Evaluation of the efficacy of insecticide treated bednets (ITBNS) control trials on some aspects of transmission dynamics of bancroftian filariasis vectors in Ebonyi State, Nigeria.

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To evaluate the efficacy of bed nets on bancroftian filariasis transmission and on its vectors, we conducted this study in selected sentinel villages of Ebonyi State, Nigeria between June 2010 and August 2011. Two cohorts were used; full coverage group (distribution of bed nets to all households) and vulnerable group coverage (pregnant women and children <5 years). Endophilic (indoor resting) mosquitoes were caught before (insecticide treated bed net), ITBN- intervention and after ITBN intervention in households twice a month by pyrethrum knockdown (PKD) technique. The mosquitoes caught were identified using standard morphological keys. The engorged and parous mosquitoes were dissected fresh to enumerate the proportion and distribution of infective larvae (L3). The result show that no significant differences were found between the cohorts after ITBN intervention. The engorged and parous mosquitoes were dissected fresh to enumerate the proportion and distribution of infective larvae (L3). The result show that no significant differences were found between the cohorts after ITBN intervention. Anopheles gambiae was the most predominant species for both cohorts. Facilitation in the couple W. bancrofti / A. gambiae continued after ITBN-intervention suggesting pyrethriod-resistance. However, A. funestus transmission of filariasis was controlled by ITBN-intervention in both cohorts. Survival rate of the infected species from both cohorts were insignificant (p > 0.05). Evaluation on entomologic indices herein offers inexpensive alternative to blood smear analysis. Possible reasons for lack of evidence on ITBN full coverage group as compared to ITBN vulnerable group in reducing infections were discussed.

Keywords: Insecticide-treated bed net, infective larvae, bancroftian filariasis vectors, anatomical distribution, transmission.

INTRODUCTION

Bancroftian filariasis or lymphatic filariasis (LF), a deforming and disabling parasitic disease caused by the filarial nematode Wuchereria bancrofti is endemic in some 80 countries with an estimated 100 million infected people (TDR, 1997). Although it does not increase mortality in endemic areas, morbidity causes psychosocial and psychosexual conditions in affected individuals (WHO, 2002). In recent years there has been great optimism about effective control and possible elimination, mainly through treatment to interrupt transmission (Ottesen et al., 1997). 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. Moreover, children below 2 years of age, severely ill persons and pregnant women (based on menstrual history) are excluded from mass drug administration (MDA) (Gyapong, 2000). Evidence also abound that despite high coverage with MDA, transmission was not interrupted suggesting synergy with other control measures (Burkot et al., 2005).

Nocturnally periodic lymphatic filariasis has been endemic in Nigeria for a very long time (Annet et al., 1901) with most cases in the densely populated South
East, Nigeria. Recent spot surveys have shown microfilariae prevalence of 45.00% and vector infectivity rate of 4.60% (Amaechi et al., 2009). Fortunately, Nigeria is among the first to join global control efforts by developing a national programme to eliminate lymphatic filariasis (NPELF) as a public health problem. Transmission of bancroftian filariasis by Anopheline vectors which also transmit malaria has been well documented (McMahorn et al., 1981; Muturi et al., 2006; Awolola et al., 2006). Insecticide-treated bed nets (ITBNs) have been promoted as effective strategy in reducing mortality and morbidity from malaria (Lengeler, 2003). Just as in malaria, ITBNs with both chemical (repellent and insecticidal effects on mosquitoes) as well as a physical barrier could supplement MDA in view of its safety and sustainability (Idowu et al., 2004; Anosike et al., 2004). The Carter Center in November 2009 issued free of charge ITBN to resident households (HHs) in parts of Ebonyi State, Nigeria where the burden of these diseases are high. Consequently, the present study was designed to evaluate the efficacy of ITBN on infective status and transmission efficiency of LF vectors.

