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Malaria burden and residual transmission: two thirds of mosquito bites may not be preventable with current vector control tools on Bioko Island, Equatorial Guinea

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ABSTRACT

Objectives: This study assesses exposure to malaria vector mosquitos that is nonpreventable through use of nets, the contribution of outdoor and indoor biting towards residual vector exposure, and the risk factors for being bitten and for being infected with malaria parasites on Bioko Island, Equatorial Guinea.
Methods: Human behavior and malaria infection data were collected from 13,735 randomly selected residents during cross-sectional surveys, concomitantly with entomological human landing catches, indoors and outdoors, in 20 locations on the Island. Self-reported time of going indoors, going to bed and whether using a net were analyzed to impute for each respondent the number of bites received outdoors and indoors during the night before the survey.
Results: On average, each person received 2.7 (95% CI: 2.6-2.8) bites per night outdoors, 8.5 (8.3 to 8.7) bites indoors if not using a net, and 4.7 (4.5 to 4.8) bites indoors if using a net. Malaria infection was associated with more bites, regardless of whether received indoors or outdoors. Older age, male gender, not using a net, rural location, and going indoors later increased the risk of being bitten. The proportion of bites not averted by using a net was estimated as 66% (61 to 71).
Conclusions: A large proportion of biting, mostly indoors, may not be preventable by bednets. Tools targeting indoor biting should be prioritized in Bioko. Novel vector control tools are urgently needed to reduce overall exposure to mosquito bites.
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Introduction

In 2022, malaria cases were estimated at 249 million globally, with about 95% occurring in sub-Saharan Africa [1]. Insecticide treated bed nets (ITNs), and indoor residual spraying (IRS) have been estimated by modelling studies to have averted 68% and 10% of clinical cases, respectively, between 2000 and 2015 [2]. However, malaria transmission continues to occur in many areas despite the use of ITNs and IRS [1]. Residual transmission, defined as "the actual maintained inoculation of *Plasmodium*, in spite of a well-designed and implemented vector control program" [3] has been identified as a major challenge for malaria control and elimination [4].

Residual malaria transmission can be attributed to several factors. One driver is the increase in recent years in the number of malaria vectors resistant to insecticides [1]. In addition, a proportion of mosquitoes may evade fatal exposure to insecticides by feeding outdoors or earlier in the evening before people have gone to sleep, resting outdoors of human houses, or by feeding on both humans and animals [5,6]. The emergence of resistance is an evolutionary consequence of continuous exposure to intensive insecticidal interventions, such as long lasting insecticidal nets (LLINs)

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and IRS [6]. Intensive insecticidal interventions have also led to changes in species composition by reducing the proportion of endophilic compared to exophilic species [7-9].

The contribution to residual transmission of the time when mosquitoes bite humans is not well understood. The number of malaria vector bites received by individuals who are not protected by LLINs and IRS can be estimated by the overlap between mosquito biting behavior and the time humans spend outdoors or indoors before going under a bed net [10-13]. A number of previous studies have linked data on human behavior with mosquito-biting data [4,14-17]. This study used nightly human biting rate (HBR) and malaria indicator survey (MIS) data to investigate how malaria infection prevalence is determined by exposure to mosquito bites as a function of self-reported time spent outdoors and indoors, and whether using a mosquito net. The aim of the study was to: (1) quantify residual biting by estimating the number of bites that users of nets are unable to avoid, compared to those who are not protected by nets; (2) determine the contributions to residual biting from indoor versus outdoor exposure to vector mosquitos; and (3) identify risk factors for biting and for malaria infection.

Methods

The study was conducted on Bioko Island, Equatorial Guinea. A description of Bioko and details of malaria control interventions deployed since the launch of the Bioko Island Malaria Elimination Project (BIMEP) are provided in the supplementary materials.

Significant reductions in malaria transmission were achieved during the initial four years of the BIMEP with an almost 70% reduction in malaria prevalence, 90% reduction in child anemia, and a 66% drop in all-cause child mortality [18,19]. There was a notable reduction in mosquito biting rates [7] and two principal vector species, *Anopheles funestus* and *An. gambiae* s.s., were eliminated from the island [9,20]. Currently, malaria transmission is sustained mostly by *An. coluzzii* with a small contribution of *An. melas* in specific areas [7]. However, progress in reducing malaria on Bioko has stalled since 2016 [21].

Bioko Island spatial decision support system (SDSS)

All household-based malaria interventions on Bioko are planned, implemented, and monitored through a bespoke mapping system consisting of two nested grids of ~2,000 1 × 1 km map-areas, each containing one hundred 100 × 100 m map-sectors [22]. This system allows close monitoring of intervention coverage and impact (malaria prevalence, vector density), and decision support [23]. The data used in this paper were identified according to the location of their map-area (Figure 1).

