Efficacy of Pyrethroid Impregnated Bed-nets on Malaria Transmission in Eastern Sudan


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Abstract - The effects of impregnated bednets in the mosquitos’ density and malaria morbidity has been evaluated in area with heterogeneity of malaria vectors and parasites in Tabaldia and Batta villages Eastern Sudan. The density of An. arabiensis, An. funestus and An. rufipes species collected from study area showed significant variation in their numbers ($\chi^2 (P) = 150.98 (<0.001)$ for Tabaldia; $164.627 (<0.001)$ for Batta and $310.471 (<0.001)$ for the total of different Anopheles mosquitoes collected from both study villages. The impact of the impregnated bednets in the density of the two malaria vectors An. arabiensis and An. funestus and their sporozoite infection rates also highly significant different (P<0.001) between intervention (Tabaldia) and non-intervention (Batta) villages.

The prevalence of malaria in the area was estimated as (8.7%, 7.6%) for Tabaldia and (14.7%, 12.6%) for Batta village at the first and second phases of the study respectively. The main predominate malaria parasite in the area was P. falciparum (98.7%), where P. vivax and P. malariae were detected with an equal rates (0.65%). Also the efficacy of impregnated bednets in the age groups within and between two villages showed significant difference mainly in the ages ranged in 6-15 years old and clear reduction observed in age 16-30 years.

Index Terms - Anopheles arabiensis, Anopheles funestus, Malaria, Impregnated bednets, Species composition.

I. INTRODUCTION
Malaria transmission in Tropical Africa occurs within the comprehensive incidence of the relationship among entomological inoculation rate, incidence and recovery rates of malaria, parasitaemia and incidence of malaria attacks in stable endemic area [1-3].

Transmission of malaria may be estimated by quantifying parameters in either the human host or the entomological measurements in Anopheles vectors [4, 5]. Some of these parameters affect the degree of transmission and other affect the frequency as well as the rate of transmission.

Impregnated bednets are being strongly promoted as malaria control in most parts of Africa as recommended by WHO which reducing human-vector contact, malaria morbidity and mortality which has been demonstrated in various epidemiological situation [6, 7].

The main vector of human malaria in Sudan is An. arabiensis with considerable peak of An. funestus [8]. Many studies were carried out in different parts of the Sudan to determine the vectorial capacity of An. arabiensis as a principal malaria vector. But there was few studies was carried to investigate the role of others malaria vectors in malaria transmission. An. funestus has considerable role in malaria transmission in Eastern Sudan. Also our studies indicated that there is interacted in malaria transmission between An. arabiensis and An. funestus. Where all the pervious studies showed that the An. arabiensis is an only principal vector in most parts of the Sudan. Anopheles funestus did not conducted in malaria research in Sudan where in most part of Africa consider as major malaria vector [9].

out of these species only An. funestus and An. rivulorum were found that have major role in malaria transmission and their vectorial capacity is similar to that of An. gambiae [10]. Also this study showed that there are different Anopheline mosquitoes in the area such as An. pharonesis and An. rufipes. Mainly An. arabiensis and An. funestus were found incriminated in malaria transmission.

This study directed towards measuring effects of impregnated bednets on malarial indices in intervention village supplied by pyrethroid impregnated bednets and non-intervention village, in incidence of the disease in human, density and abundance of mosquito’ populations.

II. MATERIALS AND METHODS

Study area
The study was carried out in two villages: Batta (12.89 N-35.13E) and Tabaldia (12.88N-35.16E) Rahad Province, Gedaref State, Eastern Sudan. The villages lie on El Rahad River eastern bank (seasonal river). The study was performed during November 1999 – November 2000.

The climate of the study area is tropical continental climate with two seasons; rainy season lasting from June-October and dry season between November to May. The average annual rainfall of the study area is 815 mm. The dry season is divided into two phases, warm winter, with average min-max temperature of 17.9-26.2°C and a hot summer, with an average min-max temperature of 34-40°C.

Plan of the bednets intervention programme
The two study villages were selected to represent the same ecology and population activities, but differ in availability of impregnated bednets. Bioassays were performed to assess killing and prevent of the natural mosquitoes population in the study area before Pyrethroid impregnated bednets, were distributed by MSF- Holland for the inhabitants of Tabaldia villages (hence the villages was identified as the intervention village) in October 1998, while the people of the Batta were not supplied by bednets in the first phase of the study (hence the village was identified as the non-intervention village) where in second phase the people of Batta were supplied by bednets in April 2000.

