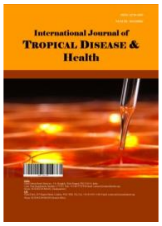


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# The Impact of Insecticide-treated Bed Nets on Parity and Malaria Sporozoite Infectivity of *Anopheles* *S*pecies in Kamuli District, Uganda

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author FGK conceived, designed and carried out the mosquito sampling, analysed the data and drafted the manuscript. Authors AMA and KJB helped to design the study and provided backstopping during the field work and provided critical comments on the manuscript. Author AWO helped to design the study and provided critical comments on the different versions of the manuscript. All the authors read and approved the final manuscript.*

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## ABSTRACT

**Background:** The main objective of treating bed nets with insecticides is to affect the mean longevity of the main vector population, and consequently the vector density and sporozoite rates.
**Objective:** This study aimed at establishing the impact of insecticide-treated bed nets (ITNs) on the longevity and ability to transmit malaria sporozoites by the vector species as an assessment of effectiveness of the ITN intervention in Kamuli district, Uganda.
**Methods:** Indoor human-biting mosquitoes were trapped in three randomly selected houses in two separate nights using battery-operated CDC light traps in both intervention (with ITNs) and non-intervention villages (without ITNs). The female anophelines were dissected and their parity rates

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and *Plasmodium falciparum* sporozoite positivity compared between the two zones. A sporozoite Enzyme-Linked Immuno Sorbent Assay, ELISA, was used to detect the presence of *P. falciparum* sporozoites in the parous vectors in both zones.
The parity and *P. falciparum* sporozoite infectivity were compared between the two zones using the Kruskal-Wallis rank sum test of the R-Statistics software.
**Results:** Out of the 166 *Anopheles* mosquitoes dissected, 37.3% (19 out of 51) and 53.9% (62 out of 115) were parous in the intervention and non-intervention zones, respectively, indicating that parity of the mosquitoes was higher in the non-intervention ($p = 0.005$). Infectivity of the vectors in the non-intervention exceeded that in the intervention zone ($p = 0.032$), with active sporozoite transmission observed before and after bed time in the non-intervention zone.
**Conclusion:** Results showed that ITNs had impacted on the survival and consequently the density of the older malaria vectors, and on their ability to transmit *Plasmodium* sporozoites. This calls for intensification of use of this effective malaria control strategy, coupled with behavioural change communications strategy to promote correct use, as well as use of other interventions like repellents to provide additional protection especially before and after bed time.

*Keywords: Mosquito survival; sporozoite rates; malaria circumsporozoite protein; ELISA.*

## 1. INTRODUCTION

The widespread use of insecticides against anophelines is expected to result in such a high mortality during each gonotrophic cycle that the proportion of females reaching the age at which malaria sporozoites may appear in their salivary glands is insignificant as far as the transmission of the infection is concerned. Therefore the main objective of treating bed nets with insecticides is to affect the mean longevity of the main vector population [1] and consequently the vector density and sporozoite rates [2].

Malaria parasites transmission is directly proportional to the density of the vector, the square of the number of times each day that the mosquito bites man, and the tenth power of the probability of the mosquito surviving for 1 day. Vector longevity is therefore important in determining transmission and focuses control measures on the adult mosquito [3]. Determination of the age (parity) of the most important vectors of malaria parasites in a locality is therefore important for the measurement of transmission and the success of control interventions [4].

Since only relatively old (≥10 days) malaria vectors are capable of transmitting malaria parasites [5], knowledge of the age distribution of the vector populations is essential for predicting the proportions of potentially infective vectors, and how this changes over time and in response to control interventions [1,5]. In addition, age determination is essential for estimation of mosquito survival, which is the most important determinant of transmission intensity [5].

In the event of Uganda's efforts to scale up use of insecticide treated bed nets as a control tool of malaria, it is important to establish their (ITNs) long-term impact on the malaria sporozoite-transmitting vectors. Little has been studied on the relationship between long-term ITN usage and the parity and malaria sporozoite infectivity of the vectors. Could there be impact of ITNs on the parity and ability to transmit malaria sporozoites by the most important malaria vectors, *Anopheles gambiae* complex and *An. funestus* species? There is also a possibility that the older (multi-parous) and malaria sporozoite-positive mosquitoes may bite earlier or later in the night than the majority of younger mosquitoes [6,7], rendering bed nets less effective, hence causing an increase in the malaria infection rates. If so, this could have serious consequences for malaria control using this method and could explain the continued morbidity and mortality due to malaria in the country.