Annual cross-sectional household surveys

Since 2004, MIS have been conducted annually on Bioko, in August and September. This study is based on the six annual MISs from 2017 to 2022, when data were collected more uniformly than previously and fully integrated in the SDSS [23]. The survey instrument was adapted from the standard Roll Back Malaria MIS [24], with additional modules added when necessary. Since 2015 the sampling frame for the MIS consisted of all households from all localities across the island. From 2019, map-areas were used as the primary sampling units (PSUs) which were categorized as urban or rural. Households were selected by stratified, simple random sampling with sampling fractions of 5% in urban and 25% in rural PSUs, resulting in an overall sampling fraction of approximately 7% of all households.

Members of selected households were tested for *Plasmodium falciparum* infection by rapid diagnostic tests (CareStart Malaria, AccessBio Inc., Monmouth, USA) subject to informed written consent from the participant or caregiver in the case of children. Participants who tested positive for malaria were referred to a survey nurse for treatment according to national guidelines.

The MIS included questions about the time individuals entered the house, the time they went to bed, and whether they slept under a bed net the night prior to the survey. See the supplementary materials for further details.

Entomological collections

Human landing catch (HLC) mosquito collections were performed one night per month in 20 entomological sentinel sites across the island (Figure 1 and Table S2). In each site, collectors worked in two households located at least 100 m apart. Collectors worked indoors and outdoors in each house between 7 pm and 6 am, switching positions at midnight. HBR were standardized to bites per-person-hour according to the number of collectors working per household.

MIS respondents were linked to HLC data as follows: (1) in urban Malabo, HLC data from each site and for each year were linked to all MIS respondents who had been sampled in the same year in the map-area corresponding to the entomological sentinel site (map-areas within the dashed box in Figure 1) based on the assumption that HLC rates were representative of the entire 1 km^2 map area in which they were collected. (2) In rural areas, HLC data were assumed to be representative of the map area in which they were collected and the immediately adjacent map-areas (Figure 1). Thus, MIS responses from the map-area of the entomological sentinel site and from immediately neighboring map-areas were linked to the HLC data for the sentinel site for the same year. (3) In rural sentinel sites, there were no populated neighboring map-areas (Figure 1).

Statistical analyses

The analysis was restricted to MIS data corresponding to map-areas containing an HLC site, or neighboring an HLC site, as explained above. For every site, there were at least 4 months of HLC data for it to be included. The human biting data were available between 7 pm and 6 am nightly and were averaged for each hour of the night for each site and year after adjusting for collection effort. The total number of bites each survey participant would have received outdoors and indoors was calculated from self-reported time of entry to the house the previous night and hourly HBR as detailed below, similar to methods documented in previous studies [10,11].

For participants who did not use a bed net, the indoor biting exposure was calculated as the cumulative hourly HBR from the time of entry to the house to 6 am. For participants who reported using a net, their indoor biting exposure was calculated as the cumulative number of bites indoors from the time of entry to the house up to the time when they went to bed. Outdoor biting exposure was calculated as the cumulative outdoor HLC biting rates from 7 pm to the hour they reportedly went indoors. It was assumed that when a person went indoors/to bed they remained there for the rest of the night.

It was further assumed that individuals not using a net were exposed to the same indoor biting rates as those of an indoor HLC collector and that those sleeping under a net were not exposed to mosquito bites once they went to bed. Individuals who remained indoors throughout the previous day or entered the house before 7 pm were considered indoor-only exposed. Those who never entered the house the previous night were categorized as outdoors-

