

## IMPACT OF IMPREGNATED-TREATED BED NET (ITBN) CONTROL TRIALS ON HUMAN LYMPHATIC FILARIASIS VECTORS, FILARIAL DISTRIBUTION AND TRANSMISSION IN EBONYI STATE, NIGERIA

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**ABSTRACT:** To assess the impact of bed nets on lymphatic filariasis (LF) control, we conducted a study in selected sentinel villages of Ebonyi State, Nigeria between July, 2007 and November 2008. Species infection prevalence data as well as Knowledge and perceived efficacy of insecticide-treated bed nets (ITBN) on LF control were also investigated. Two cohorts were used; pre-intervention households and ITBN-intervention households. Mosquitoes were caught indoors twice a month by pyrethrum knock down (PKD) technique and identified using standard morphological keys. Blood fed female mosquitoes were dissected fresh in the field for parity and infection/infectivity status with *Wuchereria bancrofti*. Of 2,970 mosquitoes caught, 50.97% (1,781/2,970) and 49.03% (1,189/2,970) represented pre-intervention and ITBN-intervention household catches respectively. *Anopheles* species and *Cx. quinquefasciatus* were found to be susceptible to developing stages (L<sub>1</sub>-L<sub>3</sub>) of *W. bancrofti* larvae. However, filarial infection/transmission progressed only in *An. gambiae* (which was the predominant species 36.67% vs 27.71%) on ITBN-intervention. The overall parous and infection/infective rates for both cohorts were not significantly different ( $p > 0.05$ ). Evaluation on entomologic measures for filarial infection reported here offers an inexpensive alternative to blood smear analysis. Findings were discussed in the context of on-going plans to eliminate LF and the respective vectors in Nigeria.

**KEYWORDS:** Lymphatic filariasis, Insecticide-treated bed nets, Vector control, filarial distribution and transmission, Nigeria.

### INTRODUCTION

Human lymphatic filariasis (LF) is a disabling, disfiguring and poverty promoting disease transmitted by mosquitoes in the tropics (Gyapong, 1999). LF has been an increasing problem in Nigeria; with the largest population at risk on African continent and the third most endemic country worldwide (after India and Indonesia). An estimated 22.1% of the population is thought to be infected (Eigege *et al.*, 2002). Although it does not increase mortality in endemic areas, morbidity causes psychosocial and psychosexual conditions in affected individuals (WHO, 2002a). The demand for LF treatment greatly exceeds the available resources. A cost-effective and sustainable strategy is clearly indicated. With tools currently available, LF in principle could be eliminated. A number of countries (including Nigeria) have completed annual rounds of mass drug administration (MDA) but their impacts are yet to be fully evaluated. There is however, evidence

from Pacific (Somalia and French Polynesia) that despite high coverage with MDA, transmission has not been interrupted (Burkrot *et al.*, 2005); thus, an indication for adjunct controls measures. In Nigeria, the impact of the MDA programme in sentinel sites will be reported elsewhere. Our focus here is on the assessment of infections in the mosquito vectors.

Vector control was the primary tool for filariasis control when effective anti-filarial drugs were unknown and even after effective anti filarials became available (Burkot and Ichimori, 2002; Burkot *et al.*, 2005). In order to demonstrate the success of a filariasis programme, careful evaluation of infection levels in human populations and vectors following intervention is important. Due to ethical reasons and reluctance on the part of humans to submit to regular blood examinations, assessment of infection in vectors offer advantages for monitoring infection after implementation of intervention. Two methods of detecting infection

