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Research Article

Long lasting insecticidal nets (LLINs) intervention impact on use, misuse and challenges in malaria and lymphatic filariasis transmission

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ABSTRACT

To assess the effectiveness and misuse status of Long Lasting Insecticidal Nets (LLINs), intervention, a study was conducted in areas of malaria and lymphatic filariasis endemicity from November 2021 to August 2022. A preliminary survey preceded a cross-sectional survey of the misuse of LLINs. Considering the outcome of the former, 57 households misusing LLINs were reported. Thirty (30) cohorts each from LLIN misusing and LLIN non misusing households served as permanent cohorts for mosquito collections (60 rooms in 60 households in 3 sentinel villages). The study also investigated the status of LLIN usage and non usage. Mosquitoes caught with Aspirators and Pyrethrum Knockdown (PKD) techniques were identified using standard morphological keys. Among those who used LLIN, 68.42% used it for other purposes than vector control while 31.58% didn't utilize it for mosquito control. About 59.65% didn't know the effect of using it for purposes other than malaria/LF control while 40.35% were familiar. Of 8,180 endophilic mosquitoes caught and assessed for parity, infection/infectivity status, 60.07% (4,914/8,180) and 39.93% (3,266/8,180) represented LLIN misusing and LLIN non-misusing catches respectively. Overall, insignificantly higher catches were made in LLIN misusing cohorts than LLIN non misusing cohorts (63.54% versus 36.45%, 58.89% versus 41.11%, 58.86% versus 41.18%) for 2015, 2016 and 2017 respectively. Specifically, malaria/LF vector densities showed that *Anopheles gambiae s.l.* (82.53% versus 74.04%) was the highest followed by *An. funestus s.l.* (10.15% versus 12.19%) and *Culex quinquefasciatus* (6.82% versus 3.83%) while other species were rarely present. The physiological, infection (0.28% versus 0.49%) and infectivity (0.00% versus 0.00%) statuses with *Wuchereria bancrofti* were insignificant between the cohorts (p>0.05). None of the *Anopheles* assessed for *Plasmodium* sporozoite were positive for both cohorts. The percentage level of LLIN household use was 43.33% while 41.03% slept under LLIN daily, 58.97% slept sometimes. Obstacles to LLIN use included hot/heat (56.86%), dislike (21.57%), lack of protection (11.86%) and ineffectiveness (9.80%). The role of significant misuse of LLIN was highlighted by the study. The results indicated the intensity of filariasis transmission by transmitting vectors was higher than that of malaria and re-emphasis the need to promote positive attitude towards the use of LLIN for control of the two diseases.

Key words*:* Malaria and lymphatic filariasis, LLIN-misusing households, LLIN-non-misusing households, Vectors, Transmission

INTRODUCTION

Malaria and Lymphatic Filariasis (LF) are Africa's most important vector-borne diseases. Currently, an estimated 90% of the 1.5-3 million deaths due to malaria occur in Africa (WHO, 2002a) and over one-third of the 146 million people are infected with LF from this same continent (Michael & Bundy, 1997) making them toughest global health challenges for scientist and policymakers. Malaria is a major public health problem in Nigeria where it accounts for more cases and deaths than any other country in the world (United States Embassy in Nigeria, 2016). Nigeria is also the third most endemic country worldwide for LF with an estimated 22.1% thought to be affected (Eigege *et al.,* 2002). The vectors of these diseases are female mosquitoes in the genus; *Anopheles*, *Culex* and *Aedes* which support filarial developments, microfilariae (mf) and *Plasmodium* sporozoite. Nigeria has an abundance of all these proven vectors in different ecological regions and the southeast including rural areas of Imo State falls within the parts (materially disadvantage) that lack adequate facilities. Thus their contribution to the uncontrolled breeding of these vectors and the continued risk of morbidity associated with both diseases. Co-infection of malaria and LF in humans and mosquitoes (Chadee *et al.,* 2003; Awolola *et al.,* 2006; Okorie *et al.,* 2011) points to the integration of a national malaria control programme where intervention remains an essential component. Numerous interventions have been implemented in endemic areas to reduce the burden of these diseases. Common among them are indoor residual spray, the distribution of drugs and treated nets (Kleinschmidt *et al.,* 2009). Of these interventions, insecticidal treated bed nets especially of long lasting insecticidal nets (LLIN) have proven to be a practical, highly effective and cost-effective intervention (Lenegler, 2004). Currently, the Roll Back Malaria (RBM) partnership aims to ensure that malaria is no longer a public health problem by 2025 while the Global Programme to Eliminate Lymphatic Filariasis (GPELF) aim to achieve a similar result for LF by 2020. Since the two diseases share common vectors, some synergy between the two programmes not only seems feasible and cost-effective but will also ensure that vector control which is currently not well defined in GPELF as it is in RBM becomes an integral part of LF.