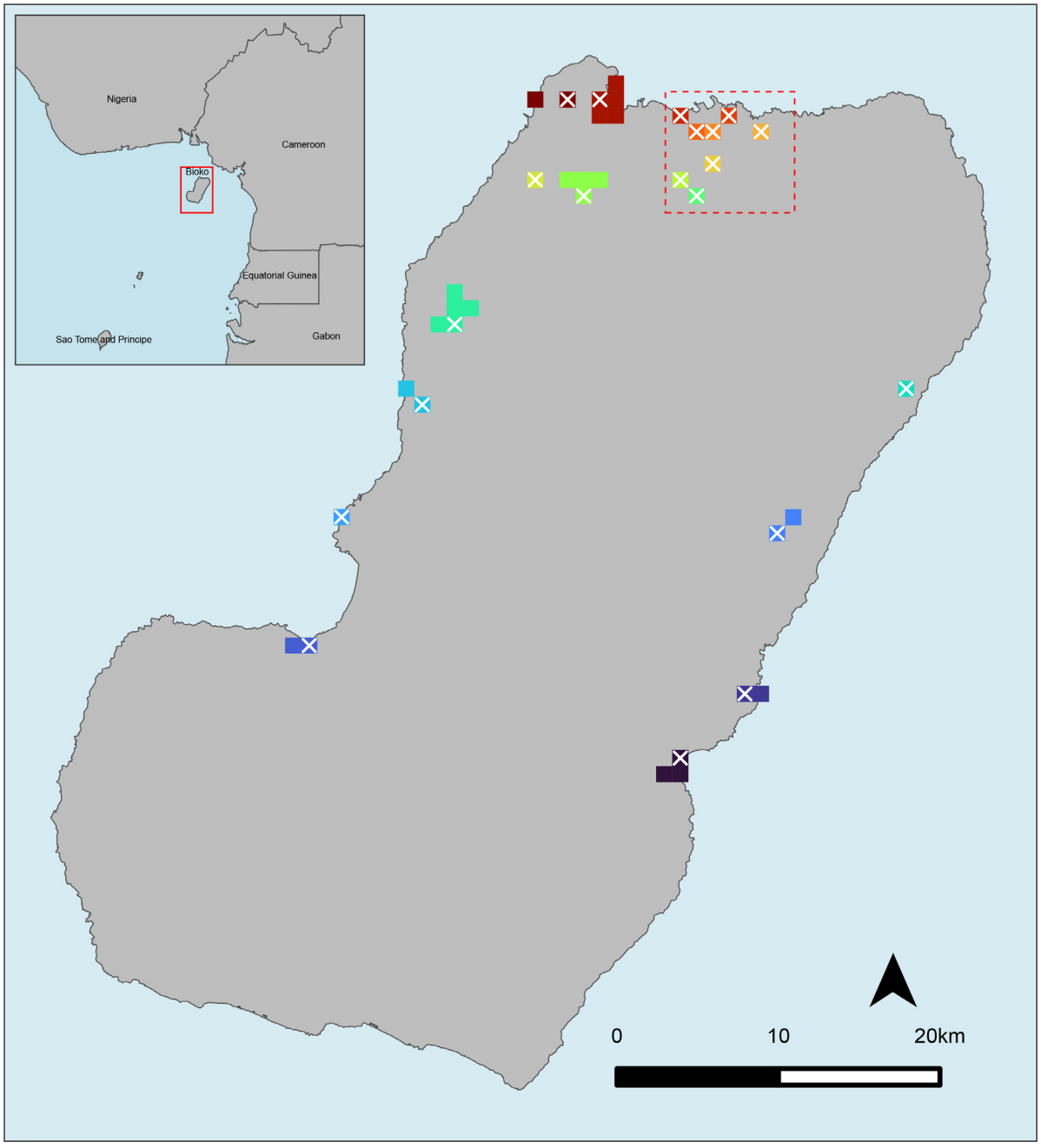

Figure 1. Map of Bioko Island showing its location in the Gulf of Guinea (red box in the inset). White crosses indicate the locations of entomological collections within map-areas; 20 in total. The colored squares represent map-areas providing MIS data to each entomological site; map-areas of the same color represent MIS "catchment areas" for the corresponding HLC location. Dashed red box roughly delineates urban Malabo.

only exposed. Individuals unsure of the time they went indoors were excluded from the analysis.

The average number of bites received outdoors and indoors for net users and nonusers was calculated by age group (under 5 years, 5 to under 15, and 15 years and older) and for urban and rural residents.

The proportion of bites that are not avoided by using a bed net (residual biting) was calculated overall for each age group and for rural and urban residents as

$$B_u/B_{nu}$$

where B_u = average total bites received by user of nets and B_{nu} = average total bites received by nonuser of nets.

The confidence interval for B_u/B_{nu} was calculated using Fieller's method [25].

Results from the sensitivity analysis showed that although the percentage of unpreventable bites increased as the percentage of feeding inhibition reduced (from 100% to 90% and 80%), the differences were not significant (Supplementary Figure 4).

Logistic regression was used to estimate the effect of biting exposure on malaria prevalence with robust standard errors to account for clustering by site. The odds ratios were adjusted for: age group, gender, net use the previous night, travel to mainland Equatorial Guinea in the eight weeks preceding the survey, rural versus urban, quintile of asset-based socio-economic status (SES) calculated by principal component analysis [26], and year. The interaction between total biting

Table 1
Demographic characteristics of participants from the annual MIS and the summary of entomological parameters. Values in brackets are percentages by characteristic.