MATERIALS AND METHODS

Study area/study design

The study was conducted in four sentinel villages; two each from Ohaukwu and Abakaliki LGA (Orijiriafor, Ndiagu Obu, Mgbabeluzor and Okarie Eehida) of Ebonyi State, Nigeria (Latitude 7° 30’ - 8° 18’N and Longitude 5° 36’-6° 15’ E). The ecology of the area has been described in details (Amaechi et al., 2011 a, b). The inhabitants are Ibos of Southeast, Nigeria. Occupationally, they are hired labourers, peasant farmers and craft men. The endemicity of the villages for W. bancrofti (Preliminary ICT survey of LF ≤ 20%) was analysed by (TCC, 2007 unpublished data). In brief the four villages had overall microfilariae (mf) prevalence of 17 - 61%. There was no evidence of prior ITBN use in these villages. 30 households randomly selected prior to ITBN intervention in each village served as cohort for the study and one room was sampled. The overall study was carried out from June 2010 to August 2011. On arrival in the study areas, field team visited the clan heads to explain the study objectives and obtain informed consent. Before ITBN-intervention, a full community census was undertaken in November 2009 and each household designated with unique identification number. This was followed by pre-intervention vector household surveys (for vector density and infection/infectivity prevalence) between July and December 2009. ITBNs were issued to all resident households in villages (Mgbabeluzor and Okarie Echida) from Abakaliki (full coverage group) while only the vulnerable people received nets in villages (Orijiriafor and Ndiagu Obu) from Ohaukwu (vulnerable group coverage). The long lasting bednets was supplied by the Carter Centre and distributed by the research team, district health and village representatives using a village register updated during the study. To ensure full compliance, direct observations were made on households while recipients were educated on how to hang the net and best way to roll it up after sleeping. This pattern of net distribution provided an opportunity to assess and compare the infective status of the vectors on chosen cohorts.

Ethical consideration

The study was approved by the Post Graduate Research Board of the Zoology Department of Imo State University Owerri, Nigeria and Ebonyi State Ministry of Health. Mosquitoes were caught in sampling households with informed oral consent from house owners.

Mosquito study

The prevalence of infective mosquitoes was assessed in the study households. Houses were visited twice a month during the morning between 7am to 11am and adult endophilic mosquitoes were caught using pyrethrum knockdown as reported elsewhere (Mboera et al., 2006), the brand used was Baygon. As much as possible the houses were of similar construction to avoid the effect of variability. 30 households in each village served as a permanent cohort and at least one sleeping room was used for mosquito collections (Mboera et al., 2006). Records on time and number of species caught, number of rooms sprayed and compound number, number of persons sleeping in a room, previous and current use of ITBN were collected. Mosquitoes caught were taken to a makeshift dissection centre and visual identification (for vector morphology) was made using different keys and characteristic features (Emukah et al., 2007). Blood fed and gravid females were assessed for parity by observing the degree of ovarian trachioles (Detinova, 1962). Recovery of larval stages of W. bancrofti was done according to Nelson and Pester (1962). Transmission efficiency (i.e. the proportion of L₃ escaping from the mosquitoes to enter the human host), involved sum of L₃ estimated as L₃ in the transmitting vectors (i.e. L₃ from the head, thorax and abdomen as calculated by Dinah (2000). All measured entomological indices for LF vectors (infectivity rate) and physiological status were compared for both cohorts.

Data analysis

The transmission efficiencies of the LF vectors were
compared by descriptive statistics and comparisons of proportions. The abundance and rate of infection was calculated using percentages and formulae respectively. Independence and relationship of entomological indices was tested using Chi-square ($\chi^2$) at 95% ($p = 0.05$) acceptance level and displayed in tables.

**RESULTS**

A total of 4,195 mosquitoes were caught and dissected for parity and infective status from ITBN-vulnerable group coverage villages. Of this, 53.33% (2,317/4,195) and 44.77% (1,878/4,195) were caught before and after ITBN-intervention respectively (as shown in the upper part of Table 1). *A. gambiae* (75.62% vs. 75.24%) was the predominant species and overall LF vector densities differed significantly (df = 4; $p < 0.05$). Only *Anopheles* species (*A. gambiae* and *A. funestus*) were susceptible with infective larvae and their differences before and after ITBN intervention were insignificant ($p > 0.05$). The distribution of $L_3$ in *A. gambiae* and *A. funestus* are shown in Table 1. Comparisons of the proportions of total $L_3$ in each body site for *A. gambiae* indicated decrease in the percentage of larvae in the thorax from 67.1% to 43.8% (53/79 vs. 35/80), respectively. However, an increase from 17.2% to 23.8% (14/79 vs. 19/80) was observed in the proportion of $L_3$ in the head and mouth parts and from 15.2% to 32.5% (12/79 vs. 35/80) abdomen. No $L_3$ was found in *A. funestus* after ITBN intervention. A chi-square test showed an overall insignificant difference in distribution of $L_3$.

The density and distribution of $L_3$ from ITBN full coverage villages were as shown in the lower part of Table 1. Vector proportions were 53.55% (1,154/2,155) and 46.45% (1,001/2,155) before and after ITBN intervention respectively. *A. gambiae* (67.74% vs. 55.44%) was the most abundant species as was found for ITBN vulnerable cohorts.. Comparisons of $L_3$ in each body site revealed an increase in $L_3$ at the thorax. However, no $L_3$ was found in *A. funestus* after ITBN intervention. Cx. *quinquefasciatus* and other species (*A. aegypti* and *M. africana*) were not susceptible to *W. bancrofti* larvae. For both trial villages there was a tendency for $L_3$ larvae to concentrate in the thorax of infected species.