in vectors that are now widely used are dissection and polymerase chain reaction (PCR). PCR, though not as common as dissection, is more sensitive for filarial detection in mosquitoes than the conventional dissection and microscopy ([Ramzy et al., 1997](#)). Nonetheless, mosquito dissection has been the gold standard against which other methods are compared in the context of the filariasis elimination programme for monitoring infection status ([Plichart et al., 2006](#)). Mosquito PCR provides an indirect measure of filarial infection rates in human population but is not a measure of transmission. Detection of filarial DNA in mosquitoes cannot differentiate infection from infective mosquitoes (as not all MF ingested survives and develop into infective, L<sub>3</sub> larvae) ([Ramzy et al., 1997](#)). The LF vectors, *Anopheles gambiae* sl, *An. funestus* sl and *Culex quinquefasciatus* in Ebonyi State (South East, Nigeria) are considered to be endophagic and to bite mostly in the evening and at night ([Amaechi, 2009](#)). Mosquitoes were generally sensitive to insecticide-treated materials ([Amaechi et al., 2010](#)). Thus, personal protection with fabrics such as insecticide-treated bed nets (ITBN) could be an effective alternative and additionally aid malaria control in Nigeria ([Idowu et al., 2004](#); [Anosike et al., 2004](#); [Adeyemi et al., 2007](#)). Results of co-infection of malaria and LF both in humans ([Ghos and Yadav, 1999](#)) and in mosquitoes playing dual roles in transmission ([Awolola et al., 2006](#)) clearly indicated that ITBN could offer protection against LF vectors and filarial infection. Currently, Nigerian Lymphatic Filariasis Elimination Programme (NLFEP) of the federal Ministry of Health assisted by the Carter Center has embarked on several pilot studies including ITBN control trials. The impact needs to be fully evaluated. No village-scale trials on the use of ITBN to prevent LF vectors and transmission have thus far been published. Accordingly, this study was conducted to assess its impact on the LF vectors, filarial distribution and transmission.

## MATERIALS AND METHODS

### 2.1. Area Study and design

This study was undertaken in 2 sentinel villages; Mgbabeluzor and Obeagu Ibom of Abakaliki Local Government Area of Ebonyi State, Nigeria (Lat. 7°30'1"-8°18'1"N and Long. 5°36'1"-6°15'1"E). The current study was conducted based on preliminary ICT-survey prior to this study ([The Carter Center, 2007](#)) indicating the presence LF in the area. These villages had microfilariae prevalence of 21% and 36%. They are typical and represented the highest filariasis-endemic

villages in Nigeria. The tropical rainforest climate favours high breeding of mosquitoes that transmit both malaria and LF. Villagers are mainly subsistence farmers and houses are predominantly constructed with mud walls and floors and thatched roofs. LF is transmitted throughout the year by these vectors ([Amaechi et al., 2010](#)). There was no evidence of prior bed net use in the villages.

On arrival in these villages, the field team visited the clan heads to explain the purpose of the study and obtain informed consent. Before intervention with ITBN, a full community census was undertaken in November 2007 and each household had a unique identification number. This was followed by a pre intervention household survey or preliminary vector surveys between July 2007 and March 2008. ITBNs were issued to ensure coverage of individual residents in April, 2008. The team with the district health service and village representatives distributed the nets using a community register which was updated during the study. They were educated on how to hang bed nets and best way to roll it up after sleeping. Compliance was monitored. For variety of reasons including absence at the time of net issue and refusal to accept a net, some residents were never issued with a net. This coverage provided opportunity to access the impact of ITBN on ITBN-intervention household surveys on LF vectors and transmission indices on study cohorts/households in areas of widespread ITBN usage.

### 2.2. Ethical Consideration

Ethical clearance and permission to conduct this study was approved by the Post Graduate Research Board of the Zoology Dept. of Imo State University Owerri, Nigeria and Ebonyi State Ministry of Health. Team members were trained on entomological methods. Residents of households gave informed verbal consent concerning mosquito collection during the course of this study.

### 2.3. Adult Mosquito Collections, Morphological Identification and Dissection

The prevalence of infected mosquitoes was assessed in the households. Houses were visited twice monthly during the mornings and endophilic mosquitoes were collected by pyrethrum knock down (PKD) ([WHO, 2002b](#)). The time and period of collection were carefully chosen to catch fully engorged endophilic vectors and reflected the seasons (Rainy and Dry) of the area. As much as possible the houses were of similar construction to avoid the effect

of variability. Endophilic mosquitoes were collected in 20 selected houses (which served as the permanent cohort). At least one sleeping room in each house was used for mosquito collections as reported elsewhere ([Mboera \*et al.\*, 2006](#)). Records were also taken on time and number of species collected, compound number and number of rooms sprayed, number of persons sleeping in a room, and use and perceived efficacy of ITBN. Mosquitoes caught were immediately taken to a temporary dissection center as time allowed. Visual identification (for morphology) was made using different keys and characteristics ([Emukah \*et al.\*, 2007](#)). Blood fed females were dissected to determine parity by observing the degree of ovarian trachioles ([Detinova, 1962](#)). Recovery of larval stages of *W. bancrofti* was done according to [Nelson and Pester, \(1962\)](#). Larval stages were categorized by sizes rather than by appearances ([Nathan, 1981](#)).