	2017	2018	2019	2020	2021	2022	2017-2022
Annual MIS surveys							
Number of participants	2768	2551	2490	2204	2234	1488	13735
Sex							
Male	1288 (46.5)	1161 (45.5)	1179 (47.3)	1054 (47.8)	1037 (46.4)	714 (48.0)	6433 (46.8)
Female	1480 (53.5)	1390 (54.5)	1311 (52.7)	1150 (52.2)	1197 (53.6)	774 (52.0)	7302 (53.2)
Age groups[a]							
0 to <5 years	536 (19.4)	444 (17.4)	395 (15.9)	375 (17.0)	316 (14.2)	196 (13.2)	2262 (16.5)
5 to <15 years	759 (27.4)	664 (26.0)	652 (26.2)	625 (28.4)	639 (28.6)	455 (30.6)	3794 (27.6)
15+ years	1473 (53.2)	1443 (56.6)	1443 (58.0)	1202 (54.6)	1276 (57.2)	836 (56.2)	7673 (55.9)
Parasite prevalence							
0 to <5 years	45 (8.4)	34 (7.7)	31 (7.8)	19 (5.1)	25 (7.9)	23 (11.7)	177 (7.8)
5 to <15 years	102 (13.4)	103 (15.5)	125 (19.2)	97 (15.5)	133 (20.8)	109 (24.0)	669 (17.6)
15+ years	131 (8.9)	150 (10.4)	210 (14.6)	149 (12.4)	187 (14.7)	174 (20.8)	1001 (13.0)
All ages	278 (10.0)	287 (11.3)	366 (14.7)	265 (12.0)	345 (15.5)	306 (20.6)	1847 (13.5)
Net use previous night[b]							
0 to <5 years	290 (54.2)	306 (69.1)	198 (50.8)	219 (58.7)	145 (45.9)	105 (53.6)	1263 (56.1)
5 to <15 years	361 (47.6)	391 (59.1)	265 (41.0)	287 (45.9)	259 (40.6)	183 (40.2)	1746 (46.1)
15+ years	661 (44.9)	799 (56.0)	576 (41.9)	501 (41.8)	508 (39.8)	338 (40.5)	3383 (44.6)
All ages	1312 (47.5)	1496 (59.1)	1039 (43.1)	1008 (45.8)	912 (40.9)	627 (42.2)	6394 (46.9)
Travelled to mainland in last 2 months[c]	219 (7.9)	151 (5.9)	253 (10.2)	23 (1.0)	26 (1.2)	24 (1.6)	696 (5.1)
Location (Urban)	1866 (67.4)	1753 (68.7)	2053 (82.4)	1893 (85.9)	1826 (81.7)	842 (56.6)	10233 (74.5)
Socio Economic Status (SES)							
1st quintile	835 (30.2)	663 (26.0)	534 (21.4)	634 (28.8)	554 (24.8)	424 (28.5)	3644 (26.5)
2nd quintile	744 (26.9)	749 (29.4)	746 (30.0)	575 (26.1)	596 (26.7)	314 (21.1)	3724 (27.1)
3rd quintile	579 (20.9)	691 (27.1)	676 (27.1)	590 (26.8)	583 (26.1)	397 (26.7)	3516 (25.6)
4th quintile	610 (22.0)	448 (17.6)	534 (21.4)	405 (18.4)	501 (22.4)	353 (23.7)	2851 (20.8)
Entomological surveillance							
Number of mosquitoes caught							
Indoors	3950	3305	6640	6531	6197	10269	36892
Outdoors	4097	3301	9198	8162	8181	19502	52441
Mean human biting rate (bites per person per night)							
Rural							
Indoors	6.2	6.0	17.2	15.3	15.3	46.2	16.3
Outdoors	6.4	6.0	23.0	18.5	20.1	84.1	24.1
Urban							
Indoors	1.8	1.1	5.5	2.8	3.8	10.2	4.5
Outdoors	2.0	0.9	8.8	4.4	5.0	26.0	8.6

[a] 6 missing values.
[b] 107 missing values.
[c] 5 missing values.

exposures and the proportion of bites received outdoors was explored.

Poisson regression was used to investigate risk factors for biting exposure in a model with total bites as the outcome, and age group, gender, net use the previous night, travel to mainland Equatorial Guinea, rural versus urban, SES, year, and time going indoors as potential explanatory variables.

Statistical analyses were done with Stata version 18.0 (StataCorp, College Station, Texas) and R version 4.2.2 (R Core Team, R: A Language and Environment for Statistical Computing).

Results

Of the 75,451 individuals sampled between 2017 and 2022 during the annual MIS, 18.2% (13,735) were surveyed from map-areas corresponding to HLC sites. In the latter subgroup, which forms the data set for this study, the prevalence of malaria infection across all ages was 13.5% (1,847/13,735), which increased from 10.0% in 2017 to 20.6% in 2022. The overall proportion of respondents of all ages overall years who reported net use the previous night was 46.9% (6,394/13,628). ITN use was higher among children under 5 years (56.1%) than those aged 5 to <15 years (46.1%) and adults (44.6%) (Table 1).

There was large variation in HBR between sites and between years (Supplementary Figures 1 and 2). The average indoor and outdoor HBR increased until 9-10 pm, plateaued and steadily declined after midnight. For most of the night, the average outdoor HBR was higher than the indoor HBR, but the two rates were similar from about 3 am onwards.

People spend more time indoors than outdoors during the night. About 52% reported being outdoors the previous night between 7 and 8 pm, which decreased until midnight, with just over 2% being outdoors between midnight and 1 am. Only 1% of the participants reported being outdoors all night (Figure 2). The proportion of people sleeping under a net increased from about 5% between 7 pm and 8 pm to 46% between 12 am and 1 am (Supplementary Figure 3).

On average, participants received 9.4 bites per person per night (range: 0-210), of which 2.7 bites (29%) were received outdoors (Figure 3b). In rural areas, the mean number of bites per person per night was 23.4 compared to 3.6 in urban areas, of which similar proportions were outdoor bites (33% and 31%, respectively) (Figure 3a). Users of nets received about half the number of indoor bites compared to nonusers (4.7 vs. 8.5 bites per person per night) (Figure 3b).