This study was aimed at establishing the impact of prolonged (≥ 5 years) use of ITNs on the longevity/lifespan and ability to transmit malaria sporozoites by *Anopheles gambiae* complex and *An. funestus* species that are known for most of the transmission in Uganda [8-12], as an assessment of effectiveness of the ITN intervention in Kamuli district. The study compares the age composition and sporozoite rates of the vector population in villages using insecticide bed nets with the age composition and sporozoite rates of the populations in untreated villages. The study further provides information on the biting times of the more dangerous older (parous) and malaria sporozoite-positive mosquitoes compared to the majority of the younger (nulliparous) mosquitoes under ITN intervention.

Results of the study will inform policy and may guide Uganda's National Malaria Control Programme in formulation of malaria control plans particularly in the event of the current efforts to scale up ITN use in malaria control.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The study area was located in Kamuli district (01º05'N 33º15' E), 68km North of the source of River Nile and divided into intervention zone (five villages using ITNs for at least five years) and non-intervention zone (five villages not using ITNs). The intervention villages were located in Kamuli Town Council and Nabwigulu Sub County, both in Bugabula County. The non-intervention villages were located in Bugaya and Buyende sub counties, both in Budiope County located in the North East of Kamuli Town Council, and well over twenty kilometers away, with households owning no bed nets before the entomological survey (Kamuli District Health Status Reports, 1999/2000-2004/2005; Kamuli District Health Sector Strategic Plan, 2005/06-2009/10-Un published; Personal House hold preliminary survey).

The ITNs coverage in five out of 18 sub counties of Kamuli district by the year 2006 was as follows: Kamuli Town Council (3051 nets in 4080 households sampled), Nabwigulu (3378 nets in 297 households sampled), Balawoli (1066 nets in 256 households sampled), Kitayunjwa (1485 nets in 136 households sampled), and Namwendwa (846 nets in 229 households sampled).

The proportion of households that were using bed nets for the last five years in the two sub counties studied (Kamuli Town Council and Nabwigulu) was at least 52%, while at the time of the study coverage stood at 74.8% and 64 % for Kamuli Town Council and Nabwigulu, respectively, with an average of 69% of the households using at least one net (Kamuli District Health Status Reports,1999/2000-2004/2005; Kamuli District Health Sector Strategic Plan 2005/06-2010; Personal survey of existing Kamuli Town Council HMIS records; Kamuli District HMIS Reports, 2005/06-2010; Kamuli Christian Children's Fund, CCF, office records- all Unpublished).

These villages were privileged with a number of Non Governmental Organizations (NGOs) like CCF and Plan-Uganda that intervened with insecticide-treated bed nets since the late 1990s, and later Long Lasting Nets to supplement government 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.

### 2.2 Mosquito Sampling and Identification

Indoor human-biting mosquitoes were collected from 19:00 to 06:00 hours using battery operated miniature CDC light traps that were set 1.5 metres from the floor and about 50 centimetres close to occupied intact untreated bed nets inside randomly selected houses [13]. Households whose heads did not permit their houses to be sampled for mosquitoes were left out and another house was selected. Volunteers were recruited from the study area. They were counseled and taught how to trap mosquitoes, and two pairs were positioned at each of the sampling sites. These were replaced in shifts every three hours in each household and were rotated between households. Trapping was carried out by the trained catchers in the three randomly selected houses in two separate nights in both intervention and non-intervention villages. The catchers connected the CDC light traps to the battery terminals at 7:00 pm and disconnected them at 7:00 am.

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, were attracted to the light and were caught by the traps [13]. 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 net and trap were absent [6].

The human-biting fractions of the mosquito population and time of biting were determined and recorded throughout the sampling nights and each hourly catch of the night was identified morphologically using a simplified key [14].

Each hourly catch was placed in a disposable polystyrene container pre-labeled with date and time of capture, and taken to laboratory for assessment [15] while feeding on a 10% sugar solution available through a cotton wick [16].

The percentage of night's biting was obtained by dividing the number of *Anopheles* mosquitoes caught during a particular third of the night ($n_t$) divided by the total caught during the whole night in the zone (N) multiplied by 100, that's, $n_t/N$ x 100.

