

Anopheles arabiensis and Anopheles funestus s.s. biting pattern in Dielmo, an area of low level exposure to malaria vectors.

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Abstract

Background: In Dielmo, Senegal, the widespread use of long lasting insecticidal nets has decreased both the incidence of malaria and the density of the anopheles population. However, persistent low-level malaria transmission may hamper efforts to eliminate the disease. Therefore, continuous monitoring of the vector population is needed in order to improve knowledge of anopheles biting behaviour and to readjust control interventions.

Methods: In 2015, anopheles were collected every month for a whole year and each specimen was identified using morphological and molecular techniques. The biting pattern of each species was analysed according to night (7pm–7am) and day (7am–11am) periods, the place of biting and the season. The ELISA CSP technique was used to evaluate the entomological inoculation rate (EIR).

Results: *An. arabiensis* and *An. funestus* s.s. were found to be the main vectors biting human in Dielmo. Overall, the biting rate was low, at 3.84 bites per night (bpn) and 1.27 bites per day (bpd), respectively. The EIR was 2.51 and 5.03 infected bites per year during the night and day, respectively. During the night, the *An. arabiensis* and *An. funestus* biting rate was 1.81 bpn and 1.71 bpn, respectively ($p=0.80$). During daylight hours, their aggressiveness decreased to 0.51 bpd and 0.73 bpd for *An. arabiensis* and *An. funestus*, respectively ($p=0.30$). During the night, depending on the seasons, only *An. funestus* displayed a significant outdoor biting behaviour both in the rainy and dry seasons, while during the day, no specific trend of indoor or outdoor biting was observed, regardless of the season for both vectors.

Conclusion: This study highlighted low level anopheles nocturnal and diurnal biting and the associated risk of malaria transmission. It showed also the influence of the season on the indoor and outdoor biting pattern, indicating that the human population could be exposed all year round to a low level of anopheles bites. Control programs should increase awareness of the use of bed-nets throughout the year, particularly shortly after dawn when people are still indoors.

Background

The incidence of malaria has decreased significantly over the last fifteen years [1]. The decrease in incidence of the disease was made possible due to combined strategies targeting both the *Plasmodium* parasites and anopheles vectors. This situation has made it possible to envisage the elimination of malaria in some endemic countries. However, malaria still remains a public health problem, as no significant progress has been made in reducing the incidence of the disease further in recent years [1]. This situation highlights the need to reinforce malaria surveillance, particularly the control of anopheles vectors. Universal coverage of long lasting insecticidal nets (LLINs) is the primary and most effective strategy for controlling anopheles vectors and consequently transmission of *Plasmodium* parasites [2]. The use of LLINs represents a powerful barrier against anopheles mosquitoes biting and resting indoors. For this reason, the LLINs could continue to be effective as long as the human populations stay indoors during the period when anopheles are seeking hosts. However, socio-environmental changes could reduce

the effectiveness of LLINs and sustain malaria transmission even at very low levels [3,4]. Changes in human behaviour, such as staying outside sleeping places where LLINs are not used could make a portion of the population vulnerable to anopheles bites [3,4]. On the other hand, the complex bio-ecology of anopheles may have an impact upon the effectiveness of LLINs. Indeed, it is well known that most of the efficient vectors of malaria in endemic areas have developed particular behaviour enabling them to avoid insecticide treated tools and to feed safely on the human population [5]. This avoidance of LLINs by anopheles currently marked by significant outdoor feeding after the introduction of bed-nets in some endemic areas [6,7]. In addition to increased outdoor biting, anopheles may adapt their biting time to specific periods during which the LLINs may not be used by the population. It is now widely observed that have crepuscular and diurnal host-seeking activity which coincide, respectively, with the periods just before people go to sleep under their bed nets and just after waking up when they move outside the bed nets [8–10]. Therefore, despite a decrease in human exposure [7,11] resulting from general coverage and a high level of bed net use, anopheles behaviour could reduce the effectiveness of LLINs as people remain unprotected when mosquitoes shift their biting times or locations[9,12]. This situation could therefore represent a challenge for malaria control, particularly in areas where vector controls have been implemented to eliminate the disease. Thus, close monitoring of vectors is needed in order to determine the particular biting behaviour of anopheles that could put the human population at risk, despite the use of LLINs.