#### 2.4. Statistical Analysis

The abundance/biting densities and rates (parous, infection/infective) was calculated using percentages and formulae respectively. Independence and relationship of entomological indices was tested using Chi-square( $X^2$ ) at 95% ( $p=0.05$ ) acceptable significant level and displayed in tables.

### RESULTS

A total of 2,970 mosquitoes (from 4 genera of 5 species) were collected and assessed for parity and infection. Of these, 59.97% (1,781/2,970) were from pre-intervention household catches and 49.03% (1,189/2,970) from ITBN-intervention household catches (Table 1). Vector densities/abundance differed significantly for both cohorts when compared ( $df=4$ ;  $p<0.05$ ). All species found were present in both cohorts with *An. gambiae* S.L (36.67% vs 27.71%) as the predominant species. *Anopheles* species and *Cx. quinquefasciatus* were positive (both infection/infective) with developing larvae (L<sub>1</sub>-L<sub>3</sub>) in pre-intervention cohorts. However, filarial transmission progressed only in *An. gambiae* on ITBN-intervention cohorts (Table 1). The proportion of infected mosquitoes with developing larvae, 1.35% (24/1,781) and 0.25% (3/1,189) from pre and ITBN intervention cohorts did not differ ( $p>0.05$ ). Similarly, the proportion with infective larvae (L<sub>3</sub> in the head; mouthparts and proboscis) 0.56% (10/1,781) and 0.00% (0/1,189) were insignificant ( $p>0.05$ ). The abdominal conditions and infection status (parous, infection/infective rates) of species caught /dissected are shown on

Table 2. Overall, 82.76% vs 50.47% and 68.39% vs 44.49% from pre-intervention and ITBN-intervention households were blood fed and gravid respectively. However, greater number of nulliparous (55.51% vs 31.61%) and unfed mosquitoes (51.47% vs 17.24%) were caught from ITBN-intervention households. Survival rates of infected vectors from both cohorts as assessed by parous, infection/infectivity rates were insignificant ( $p>0.05$ ).

### DISCUSSION

For the first time, data have been provided on the impact of ITBNs on LF in Ebonyi State, Nigeria in view of careful evaluation of vector density and filarial transmission which is imperative to assist with certification of elimination. In human, assessing infection can be considered a "lagging-indicator" because the pre patent period may extend for months after treatment ([Goodman \*et al.\*, 2003](#)). In contrast, entomologic measures provide "real-time" estimates of filarial transmission ([Williams \*et al.\*, 2002](#)). We explored the possible effect of wide-scale ITBN use upon LF vectors and transmission indices. However, the number of each mosquito vector caught does not reflect the geographical abundance of each species such as those that are exophilic but endophagic. Despite this our data indicates that ITBN had a protective effect against transmission to the respective household residents as found in previous studies ([Robert and Carnavale, 1991](#); [Kere \*et al.\*, 1993](#); [Jana-Kara \*et al.\*, 1995](#)). The observed variations in species densities could be due to samples collected from different villages with contrasting proportions of the filarial vectors and smaller than desired proportions of vectors (59.97% vs 49.03%) dissected. There is also difference in filarial-frequency in the two areas; in Mgbabeluzor it is 21% and in Obeagu Ibom it is 36%. Nonetheless, it further suggests that mass distribution of nets is a better determinant than individual net use ([Amaechi \*et al.\*, 2010](#)). This is supported by observed interruption of filarial infection by *An. funestus* and *Cx. quinquefasciatus*. However, the disparity found on the impact of ITBN on *Anopheles* species (*An. gambiae* and *An. funestus*) is not unusual. These species show similar behaviour; both are highly endophilic and anthropophilic ([Mnzava \*et al.\*, 1989](#)). Species often elicit different sets of behaviour which must be taken into account in planning control options. *Anopheles gambiae* like *Aedes aegypti* are opportunistic feeders ([Caryon \*et al.\*, 1998](#)) due to increase host seeking rates with host availability. Unlike other species however, it

