The impact of insecticide-treated bed nets on malaria sporozoite-infective biting hours and transmission intensities in Kamuli District, Uganda: A longitudinal study

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Abstract

Background: Despite the wide coverage and prolonged use of insecticide-treated bed nets (ITNs) or long lasting insecticide-treated nets (LLINs) in combination with other interventions, malaria has remained the leading cause of illness in Uganda especially among young children. The biting hours of the parous and potentially infectious vectors is thought to have changed following prolonged use of ITNs/LLINs, rendering this intervention less effective. This could explain the continued morbidity and mortality due to malaria in Uganda.

Methods: A Plasmodium falciparum circum-sporozoite protein Enzyme-Linked Immunosorbent Assay, ELISA, was performed on 551 (112 pools) and 1640 (331 pools) Anopheles gambiae s.l. and An. funestus group caught at different hours of the night in intervention (with ITNs) and non-intervention (without ITNs) zones, respectively, in Kamuli district. The circumsporozoite positivity of the vectors was related to the time of biting humans, while the annual entomological inoculation rates (AEIRs) were obtained by multiplying the average annual human biting rate by the sporozoite rate. Data were analysed by one-way analysis of variance (ANOVA). The Plasmodium falciparum sporozoite-infective biting hours of the night and the parasite transmission intensities in both zones are reported.

Results: There was no impact of ITNs/LLINs on the sporozoite-infective biting hours (P = 0.9547), but hour-to-hour differences in sporozoite concentrations were significant in both zones (P = 0.0001). There was no significant difference in the sporozoite infection rates between the two zones (P = 0.6638). Infective biting by the vectors occurred throughout the night, with peak infective bites occurring between 20:00 and 04:00 hours. Many people were, however, exposed to infectious bites before and after bed time in both zones. In both zones, the malaria transmission potential was higher outdoors than indoors, although without significant differences in indoor and outdoor AEIRs between the two zones (Indoor: P = 0.5137; Outdoor: P = 0.4077). Generally, the annual transmission potential was several fold higher in the non-intervention than in the intervention zone.

Conclusion: ITNs/LLINs were still an effective malaria control tool which should be widely promoted in the study area. Other protective interventions when people are not in bed are also recommended.

Key Words: Plasmodium falciparum circumsporozoites, ELISA, biting cycle, Anopheles gambiae, An. funestus

Background

Uganda has one of the world’s highest malaria incidences with a rate of 478 cases per 1000 people per year, with an estimated 70,000 to 110,000 malaria deaths each year [1 - 3]. Malaria is highly endemic in Uganda and is the leading cause of morbidity and mortality especially among young children [4 - 6].
Anopheles gambiae sensu lato and Anopheles funestus are the principal malaria vectors in Uganda. These species have for long been known to bite between 10:00pm and 5:00am [7, 8] as in most parts of Africa. These are hours of the night when most people are in bed and under bed nets if they have them, and were the main entomological justification for the current use of insecticide-treated bed nets for malaria control in Africa [9].

Use of insecticide-treated bed nets/LLINs is one of the main malaria vector control methods being promoted under the World Health Organisation’s Roll Back Malaria (RBM) policy [10, 11, 12] and the Uganda National Malaria Control Programme [13]. In Uganda, ITN use has exceeded five years in several areas. Despite this, malaria related deaths continue to be high even in these areas [14, 15]. Extensive use of impregnated bed nets could have resulted in a greater proportion of the parous and potentially infectious Anopheles mosquitoes changing their biting pattern - biting earlier or later in the night when many people are not in bed [9, 16]. This, in addition to social variables in the different human settings, would render bed nets less effective and could explain the continued high rates of morbidity and mortality due to malaria in Kamuli district [8], and possibly other parts of the country [14].

A study in Kenya showed that biting by the malaria vectors occurred earlier in the evening following ITN use [17]. Studies in Papua New Guinea and Tanzania showed shifts in time of biting, with vector biting occurring earlier in the evening as hosts had not yet gone to bed and were easily accessible [18].