Materials And Methods

Study Area

The village of Dielmo is located 280km southeast of Dakar. The climate is typical Sudanese-Savanna and the rainy season occurs from June/July to October/November. LLINs (active ingredient: deltamethrin; PermaNet® 2.0) universal coverage strategy began in 2008 with three general renewal operations in 2011, 2014, 2016 and 2019.

Field study and laboratory processing of mosquitoes

The human landing catch (HLC) technique was used to collect anopheles mosquitoes every month between January 2015 and December 2015. Every month, mosquitoes were trapped at two sites over three consecutive nights. For each site, the collections were made both indoors and outdoors from 7pm to 11am. The mosquitoes trapped from 7pm to 7am and from 7am to 11am represent the night and the day-time collection, respectively. All mosquitoes were identified morphologically using the Giles and DeMeillon keys. In addition, a one-step PCR method using intentional mismatch primers (IMPs) [13] was used to identify sibling species of *An. gambiae* s.l. complex and *An. funestus* group collected during the entire study. The crushed head and thorax was used to detect the presence of *Plasmodium falciparum* circumsporozoite protein (CSP) antigen in each anopheles specimen using the ELISA-CSP technique [14].

Rainfall data collection

During the entire study period, daily rainfall was recorded to define a mean level of rainfall during the rainy and dry seasons.

Data analysis

The human biting rate (HBR), which represents the aggressiveness of the anopheles was calculated by dividing the number of mosquitoes collected by the number of person-night/day during the sampling period. Thus, throughout the course of the study, total anopheles aggressiveness during the night and day was evaluated as well as the hourly biting rate. The entomological inoculation rate (EIR) was obtained by multiplying the HBR by the ratio of the number of infected mosquitoes by the number of total mosquitoes screened for the presence of *Pl. falciparum*.

The aggressiveness of the anopheles was analysed according to the time of biting (night/day), the place of biting (indoor/outdoor) and the season (dry/rainy) using a GLM (generalised linear model) with a Poisson distribution. Analyses were performed using Stata Software, version 11.0 (College Station, Texas, USA).

Results

Anopheles species composition

From January to December 2015, a total of 680 female anopheles were collected according the following distribution: 588 (86.49%) specimens in the night and 92 (13.50%) in the day. Morphological identification enabled us to highlight the presence of *An. ziemanni*, *An. pharaonis* and species which belong to the *An. gambiae.s.l* complex and *An. funestus* group. In order to have the precise species composition of the anopheles population, molecular identification was carried out on *An. gambiae s.l* complex and *An. funestus* group specimens. During the night, *An. arabiensis* and *An. funestus* represented 261 (44.38%) and 247 (42%) of the collection, respectively. At the same time *An. coluzzii* and *An. gambiae s.s* species, which belong to the *An. gambiae s.l* complex, were found in the proportions of 26 (4.42%) and 20 (3.40%), respectively. However, PCR did not allow us to identify nine (1.53%) specimens of *An. gambiae s.l* caught during the night. *An. ziemanni* and *An. pharaonis* represented 16 (2.72%) and nine (1.53%), respectively, of the entire night-time collection. In the daylight collection, 53 *An. funestus* (57.60%), 37 *An. arabiensis* (40.21%) and two *An. coluzzii* (2.17%) were collected.

Anopheles biting pattern

Overall, from 7pm to 11am, the biting rate was at 2.56 bites/person. The HBR was significantly different during the night and the day with 3.84 bites per night (bpn) and 1.27 bites per day (bpd) ($p<0.001$), respectively. *An. arabiensis* and *An. funestus* were the main species biting humans both at night and day. During the night, *An. arabiensis* and *An. funestus* had almost the same biting rate, 1.81 bpn and 1.71 bpn, respectively (figure 1) ($p=0.80$). During the day, the biting rate of *An. funestus* (0.73 bpd) was slightly higher than that of *An. arabiensis* (0.51 bpd) (Figure 1) ($p=0.30$). However, during the whole study period

and both during the day and the night, there was no significant difference between the biting rate of *An. arabiensis* and *An. funestus* ($p=0.84$), and the aggressiveness of *An. coluzzii* and *An. gambiae s.s.* was significantly lower than that of *An. arabiensis* and *An. funestus* ($p<0.001$). The *An. coluzzii* biting rates was 0.18 bpn and 0.02 bpd, whereas *An. gambiae s.s.* biting activity was noticed only during the night (0.13 bpn) (Figure 1).