Previous studies found that sleeping in bed nets treated with insecticides greatly reduces death by malaria (WHO, 2002b), especially in children (WHO, 2008) and morbidity associated with LF (Emukah *et al.,* 2009; Amaechi *et al.,* 2017). However, the impact of the misuse of the net as a growing concern together with the combined effects of the net on the two diseases remains speculative. LLIN has a life span of 3-4 years and was issued free of charge to households (HHs) during the immunization Plus days in parts of Imo State to reduce the burden of these diseases. The benefiting villages included Opuoma, Etioha and Umukene in Ohaji/Egbema LGA of Imo State, Nigeria. No data exist on misuse, efficacy and challenges of LLIN in these areas of intense, perennial malaria and LF transmission. Specifically after LLIN intervention, it becomes imperative to assess the extent of misuse, and compare the effectiveness and possible challenges in reducing malaria and LF transmission.

MATERIALS AND METHODS

Study area/design

The study was conducted in three (3) sentinel villages (Opuoma, Etioha and Umukene) in Ohaji/Egbema LGA (Lat. 5°10' N - 5°5' N and Long. 6°35' E - 7°28' E, Figure 1). The ecology has been described in detail (Amaechi *et al.,* 2017). The climate and topography together with human activities encourage malaria and LF transmissions. The inhabitants are technically ibos of Nigeria. Occupationally, they are peasant farmers, craftmen, etc. The villages were selected based on preliminary malaria and LF surveys and LLIN intervention (The Carter Center, 2009; Amaechi *et al.,* 2017).

The study protocol involved a full village census undertaken in November 2016 where each household had a unique

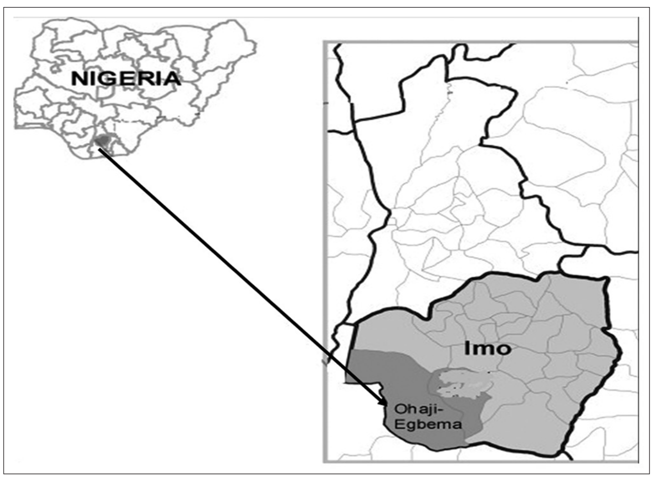

Figure 1: Map showing the location of the study area

identification number. LLIN, permanent 2.0, an insecticide treated bed net impregnated with 5.5 mg of deltamethrin per square meter (Vestergaard Frandsen) were issued in 2015 to all residents. A survey on bed net use prior to this distribution was assessed. This was followed by a preliminary survey before the beginning of the study on the status of bed net misuse from January to March 2017. Considering the outcome of our preliminary investigation households misusing nets (57 households) for purposes other than malaria and LF control were identified. The household heads gave consent and were subsequently interviewed (key individual interview, KII) on the extent of net misuse through a standardized household questionnaire. Thereafter thirty households randomly chosen from both misusing and non-misusing were used as study cohorts for entomological study. A pre-tested questionnaire was designed to obtain information on the level of LLIN usage and reasons for non use. It was self administered and interpreted by the inhabitants in their native language (ibo). Compliance was monitored and this pattern of net use provided an opportunity to assess species composition, densities and parasite infectivity status in the cohorts.