In a study to test bed net traps for monitoring mosquito populations and time of biting in Tanzania and possible impact of prolonged use of insecticide treated bed nets, it was observed that somewhat more of the Anopheles biting occurred early and late in villages with ITNs, whereas in villages with no history of ITN use, biting was concentrated in the middle of the night. This suggested that behavioural adaptation to avoid contact with ITNs could have begun.
to evolve in those ITN villages [19]. In a study on the South eastern coast of Kenya, the malaria transmission intensity of the vectors was reduced following more than five years of 60 to 80% coverage with ITNs [20].

Similar changes could have possibly occurred in several areas in Uganda where ITNs/LLINs have been in use for some time. This study therefore aimed to investigate if the malaria sporozoite infective-biting hours of the night and the transmission intensities by the vector mosquitoes had changed in Kamuli district, Uganda following extensive and prolonged (≥ 5 years) use of ITNs/LLINs. The *Plasmodium falciparum* sporozoite-infective biting hours of the night and the parasite transmission intensities in both zones are reported. These results may give guidance in determining suitable times for deploying the most effective malaria vector control interventions, when the vectors are most active and transmission is at its peak. The results will also assess the impact of the ITNs/LLINs intervention on the Entomological inoculation rates of the major *Anopheles* mosquito vectors in the study area, and possibly other areas in the country with a modest ITN/LLINs coverage of 35-65% or at least 80% coverage known to provide equitable community-wide protection [11, 21].

**Methods**

**Study sites**

Mosquito collections were made in 48 households randomly selected from 10 villages in intervention (Five villages with insecticide-treated bed nets, ITNs) and non-intervention zones (Five villages without ITNs) in Kamuli district. The intervention villages, with 69% of the households using at least one ITN, were located in Kamuli Town Council and Nabwigulu Sub County, while the non-intervention villages were located in Bugaya and Buyende sub counties in the North East of Kamuli Town Council and well over 20 kilometers away from the intervention villages. The intervention villages were privileged with a number of Non Governmental
Organizations (NGOs) such as the Christian Child Fund and Plan-Uganda that intervened with insecticide-treated bed nets since the late 1990s and later with the supply of Long Lasting Nets to supplement the Ministry of Health efforts in the control of malaria targeting pregnant mothers, children under five years and People Living with HIV/AIDS. The NGOs also carried out several community sensitizations in conjunction with the District Health department aimed at promoting ITN use. This is why Kamuli district was chosen for the study.

**Mosquito sampling and identification**

Each month two households from each of the sampling zones were randomly sampled for mosquitoes using human-baited bed net traps [2]. The bed net trap was made by making four to six holes (3 x 3 inches each) on an untreated bed net. This gave some protection to the human-baits sitting inside the trap. The trap permitted the entrance of mosquitoes but prevented their escape. This method was preferred to the CDC light trap because most of the mosquitoes remained alive, while most of the mosquitoes caught in the light trap (used initially) were found dead and brittle, making morphological identification of the samples difficult.

People living in a room were protected with a net each, and as hungry mosquitoes persisted in their attempts to look for a blood meal, they got near to the trap and were caught by it – nets improve the efficiency of the traps [22]. It was assumed that the mosquitoes which entered a trap during any hour were those actively seeking hosts and, in most cases, would bite human hosts in the same hour and room/house if the bed net trap was absent [9].

The human-biting fractions of the mosquito population and time of biting were determined and recorded throughout four repeated nights. Each hourly catch was placed in a disposable polystyrene container pre-labeled with date and time of capture, and taken to laboratory for assessment [23] while feeding on a 10% sugar solution available through a cotton wick [24].
Each hourly catch of the night was identified morphologically using a simplified key [25], while the morphological identifications were confirmed by an Entomologist at the Vector Control Division Laboratory, Ministry of Health, Kampala, Uganda.

**Determination of malaria sporozoite-infected biting hours of the night by *Anopheles* species mosquitoes**

The heads and thoraces of a pool of five mosquitoes were tested for *Plasmodium falciparum* sporozoites by the Enzyme-Linked Immuno-Sorbent Assay, ELISA, method using monoclonal antibodies [9, 23, 26-29] for *Plasmodium falciparum* circum sporozoite protein (CSP) [30]. Five mosquitoes were used per pool to ensure 99% confidence of detecting at least one infected mosquito per pool [31].