Table 2 shows that parous rates and transmission indices of infected species (infection, infective rates and transmission efficiencies) from both trial villages were insignificant ($p > 0.05$). Comparisons of proportions of total $L_3$ in each body sites of LF vectors before and after intervention indicates that infective rates decreased by almost half (from 20.78% to 12.96%) for *A. gambiae* from villages where vulnerable groups had ITBNs. However, no transmission of filariasis was found from villages with total ITBN intervention. Similarly, no transmission was found in *A. funestus* from both trial villages upon ITBN intervention.

**Discussion**

We have explored possible effects of ITBN on infective larvae from both cohorts; full coverage group and vulnerable group coverage on filariasis vectors and filarial transmission to assist with certification of elimination. Our result supports previous studies Anosike et al. (2003), Mbah and Njoku (2004) which established transmission, mf-$L_3$, of *W. bancrofti* (vector-specific parasites) in *Anopheles* mosquitoes in rural Nigeria. It should be noted that transmission dynamics is largely a function of the mf becoming infective ($L_3$) in a mosquito vector and perhaps, the microfilarial density of the donor’s blood (Southgate, 1992). Despite the absence of parasitological examination of microfilaria densities of carriers, our result confirmed that *A. gambiae* was susceptible to the local strain of *W. bancrofti*. However, the main finding is that the study did not provide any evidence for the ITBN full coverage group use as compared to vulnerable group use to be superior in reducing or clearing infection after intervention. For LF transmission to be interrupted, vector density or mf intensity needs to be driven below a threshold that ensures no infection occurs. The fact that parity in infective larvae ($L_3$) was found from both cohorts is noteworthy. Reducing the longevity of LF vectors leads to reduction in transmission when the vectors are endophilic and not mere reduction in proportions. It showed that infected mosquito can infect susceptible individuals and also indicated marked affinities of *Anopheles* species to human and their dwellings. This would increase their chances of becoming infected (White, 1976) and even re-infected during subsequent feeding (Service, 1976). It showcases how human populations are constantly exposed to infective bites with epidemiological significance in LF transmission dynamics. This is because severity of disease might hasten patent infection Nonetheless, reduction in proportion of *A. funestus* due to ITBN intervention (Darriet et al., 1984; Lines et al., 1985) is strong evidence in support of the notion that ITBNs confer protective effect and could add sustainability to strategies of preventive chemotherapy. However, the reverse was the case for *Cq. quinquefasciatus* and probably suggests different behaviour of species which must be considered in vector control options. We are unaware of previous studies on ITBN control of LF on transmission potentials and suggest further investigation to unveil the significance of this interesting finding.

The variation in vectorial capacity on the impact of ITBN for *A. gambiae* and *A. funestus* is paradoxical and may not be fully justified since both species show similar behaviour; endophilic and anthropophilic. Our observation and Lines et al., (1985) in Tanzania on treated materials found that *A. gambiae* was the
Table 1. Overall vector densities and distribution of infective larvae (L₃) in vectors from the study area/villages.

<table>
<thead>
<tr>
<th>Species</th>
<th>Before ITBN-intervention</th>
<th>After ITBN-intervention</th>
<th>Distribution of L₃</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.(%)/ mosquito recovered</td>
<td>No.(%)/ mosquito recovered</td>
<td></td>
</tr>
<tr>
<td></td>
<td>caught/dissected</td>
<td>caught/dissected</td>
<td>H/M</td>
</tr>
<tr>
<td>A. gambiae sl</td>
<td>1,752 (75.62)</td>
<td>1,413 (75.24)</td>
<td>14</td>
</tr>
<tr>
<td>A. funestus sl</td>
<td>463 (19.98)</td>
<td>26 (1.38)</td>
<td>4</td>
</tr>
<tr>
<td>Cx. quinquefasciatus</td>
<td>98 (4.23)</td>
<td>425 (22.63)</td>
<td>0</td>
</tr>
<tr>
<td>Mn. africana</td>
<td>3 (0.13)</td>
<td>7 (0.37)</td>
<td>0</td>
</tr>
<tr>
<td>M. uniformis</td>
<td>0 (0.00)</td>
<td>2 (0.11)</td>
<td>0</td>
</tr>
<tr>
<td>A. aegypti</td>
<td>1 (0.04)</td>
<td>5 (0.27)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>2,317 (53.23)</td>
<td>1,878 (44.77)</td>
<td>14 (0.75)</td>
</tr>
</tbody>
</table>

Table 2. Overall Abdominal condition and transmission efficiencies of infected species in the study area/villages.