Ethical consideration

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

Adult mosquito sampling

Mosquito collection was by day resting indoor collection (DRI) by Aspirators and pyrethrum spray catch (PSC) as described by WHO (1975). These two methods were concurrently used to increase the catch in terms of physiological status and differences in feeding and resting habits. In each cohort, a room that was slept in the previous night was used for mosquito collection. Before the day, the house owners were informed not to open their windows in the morning. Records were also taken on time and number of species collected, household number and number of rooms sprayed, number of persons sleeping in a room and use of LLINs. Collected species were preserved in Petri dishes lined with moist cotton wool and transported to the temporary dissection center for further processing individually to determine infection status.

Morphological identification/dissection

Mosquitoes that were still alive (caught by DRI) were killed by chloroform. Dead mosquitoes were then sorted out into species based on morphological criteria (Gillet, 1972; Amaechi & Nwoke, 2015). Physiological conditions of female mosquitoes were examined to classify them into fed, unfed and gravid (Detinova, 1962). Randomly selected *Anopheles* was assessed for *Plasmoduim* sporozoites while all parous mosquitoes were processed for larval stages of *Wuchereria bancrofti* which were categorized by sizes rather than by appearance (Nathan, 1981). The filarial infection profile was assessed by examining each of the teased parts (head, thorax and abdomen) of the mosquitoes under a compound microscope. Infection and infectivity rates were calculated using the formulae of Kasili *et al.* (2009).

Data analysis

Data on questionnaires were entered in Microsoft Excel data sheets, cross checked, transferred and analysed using table and percentage distribution. Chi Sqaure (X^2) using Epi Info 6 computer software statistical analysis programme compared the physiological and infectivity rates of malaria and LF vectors between the cohorts, the study areas and mosquito density/abundance were calculated using percentages.

RESULTS

Most (68.42%) of the respondents use LLIN for purposes other than malaria/LF control. Likewise, 31.58% reported that they didn't utilize LLIN for mosquito control. Prominent among the reasons for misuse of the net was to cover household problems (64.91%). Susceptible malaria outbreak (33.33%) was the most acclaimed post distribution advice by health workers on the proper utilization of nets. About 59.65% didn't know the effect of using treated net for purposes other than malaria/LF control. Majority (68.42%) reported that net usage was limited to sometimes than utilizing on a regular basis (31.58%) to prevent mosquito bites. The important breeding sites of mosquitoes along with their preferences are summarized (Table 1).

Of the 8,180 mosquitoes caught and assessed for parity, infection and infectivity status 60.07% (4,914/8,180) and 39.93% (3,266/8,180) were caught in LLIN misusing and LLIN non misusing households respectively. Five species were found and their abundance differed significantly (df=4; p<0.05). An insignificantly greater proportion of vectors were caught in LLIN non misusing cohorts than in LLIN misusing cohorts (63.54% versus 36.45%, 58.89% versus 41.11%, 58.86% versus 41.18%) for 2017, 2018 and 2019 respectively (Table 2 and Figure 2). Specific malaria and LF vector densities showed that *An. gambiae* (82.53% versus 74.04%) was the highest followed by *An. funestus* (10.15% versus 12.19%) and *Cx. quinquefasciatus* (6.82% versus 3.83%) while other species were rarely present (Figure 3). The result of physiological status showed no significant difference in the collections from both cohorts (p<0.05). *An. gambiae* collected were fed (90.06% versus 73.28%) while (66.01% versus 58.78%) and (43.40% versus 75.37%) were unfed and gravid correspondingly. In *An. funestus* fed population were (7.86% versus 11.56%) while unfed and gravid were (21.27% versus 16.89%) and (8.08% versus 2.685). In *Cx. quinquefasciatus*, unfed population were the most abundant (21.27% versus 16.89%) while (1.58% versus 4.47%) and (8.08% versus 2.685) were fed and gravid status (Table 3). Table 4 summarizes the infection and infectivity rates of LF species. Infection rates

