ASSESSMENT OF INSECTICIDE TREATED BED NETS (ITBNS) ON ENTOMOLOGICAL INDICES IN LYMPHATIC FILARIAISIS TRANSMISSION IN EBONYI STATE, NIGERIA

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ABSTRACT
The effect of insecticide treated bed nets (ITBNs) on lymphatic filariasis vectors was studied as part of a large scale trial in Ohaukwu, Ebonyi State Nigeria in 2016. Two cohorts were used; ITBN-intervention and non ITBN-intervention households. Mosquitoes were caught monthly by Pyrethrum knockdown (PKD) and mechanical aspirator and identified using standard morphological criteria. A total of 1,693 mosquitoes caught were assessed for entomological indices, parous, infection and infectivity status with W. bancrofti larvae. Mosquitoes from non ITN- intervention households 1,456 (86.00%) were insignificantly higher than ITN- intervention households, 237 (14.00%). An. gambiae (12.97% versus 82.69%) and Cx. quinquefasciatus (1.00% versus 3.07%) were present in both cohorts. However, Aedes aegypti and An. funestus were present only on non ITN-intervention households. The physiological, infection and infectivity status were comparable between the cohorts (P>0.05). No significant difference in the average/total transmission potentials (TP+) were found between the cohorts (P>0.05). The study highlights the need for ITBNs in synergy with drugs for filariasis control in view of the involvement of An. gambiae as the only transmitting vector.

Keywords: Lymphatic filariasis transmission, Insecticide treated bed net, Entomologic indices, Nigeria.

INTRODUCTION
The main intervention measure recommend for the control of Lymphatic Filariasis (LF) is mass drug treatment of the human population (with combination of ivermectin and albendazole) with vector control serving a supporting role when feasible and affordable (Ottensen et al 1997). In recent years, there has been great optimum about effective control and possible elimination mainly through treatment to interrupt transmission. However, the crucial unresolved issue in chemotherapy-based elimination programme is the level to which the parasite population density must be reduced in order to stop the intervention with minimal recrudescence.

Among the available vector control measures protection from mosquito bites by bed nets has received a lot of attention probably as a familiar concept that is effective and simple to implement (Ukaga C.N personal communication). Insecticide treated bed nets (ITBNs) have been found to impact on malaria transmission in experimental trials in sub-Saharan Africa, as shown by reduction of various entomologic indices (Lindsay et al 1989; Mbogo et al., 1996; Bogh et al., 1998) including reduction in mortality and morbidity in humans (Binka et al., 1996; Nevill et al., 1996). ITBNs have also been found useful in
controlling vector-borne infections; sand flies and anthropoponic cutaneous leishmaniasis (Reyburn et al., 2000) *Culicoides* (Shreck and Kline, 1993), and house flies mediated infections (Cakir et al., 2008).

Despite the growing evidence that ITBNs reduce malaria morbidity and mortality in various epidemiological conditions (Choi *et al*., 1995) and that *Plasmodium* carrying mosquito (*Anopheles*) also transmit LF (Pederson and Mukoko, 2002; Awolola *et al*., 2006), their value against LF infection and disease has been rarely evaluated. As part of the efficacy trial, Nigeria Lymphatic Filariasis Elimination Programme (NLFEP) of the Federal Ministry of Health assisted by the Carter Centre under the grant provided by Smith Kline Beecham has set 2020AD as the year to eliminate LF. This study is part of aid programme to identify control strategy, assess and compare the effects of ITBNs coverage on the rates of LF transmission in parts of Ebonyi State where *Loa-loa* co-endemicity has up to now prevented mass drug administration (MDA).

**MATERIALS AND METHODS**

**Study Area**
The study was conducted in three (3) sentinel villages Orijiriafor, Okpochiri and Ndiagu Obu in Ohaukwu LGA of Ebonyi State Nigeria (7°31’ – 8°18’N and 5°36’ – 6°15’ E). The LGA is among the *L.loa* endemic areas and were not Onchocerciasis hyper/mesoendemic, thus not on MDA. These villages had (MF) prevalence of 31-61% (Carter Center 2009 unpublished data). They are typical and represented the highest filariasis endemic villages in Nigeria. The sentinel villages consist of 2,709 scattered mud houses with few brick houses. There was no evidence of ITBNs use in these areas. The ecology of the area has been described in details (Amaechi *et al*., 2010; Richards *et al*., 2013). Follow up parasitological survey Immunochromatographic Card Test (blood slide or ICT) were not carried out, but entomological monitoring of ITBNs intervention were employed.