Anopheles hourly aggressiveness

An. funestus and *An. arabiensis* aggressiveness increased progressively throughout the first half of the night (7pm-12 midnight) reaching 0.20 and 0.17 bites per hour, respectively. During the second part of the night (12 midnight-7am), two peaks of aggressiveness were observed for both vectors (Figure 2). The first peak was recorded between 1am and 2am with an HBR of 0.29 and 0.31 bites per hour for *An. funestus* and *An. arabiensis*, respectively. The second peak was observed at the end of the second part of the night, between 4am-5am for *An. funestus* (0.24 bph) and between 5am-6am for *An. arabiensis* (0.17 bph). The HBR of *An. coluzzii* and *An. gambiae s.s.* was very low and constant, despite weak peaks of aggressiveness occurring between 11pm-12 midnight, between 6am-7am for *An. coluzzii*, and between 1am-2am for *An. gambiae s.s.*. However, there was no significant difference in the level of aggressiveness between the first and the second part of the night ($p= 0.45$) (Figure 2). The peak of aggressiveness in daylight was observed between 7am and 8am with an HBR of 0.17 bites per hour and 0.09 bite per hour for *An. funestus s.s.* and *An. arabiensis*, respectively (Figure 2).

Anopheles indoor and outdoor biting activity

The influence of the season (dry season: 0.5 mm of rainfall; rainy season: 813.38 mm of rainfall) on anopheles biting patterns was also evaluated by taking into account the place of biting (indoor/outdoor), the species and period (night/day).

Overall during the study, in the night, the HBR was higher during the rainy season (2.520 bpn) compared to the dry season (1.32 bpn) ($p<0.001$). Regardless of the season, the outdoor biting rate (5.01 bpn) was significantly higher than indoor aggressiveness (2.68 bpn) ($p= 0.004$). Depending on the place of the biting, the *An. arabiensis* outdoor biting rate (2.19 bpn) was higher than that recorded indoor (1.43 bpn) despite the fact that this trend of exophagic behaviour was not significant ($p>0.168$). *An. funestus* had a HBR of 0.95 bpn and 2.47 bpn indoors and outdoors, respectively ($p=0.006$). The *An. coluzzii* biting rate was identical indoors and outdoors (0.18 bpn); *An. gambiae s.s* showed nearly the same biting rates indoors (0.11 bpn) and outdoors (0.16 bpn) (figure 3a). When the season and the biting place are combined, only *An. funestus* displayed significant exophagic behaviour both in the rainy and dry seasons. *An. funestus* HBR was at 1.08 bpn and 0.34 bpn outdoors and indoors, respectively in the dry season ($p <0.043$) (Table 1a). In the rainy season, outdoor and indoor aggressiveness was at 1.38 bpn and 0.61 bpn, respectively ($p=0.06$) (Table 1a). The vectors belonging to the *An. gambiae s.l.* complex did not show any significant difference. During the rainy season, the aggressiveness of *An. arabiensis* was 1.02 and 1.54 bpn indoors and outdoors, respectively ($p=0.27$) while it was 0.40 bpn indoors and 0.65 bpn outdoors ($p=0.40$) in the dry season (Table 1a). The *An. coluzzii* feeding rate was at 0.13 bpn and

0.06 bpn outdoors and indoors, respectively during the rainy season ($p=0.61$), while the opposite trend was observed in the dry season (0.11 bpn indoors and 0.04 bpn outdoors) ($p=0.55$) (Table 1). In the rainy season, *An. gambiae* s.s. displayed a biting rate of 0.09 bpn and 0.16 bpn indoors and outdoors, respectively ($p=0.64$). In the dry season, aggressiveness was only observed indoors (0.01 bpn) (Table 1a).