Table 1: Status and Categorization of LLIN misuse by the households (N=57)

	Variables	Frequency	Percentage (%)
1	Status		
	Protecting other insects	27	47.37
	Covering household items	7	12.28
	Protection of crops (seedlings)	15	8.77
	Not used for mosquito control	18	31.58
2	Reasons for misuse		
	Cover household problems	37	64.91
	Misunderstanding of its importance	8	14.04
	Technical limitations of its use	7	12.28
3	Time of advices on use		
	Susceptible outbreak of malaria	19	33.33
	During reported	7	12.28
	cases of malaria	7	12.28
	Could be used		
	for filariasis (LF)	13	22.81
	During preparedness program for mosquito borne disease		
	Others (public meeting and social affairs)	11	19.30
4	Perception on the effects		
	Spread of malaria	22	38.60
	Spread of LF	3	5.26
	Economy decline	7	12.28
	No adverse effect	23	40.35
	Others	2	3.51
5	Numbers of LLIN ownership		
	1	1	12.28
	2	19	33.33
	3	18	31.58
	>3	11	19.30
6	Frequency of use		
	Regular	18	31.58
	Sometimes	19	68.42
7	Knowledge of mosquito breeding habitat		
	Nearby home gardens	16	28.07
	Household dust bins	13	22.81
	Open household equipments/containers with water in the gardens	25	43.86
	Others	3	5.26

(0.28% versus 0.49%) and infective rates, nil in either cohort did not differ for both cohorts (p<0.05). Considering the infection rates independently, the order of vector importance for the three main vectors was *An. gambiae, An. funestus* and *Cx. quinquefasciatus* without infection on LLIN misusing cohorts. None of the Anopheles assessed for *Plasmodium* sporozoite had an infection in both cohorts (Table 5). It was observed that 43.33% of the households (HHs) used LLINs (Table 6). Analyses showed a relationship between the use and non use of LLIN in the communities (p<0.05). Among the

Table 2: Overall composition and relative abundance of mosquitoes collected from the households during the selected years

Species	2017		2018		2019		Total	
	X	Y	X	Y	X	Y	X	Y
An. gambiae s.l.	474 (31.90)	1,012 (68.10)	1,165 (38.89)	1,831 (61.11)	779 (39.11)	1,213 (60.89)	2,418 (37.35)	4,056 (62.65)
An. funestus s.l.	176 (47.95)	191 (52.04)	179 (39.60)	131 (63.59)	43 (55.13)	35 (44.87)	398 (44.37)	499 (55.63)
Cx. Quinquefasciatus	20 (13.07)	133 (86.93)	75 (36.41)	131 (63.59)	30 (42.25)	71 (70.30)	125 (27.17)	335 (72.83)
Ae. Aegypti	02 (100.00)	0 (0.00)	13 (72.22)	05 (27.78)	03 (16.67)	15 (83.33)	18 (47.37)	20 (52.63)
Mn. Africana	95 (98.96)	01 (1.04)	133 (98.52)	02 (1.48)	79 (98.75)	01 (1.25)	307 (98.71)	04 (1.29)
Total	767 (36.45)	1,337 (63.54)	1,565 (41.11)	2,242 (58.89)	934 (41.18)	1,335 (58.86)	3,266 (39.93)	4,917 (60.07)

X=LLIN Non-misusing households, Y=LLIN misusing households

Table 3: Physiological status of mosquitoes in the study area

Parity		*An. gambiae s.l.*	*An. funestus s.l.*	*Cx. quinquefasciatus*	*Ae. aegypti*	*Mn. africana*
Fed	X	73.28	11.56	4.47	0.65	10.03
	Y	90.06	7.86	1.58	0.15	6.25
Unfed	X	58.78	5.41	16.89	4.73	14.19
	Y	66.01	8.99	21.27	3.73	0.00
Gravidy	X	75.37	12.90	2.68	0.23	8.81
	Y	43.40	12.19	8.08	0.00	0.12