The bednets distributed to the population, were 150 x 180 x 75 cm Dinier mesh, supplied by Siam dutch Mosquito Netting Co. Ltd. (Bangkok, Thailand) which was re-impregnated by deltamethrin insecticide (10 mg per m²).

At the beginning of the study a census was carried out in two villages where all people were registered and given identification number classified by houses, families, sex and age. Ethical consideration was taken from national ethical committee of the Federal ministry of Health for the large project of impregnated bednets used in visceral leishmaniasis and malaria control in Eastern Sudan, also written informed consent was obtained from all participants.

Mosquitoes Collection and identification
The mosquitoes collected by knock down technique following the standard WHO method [11], were identified morphologically according to Gillies, M.T. & Cotezee, M. (1987) and Gillies, M. T. & De Meillon, B. (1968) the key of [9, 12].

Estimation of sporozoite rate within collected anopheline mosquitoes
Sporozoite rates in natural vector population of both An. arabiensis and An. funestus estimated by using ELISA technique as described by Donnelly MJ, et al. (2001) [13].

Screening of Malaria in intervention and non-intervention villages
Two cross-sectional surveys took place in the two study villages during November 1999 and October-November 2000 to determine the prevalence of malaria in intervention and non-intervention villages. In the first survey a total of 715 individual from (intervention village) and 633 from non-intervention village, were screened clinically and parasitologically.

In the second survey, in October – November 2000, all the above-mentioned populations were also screened for malaria situation. Each positive patient in all surveyed was supplied by anti-malarial by physician.

The data obtained from this study was analyzed using Minitab and EPI_ info computer software to compare between entomological parameters and malaria prevalence in two study villages.

III. RESULTS AND DISCUSSION

Composition of Adult Anopheline mosquitoes collected in study area
During three entomological surveys carried out in the study area: three common species of Anopheles mosquitoes were identified that include An. arabiensis, An. funestus and An. rufipes. These species shows significant variation in their numbers ($\chi^2 (P) = 150.98 (<0.001)$ for Tabaldia; 164.627 ($<0.001$) for Batta and 310.471 ($<0.001$) for the total of different Anopheles mosquitoes collected from
two study areas. Only few (13) mosquitoes of An. pharoensis species were collected (Fig. 1).

![Graph showing abundance of Anopheline mosquitoes collected from Tabaldia and Batta (Rahad Province, Gadaref State, Eastern Sudan) during November 1999 - November 2000.](image)

**Fig. 1:** Effects of impregnated bednets on the density of malaria vectors during study period in two study villages

In the first phase, where non-intervention village not supplied by impregnated bednets the density of An. arabiensis and An. funestus was significantly higher in Tabaldia (the intervention) than in Batta (non-intervention) $\chi^2 (P) = 144.77 (<0.001)$; $174.22 (<0.001)$ respectively for the two above species.

In second year where the non-intervention village was supplied by bednets the density of An. arabiensis dropped significantly to a level less than that observed in the original intervention village (Tabaldia) $\chi^2 (P) = 4.87 (0.027)$, where the density of An. funestus was similar in the two villages $\chi^2 (P) = 0.00 (1)$ (Table 1).

**Table 1:** Effects of impregnated bednets on density of malaria vectors in Tabaldia and Batta villages (Rahad Province, Gadaref State, Eastern Sudan) during November 1999- November 2000

<table>
<thead>
<tr>
<th>Time of survey</th>
<th>Anopheles species</th>
<th>No. of mosquitoes collected from each village</th>
<th>$\chi^2$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nov.99-Feb.2000</td>
<td>An. arabiensis</td>
<td>Tabaldia 269  Batta 507</td>
<td>144.77</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(a)*</td>
<td>An. funestus</td>
<td>Tabaldia 42  Batta 183</td>
<td>174.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Oct.-Nov.2000</td>
<td>An. arabiensis</td>
<td>Tabaldia 76  Batta 57</td>
<td>4.87</td>
<td>0.027</td>
</tr>
<tr>
<td>(b)*</td>
<td>An. funestus</td>
<td>Tabaldia 92  Batta 91</td>
<td>0.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

(a): before introduction bednets in non-intervention (Batta) village.
(b): following supplying bednets in non-intervention village.
* number of huts sprayed in (a) were 42 for each village, where in (b) 28 huts were sprayed in each village.