The average total number of bites (outdoors plus indoors) received was 7.4 for users of nets compared to 11.2 for non-users. Therefore, the proportion of bites that cannot be avoided by using a net (residual biting) was 66% (95% CI: 61.4-71.0). For children under 5, this proportion was lower at 52% (Figure 3d).

Infection prevalence was related to the number of bites received, irrespective of whether the bites were predominantly outdoors or indoors. However, prevalence of infection was higher for those receiving 10 or more bites per night outdoors compared to

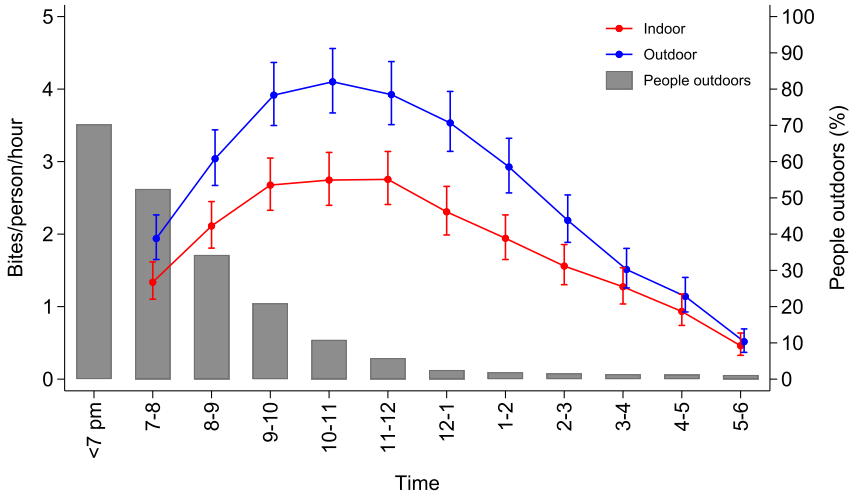

Figure 2. Human biting rate and proportion of individuals outdoors by hour, all sites (2017-2022). The error bars correspond to the 95% confidence interval.