The test results were read visually for positivity and scored 30-60 minutes after the substrate was added and then measured spectrophotometrically at 405 nm. Each of the five-mosquito sample pools was considered positive if the colour changed to green and had absorbance values of twice the average of the optical density values of the negative samples. All the positive pools and 10% of the negatives were repeated to confirm the initial results. Sporozoite positivity of the human-biting mosquito proportions were then related to their times of biting.

A standard curve of the optical densities of 8 serial dilutions of the *P. falciparum* CSP positive control versus the respective concentrations was constructed and used to determine concentrations of the various test samples (Figure 1).

**Determination of *Anopheles* sporozoite rates**

Sporozoite rates of the malaria vectors were assessed by processing pooled samples (Five mosquitoes) as it is a highly efficient and economic method. A total of 511 (112 sample pools) and 1640 (331 sample pools) mosquitoes collected from intervention and non-intervention zones,
respectively, were processed for *P. falciparum* sporozoite infection. All positive pools were regarded as undoubtedly *Plasmodium falciparum* sporozoite- infective because only thoraces and heads of the test samples were exclusively used [32, 33]. The sporozoite rate (S) was calculated as the percentage of *Plasmodium* sporozoite positive mosquito sample pools out of the total number of mosquitoes analysed, with the assumption that each positive pool had at least one infective mosquito.

**Determination of entomological inoculation rates and *Plasmodium* transmission intensity**

The average number of sporozoite-positive mosquitoes caught at each hour of the night [Daily Entomological inoculation rate, EIR] were obtained from EIR= mas (where ma = Human-Biting Rate, HBR, and S = sporozoite rate) for the whole sampling period, while the Annual Entomological Inoculation Rate, AEIR, (i.e. the number of sporozoite-positive bites per person per year in intervention and non-intervention zones) were obtained by multiplying the average annual HBR by the sporozoite rate [17, 31], i.e., AEIR = Ma (HBR) x S.

**Data analysis**

The proportions of *Plasmodium falciparum* circum-sporozoite protein positive *Anopheles* bites, differences in sporozoite concentrations between different hours of the night, sporozoite rates and the indoor and outdoor annual entomological inoculation rates in intervention and non-intervention zones were compared by one-way analysis of variance using the Graph Pad Prism software, version 5 [34].

**Ethical Issues**

Prior to start of the study, approval was obtained from the Uganda National Council for Science
and Technology and Health Research Ethics Committee (Reference Number: HS 263). House
hold owners, village and district authorities were sensitized prior to the study and their
permission obtained, while the privacy and psycho-social needs of the individual participants and
household members were highly protected. Catchers were selected from the local community to
facilitate acceptance from residents. Informed consent was obtained from each catcher.
The catchers were trained to collect landing mosquitoes prior to blood feeding to minimize the
risk of malaria transmission. They were given anti-malarial drugs as this geographical area has
high transmission of *Plasmodium falciparum* with resistance to anti-malarial drugs [Dr. Lopita
Micah, Pers. Communication]. At least two bed nets (LLINs) were donated to each participating
household and findings were disseminated to the community in a meeting following the study.

**Results**

**Malaria sporozoite-infective biting and peak infection hours of the night by *Anopheles*
species**

Results indicated that biting by infective mosquitoes occurred during the hours 20:00 to 05:00
and 19:00 to 06:00 hours in the intervention and non-intervention zones, respectively (Table 1).
The distribution of sporozoite positive mosquitoes in three four-hour periods of the night (19:00-
22:00, 23:00-02:00 & 03:00-06:00) showed that infective-biting occurred during all the three
periods of the night (Table 2), demonstrating that generally all human bites by the *Anopheles*
mosquitoes in the study area were potentially infectious with *Plasmodium falciparum*
sporozoites and possibly other *Plasmodium* species [35]. However, three distinct peaks of
infective-biting occurred during the period between 20:00 and 04:00 hours in both intervention
and non-intervention zones, i.e., peak exposure occurred at 21:00, 23:00 and 03:00 hours in the
intervention and at 21:00, 23:00 and 01:00 hours in the non-intervention zone, differing only at
01:00 hour and 03.00 hour in the non-intervention and intervention zones, respectively (Table 2).