During the daytime there was no significant difference ($p=0.81$) between aggressiveness during the rainy (0.51 bpd) and the dry season (0.76 bpd) and the indoor aggressiveness (1.77 b/p/d) was significantly higher than that recorded outdoor (0.75 bpd) ($p=0.03$). The *An. arabiensis* indoor and outdoor HBR was at 0.75 bpd and 0.25 bpd, respectively ($p=0.099$) (Figure 3b) and no significant difference was found ($p=0.186$) between the indoors (0.97 bpd) and the outdoors (0.5 bpd) regarding the aggressiveness of *An. funestus* (Figure 3b). During daylight during the dry season, *An. arabiensis* biting activity was only observed indoors (0.36 bpd) area while in the rainy season it was 0.38 bpd and 0.25 bpd indoors and outdoors, respectively ($p=0.55$) (Table 1b). In the dry season, the indoor and outdoor aggressiveness of *An. funestus* was 0.83 bpd and 0.27 bpd ($p=0.082$), respectively. In the rainy season, its aggressiveness was at 0.13 bpd and 0.22 bpd indoors and outdoors, respectively ($p=0.634$) (Table 1b). *An. coluzzii* aggressiveness was only observed indoors and only during the dry season (0.055 bpd) (Table 1b).

Anopheles EIR

Throughout the study, two anopheles specimens were found to be positive using the ELISA-CSP test and were collected during the night and the day. Throughout the whole study, only two specimens were found to be using to the ELISA-CSP test: one *An. arabiensis* and one *An. funestus* collected during the night and the day, respectively. Both positive specimens were caught outdoors, during the dry season. The EIR was, therefore, estimated to be 2.51 infected bites per person per year during the night compared to 5.03 infected bites per person per year during the day.

Discussion

In Dielmo, malaria vectors have been monitored since 1990 [15]. The results of this investigation confirm the dominance of *An. arabiensis* and *An. funestus* as the main vectors of malaria and the collapse of the anopheles population that has been observed in Dielmo since the introduction of universal LLIN coverage [7]. In addition to *An. funestus* biting humans during daylight, as previously described [9], this study shows, for the first time, the diurnal host-seeking activity of *An. arabiensis* and *An. coluzzii* in Dielmo. Therefore, in Dielmo, three main malaria vectors now demonstrate diurnal host-seeking behaviour. The widespread introduction of insecticide-based control explains the host-seeking activity of anopheles shortly before dusk and after dawn [9,10,16,17]. In Dielmo, universal LLIN coverage has been the only strategy implemented to control the anopheles vector since 2008 and has been suspected of contributing to the daytime behaviour of *An. funestus* [9]. To date, there has been no thorough investigation into the daytime behaviour of anopheles vectors. It is possible that this behaviour is due to the plasticity of vectors that continue their host seeking activity during daylight when they could not feed at night due to the use of LLINs. On the other hand, anopheles mosquitoes have a specific circadian rhythm in which

blood-feeding activities are preferentially performed during the night [18]. Thus, this daytime biting activity may imply a change in anopheles circadian rhythms, despite the fact that experimental exposure to light can alter their biting ability [19]. Further investigations are needed to assess the basis of this diurnal biting behaviour, as it is now becoming widespread in Dielmo.

In Dielmo, the *An.gambiae s.l.* complex and *An. funestus* groups are the only malaria vectors involved in *Plasmodium* transmission, therefore, the HBR and the EIR were evaluated accordingly. *Anopheles* daylight biting activity is marginal compared to the night-time vector aggressiveness which is three times higher. Paradoxically, the level of EIR during the day is twice as high compared to the night. The same trend was observed in Dielmo in 2011 when *An. funestus* was the sole vector biting humans during the day [9]. However, in this previous study, the level of exposure to vector bites was higher than that observed in our study, during which the incidence of the disease was very low and transmission remained seasonal [20]. Therefore, the EIR evaluated during this study should be interpreted with caution, as the level of exposure to anopheles during the day and the night is very low. This study therefore questions the value that should be given to entomological data and the problem of the level of vector surveillance required in areas dealing with low level of exposure to vector bites and residual malaria transmission [21,22]. However, incorporating a socio-demographic aspect into control strategies could help to provide better containment for residual transmission as human behaviour contributes greatly to malaria outbreaks in this situation [3,23] and malaria transmission could persist at very low level even during the dry season [24].