**Entomological Surveillance / Laboratory Processing**
After ethical approval by Post Graduate Board of the Zoology Department of Imo State University and Ebonyi State Ministry of Health, there was census of households where they had a unique identification numbers. The prevalence of infected mosquitoes was assessed in the households. Houses were visited twice monthly during the mornings between 7.am and 12.pm. Endophilic mosquitoes were caught by Pyrethrum knock down (PKD) (WHO, 2002) and mechanical aspirator concurrently to increase the catch in terms of physiological status and different feeding and resting habits. Two rooms that was slept in the previous night, one for each method in non-ITBNs intervention and one for both methods in ITBNs-intervention cohorts were used for mosquito collections (Amaechi *et al*., 2013). Records were made of time and number of species collected, compound number and number of persons sleeping in a room. As much as possible, houses chosen were of similar construction to avoid the effect of variability. Collected mosquitoes were preserved in Petri-dishes lined with moist cotton wool and taken to temporary dissection center. Visual identification was made using different keys and characteristics (Nwoke *et al*., 2010) and sorted by abdominal status (fed, unfed, gravid and half gravid). Blood fed mosquitoes were dissected to determine parity by observing the degree of ovarian trachioles (Detinova, 1962). Recovery of larval stages of *W. bancrofti* was by Nelson and Pester (1962) and categorization of larvae was by sizes rather than appearance (Nathan, 1981). The transmission potentials were estimated following the method of Atting *et al* (2005).
Study Cohorts
Upon ITN (Pemanet 2.0, Vestergaard Frandgen) intervention, recipients were educated (hanging and rolling up nets after sleeping) while compliance was monitored. Two cohorts were used; ITN intervention house-holds (with vulnerable groups or ITBN-users) and households without ITN coverage or non ITN users/intervention. The household with vulnerable groups consists of pregnant women and children <5 years (Federal Ministry of Health, FMOH malaria strategy) while the non-ITBN coverage were those households without vulnerable members at the time of net issuance. Due to logistic difficulties (proportions that received nets from the households, migrations, refusal and absence at sometimes of collections) sampling were uneven in the households 20 households and 30 households respectively were from vulnerable and non-ITBN cohorts which served as permanent cohorts.

Statistical Analysis
Data were analysed by Chi Square using Epi-Info 6 computer software statistical analysis programme (version 2003) to compare the indices of transmission. Also mosquito density and monthly variations were calculated using percentages.

RESULTS
Cumulatively 1,693 female mosquitoes were caught / dissected from two cohorts for parity and transmission indices. Of this, 14.00% (237/1,693) and 86.00% (1456/1693) were from ITBN-intervention and non ITBN-intervention households respectively (Table 1 and Figure 1). Four mosquito species were found and their abundance differed significantly (Figure 2). Overall, significant greater number were caught in ITN-intervention cohorts than non ITBN-intervention cohorts (P>0.05). Whereas in both cohorts, An. gambiae sl were the predominant LF vector (12.99% versus 82.69%), An funestus were not found in ITBNs cohorts followed by Cx quinquefasciatus (1.00% versus 3.07%) were found also in both cohorts while other species were rarely present (Table 1). Species densities decreased proportionally from May to September for ITN intervention houses while it fluctuated with peak in July with a drop in August for non ITBN intervention houses. Physiological status (4.09% versus 4.50%), infection/infectivity rates (5.70% versus 8.40%) and (1.90% versus 2.93%) did not differ for both cohorts (P>0.05). similarly, the total/average transmission potentials (4/1 versus 65/13) for ITBNs-intervention and non ITBNs-intervention cohorts were insignificant (P>0.05).
**Table 1: Overall Biting Densities and Transmission Potentials of W. bancrofti in the Study Area.**

<table>
<thead>
<tr>
<th>Classification of Data</th>
<th>ITN- Intervention</th>
<th>Non ITN-Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Months of collection and Households</td>
<td>M</td>
<td>J</td>
</tr>
<tr>
<td>i. Species and number dissected/ Examined (%)</td>
<td>(4.08)</td>
<td>(2.95)</td>
</tr>
<tr>
<td>*An. gambiae Sl</td>
<td>(0.00)</td>
<td>(0.00)</td>
</tr>
<tr>
<td>An. funestus Sl</td>
<td>(0.59)</td>
<td>(0.12)</td>
</tr>
<tr>
<td>C(p). quinquefasciatus</td>
<td>(0.00)</td>
<td>(0.00)</td>
</tr>
<tr>
<td>Ae. Aegypti</td>
<td>(0.00)</td>
<td>(0.00)</td>
</tr>
<tr>
<td>ii. Infection Types:</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Infected mosquitoes (L1, L2 or L3)</strong></td>
<td>(0.00)</td>
<td>(0.00)</td>
</tr>
<tr>
<td>(with L3 larvae)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iii. Infective mosquitoes</td>
<td>(0.00)</td>
<td>(0.00)</td>
</tr>
<tr>
<td>iv. Intensity of infection</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>V. Overall parous and parity of infected species:</td>
<td>48</td>
<td>44</td>
</tr>
<tr>
<td>Gravid (NG &amp; PG)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Not gravid (P&amp;N)</td>
<td>(0.00)</td>
<td>(0.00)</td>
</tr>
<tr>
<td>Blood meal of infected species:</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Bloodfed (FF &amp; PF)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bloodfed</td>
<td>7.25</td>
<td>10.42</td>
</tr>
<tr>
<td>Rate (percentages):</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>i. Parous</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>ii. Infection</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>iii. Infective</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transmission Potential +</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Species infected; M,J,J,A,S= months of the year (May - September)
L3  L2 = larval stage; NG = Nulliparous gravid; PG = parous gravid;
P = parous N = nulliparous; FF = fresh fed; PF = previously fed.
**Average number of infective larvae per infective mosquito in parenthesis
+Total number of infective biting density and average number of infective larvae per infective mosquito.**
Figure 1: Overall mosquito density by households