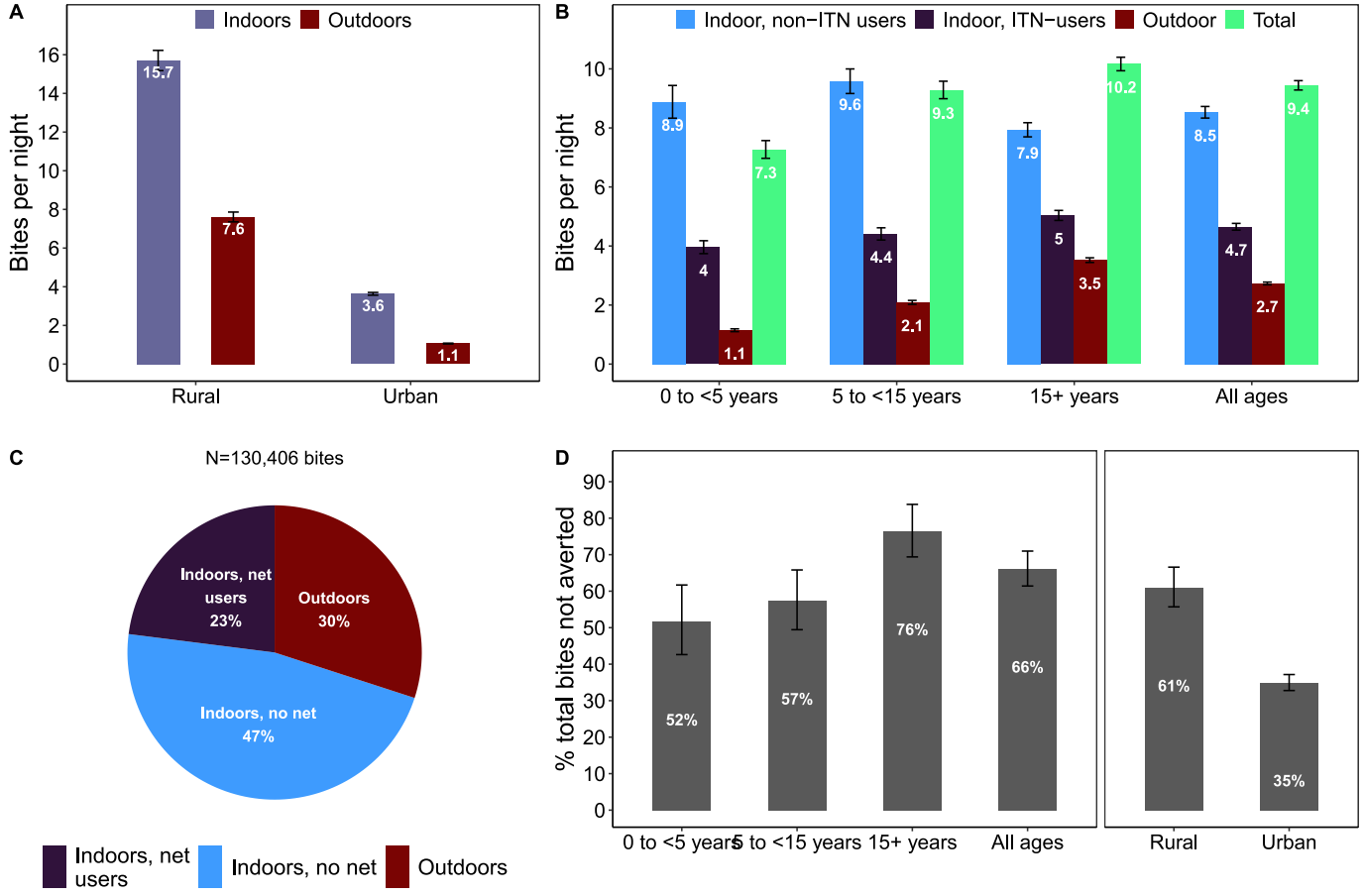

Figure 3. The average indoor, outdoor, and total bites received in Bioko Island. (a) Bites received by type of location of residence. (b) Bites received by age and LLIN use. (c) Total bites received the night before the survey. (d) Proportion of total bites not averted by LLINs.

those receiving the same number of bites indoors (31% vs. 20%) (Figure 4). The odds ratio of malaria infection per additional bite was 1.09 (95% CI: 1.02-1.18; $P < .016$) after adjusting for confounders.

The prevalence of malaria infection among individuals who received more than the median number of bites (2.8 bites per night) was 17.2%, while for those receiving less than the median number of bites, was 9.7%. (Odds ratio = 1.36 [95% CI: 1.03-1.80; $P = .031$]) (Table 2). There was no evidence ($P = .51$) of an association between malaria infection and the proportion of bites received outdoors.

Risk factors associated with biting exposure are presented in Table 3. In univariable analysis, females, ITN use the previous night, and living in urban areas were associated with reduced biting. Be-

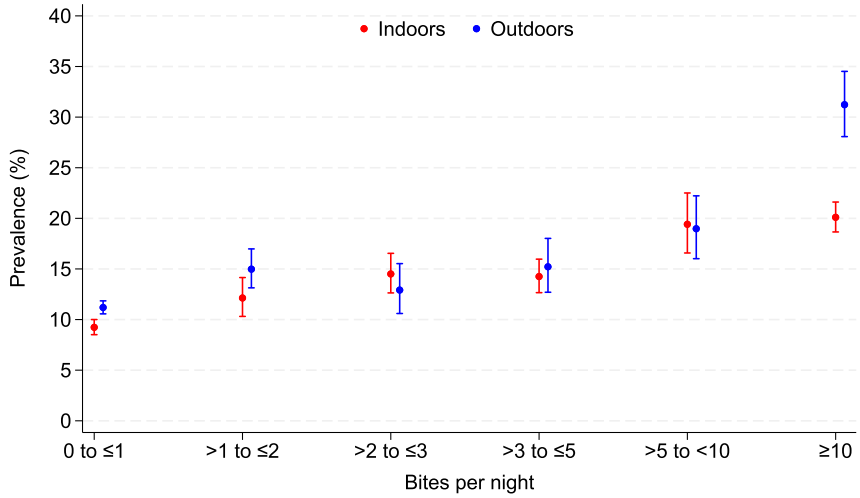

Figure 4. The prevalence of malaria infection by the number of bites received. The error bars correspond to the 95% confidence interval.

Table 2
Effect of total biting on malaria infection.

		Infection prevalence, % (*N*)	Unadjusted OR	95% CI	*P* value	Adjusted OR[a]	95% CI	*P* value
Total exposure, average bites per night	Low (<2.8 bites)	9.7 (6852)	1		.001	1		.031
	High (≥2.8 bites)	17.2 (6883)	1.92	1.29-2.87		1.36	1.03-1.80	
Proportion of bites outdoors	<60%	13.5 (11782)	1		.61	1		.51
	≥60%	12.9 (1953)	0.95	0.77-1.17		1.06	0.90-1.23	

[a] Adjusted for age group, gender, net use the previous night, travel to the mainland, urban versus rural, socioeconomic status and year.

Table 3
Risk factors associated with total biting exposure.