On the other hand, during the night, the peak of hourly aggressiveness observed did not change compared to a previous study in Dielmo and remains confined to the second part of the night [7]. This indicates that the population could be protected while resting indoor, as the peaks observed during the night correspond to moments when the population is asleep [7], and Dielmo area is marked by a relatively high compliance rate in the use of LLINs [4], unlike the peak that occurs during the day, when people are awake and remained unprotected by the LLINs. Thus, in areas striving to eliminate malaria, more attention should be paid to the anopheles daylight biting activity which essentially takes place indoors and throughout the year, which could maintain residual levels of *Plasmodium* transmission. In Dielmo, the introduction of a significant healthcare system allowing for rapid diagnosis and malaria case management [25], combined with close entomological monitoring are used to detect and/or prevent outbreaks that can result from this low level of transmission. The increase in anopheles outdoor biting which was observed after the implementation of LLINs or IRS in endemic malaria areas [6,7,26], could also sustain the residual transmission of malaria. Indeed, despite the fact that LLINs and IRS significantly reduce the density of the vectors, the shift of the anopheles to outdoor, combined with the socio-environmental upheaval of changing human behaviour could sustainably maintain residual malaria transmission and the occurrence of outbreaks [3,22]. Therefore, controlling outdoor exposure is the current challenge facing malaria control programs. However, a more comprehensive understanding of the behaviour of anopheles is required to address this issue. In the present study, the total outdoor biting rate is higher than indoor aggressiveness, which is the main trend observed in anopheles biting behaviour. However, in rural areas, the changing seasons has an impact both on vector densities and on humans

resting behaviour (outdoor or indoor). Hence, an understanding of the spatio-temporal biting behaviour of vectors may help to identify when and where people are exposed. In Dielmo, it appears that the main malaria vectors, *An. arabiensis* and *An. funestus* do not have the same behaviour in the wet and dry seasons. During the night and both in the dry and rainy season, *An. Arabiensis* showed no preference for biting outdoor or indoor. At the same time, *An. funestus* showed a marked presence for outdoor biting regardless of the season. This shows that, despite marked outdoor feeding behaviour that has been observed in malaria endemic areas after the implementation of LLINs, indoor feeding should always be taken into account, as it could sustain malaria transmission despite the presence of LLINs [27]. Hence, whatever the season, similar attention should be paid to both indoor and outdoor biting, as *An. arabiensis*, a major malaria vector, has the same biting level in both locations, despite very low level exposure. It also shows that not all vector species from the same malaria transmission area have the similar biting behaviour, thereby revealing the complexity of human exposure to anopheles bites when LLINs are in use. It becomes therefore important to consider anopheles adaptive response to insecticide-treated tools as this could help identify the occurrence of residual exposure to their bites when LLIN universal coverage is implemented over a long period as is the case in Dielmo. In this area, previous studies showed that the introduction of LLINs induced a shift in the anopheles population in favour of *An. arabiensis* which became the dominant species [28]. Furthermore, it induced a temporal and spatial structuration of the *An. arabiensis* population [29] suggesting a different subpopulation that may have a different pattern of biting. In Dielmo, the adults *An. arabiensis* issued from a larval population carry kdr alleles despite the fact that this was not associated with resistance to pyrethroids [30], while no data regarding *An. funestus* is available, although its resistance to lambda-cyhalothrin has been recorded elsewhere in Senegal [31].

Conclusion

In Dielmo, the human population is exposed to low level bites from three anopheles vectors both during the night and during the day. Despite this low level of exposure, awareness should be reinforced for LLIN use all year round. Taken as a whole, these results suggest that, in addition to the objective of developing complimentary tools to control the outdoor biting of anopheles, greater effort must be made to annihilate the residual daylight aggressiveness that occurs during both the dry and rainy seasons.

Declarations

Authors' contributions

SD, ND, and CS designed the study. SD and ND supervised the study. CB carried out the field collections. OT did the experiments. SD and ANW analysed the data. SD wrote the manuscript. SD, ND, ANW and PP reviewed the final draft. All authors read and approved the final manuscript

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Ethics approval and consent to participate

The study was approved by the Ministry of Health of Senegal and the assembly of the Dielmo population.