**Anopheles sporozoite rates**

Altogether, 38.4% (43 out of 112) and 35.4% (117 out of 331) of the test sample pools were positive for *Plasmodium falciparum* circum-sporozoite protein in the intervention and non-intervention zones, respectively. Assuming that there was only one infected mosquito in each positive sample pool, the minimum infection rate, MIR, was calculated as the ratio of the number of positive pools to the total number of tested mosquitoes [31, 36, 37]. The minimum infection rates (MIR) in the intervention and non-intervention zones were 0.078 and 0.071, respectively. There was no significant difference in the proportions of sporozoite-positive anophelines between the two zones (p = 0.6638, t = 0.4540, df = 22, 95% CI = -0.03093- 0.04836).

In both zones, differences in sporozoite concentrations between the different hours of human-biting were significant (p = 0.0001) (Figures 3a and 3b). *Plasmodium falciparum* sporozoite concentrations were observed to be significantly higher in the intervention than the non-intervention zone (Figure 4) (p = 0.0018, t = 3.553, df = 22, 95% CI = 0.6672- 2.538), an indication of higher malaria sporozoite loads in the salivary glands of infected mosquitoes in the intervention zone [27, 38].

**Entomological inoculation rates and Plasmodium transmission intensity**

The Minimum infection rates, 0.078 and 0.071 (as calculated in sporozoite rates section and table 2 above) were taken as the sporozoite rates for intervention and non-intervention zones, respectively.

These results indicated that generally, both indoor and outdoor malaria parasites transmission intensities were higher in the non-intervention than in the intervention zone (Table 3), the higher
transmission intensities being influenced by the higher human biting rates in the non-intervention zone. However, there was no significant difference in the indoor and outdoor AEIRs between the two zones (Indoor: p= 0.5137, t = 0.6939, df= 6, 95% CI = -3.191-1.781; Outdoor: p= 0.4077, t= 0.8901, df= 6, 95% CI= -10.26-4.787). In the intervention zone, the indoor and outdoor EIRs were shown to be similar [(Table 3), (p = 0.914, t = 0.1126, df= 6, 95% CI = -1.079-0.9844)]. In the non-intervention zone, the outdoor EIR was about four times higher than the indoor EIR (Table 3); however, there was no significant difference between the indoor and outdoor EIRs (p = 0.5412, t = 0.6477, df =6, 95% CI = -9.936-5.777).

**Discussion**

**Malaria sporozoite-infective biting and peak infection hours of the night by Anopheles mosquitoes**

The data presented show the proportions of mosquito test sample pools that were positive for *Plasmodium falciparum* circum-sporozoite protein and the infective-biting hours of the night, and the malaria transmission intensities (AEIRs) by the vector mosquitoes in both intervention and non-intervention zones. The presence of infective mosquitoes was an indicator that transmission of malaria parasites actively occurred in the households of both zones. These results are consistent with earlier reports that *P. falciparum* is the main species of malaria parasites prevalent in Uganda [12, 39, 40] and possibly responsible for most of the morbidity and mortality in this part of the country.

The results showed that the hour-by-hour pooled samples of the-all-night biting Anopheles mosquitoes collected from both zones were circum sporozoite protein-positive, with equally significant differences in sporozoite-positivity at the different hours of the night (p = 0.9547), indicating that use of ITNs in the intervention zone did not affect the malaria sporozoite-infesting hours of the night. In both zones malaria sporozoite infective biting occurred generally
throughout the night, i.e., from 20:00 to 05:00 hours in the intervention and from 19:00 to 06:00 hours in the non-intervention zone. Therefore, although people can still be protected from the night peak *Plasmodium*-infective bites, they are still at a risk of receiving infective bites at hours before and after bed particularly in the non-intervention zones.

*Anopheles* sporozoite infective rates

Although the method used is most suitable when vector infective rates are low [30, 41, 42], the results may help to cost-effectively estimate the transmission dynamics of *P. falciparum* malaria in Kamuli district.