Figure 2: Abundance of mosquito species in the Study Area by households
DISCUSSION

In order to demonstrate the success of a filariasis programme, careful evaluation of infection levels in both human and vector is important after intervention. Due to ethical reasons and reluctance to submit to regular blood check, assessment of infection in vectors offer advantage for monitoring infection after implementation. There was a clear impact of ITBNs upon the endophlic LF vectors densities. Overall, we observed reduction in the proportions from the ITBNs intervention households when compared with the non intervention cohorts (14.00% versus 86.00%). Studies in the Gambia (Lindsay et al., 1989), Burkina Faso (Cuzin-Quattora et al., 1999) and Coastal Kenya (Mbogo et al., 1996; Bogh et al., 1998) have reported reductions with indoor Anopheline densities by more than 80% with ITN or curtains intervention. Comparism with other studies were however difficult due to the different sampling methods used in each. Estimated changes in mosquito densities or biting rates vary based upon collection method. In the Gambia and Sierra Lone, studies have reported reduction in endophilic densities in houses with ITN based on PSC while comparisons based upon light traps or human baits showed little or no change in vector densities (Magbity et al., 1997; Quiones et al., 1998). Endophilic collections may be based estimates of mosquitoes abundance using ITBNs because the exito-repellency of permethrin may cause them to exit early (Line et al., 1987; Miller et al., 1991).

The ITN was observed not to affect all species to the same degree with An. funestus the most sensitive and Cx quinquefasciatus the least sensitive. An. funestus has been reported to be very susceptible to chemicals and spraying campaign has found apparent eradication of this species from small foci (Gillies and De-Meillon, 1968). This species was most strongly affected by the presence of ITN probably due to feeding habit as well as resting habit (deliberate exophily). Kurihara et al., (1985) made similar observation. The ratio of An. gambiae clearly showed that they have wide range of tolerance and ability to resist diectamethrin use for net treatment (Basilu-Kanza et al., 2013). This probably accounted for their role in continued transmission.

Further to these observations, the reduction on vectors densities could not affect LF transmission. Changes in these indices should be interpreted as evidence that ITBNs have a community level effect upon the vector population. The absence of significant difference in entomologic indices (parous, infection and infectivity status) was however, unexpected. It suggests that in both cohorts, vectors were imbibing microfilariemic blood with resultant larvae developments due to exposure related factors. Besides, no house was found screened with insect nets. This habit together with human behaviour is critical in transmission, hence suggestion herein that the widespread use of ITN could have an impact on the vector population.

The results of this study also revealed that ITBNs alone could not halt transmission to a state where infective larvae (L3) remained undictatable by dissection. One important way ITBNs reduce LF transmission is by providing a barrier between infected (microfilaremic) human carrier and the vector. The continued human infection calls for sufficient ITBNs ownership and use for longer time when W. bancrofti worms may die or can no longer produce MF. Going by Pederson et al (2009) estimation of transmission threshold as being below an overall mosquito larval infection rate of 0.65%. infection rates found here in (Table 1) were higher. Granted all factors including efficacy of nets are to be assumed, poor compliance, human behaviour and activities could have played central role. Misconception and influence of previous studies which centered on malaria as well as personal discomfort probably contributed. However, until verified by longitudinal studies this finding should be treated with caution. The numbers of households used and months covered were too few to support any statements about
evidence of ITBNs on LF transmission. Interestingly, these sentinel villages were later converted to full ITN coverage and follow up study is on-going.

The monthly transmission potentials (TP⁺) for the cohorts had mix distribution; the potentials were comparable and clearly revealed no changes in vector contact rates among the infected people. These findings have major implications for global programme for the elimination of LF. It shows that the effectiveness of ITBNs may be determined not only by convincing people to sleep under net but consistent use of net and also on the vector species present in a given area. The study have finally show cases that in areas where An. funestus is the predominant vector, the mere presence of bed net may be enough to significantly interrupt transmission. Where as in areas where An. gambiae is the predominant vector, nets in synergy with drugs is likely to achieve greater impact than mere net.

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