Consent for publication

Not applicable

Abbreviations

bpn bite per night

bbpd bite per day

bph bite per hour

CSP circumsporozoite Protein

ELISA Enzyme Linked Immunosorbent Assay

EIR entomological Inoculation Rate

HBR human Biting Rate

HLC human landing catch

LLINs long lasting insecticidal nets

IMPs Intentional mismatch primers

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Table

Table1 a: Anopheline indoor and outdoor biting rate during the night according the season. Each value represents the number of bites per person per night, indoor or outdoor. Only *An. funestus* displayed significant outdoor feeding both during the dry ($p < 0,043$) and the rainy season ($p=0.06$)

Night

	Dry Season				Rainy Season			
	<i>An. arabiensis</i>	<i>An. funestus</i>	<i>An. coluzzii</i>	<i>An. gambiae</i>	<i>An. arabiensis</i>	<i>An. funestus</i>	<i>An. coluzzii</i>	<i>An. gambiae</i>
indoor	0,40	0,34	0,11	0,01	1,02	0,61	0,06	0,09
outdoor	0,65	1,08	0,04	0	1,54	1,38	0,13	0,16

Table1 b: Anopheline indoor and outdoor biting rate during the day according the season. Each value represents the number of bites per person per day, indoor or outdoor. No significant feeding behaviour according the place of biting was observed for both vector during the dry and the rainy season.

	Day							
	Dry Season				Rainy Season			
<i>An. arabiensis</i>	<i>An. funestus</i>	<i>An. coluzzii</i>	<i>An. gambiae</i>	<i>An. arabiensis</i>	<i>An. funestus</i>	<i>An. coluzzii</i>	<i>An. gambiae</i>	
indoor	0,36	0,83	0,05	0	0,38	0,13	0	0
outdoor	0	0,27	0	0	0,25	0,22	0	0

