4	0.76
<i>Aedes gr. palpalis</i>	3	0.57
<i>Aedes gr. tarsalis</i>	2	0.38
<i>Culex quinquefasciatus</i>	88	16.67
<i>Culex gr. decens</i>	9	1.70
<i>Culex nebulosus</i>	8	1.52
<i>Culex fatigans</i>	44	8.33
<i>Mansonia africana</i>	36	6.82
Total	528	

Malaria vector species composition and abundance and indoor/outdoor density

A total of 271 female of malaria vectors mosquitoes belonging to four species were collected during the study

period (Fig. 2). There was a significant difference in anopheline mosquito species co-occurrence between indoor and outdoor locations ($p < 0.0001$). The proportion of all vector species was higher outdoors than indoors.

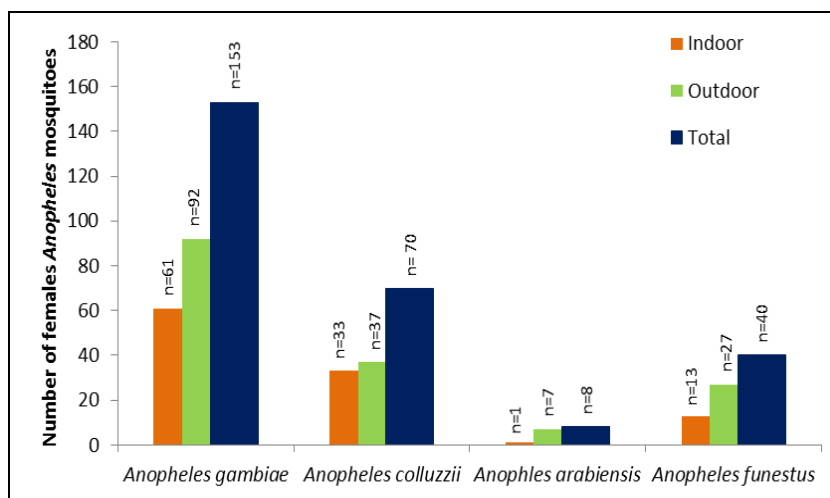


Fig 2: Summary of malaria vector species collected from indoor and outdoor in Kouandé district from January to December 2016.

Hourly activity of malaria vectors

The majority of biting occurred late at night between 2am and 5am with similar trends both indoors and outdoors (fig. 3).

However, a considerable biting occurred also early in the evening from 8pm to 10pm with a minor peak.

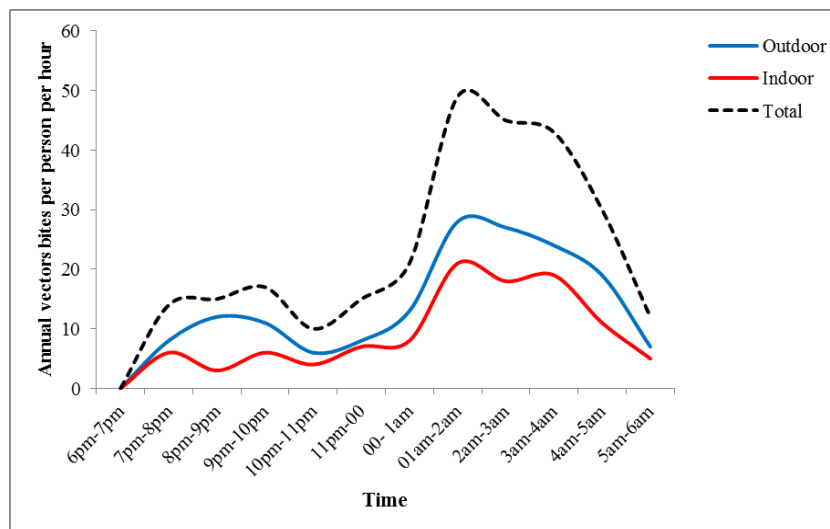


Fig 3: Biting times of malaria vectors (*An. gambiae s.l* and *An. funestus*) in kouande district.

Infectivity rate for malaria vector species

Sporozoites rates for all malaria vector species are presented in table 2. *An. Gambiae*, *An. Colluzzii*, *An. Arabiensis* and *An. Funestus* are all involved in malaria transmission in the study

area. The mean sporozoite rate was 20.26% for *An. gambiae*, 10% for *An. colluzzii*, 12.5% for *An. arabiensis* and *An. funestus*.