**Effects of impregnated bednets on sporozoite rate detected in An. arabiensis and An. funestus collected from Tabaldia and Batta during study period**

Malaria parasites of Plasmodium falciparum, P. vivax and P. malariae were detected in An. arabiensis in both intervention and non-intervention villages. In the first year a significant reduction of the sporozoite rates of different malaria parasites in intervention village was observed. Whereas, in Batta village 4.1% infection rate of P. falciparum was estimated, 6.1% of P. malariae, 8.1% of P. vivax and also mix infection of the first two mentioned species determined as 3.1%. Whereas in Tabaldia village only 1.9% for P. malariae and 1.3% for P. vivax were estimated and there was no P. falciparum or mix infection was detected.

Four out of 15 An. funestus collected from Batta only, tested for sporozoite rate were found infected by P. Malariae (Table 2).

**Sporozoite rate after the intervention of both villages**

All An. arabiensis collected in second year after covering the two study villages by impregnated bednets were found to be negative for all malaria parasites. Only 3 out of 77 (4.2) of An. funestus samples detected from Batta were found to be positive for P. malariae and there is no sporozoite was detected in all An. funestus collected from Tabaldia in this phase (Table 2).
Table 2: Effects of impregnated bednets on sporozoite rates of An. arabiensis collected from Tabaldia (intervention) and Batta (non-intervention) villages (Rahad Province, Gadaaref State, Eastern Sudan) during Jan-February 2000

<table>
<thead>
<tr>
<th>Parasite species</th>
<th>Tabaldia + ve No.</th>
<th>% of infection</th>
<th>Batta + ve No.</th>
<th>% of infection</th>
<th>Reduction rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. falciparum</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>4.1</td>
<td>100 %</td>
</tr>
<tr>
<td>P. malariae</td>
<td>2</td>
<td>1.9</td>
<td>6</td>
<td>6.1</td>
<td>52.5 %</td>
</tr>
<tr>
<td>P. vivax</td>
<td>1</td>
<td>1.3</td>
<td>8</td>
<td>8.1</td>
<td>72.3 %</td>
</tr>
<tr>
<td>P. mix infection*</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3.1</td>
<td>100 %</td>
</tr>
</tbody>
</table>

- Mix infection was P. falciparum and P. malariae.

Prevalence of malaria in Tabaldia and Batta villages’ population during November 1999 and November 2000

During cross-sectional survey done in November 1999 in the two villages, among 1348 individuals screened for malaria, 153 (11.35%) were found positive for P. falciparum and 1 (0.07%) of the population were found positive for each one of the P. vivax and P. malariae.

A significant low parasite rates was observed in intervention village (62/715= 8.7%) where in non-intervention village was found as (93/633 = 14.7%), ($\chi^2$ ($P$) = 11.38 (<0.001) (Table 3).

The same finding also was observed in the second phase after supplied Batta population by impregnated bednets with marked reduction in malaria rates ($\chi^2$ ($P$), 4.5(0.023).

Table 3: Effects of impregnated bednets on malaria prevalence in two study villages during November 1999 and November 2000

<table>
<thead>
<tr>
<th>Village</th>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of People screened</td>
<td>Positive (%)</td>
</tr>
<tr>
<td>Tabaldia</td>
<td>715</td>
<td>62(8.7)</td>
</tr>
<tr>
<td>Batta</td>
<td>633</td>
<td>93(14.7)</td>
</tr>
<tr>
<td>Total</td>
<td>1348</td>
<td>155(11.5)</td>
</tr>
<tr>
<td>$\chi^2$</td>
<td>-</td>
<td>11.38</td>
</tr>
<tr>
<td>$P$ - value</td>
<td>-</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

Phase I: before supplied Batta by impregnated bednets.
Phase II after intervention of two villages.

* Significant difference ** Highly significant different

The incidence of malaria in the non-intervention village (Batta) found with high prevalence in children aged between 5-16 years, this was followed by remarked reduction in the parasite rates for young adults whom were 16-30 years old.