### 2.3 *Anopheles* Mosquito Dissection for Ovarian Age-grading

Mosquitoes were dissected and checked for parity to determine their age structure [1,17-19]. The mosquitoes were classified as nulliparous (≤ 4 days) or parous (≥10 days) [5] by the tracheation of the ovarioles [1,4,15,17]. In nulliparous females the tracheoles were tightly wound coils called 'skeins', while parous females were indicated by a thread-like net work of tracheoles [17,19]. Parous females had tracheoles that had distended (stretched), with dilatations depending on the number of times the female had fed and oviposited [1,17,19–22].

In females with degenerated follicles, dilatations filled with products of degeneration were included in the total number of dilatations determining physiological age. A number of follicular tubes were examined in each ovary to avoid mistakes in determining physiological age. Counting of dilatations was performed only in tubes which were normally connected with the internal oviduct.

A total of 166 of the human-biting *Anopheles* mosquito catch were dissected and checked for parity. The percentage of parous *Anopheles* mosquitoes was obtained by dividing the number of parous females in a particular third of the night by the number of female *Anopheles* mosquitoes dissected multiplied by 100.

### 2.4 Determination of Malaria Sporozoite Infectivity of the Anopheline Mosquitoes by Direct ELISA

A sporozoite Enzyme-Linked Immuno Sorbent Assay, ELISA technique was used to detect the presence of *Plasmodium falciparum* sporozoites in the dangerous older (Parous) *Anopheles gambiae s.l.* and *An. funestus mosquitoes* in both intervention and non-intervention zones [23].

Ten out of the 19 and 40 out of the 62 parous mosquitoes caught in the intervention and non-intervention zones respectively were tested for *P. falciparum* circumsporozoite protein in pools of a maximum of five heads and thoraces of the anophelines per sample pool taken from all the three 4-hour periods of the night. Positive pools were confirmed by a second ELISA. The percentage of mosquitoes that were sporozoite-positive was obtained by dividing the number of test sample pools positive for *Plasmodium falciparum* circum-sprozoite protein in a particular period of night's biting by the total number of tested mosquitoes for that particular period, multiplied by 100. This gave the infection rate, (IR), of the anophelines analysed by zone, with the assumption that there was only one infective mosquito in each positive pool [24,25].

The parity and sporozoite infectivity of the human-biting proportions of the parous anopheles mosquitoes in the two zones were later related to their times of biting [26].

### 2.5 Data Analysis

The parity and *P. falciparum* sporozoite infectivity of the *Anopheles* species were compared between the two zones using the Kruskal-Wallis rank sum test of the R-Statistics soft ware, version 2.15.0 (2012) [27].

## 3. RESULTS

### 3.1 Parity of the *Anopheles* Mosquitoes

Out of the 166 *An. gambiae s.l.* and *An. funestus* mosquitoes dissected, 37.3% (19 out of 51) and 53.9% (62 out of 115) were parous in the intervention and non-intervention zones, respectively. This indicated that the proportion of the parous *Anopheles* mosquitoes caught in the non-intervention zone was higher than the parous proportion in the intervention zone (Chi-squared = 22.2065, df = 1, p = 0.005). Parity of the malaria mosquitoes was also found to correspondingly increase during the night, peaking between 23:00 and 06:00 hours in both intervention and non-intervention zones (Table 1).

### 3.2 Infectivity of the Parous *Anopheles* Mosquitoes

Ability to transmit *Plasmodium falciparum* sporozoites by *Anopheles gambiae s.l.* and *An. funestus mosquitoes* was observed in the human biting mosquitoes caught in the last two four-hour periods of the night (23:00-02:00 and 03:00-06:00 hours) in the intervention zone. This was shown by the proportions of *Plasmodium falciparum* CSP positive mosquito sample pools observed, i.e., 0%, 10.0% and 10.0% of the sample pools in the first, second and last thirds of the night, respectively. This corresponded to the periods of the night during which parous anophelines were observed. In the non-intervention zone, infectivity of the bites by parous anophelines was observed to occur throughout the night, with peak infective biting in the middle third of the night as evidenced by the proportions of anopheline sample pools that were positive for *Plasmodium falciparum* circum-sporozoite protein, i.e., 2.5%, 10.0% and 7.5% in the first, middle and last thirds of the night, respectively.