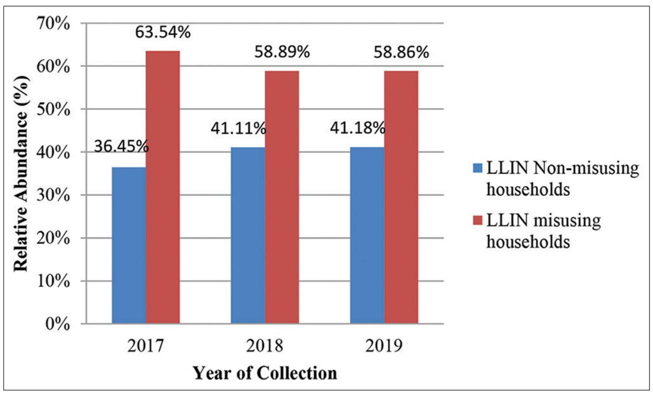

Figure 2: Overall relative abundance of mosquito during the selected years

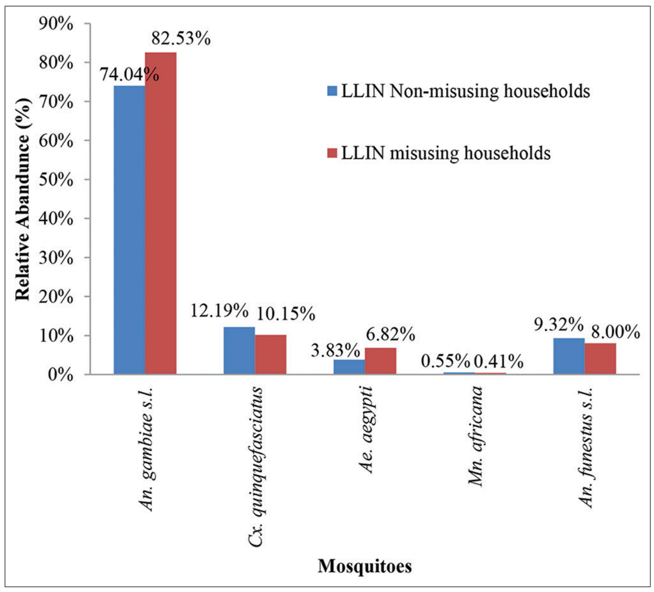

Figure 3: Overall Composition and relative Abundance of mosquito species from the sampling households

43.33% that use LLIN, 16 (41.03%) slept under it daily while 23 (58.97%) slept sometimes. The level of use and proportions that use LLINs were insignificant ($p>0.05$). Reasons for non use of LLIN varied among the inhabitants; 56.86% attributed theirs to heat/hot, 21.57% disliked LLIN, 11.76% viewed LLIN as that which can hardly protect them from mosquito bites and 9.8% reported non effectiveness. There existed a relationship between reported reasons for the use and non-use of LLIN ($p>0.05$).

DISCUSSION

The impact of LLIN on both households (misusing and non-misusing) on malaria and LF transmissions was explored to assist with certification of elimination. LLIN affected vector proportions as evidenced by cohort ratios (39.93% versus 60.02%) and suggested protective effect. Specifically, this reduction seems to affect only malaria transmission but not LF transmission. Interestingly, the study could not provide enough evidence for LLIN misusing cohorts as compared with non misusing cohorts to be superior in reducing infections. This is an encouraging result because in theory, the converse could have been the case. Both cohorts had mixed occupants (adults and children) and probably exhibited similar habits that could expose them to vector bites. The observed insignificant vector contact rates are suggestive of an even distribution of bites and vector competence (imbibing blood meal with resultant parasite development).