probably had the ability to defy or physiologically resist insecticide treatment used in nets ([Guillet et al., 2001](#)). The continued facilitation in the couple *W. bancrofti* /*An. gambiae* and limitation in *Culex* could be associated with density dependence and survival probability. Critical density of mf, a pre larval form, required to sustain transmission in *Culex* is less than in *Anopheles* and these could also determine the possibility of recrudescence of infection in (mass drug administration), MDA –implemented communities ([Ramaiah and Rajendran, 2005](#)).

Our dissection results, revealed tendency of filarial larvae (especially L<sub>3</sub>) to concentrate in the thorax rather than mouthparts and proboscis. This relative rarity of identifying L<sub>3</sub> mosquitoes implies that transmission monitoring based on L<sub>3</sub> may not be feasible within the scope of LF elimination programmes in the locality. Thus, decline in filarial infected vectors demands dissection of increasing number of mosquitoes to demonstrate significant decline in infection prevalence ([Burkot and Ichimori, 2002](#)). PCR techniques with the ability to detect one mf in pools of mosquitoes could be an alternative method.

The parity found for filarial positive vectors as assessed by parous (age-structure) which are possible carriers of parasites and infection/infective rates were however, unexpected. It suggests endophilic-endophagic tendencies of LF vectors (vectoral capacity) and even distribution of their bites due to exposure-related factors by the inhabitants. This habit coupled with human behaviour is critical in transmission. Besides, the observed familial clustering of the inhabitants in the study area could create synchronizing condition for the vectors. This when combined with reported non seasonal variation of LF transmission ([Amaechi et al., 2010](#)) could be another contributory factor as *W. bancrofti* is known to be of medical importance in densely populated areas of eastern Nigeria ([Nwoke et al., 2000](#); [Anosike et al., 2005](#)). These results are of practical importance in the transmission mechanism and control of human LF. Its implication in LF transmission is that microfilariae inoculation (worm burden) by the infective vectors within short space of time or spread out over years is enhanced and severity might hasten patent human infection. Possibility of cumulative disease (i.e., secondary bacterial infections due to itching and scratching) is also increased. However, our result which corroborates ([Snow et al., 1987](#); [Amaechi et al., 2010](#)) on users and non-ITBN users and ([Lindsay et al., 1993](#);

[Quinones et al., 1998](#)) on treated and untreated nets is encouraging since ITBN provide both physical and chemical barriers to LF vector bites and in theory the converse would have been the case. The observed gravid status of all infected species from both cohorts is remarkable. This is because female vectors must take high protein meal in form of blood from vertebrates to develop a clutch of eggs (autogenous) as is true for most species ([Packer and Gorbert, 1989](#)). Available resources and reluctance to submit blood for examination did not permit parasitological explanations. In the areas studied and elsewhere, poor compliance of ITBN observed and reported included; lack of perceived efficacy, personal discomfort (due to high inside temperature) and social habits (sleeping some night elsewhere or not sleeping at all). Some claim to use ITBN during perceived time of high mosquito densities while others do not regularly use it. Those who hardly believe in preventive effectiveness are probably those that are also exposed to infective bites outside bed time which is common in endemic areas. It may as well include those who do not consistently use ITBN and are infected. These could perhaps account for proportions nulliparous (blood thirsty) and explain the parity status among the cohorts in the study. MDA compliant status of these subjects may be validated through subsequent research.