Table 2: *Plasmodium falciparum* sporozoite rates for malaria vectors in Kouané district.

Vector species	Number tested	Number sporozoite positive	Sporozoite Index (%)	Confidence Interval
<i>An. gambiae</i>	153	31	20.26	14.2 – 27.51
<i>An. colluzzii</i>	70	7	10	4.12 – 19.52
<i>An. arabiensis</i>	8	1	12.5	0.32 – 52.65
<i>An. funestus</i>	40	5	12.5	4.19 – 26.80

Time of malaria-infective biting and peak infection by malaria vectors

Table 3 shows the dynamic of hour-by-hour pooled samples of the malaria vectors, sporozoite indice (SI), human biting rate (HBR) and entomological inoculation rate (TIE), and their overall estimated contribution to malaria transmission indoor and outdoor from January to December 2016.

The estimated mean daily *P. falciparum* EIR or the mean number of infective bites per man per night (ib/m/n) was 0.229 (Table 3). Thus within a month, inhabitants in this area would have received an average of 6.88 infective bites at Kouandé. Biting by malaria infective mosquitoes occurred during the hours 7pm to 6 am. The majority of sporozoite-positive bites as the EIR occurred in the periods between 11pm and 05am, with more sporozoite-positive mosquitoes observed outdoor than indoor (Fig. 4). The proportion of annual entomological malaria transmission occurring in Kouandé district is estimated at 82.88 infected bites/person/year (ib/p/year) and 13.66% (11.28 ib/p/year) of this transmission happened during the early night time (6pm-10pm) (Fig. 5)

Discussion

Broadly defined as all forms of transmission that can persist after achieving full universal coverage with effective LLINs (long-lasting insecticidal nets) and/or IRS (indoor residual spraying), residual malaria transmission deserves to be quantified in order to better understand the current challenges for malaria controlling and eliminating.

Despite a large scale Indoor Residual Spraying (IRS) and ITNs distribution, the transmission of malaria is high in kouandé district. In this study, three members of *An. Gambiae complex* were found in sympatry (*An. gambiae*, *An. coluzzii* and *An. arabiensis*) with the predominance of *An. gambiae* and, concurs with previous findings in Benin that reported both M and S forms with the predominance of S forms in savannah areas [15]. *Anopheles funestus* s.s. was the only specie among *Anopheles funestus* group in the study area. Similar Findings were Reported in the Same area and in

Tanzania [16, 17]. Other anophelines recorded included *An. pharoensis* and *An. ziemanni*. However, *An. gambiae* s.l and *An. funestus* are identified as the two major malaria vectors and are both involved in malaria transmission in the study area.

The EIR data showed that the majority of malaria transmission by *An. gambiae* s.l. and *An. funestus* occurred outdoors. In the mean-time, the magnitude of the indoor EIRs was also considerable despite high ITNs and IRS coverage. This situation could be attributed to inconsistent ITN use [18], increasing insecticide resistance among vectors [19, 20], and shifts in malaria vector biting times from mid-night to early evening and morning when people are still indoor but unprotected by ITNs [21, 22]. Malaria transmission in Kouandé district started soon after dusk and continued till daybreak. The major peak observed in the second half of the night suggests that correct use of ITNs in this area, and sleeping in treated houses may be effective for vector control. However, additional protective tools targeting outdoor and early biting that can accessible by rural populations are required to avoid pre-bedtime and outdoor exposure to the bites of infected anophelines [23]. The proportion of malaria transmission occurring before sleeping hours and outdoors is compromises the efforts towards malaria control. The high degree of outdoor behavior contrasts with a greater endophilic tendency displayed by this species in previous studies in different areas of Ghana [24, 25]. Increases in exophagy in malaria vectors have been already observed following indoor interventions [26, 27, 28]. This study highlights that in Kouandé district, shifts in biting behavior can have detrimental impacts on the personal protection provided by LLINs and IRS. More structured data collections would make possible a larger sample area and thus provide a stronger basis for generalization.

Finally, documenting human activity at night through sociological and anthropological research would be useful in this area to understanding human-vector interaction in order to identify malaria prevention strategies compatible with human behaviour.