Figures

Figure 1

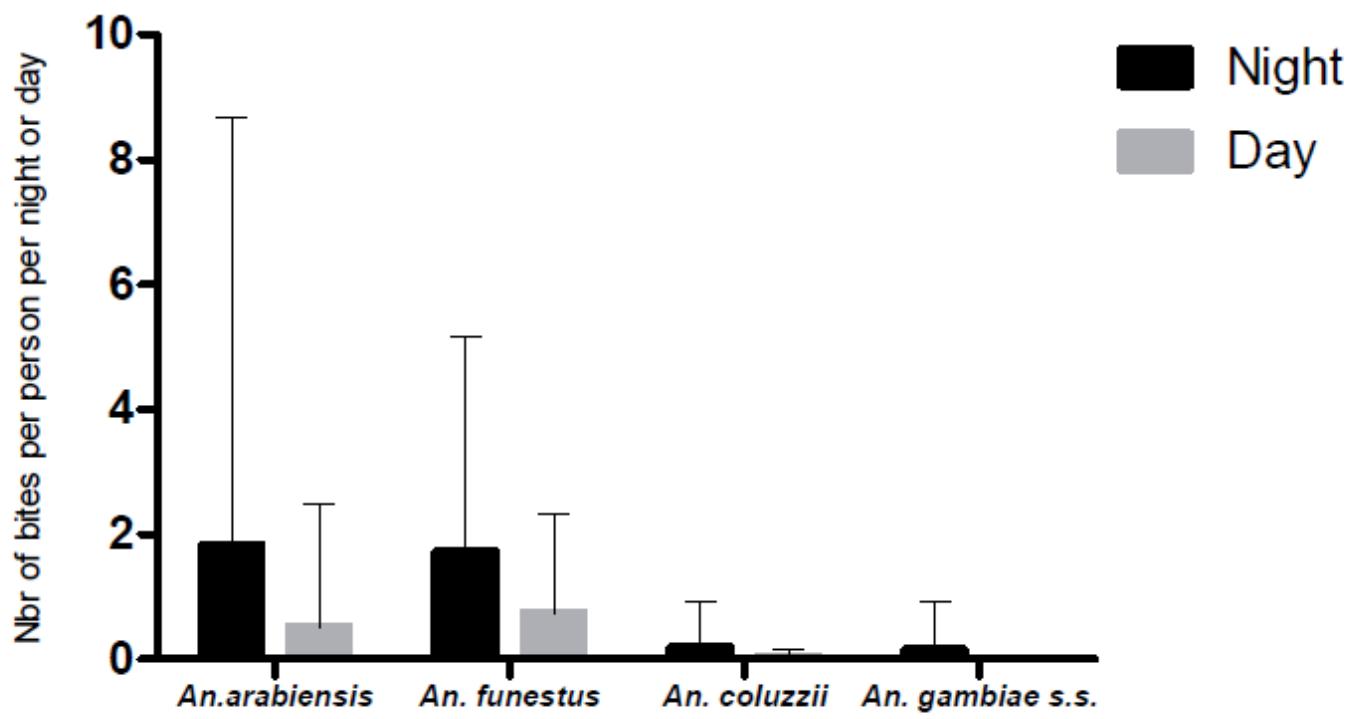


Figure 1

the level of human exposure to anopheles bites during the night and the day An. arabiensis and An. funestus were the main species biting humans both at night and day with no significant difference between their biting rate ($p=0,84$). An. coluzzii and An. gambiae s.s. aggressiveness was marginal.