Sporozoite rates appeared to be unaffected by the ITN/LLIN intervention. However, it is very possible that the *Anopheles* infective rates and sporozoite loads could have been even much higher before intervention with ITNs/LLINs in this zone. The variances in sporozoite concentrations between the different hours in both zones were very significant (p = 0.0001), and sporozoite concentrations were seen to be higher in the intervention than in the non-intervention zone. This was also visually observed in the ELISA plates and confirmed by the higher absorbance values corresponding to the higher sporozoite concentrations. Intensity of colour is directly proportional to the amount of circum sporozoite protein present in the test sample [32]. This surprisingly implied higher sporozoite loads in the intervention zone than in the non-intervention zone [43].

The present study is comparable to other studies on effects of ITNs on mosquitoes and malaria transmission potential conducted in The Gambia, Democratic Republic of Congo, Kenya and Ivory Coast that showed sporozoite rates unaffected by ITN use [17, 18]. Similar studies in Ivory Coast, Kenya, Tanzania, Solomon Islands, Senegal, and Burkina Faso showed reduced sporozoite rates [10, 18, 44, 45]. This trend was expected in Kamuli district and other areas
using ITNs/LLINs, and may be realized in the near future upon consistent and intensified
ITNs/LLINs ownership and use.

Entomological inoculation rates and *Plasmodium* transmission intensity

Minimum infection rates were used to calculate the entomological inoculation rates. Therefore,
the results under discussion are just at the minimum level. The results showed that people in the
non-intervention zone received more *Plasmodium falciparum* sporozoite-positive bites in a year
than those in the intervention zone, despite the observed higher sporozoite loads in the
intervention zone. That is, malaria transmission intensity and potential were higher in the non-
intervention (influenced by the higher human biting rates) than in the intervention zone, a
possibility that the lower EIR in the intervention zone was an effect of the ITNs on the human
biting *Anopheles* density in this area.

The number of positive pools is an approximation of the number of infective mosquitoes in a
given sample collection [42], and thus the density of infective mosquitoes being an indicator of
the parasite transmission intensity [36]. Therefore, the apparently higher density of infective
mosquitoes in the intervention zone would be an indicator of higher transmission intensity in
this zone, although the actual number of sporozoites transmitted during blood-feeding may be
quite low [43]. The higher density of infective mosquitoes in the intervention zone would
probably be attributed to the higher human population density which may have facilitated higher
vector-human interactions, hence the vectors picking *Plasmodium* infections faster in this zone
compared to the non-intervention zone.

Similar studies to assess impact of ITNs on malaria transmission and elimination in Tanzania and
the Solomon Islands showed reduced annual entomological inoculation rates, although none
reduced it to zero [10, 44, 46]. In the present study, both the indoor and outdoor EIRs,
influenced by the annual human biting rates in the intervention and non-intervention zones exceeded one, i.e., each person in the area received more than one *P. falciparum*-positive mosquito bites in a year. Therefore, despite the available malaria control efforts, like effective case management and vector control with ITNS/LLINs [7, 13], a lot is still required to attain a manageable level of the disease, i.e., annual EIRs less than one, to reduce parasite rates to levels that could interrupt *P. falciparum* malaria transmission.

According to the 2012 World Health Organisation report [12], Uganda is one of the African countries that are still in a control or pre-elimination phase as evidenced by the EIRs greater than one sporozoite-infective bite per person per year. Kamuli and most parts of the country are at levels of EIRs of more than one hundred [15] that must be reduced in order to substantially reduce the prevalence of malaria infection as shown by earlier studies [46, 47].

**Conclusion and recommendations**

The study aimed at determining the *Plasmodium falciparum* sporozoite-infective biting hours of the night and transmission intensities and thus the annual transmission potential of the vector mosquitoes under prolonged use of ITNs/LLINs in Kamuli district, Uganda. This aimed at establishing whether or not ITNs were still protective against malaria-infective biting by the vector mosquitoes. The results showed that ITNs/LLINs apparently did not yet have an impact on the mosquito infection rates. The results further indicated that infective biting by *Anopheles gambiae sensu lato* and *An. funestus* group mosquitoes in this part of the country occurred throughout all night hours, i.e., in the period 19:00 to 06:00 hours, with peak infective bites occurring in the period 20:00 to 04:00 hours. These results therefore showed that ITNs/LLINs did not have an impact on the sporozoite-infective biting hours of the night and so were still protective against malaria sporozoite-infective biting by the mosquito vectors. This is because most infective biting occurred at hours of the night when people were expected to be under bed
nets. Many people were, however, shown to be exposed to infectious bites before and after bed
time, depending on human activity and/or behaviour patterns at dusk and dawn. This calls for
development of other protective methods for integration into the available vector control
interventions.