Table 3: Détails about the hour-by-hour pooled samples of the-all-night biting malaria vector mosquitoes, human biting rate (HBR), sporozoite index (SI) and Entomological Inoculation rate (EIR)

Parameters	Location	6pm-7pm	7pm-8pm	8pm-9pm	9pm-10pm	10pm-11pm	11pm-00	00- 1am	01am-2am	2am-3am	3am-4am	4am-5am	5am-6am	Total night	Early night (6pm-10pm)
Number of mosquitoes captured	Outdoor	0	8	12	11	6	8	13	28	27	24	19	7	163	31
	Indoor	0	6	3	6	4	7	8	21	18	19	11	5	108	15
	Total	0	14	15	17	10	15	21	49	45	43	30	12	271	46
Number of infected mosquitoes	Outdoor	0	1	1	2	1	3	4	6	7	4	3	1	33	5
	Indoor	0	0	0	0	1	1	2	3	2	1	1	0	11	1
	Total	0	1	1	2	2	4	6	9	9	5	4	1	44	6
SI (%)	Outdoor	0	0.125	0.083	0.181	0.167	0.375	0.308	0.214	0.259	0.167	0.158	0.142	0.202	0.160
	Indoor	0	0	0	0	0.25	0.143	0.25	0.143	0.111	0.053	0.090	0	0.102	0.07
	Total	0	0.071	0.067	0.118	0.20	0.267	0.286	0.184	0.2	0.116	0.133	0.083	0.162	0.13
Person night	Outdoor	96	96	96	96	96	96	96	96	96	96	96	96	96	96
	Indoor	96	96	96	96	96	96	96	96	96	96	96	96	96	96
	Total	192	192	192	192	192	192	192	192	192	192	192	192	192	192
Mean HBR/night	Outdoor	0	0.083	0.125	0.115	0.063	0.083	0.135	0.292	0.281	0.25	0.198	0.073	1.698	0.32
	Indoor	0	0.063	0.031	0.063	0.042	0.073	0.083	0.219	0.188	0.198	0.115	0.052	1.125	0.160
	Total	0	0.078	0.078	0.089	0.052	0.078	0.109	0.255	0.469	0.224	0.156	0.063	1.411	0.240
EIR/night	Outdoor	0	0.010	0.010	0.020	0.010	0.031	0.041	0.063	0.073	0.042	0.031	0.010	0.344	0.05
	Indoor	0	0	0	0	0.010	0.010	0.020	0.031	0.020	0.010	0.010	0	0.115	0.010
	Total	0	0.005	0.005	0.010	0.010	0.020	0.031	0.046	0.094	0.026	0.020	0.005	0.229	0.030
EIR/month	Outdoor	0	0.31	0.31	0.63	0.31	0.94	1.25	1.88	2.19	1.25	0.94	0.31	10.31	1.56
	Indoor	0	0.00	0.00	0.00	0.31	0.31	0.63	0.94	0.63	0.31	0.31	0.00	3.44	0.31
	Total	0	0.16	0.16	0.31	0.31	0.63	0.94	1.41	2.81	0.78	0.63	0.16	6.88	0.94

Conclusion

There is still significant residual transmission across Atacora region, particularly in Koundé district despite the conventional vector control interventions implemented in this area. The plasticity observed in biting patterns, especially the combined outdoor and early biting behavior of the vector has important threats for the success of the widely used insecticide-based strategies using ITN and IRS. To curtail residual malaria transmission, additional interventions able to target vectors escaping conventional vector control interventions should be prioritized.

Competing interests: No competing interests.

Authors' contributions

RA, RO, IA, FD, LL, FT and MA designed the study. RA and RO carried out the experiments. RA drafted the manuscript. FD, LL, MA critically revised the manuscript.

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Availability of data and materials

The data used and/or analyzed in this study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The protocol of this study was reviewed and approved by the Institutional Ethics Committee of CREC (IECC). Before mosquito collectors were involved in this study, they gave their consent to participate. They were vaccinated against yellow fever, regularly checked up by a medical doctor and taken care in case of confirmed malaria case.

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Ethical approval

This study was approved by the Institutional Ethics Committee of CREC (IECC) (N°338/MS/DC/SGM/DRFMT/CREC/CEI-CREC/SA).

Mosquito collectors gave their consent before participating in the study.

The risks of malaria were minimized by using local collectors who already had some immunity due to their prolonged exposure to mosquitoes. Mosquito collectors were also regularly monitored. In case of confirmed malaria, they were immediately taken care of by the medical doctor of the team according to protocol. All mosquito collectors were vaccinated against yellow fever.

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