In the intervention village (Tabldia), the rate remained stable at low levels in the young age groups till the age of 20 years, increased gradually for those above 31 years old. The parasite rate showed significantly high level in the age groups 6-10 years ($\chi^2$ ($P$) = 11.59 (0.001) (Fig. 3).

This study shed light on the composition of the Anopheline mosquitoes and their contribution to malaria transmission in the area. Also the present study explored the impact of the impregnated bednets in the density of malaria vectors and their sporozoite infection rates, whereas the prevalence of the malaria in human population was studied.

Anopheles arabiensis was found as predominant Anopheline mosquito in the area, followed by the An. rufipes, An. funestus and low density of An. pharoensis. These results are similar to the previous studies reported in the Eastern Sudan [14-16].

The abundance of the Anopheline mosquitoes in this area may be attributed to the ecological factors, which include: long heavy rain season, high temperature, diversity of vegetation and availability of numerous breeding sites (seasonal rainy pools and separate pools of El Rahad seasonal River), this finding also similar to the most Africa tropical regions where An. gambiae complex and An. funestus population found sympatrically and predominant throughout the year [17, 18].
An. arabiensis and An. funestus were found as primary malaria vectors in the area and Plasmodium falciparum, P. vivax and P. malariae were identified as main malaria parasites in this area [2].

This study shows clear impact in reduction of mosquitoes’ density in the area because used of impregnated bednets has been found kill some mosquitoes; prevent other from feeding on human and to drive mosquitoes out of the human dwellings [19, 20]. Studies in Burkina Faso, Gambia, Cameroon, Kenya and Tanzania demonstrated more than 80% reduction in the Anopheline densities in the presence of the impregnated bednets and curtains [7]. Interestingly that the significant different in mosquitoes reduction densities may attributed to effects of the impregnated bednets coverage in intervention and non-intervention villages as similar results was reported in Kenya [21].

Variation in sporozoite rates between control (Batta) village and intervention village (Tabaldia) also observed in both malaria vectors An. arabiensis and An. funestus has been found reduced for different malaria parasites. The reduction was estimated as (100%, 72.3% and
52.5%) for *P. falciparum*, *P. vivax* and *P. malariae* respectively. This findings match different studies conducted to evaluate impact of impregnated bednets in sporozoite rates. In Tanzania [22] reported that the impregnated bednets reduced sporozoite rate from 5.18% to 0.99% also reducing the numbers of mosquitoes’ bites human and sporozoite rate was noted in Burkina Faso and Papua New Guinea [23]. Malaria in Sudan show different epidemiological patterns which ranged from hypo- meso-hyper endemic from most Northern – central – to the most Southern part of the Sudan and seasonally in the same area. The incidence of malaria in the present study was estimated as (8.7%, 7.6%) for Tabaldia and (14.7%, 12.6%) for Batta village at the first and second phases of the study respectively, and the main predominate malaria parasite in the area was found as *P. falciparum* (98.7%), where *P. vivax* and *P. malariae* were detected with an equal low rates (0.65%). These results are in the same range of most pervasive studies which carried in different part of the Sudan mainly in Eastern Sudan [24, 25]. Change in the malaria prevalence which noted within and between control and intervention villages population showed significance reduction (<0.001, 0.023) in the first and second phases of the study periods respectively between two villages. This output of the treated net beneficial was confirmed by number of investigators whom found that the impregnated bednets reduced malaria morbidity and mortality which has been demonstrated in various epidemiological studies [6, 7]. Although impregnated bednets was showed clear reduction in malaria prevalence in human population but different fluctuations in incidence pattern between different age groups has been observed. This may attributed to the usage of the bednets between different age groups or immunity level [1] The most interesting that the age group 5-15 years (susceptible) show high significant different between intervention and non-intervention village (< 0.001), the same finding was reported in Tanzania where parasite rate, in children was reduced from 60% to 20% in two years after introduction of impregnated bednets [26].

**IV. CONCLUSIONS**

In conclusion the study revealed present of different species of Anopheleline mosquitoes in the area with clear dominancy for *An. arabiensis* in abundance and its potential in malaria transmission. The results explore the impact of the impregnated bednets on mosquito densities and their sporozoite rate in intervention and non-intervention villages. Further study is needed to investigate bionomic of these mosquitoes in area with heterogeneity of malaria vectors and parasites.

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**REFERENCES**