Figure 2

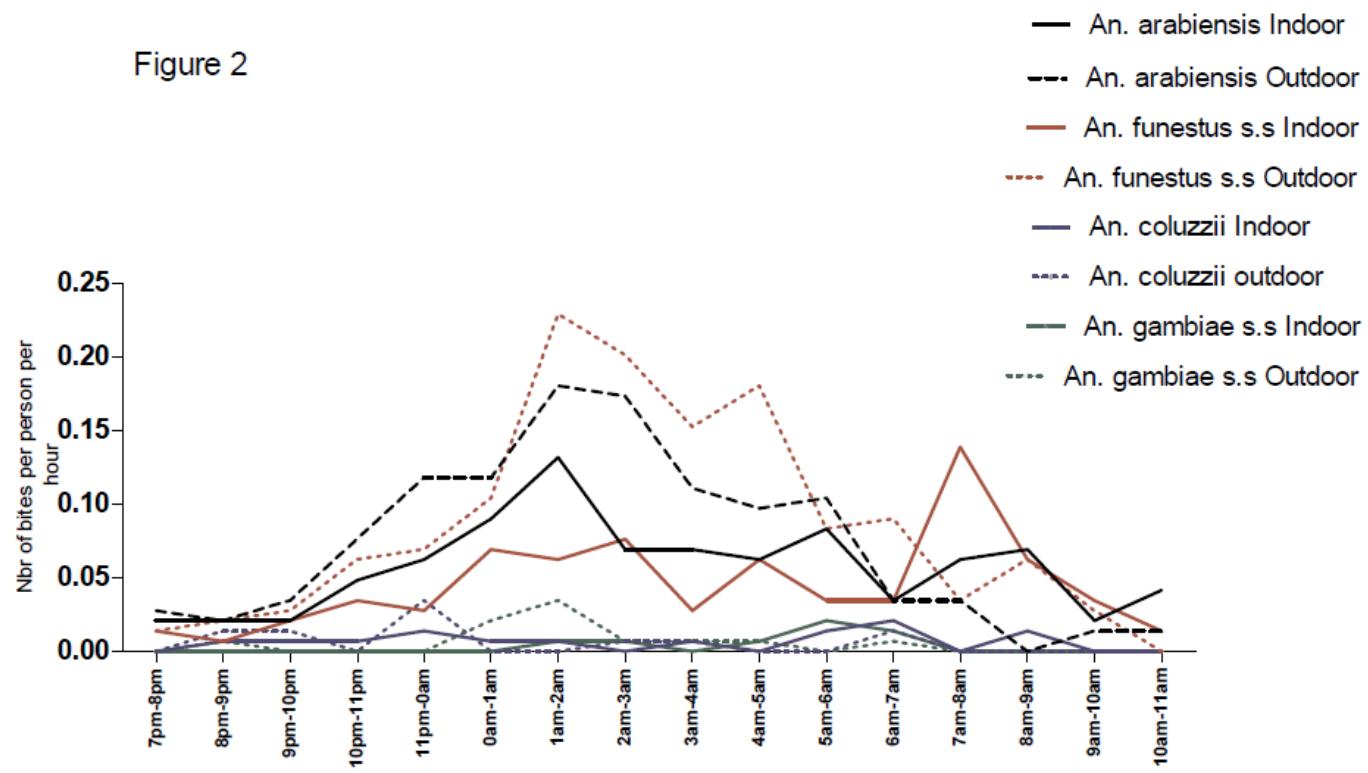


Figure 2

Anopheles hourly aggressiveness from 7pm to 11am Two peaks of aggressiveness involving *An. arabiensis* and *An. funestus* were observed during the first and second parts of the night. A third peak was also observed during the day.

Figure 3 b

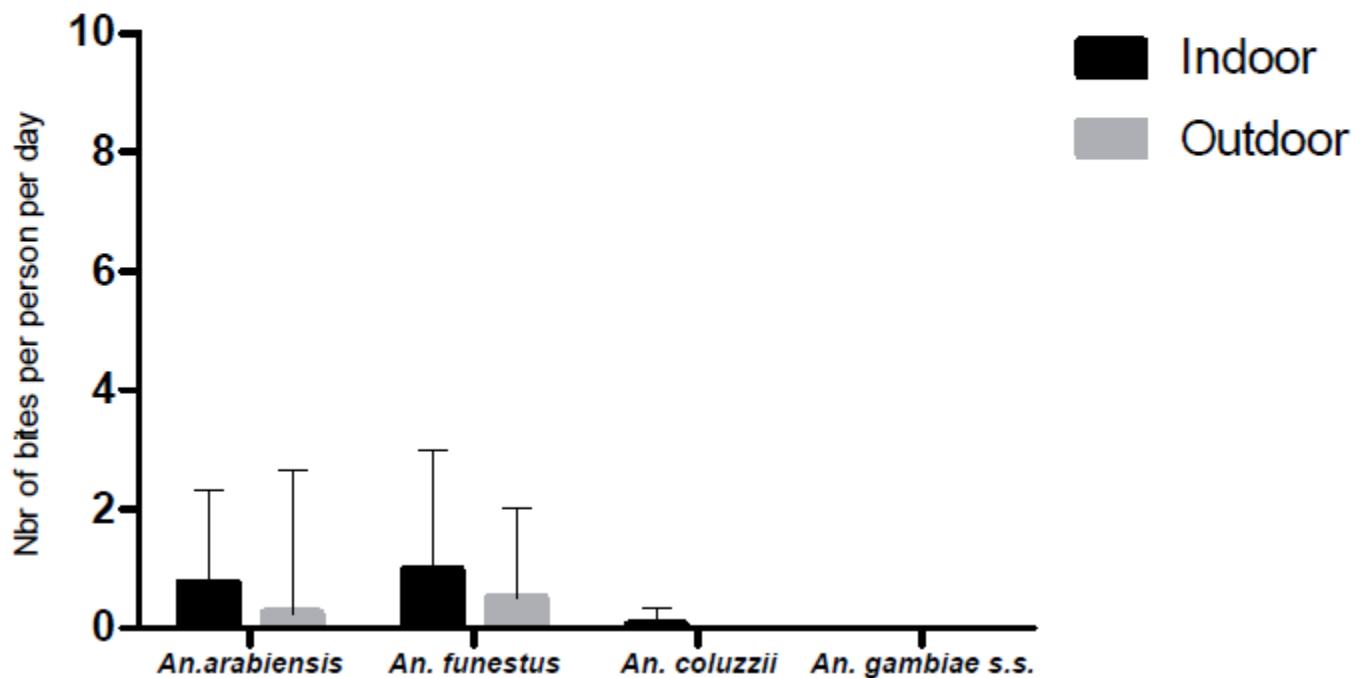


Figure 3

Figure 3: (b) Anopheles indoor and outdoor biting rates during the day During the day, for *An. arabiensis* ($p=0.099$) and *An. funestus* ($p=0.186$) the indoor aggressiveness of both vectors was greater compared to outdoor. (Note: Figure 3A was not provided by the author.)