However, with the economic depression, and favourable environment for the consummation of the vector-host relationship, the infection status could have considerable adverse short and long term consequences on them. Thus, it is desirable that the control of LF be given high priority in the implementation of primary health care programmes. These may be resolved by adjunct control measures such as combination of ITBNs and other vector control approaches. [Cartel et al., \(1991\)](#) had observed high mortality rates on mosquitoes which fed on carriers treated with microfilaricidal drugs. Thus, by either decreasing the number of infective mosquitoes or reducing the rate of mf transfer to other people through the vector with drugs and prevention of their bites with ITBN with time might represent an additional advantage in LF control programmes. Personal discomfort and perceived mosquito density could be addressed by behavioural change and mass education campaigns. Studies have shown that ITBN use increased with health promotional campaigns ([Anosike et al., 2004](#)). At the present time in the absence of suitable vaccine, personal protection in synergy with drugs is likely to achieve greater impact than either trial. We feel the results of

this trial justify the need for sustain health education towards the right perception and adoption of all LF measures. It is suggested that linking well-funded malaria control programme to other community directed health initiatives like LF control could greatly accelerate and improve the effectiveness.

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**Table 1:** overall vector densities, anatomical and transmission of *W. bancrofti* in vector population in the study area

Species	Pre-invention survey							ITBN-invention survey										
	No. (%) caught/dissected	L <sub>1</sub> /L <sub>2</sub>			L <sub>3</sub>			No. (%) infected	No. (%) infective	No. (%) caught/dissected	L <sub>1</sub> /L <sub>2</sub>			L <sub>3</sub>			No. (%) infected	No. (%) infective
		H	T	A	H	T	A				H	T	A	H	T	A		
<i>An.gambiae sl</i>	1,089(36.67)	1	14	0	10	6	0	16(1.47)	9(0.83)	823(27.71)	0	0	0	0	9	0	3(0.36)	0(0.00)
<i>An. funestus sl</i>	302(10.17)	2	2	0	0	15	0	7(2.32)	0(0.00)	27(0.091)	0	0	0	0	0	0	0(0.00)	0(0.00)
<i>Cx quinquefasciatus</i>	299(10.07)	0	0	0	1	0	0	1(0.33)	1(0.33)	244(8.22)	0	0	0	0	0	0	0(0.00)	0(0.00)
Other species																		
<i>Mn. africana</i>	90(3.03)	0	0	0	0	0	0	0(0.00)	0(0.00)	94(3.16)	0	0	0	0	0	0	0(0.00)	0(0.00)
<i>An. aegypti</i>	1(0.03)	0	0	0	0	0	0	0(0.00)	0(0.00)	1(0.03)	0	0	0	0	0	0	0(0.00)	0(0.00)
Total	1,781(59.97)	3(0.17)	16(0.90)	0(0.00)	11(0.62)	21(1.18)	0(0.00)	24(1.35)	10(0.56)	1,189(49.03)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	9(0.00)	0(0.00)	3(0.25)	0(0.00)

p>0.05; df=4; X<sup>2</sup>=0.87; No difference in the infection of vectors; \*= infected species; L1-L3=larval stages (infection); H=Head; T=Thorax;

p>0.05; df=4; X<sup>2</sup>=1.13; No difference in the infective vectors

A=abdomen; ITBN=insecticide treated bed net

**Table 2:** Abdominal conditions and Infection status compared in the study area

Classification of Data	Pre-Intervention (July, 2007-March,2008)	ITBN-Intervention (April-Nov,2008)
<b>i. Abdominal conditions (parity &amp; blood meal)</b>		
(a) overall parous	1,218(68.39)	519(44.49)
(b) overall nulliparous	563,(31.61)	660(55.51)
(c) Blood fed (FF&PF)	1,008(82.76)	267(50.47)
(d) Unfed	210(17.24)	272(51.42)
<b>ii. Infection status</b>		
(a) Microfilariae (mf)	7	2
(b) Infected mosquitoes (L <sub>1</sub> ,L <sub>2</sub> &L <sub>3</sub> )	24	3
(c) Infective mosquitoes (L <sub>3</sub> in the head)	10	0
<b>iii. Parity &amp; Blood meal of Infected species</b>		
(a) Gravid (NG&PF)	24	3
(b) Not gravid (P&N)	0	0
(c) Blood fed (FF&PF)	24	3
(d) Unfed	0	0
<b>iv. Rate (percentage)</b>		
i. Parous	0.68	0.44
ii. Infection	1.97	0.57
iii. Infective	0.82	0.00

FF= freshly fed; PF=previously fed

NG= Nulliparous gravid; PG= parous gravid; P=parous; N= nulliparous

L1-L3= larval stages (infection).