		Infection prevalence, % (*N*)	Mean bites per night	Unadjusted rate ratio	95% CI	*P* value	Adjusted rate ratio[a]	95% CI	*P* value
Age group	0 to <5 years	7.8 (2262)	7.3	1		< .001	1		.027
	5 to <15 years	17.6 (3794)	9.3	1.27	1.14, 1.43		1.03	0.96, 1.10	
	15+ years	13.0 (7673)	10.2	1.41	1.29, 1.53		1.05	1.01, 1.09	
Gender	Male	15.1 (6433)	10.4	1		< .001	1		.029
	Female	12.0 (7302)	8.7	0.83	0.78, 0.89		0.95	0.91, 0.99	
Net use previous night	No	14.8 (7234)	11.2	1		.006	1		< .001
	Yes	11.9 (6394)	7.4	0.66	0.49, 0.89		0.57	0.47, 0.69	
Travelled to mainland in last 2 months	No	12.8 (13034)	9.6	1		.057	1		.24
	Yes	26.6 (696)	7.5	0.78	0.61, 1.01		1.11	0.94, 1.30	
Location	Rural	20.7 (3502)	23.4	1		.002	1		< .001
	Urban	11.0 (10233)	4.7	0.20	0.07, 0.55		0.17	0.08, 0.39	
Socio Economic Status (SES)	1st quintile	17.5 (3644)	12.5	1		.17	1		.78
	2nd quintile	14.2 (3724)	9.5	0.76	0.55, 1.03		0.96	0.78, 1.18	
	3rd quintile	11.6 (3516)	8.1	0.65	0.43, 0.98		0.90	0.70, 1.15	
	4th quintile	9.5 (2851)	7.4	0.59	0.35, 0.98		0.91	0.67, 1.23	
Year	2017	10.0 (2768)	3.6	1		< .001	1		< .001
	2018	11.3 (2551)	3.1	0.87	0.66, 1.15		0.94	0.71, 1.24	
	2019	14.7 (2490)	13.9	3.86	2.43, 6.14		5.17	3.91, 6.83	
	2020	12.0 (2204)	7.8	2.16	1.38, 3.40		3.35	2.42, 4.64	
	2021	15.4 (2234)	10.1	2.83	1.69, 4.74		3.83	2.64, 5.55	
	2022	20.6 (1488)	25.7	7.15	2.90, 17.62		5.92	3.30, 10.63	
Time indoors categories	Didn't leave/<6 pm	10.3 (2442)	6.6	1		< .001	1		< .001
	6 pm-<10 pm	14.3 (8419)	9.9	1.52	1.17, 1.96		1.31	1.16, 1.47	
	10 pm-6 am	13.5 (2874)	10.7	1.63	1.38, 1.94		1.52	1.33, 1.73	

N = Number of participants, CI = Confidence interval.
[a] Adjusted for all the variables on the table.

ing older and going indoors later at night were associated with higher biting exposure (Table 3). In adjusted analysis lower biting exposure was associated with living in urban areas (rate ratio (RR) = 0.17, 95% CI: 0.08-0.39), and with ITN use (RR = 0.57, 95% CI: 0.47-0.69). Biting exposure was associated with going indoors between 6 pm and 10 pm (RR = 1.31, 95% CI: 1.16-1.47) and with going indoors between 10 pm and 6 am (RR = 1.52, 95% CI: 1.33-1.73), compared to those who did not leave the house (Table 3).

Discussion

A recent systematic review on mosquito feeding behavior [4] concluded that "residual transmission is likely to become a principal challenge to malaria control and elimination" since the effectiveness of LLINs, the core protection against vector mosquito bites, relies on people being under a net during the hours of intense mosquito biting. Based on a limited set of human behavior

data that were then extrapolated across Africa, the review estimated that overall, 79% of bites occurred when people are in bed and hence potentially protected by LLINs, a proportion that was estimated to have declined by 10% over the period since 2003. Based on this review, therefore, across the continent 21% of biting could not be prevented by current vector control tools. Combining HLC and human behavior data, linked by location and year, our results showed that, on average, 66% of bites were not amenable to being averted by sleeping under a bed net (residual biting). This estimate should be regarded as conservative, as it assumes no bites occur while sleeping under a bed net and does not take account of daytime biting, which may be substantial, as has been shown in other studies [27].

On Bioko, indoor biting contributes 63% to residual biting (4.7 bites indoors vs. 2.7 bites outdoors, per person per night), largely due to the early biting peak of mosquitos and relatively long period of humans spending time indoors before going to bed (approximately 2 hours on average). The balance between indoor and outdoor components of residual transmission will vary across Africa, as it is determined by the behavior of different vector species, and by varying human behavior, determined by location, custom, climate, and availability of electricity. Knowing the relative contributions of indoor and outdoor residual biting is crucial to choosing what supplementary interventions to deploy in each setting. In most settings, the proportion of bites that are '"residual," i.e., not amenable to be averted by the use of LLINs, is at a minimum the outdoor bites that a person receives on average. On Bioko, if indoor vector control could fully eliminate indoor biting, this would still leave individuals exposed to 2.7 bites per night outdoors on average, corresponding to 24% of biting that cannot be prevented by sleeping under a bed net. This fraction is similar to the one reported by Sherrard–Smith [4] but excludes residual transmission driven by indoor bites not averted by existing indoor interventions. The large resulting fraction of residual biting occurring both indoors and outdoors could explain the lack of progress in further reducing malaria on Bioko.