*Plasmodium falciparum* sporozoite infectivity of the malaria vectors in the non-intervention was shown to exceed that in the intervention zone (Chi-squared =3.8418, df = 1, p-value = 0.032), although the minimum infection rate, MIR, of 20.0% was observed in both zones.

### 3.3 Time of Biting by the Parous and Sporozoite Infective *Anopheles* Mosquitoes

The proportion of the night's biting of the mosquitoes was observed to increase through the night, peaking between 23:00 and 06:00 hours in both intervention and non-intervention zones.

Human biting by the parous *Anopheles* mosquitoes occurred between 23:00 and 06:00 hours in the intervention zone, while biting occurred throughout the night (19:00 to 06:00 hours) in the non-intervention zone. This showed that people in the non- intervention zone were exposed to potentially dangerous bites before and after bed time. Peak biting by the

**Table 1. Variation in parity and sporozoite rates in *Anopheles m*osquitoes caught biting humans during the first, middle and last third of the night. A comparison between intervention and non-intervention zones in Kamuli district**

| | Time of biting | | | |
|---|---|---|---|---|
| | 19:00 -22:00 | 23:00-02:00 | 03:00-06:00 | 19:00-06:00 (Total) |
| **Intervention zone** | | | | |
| Total dissected | 3.0 | 27.0 | 21.0 | 51 |
| % of night's biting | 5.9 | 52.9 | 41.2 | 100.0 |
| % parous (≥10 days) | 0 (0) | 25.5 (13) | 11.8 (6) | 37.3 (19) |
| % Parous sporozoite +ve | 0 (0) | 10.0 (1 /10) | 10.0 (1/10) | 20.0 (2/10) |
| **Non-intervention zone** | | | | |
| Total dissected | 12.0 | 41.0 | 62 | 115 |
| % of night's biting | 10.4 | 35.7 | 53.9 | 100.0 |
| % parous (≥10 days) | 6.9 (8) | 18.3 (21) | 28.7 (33) | 53.9 (62 ) |
| % Parous sporozoite +ve | 2.5 (1/40) | 10.0 (4/40) | 7.5 (3/40) | 20.0 (8/40) |

*The numbers of parous and sporozoite-positive test sample pools in each third of the night in Parentheses*

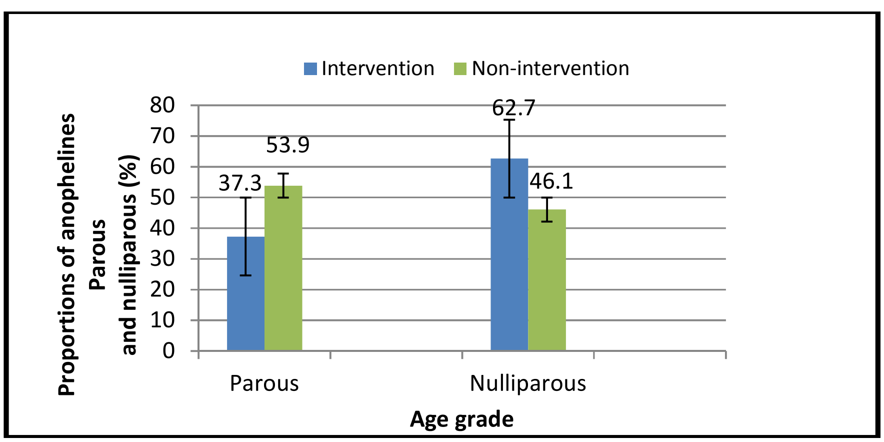


**Fig. 1. Comparison of parous and nulliparous anopheline mosquitoes (with standard error) between intervention and non-intervention zones in Kamuli district**

epidemiologically important parous anopheline proportions was observed during the middle and last third of the night in the intervention and non-intervention zones, respectively.

## 4. DISCUSSION

### 4.1 Survival Rates of the Malaria Vectors

A higher survival rate (53.9%) of *Anopheles* species was observed in the non-intervention zone compared to the intervention zone (37.3%). This indicated that use of ITNs/LLINs had an effect on the longevity and thus survival of the *Anopheles* species in the intervention zone as evidenced by the lower parity rates and lower human biting densities in this zone where 69% of the households used at least one ITN/ LLIN. Results of this study show an achievement of the main objective of treating bed nets with insecticides, that is, to affect the mean longevity of the main vector population [1] and consequently the vector density [2].