Results further evidently showed that ITNs/LLINs reduced the indoor and outdoor EIRs more
than three- and twelve-fold respectively in the intervention zone. Intensification and proper use
of this effective intervention for control of *Plasmodium* vectors as well as its integration with
other proven ecologically feasible methods is called for. Wider or universal coverage of
ITNs/LLINs and the use of insecticide-treated curtains, blankets, and other materials, to benefit
from mass killing effect are recommended. IRS and environmental management are also
recommended, while house improvements, including the use of screens in windows, doors, eaves
and ventilators may also reduce on the indoor human-biting mosquito densities as this reduces on
the entry points of the mosquitoes into the houses [48].

The use of mosquito repellents [N, N-diethyl-methylbenzamide, DEET, and proven insect
repellent plants like the cat nip plant (*Nepeta cataria*, family: *Lamiaceae*)] or its products [49],
where affordable, and long-sleeved clothing, for all those exposed to anopheline bites indoors
and outdoors [35], are recommended. Other interventions targeting outdoor and earlier biting
*Plasmodium* vectors [50, 51] are also being recommended in this area.

Mass education campaigns through the Behavioural Change Communication approaches should
be strengthened. This should be aimed at changing human behaviour and encouraging such good
practices as closing windows and doors early in the evening to avoid entry of indoor feeding
*Anopheles* mosquitoes, proper and consistent use of insecticide-treated materials and
environmental hygiene.
All the available cost effective interventions should be employed while considering the vectors’ behaviour and the environment [35, 46, 50, 52-54] in the different malaria-endemic zones in the country. This integrated approach could reduce the risk of human-mosquito contact and mitigate transmission risk by the highly anthropophilic, endophagic and endophilic Anopheles mosquitoes. This may subsequently reduce the entomological inoculation rates to less than one, and possibly drive Uganda into or close to the malaria elimination phase (zero case incidence), the global vision for the next decade [12, 50].

Since elimination of malaria is not yet ripe, fragmentation of available tools should be avoided, calling for universal coverage of the proven interventions in the context of a strengthened health system [55]. This will require careful planning, trained staff, rigorous supervision and evaluation [46].

Future studies should include establishing whether or not other Plasmodium species, i.e. P. vivax, P. ovale and P. malariae do exist in this part of the country. It is well known that these species have different life-cycles, hence complicating treatment of disease particularly in cases of mixed infections. For example, P. malariae mixed infections with P. falciparum have been found in other parts of Uganda, especially among children [40].

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

FGK conceived, designed and carried out the mosquito collections, carried out ELISA and statistical analyses of the data and drafted the manuscript. AMA, AK M and JBK helped to design the study and provided backstopping during the field work and provided critical comments on the manuscript. EM and AWO helped to design the study and provided critical
Acknowledgements

The study was funded by UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) and The African Doctoral Dissertation Research Fellowship (ADDRF) through The African Population and Health Research Centre (APHRC) in partnership with the International Development Research Centre (IDRC) and Ford Foundation. WHO/ CDC Atlanta, USA, provided the necessary antibodies and Plasmodium positive controls.

We gratefully thank all the Sixteen Entomological Attendants in Kamuli who caught mosquitoes during the whole 12-month sampling period. Special thanks go to the Local leaders and all the forty-eight household heads in Kamuli and Buyende Town Councils, Nabwigulu and Bugaya Sub Counties for the permission to sample their houses moreover at night, and to Mr. Waiswa Samuel and the late Edward Waiswa with whom we did the morphological identification, coding and preservation of the Anopheles mosquitoes in Kamuli Veterinary Laboratory. We thank Ms Dianah Katiti and Mr. Ssebyatika George of Molecular Biology Laboratory, Department of Molecular Biology, College of Veterinary Medicine and Animal Biosecurity for giving a hand in the Sporozoite ELISA work and the respective statistical computations.