As has been reported in previous studies, outdoor biting rates on Bioko exceed indoor biting rates [7]. This study quantified the actual exposure of humans to indoor and outdoor biting in relation to their behavior, thereby assigning to 13,735 respondents the average number of nightly indoor and outdoor anopheles vector bites they would have received. Both outdoor and indoor biting rates are at their highest between 9 pm and midnight, by which time most residents (>80%) reported being indoors. The large sample size of this study leads to the robust overall conclusion that most bites and hence most malaria transmission occurs indoors (Figure 3c). This is in part due to the low reported use of nets (47%). Using a net on average, nearly halved the number of indoor bites an individual was exposed to compared to someone not using a net, but even a net user received most of their bites indoors. If net use were higher, the overall number of indoor bites would be reduced, but indoor biting would still exceed outdoor biting. This is because individuals spend time indoors before going under a bed net and because indoor biting rates are close to their peak during the part of the evening when people are indoors but not under a bed net. Higher rates of use of dual active ingredient [28] nets which are effective in killing blood seeking pyrethroid resistant mosquitoes, would reduce mosquito numbers overall, thereby increasing the community (indirect) effect, and thus reduce malaria. Nevertheless, the gap in indoor biting protection before people go to bed can only be addressed by methods that prevent indoor biting overall, such as spatial repellents or effective house screening [29,30], or methods that reduce overall vector mosquito populations, for example, larviciding [31], or in future, genetic approaches [32].

Children on average receive fewer bites than adults (7.3 vs. 10.2 bites per night), but they receive a higher proportion of bites indoors since they spend less time outdoors than adults. On average they spend more time under a bed net than adults. Prioritizing children to sleep under LLINs, therefore, makes sense because it is a more efficient use of nets, and because they are more vulnerable to severe disease.

Since outdoor biting rates are higher than indoor biting rates, staying outdoors longer at night was clearly a risk factor for biting exposure (Table 3). The biggest risk factor for biting exposure was where people live, with those in rural sites on average getting five times as many bites as their urban counterparts. There was large between-year variation in biting rates, and hence in biting exposure (Table 3).

Receiving more anopheles mosquito bites increased risk of malaria infection regardless of whether bites were received indoors or outdoors (Table 2). It underscores the importance of reducing overall biting exposure to prevent malaria infection. Other risk factors for infection are those that have previously been identified from Bioko MIS [26]: age 10 to <20 years, not using a mosquito net, recent travel to the mainland, rural location, lower socioeconomic status, and year of the survey. Travel to the mainland remains an important risk factor which is not amenable to being affected by any additional vector control measures and hence biting exposure on Bioko [33].

Previous studies have shown that HLC outdoor biting rates are as high or exceed indoor biting rates [34–36] thus focusing on the need for reducing outdoor transmission. Whilst outdoor biting is clearly an important component of biting exposure, our results show that in this setting many people are exposed to more bites indoors than outdoors. Providing more comprehensive protection against indoor biting or against mosquito bites overall, therefore presents an urgent priority that malaria control programs need to address to reduce transmission and ultimately eliminate malaria.

Limitations

This study did not collect biting data indoors and outdoors outside the night-time period from 7 pm to 6 am. Recent studies [27,37] showed that whilst outdoor biting was restricted to the hours between sunset and sunrise, indoor biting continued during daytime. In addition, it was assumed that LLINs offer 100% protection against mosquito bites, yet studies show that they do not prevent all bites when people use them [38]. Residual biting indoors as a proportion of all biting may, therefore, be even higher than our results show.

The estimates of total biting exposure computed for survey respondents in this study assume that they experience the same hourly biting rate, either indoors or outdoors, as HLC collectors at the same site during a particular hour of the night and in a particular year. Biting rates vary substantially between nights, and over short distances. Hence, using the annual average HLC at a sentinel entomological site to impute an individual's exposure to bites on a particular night, at a location several hundred meters away will not be a precise estimate of the actual number of bites they received. By using data from several years and many locations representing variation in both human and vector behavior across Bioko, we believe this study provides reasonable estimates of exposure at population level.

We did not have separate indoor HLC biting rates for different types of houses. Future studies could investigate the effect of housing type on indoor biting exposure, by conducting indoor HLCs in a variety of housing types.

As mentioned above, this study was not able to take account of the community effects of net use.

Conclusion

LLINs remain an important intervention, which can be made more effective in Bioko. This study demonstrated on the basis of a large data set of human behavior and biting rate data indoors and outdoors that indoor residual biting is greater than outdoor biting, even for users of LLINs. A high proportion of biting, mostly indoors, may not be preventable by LLINs. New vector control tools are urgently needed to provide protection against malaria indoors before going to bed, and outdoors.

Declarations of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical approval

The ethics committee of the Ministry of Health and Social Welfare of Equatorial Guinea provided ethics approval to implement the annual Malaria Indicator Survey.

Author contributions

CAG, GAG, and IK conceived and designed the study. MO conducted all statistical analyses. MO, IK, and CAG drafted the manuscript. DSG and OTD were responsible for the design and execution of the household surveys. NRB, ML, VONM, PBEE, and WE were responsible for the entomological data collection. KRD contributed and reviewed the methods on entomological collections. MRR and GAG were responsible for providing the overall framework in which the study was conducted. All authors critically reviewed the text and approved the final manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2024.107197.

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