A similar study [28] on the impact of permethrin-impregnated bed nets on malaria vectors of the Kenyan coast showed unaltered vector parous rates, implying that the survival rates of the malaria vectors were not affected.

### 4.2 Infectivity of the Parous *Anopheles* Mosquitoes

Results of the study showed a statistically significant difference between the two zones in the ability of the parous *Anopheles* species to transmit *Plasmodium falciparum* parasites to humans. *Plasmodium falciparum* sporozoite infectivity of the malaria vectors in the non-intervention was shown to exceed that in the intervention zone (Chi-squared =3.8418, df = 1, p-value = 0.032). This suggested that ITN use could have possibly had an impact on the infection rates of the *Anopheles* vectors. Considering the higher human population in the intervention villages, more vector-human interactions were expected to have been going on then, with higher chances of vector-parasite interactions which would subsequently result in higher transmission intensity in this zone than in the non-intervention zone. However, a lower infectivity (number of sporozoite-positive anopheline sample pools) was still observed in the intervention zone, particularly in the first third of the night, despite the observed overall equal minimum infection rates, MIR, (20%) in both zones. These results indicate reduced sporozoite rates probably arising from the use of ITNs/LLINs. Similar studies in Ivory Coast, Kenya, Tanzania, Solomon Islands, Senegal and Burkina Faso showed reduced sporozoite rates of the malaria vectors, while studies conducted in The Gambia, Democratic Republic of Congo, Kenya and Ivory Coast showed unaffected sporozoite rates [29].

A similar study [28,29] on the impact of permethrin-impregnated bed nets on malaria vectors of the Kenyan coast showed a reduction in the human-biting rates, but the *Plasmodium falciparum* sporozoite rate remained unaffected. According to Takken [30], the overall effects of both immediate and long-term use of ITNs on mosquitoes are variable, with a reduced survival as well as reduced sporozoite rates observed in many cases.

Since the density of infected mosquitoes is an indicator of transmission intensity in an area [31], people in the non-intervention zone were at more risk of getting malaria [29] and other *Anopheles*-transmitted infections [32] since they received more bites by the more dangerous older *Anopheles* mosquitoes and due to the occurrence of more infective biting earlier in the night before going to bed. The results of the study therefore emphasize the importance of vector longevity in determining transmission and focusing control measures on the adult mosquito [3].

### 4.3 Human Biting Time by the Parous and Sporozoite-infective Anophelines

Several studies undertaken in Kenya and other countries in East Africa have shown changes in *Anopheles* feeding patterns, with vectors feeding early and late following ITNs/LLINs use, while other studies showed no change in biting time of the malaria vectors but with reduced human-vector contact and blood feeding success [29]. This study showed that peak human biting activity of the malaria vectors occurred during 23:00 to 06:00 hours in both intervention and non-intervention zones. This result corresponded to the known period of peak activity of anophelines in Uganda, that is, 23:00 to 05:00 hours, an indication that the use of ITNs/LLINs had not impacted on the biting times of the malaria sporozoite vectors. This showed that ITNs were still protective against night biting by the malaria vectors as most biting occurred at hours of the night when people were expected to be in bed and under bed nets [29].

## 5. CONCLUSION

This study aimed at establishing whether or not prolonged use of ITNs impacted on the longevity/survival of *Anopheles gambiae* complex and *Anopheles funestus* mosquitoes, and their ability to transmit malaria parasites in Kamuli district.

Results showed that ITNs had impacted on the survival and consequently the density of the older dangerous malaria vectors, and on their ability to transmit *Plasmodium* sporozoites. Results also revealed that the time of biting by the older/ parous and CSP-Positive vectors remained unchanged (23:00 to 06:00 Hours), although it was observed that sporozoite transmission actively went on before and after bed time in the non-intervention zone. This calls for intensification of ownership and correct use of ITNs/LLINs which have been shown to be an effective malaria control strategy [29,33], putting into consideration the observed time of peak transmissions. This should be coupled with behavioral change communications strategy to promote correct use. The use of other interventions like repellents can provide additional protection especially before and after bed time.

## DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## CONSENT AND ETHICAL APPROVAL

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 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. 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.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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