<table>
<thead>
<tr>
<th>Classification of data</th>
<th>Before ITBN Intervention</th>
<th>After ITBN Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X₁</td>
<td>Y₁</td>
</tr>
<tr>
<td>(i) Abdominal conditions:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Overall parous mosquitoes</td>
<td>2,096</td>
<td>811</td>
</tr>
<tr>
<td>(b) Overall nulliparous mosquitoes</td>
<td>119</td>
<td>152</td>
</tr>
<tr>
<td>(ii) Parity and Bloodmeal of Infected species:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Gravid</td>
<td>86</td>
<td>28</td>
</tr>
<tr>
<td>(b) Not gravid</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>(c) Blood fed</td>
<td>88</td>
<td>29</td>
</tr>
<tr>
<td>(d) Unfed</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>(iii) Infection status:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Infected mosquitoes:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. gambiae</td>
<td>71</td>
<td>99</td>
</tr>
<tr>
<td>A. funestus</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>(b) Infective mosquitoes (L₃ in the head):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. gambiae</td>
<td>16</td>
<td>10</td>
</tr>
</tbody>
</table>
predominant and most aggressive. The emergence of resistance to pyrethrum could limit the efficaciousness of ITBN. It probably had the ability to resist physiologically chemical treatment used in nets (Malcolm, 1988; Nwane et al., 2013) which increased survival probability by the microfilariae hence, persistence transmission with continued facilitation of the couple *W. bancrofti/*A. gambiae in both cohorts.

However, *A. funestus* found to be non-persistence with infective larvae on comparison from the cohorts (transmission drops to zero). Probably the effect of pyrethrum used for ITBN made them to be less readily irritated, hence deliberate exophily (Kurihara et al., 1995) an indication that this type of intervention has a more limited impact. They may and may be feeding on non-human hosts (Githeko et al., 1994) and the possibility of contact outdoors where they still remain efficient vectors demands complementary protections.

Interestingly *Cx. quinquefasciatus* which showed increased endophily and endophagy for both cohorts were not implicated with L₃. Their epidemiologic significance is likely limited because of life cycle parameter (anatomical variations). *Aedes aegypti* exhibited exophily and diurnal tendency (Scott et al., 1993) and *Mansonia* species (non filarial vector in Nigeria) were all present, indicative of the level of nuisance that the inhabitants of the area get from these mosquitoes.

Further observations and comparisons of transmission indices of L₃ positive vectors revealed that older mosquitoes (parous and blood fed) tend to accumulate with time and allow for increased feeding frequencies. The transmission of *W. bancrofti* is highly dependent on the life span of female mosquitoes as well as temperature and humidity (Clement, 1963). Studies on larve densities and anatomical distribution of infective larvae in mosquito posited that interrelationships could influence L₃ escape (Lavoipierre and Ho, 1966) from mosquitoes to enter the definitive host; with tendency to concentrate in the head and proboscis. These L₃ escape have epidemiological significance in understanding the transmission dynamics of filariasis. In the present study, high concentration of L₃ in the thorax rather than head and proboscis have further justified that the L₃ in the vectors and the number that would escape to enter definite host is not well characterized. This may be due to host parasite adaptation and non significant difference for both cohorts in L₃ distribution attributable to redistribution following a substantial escape of L₃ larvae. It remains to be established how the number of L₃ would affect the rate of escape and how this would influences the number of L₃ that eventually gain access to the hosts. Infective larvae have been found to fluctuate over time, as L₃ in mosquito move back and forth from the head to abdomen (Dinah, 2000). However, the fact that not all L₃ which escaped from infected vectors from both cohorts are likely to succeed in entering the human host justifies the transmission inefficiency. However, as lymphatic filariasis programme moves towards elimination, the use of molecular tools in certifying absence of LF transmission cannot be totally ignored. Granted all factors including efficacy of nets are assumed, probably non-perceived efficacy and human behaviour could account for
no change in the vector contact rates from both trial villages. Two categories ITBN non users; those living in households owing, but not hanging nets and those living in households with nets hanging but hardly sleep under it abound in these area. Also misconception and influence of previous trial/pilot studies which centered on malaria probably contributed to the challenges of ITBN control trials. Resulting studies should therefore be tailored towards encouraging the hanging of nets, its use and inclusion of all vector borne diseases in further studies.

Based on the findings of our study, the disparity in the transmission efficiency by the Anopheles species exposes the ineffectiveness of only ITBN use against LF. Since the same mosquitoes can also transmit malaria, transmission could be interrupted by the use of adjunct control measures like combination of ITBN and application of repellents (reducing vector-human contact) or the use of drugs and outdoor protections(wearing clothing that fully cover the skin). Cartel et al (1991) had found high mortality rates on mosquitoes fed on carriers treated with microfilaricide drugs, by extension decreasing the effective mosquitoes or reducing microfilariae rate transferred to human with drugs and prevention of their bites with ITBN with time might bring additional benefit in filariasis control programmes.

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