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Table 1. Proportions of *Anopheles* mosquitoes positive for *Plasmodium falciparum* Circumsporozoite protein during the night in Kamuli district

<table>
<thead>
<tr>
<th>Period of the night</th>
<th>Intervention Zone</th>
<th>Non-intervention Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. caught</td>
<td>No. tested</td>
</tr>
<tr>
<td>19.00-22.00 Hours</td>
<td>19.00</td>
<td>28</td>
</tr>
<tr>
<td>23.00-06.00 Hours</td>
<td>23.00</td>
<td>43</td>
</tr>
<tr>
<td>07.00-10.00 Hours</td>
<td>07.00</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>08.00</td>
<td>86</td>
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<tr>
<td>09.00-12.00 Hours</td>
<td>09.00</td>
<td>87</td>
</tr>
<tr>
<td>12.00-15.00 Hours</td>
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<td>107</td>
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<td>15.00-18.00 Hours</td>
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<td>109</td>
</tr>
<tr>
<td></td>
<td>19.00-06.00</td>
<td>723</td>
</tr>
</tbody>
</table>

Number of sample pools in parentheses

Table 2. Human-biting *Anopheles* mosquito catches during the first, middle and last thirds of the night and the proportions positive for *P. falciparum* CSP in Kamuli district

<table>
<thead>
<tr>
<th>Period of the night</th>
<th>Intervention Zone</th>
<th>Non-intervention Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. caught</td>
<td>No. tested</td>
</tr>
<tr>
<td>19.00-22.00 Hours</td>
<td>19.00</td>
<td>21</td>
</tr>
<tr>
<td>23.00-02.00 Hours</td>
<td>23.00</td>
<td>45</td>
</tr>
<tr>
<td>03.00-06.00 Hours</td>
<td>03.00</td>
<td>87</td>
</tr>
<tr>
<td>04.00-07.00 Hours</td>
<td>04.00</td>
<td>86</td>
</tr>
<tr>
<td>19.00-06.00 Hours</td>
<td>19.00-06.00</td>
<td>723</td>
</tr>
</tbody>
</table>

Minimum Infection Rate, MIR\* = Number of CSP positive sample pools/Total number of samples tested

Number of sample pools in parentheses

MIR\* = Number of CSP positive sample pools/Total number of samples tested
Table 3. Comparison of Annual Entomological Inoculation Rates between Intervention and Non-intervention Zones

<table>
<thead>
<tr>
<th>Intervention zone</th>
<th>Non-intervention zone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Daily HBR</td>
</tr>
<tr>
<td>Indoor</td>
<td>0.0384</td>
</tr>
<tr>
<td>Outdoor</td>
<td>0.0451</td>
</tr>
</tbody>
</table>

\*AHBR = Mean Daily Human Biting Rate (calculated as mean bites per person per night in chapter five above) x 365 days; \*\*AEIR = Annual Human Biting Rate (Ma) x Sporozoite Rate (S).

Figure 1. Standard Curve for calculating sporozoite concentrations per sample

Figure 2. Comparison of peak sporozoite positive-biting (with standard error) by Anopheles gambiae s.l. and An. funestus mosquitoes in Kamuli district

Figure 3 (a-b). Sporozoite concentrations (pg/50µl) at different hours of the night in intervention zone (3a) and non-intervention zone (3b)

Figure 4. Comparison of sporozoite concentrations of Anopheles mosquitoes collected at different hours between intervention and non-intervention zones
Figure 1

The graph shows a plot of Average OD against Concentration (pg/50ul). The equation for the fitted line is $y = 0.0038 \ln(x) + 0.0522$ with an $R^2 = 0.9674$. The data points are represented as 'Av OD' and the logarithmic fit as 'Log (Av OD)'.
Figure 2

Proportions of P.f. CSP positive samples (%)

Time of Human-biting

Intervention zone
Non-intervention zone
Figure 3 (a)

Figure 3 (b)
Figure 4