Malaria and LF vectors caught and their roles in disease transmission have been documented (Muturi *et al.*,

Table 4: Infection status of mosquitoes for filarial larvae

LLN Coverage	Mosquito species	No. Caught/ Dissected (%)	No. containing larvae (L_1-L_3)	No. infective (L_3 in head)	Infection rate (%)	Infective rate (%)
LLIN Non-misusing households	*An. gambiae sl*	2,418 (74.04)	13	0	0.54	0.00
	An. funestus sl	398 (12.19)	1	0	0.25	0.00
	Cx. Quinquefasciatus	125 (3.83)	2	0	1.60	0.00
	Ae. Aegypti	307 (9.40)	0	0	0.00	0.00
	Mn. Africana	18 (0.55)	0	0	0.00	0.00
	Total	3,266 (39.92)	16	0	0.49	0.00
LLIN misusing households	*An. gambiae sl*	4,056 (82.54)	11	0	0.27	0.00
	An. funestus sl	499 (10.15)	3	0	0.60	0.00
	Cx. Quinquefasciatus	335 (6.82)	0	0	0.00	0.00
	Ae. Aegypti	20 (0.41)	0	0	0.00	0.00
	Mn. Africana	04 (0.08)	0	0	0.00	0.00
	Total	4,914 (60.07)	14	0	0.28	0.00

Table 5: Infection status of *Anopheles* with *Plasmodium* sporozoite

LLIN Coverage	Total No Caught	No Dissected (%)	No infected (%)
LLIN non-misusing households	2,816	73 (2.59)	0 (0.00)
LLIN misusing households	4,555	93 (2.04)	0 (0.00)

2006; Okorie *et al.,* 2011). The ratios clearly showed that *An. gambiae* was the predominant and exhibited strong endophagic-endophilic behavior in both cohorts. Adaptive and host seeking habits, resistance to insecticides (Basilua Kanza *et al.,* 2013) could be attributed to these habits which reflected to survival probability of the parasites. *An. funestus* and *Cx. quinquefasciatus* had altered biting habits (Kurihara *et al.,* 1985) or deliberate exophily. The implications in the on-going elimination programme is that the mere distribution of nets on either cohort in an endemic area could significantly increase (or impact upon) their risk of developing infection due to vector habit. Other species (*Aedes* and *Mansonia*) were rather insignificant probably due to anatomic variations or genetic influence.

Considering the vector's effectiveness in both disease transmissions, the results (proportions, gravid and blood fed) are not encouraging. LLIN ought to have repellent and insecticidal effects on the vectors (reduce human factor contact by physically excluding vectors). The indoor resting densities especially *An. gambiae* (26.9% versus 45.1%) and *An. funestus* (4.4% versus 5.5%) in non-misusing and misusing households is a vivid reflection of the situation. Due to logistic difficulties, we could not conduct blood meal analysis and identification of sibling species of *An. gambiae* complex. We assumed all blood fed and gravid *Anopheles* species caught had taken their blood meals from humans. Previous reports in Nigeria have posited a high human blood index among *An. gambiae* (Okwa *et al.,* 2006). The study could not establish any case of *Plasmoduim* sporoziotes in *Anopheles* dissected. However, there was continued facilitation in the couple (*W. bancrofti/ Anopheles*) in both cohorts. This is hard to explain.

The zero sporozoite rate probably points to suppression rather than interruption attributable to cross sectional nature of the study and a low number of samples. This therefore must not be misunderstood for lack of malaria transmission in these areas. Despite this, other malaria transmission parameters or indices (blood meal origin and indoor resting densities, etc.,) are evidence. It is well known that the latent period of *W. bancrofti* in the vector is usually long in relation to the vector life expectancy (Dye, 1992). In contrast, the extrinsic cycles of malaria parasites lasts 9-10 days but can sometimes last for only 5 days (Bradley *et al.,* 1987). Consequently, the dynamics of filarial-infected mosquitoes and malarial-infected mosquitoes likely to die before the parasite matures need further study. Previous reports had posited that in areas where the two diseases co-exist, the life span of *Anopheles* mosquitoes that pick up both parasites concurrently seems to be greatly reduced to allow for simultaneous transmission of the parasites (Muturi *et al.,* 2007). In this study, co-infection was not found in mosquito species and may explain the density of *An. gambiae* and filarial infection status. Thus control of LF alone may lead to an increase in mosquito survival probability resulting in intense malaria transmission. This finding supports the need for integrated control of the two diseases.

Maxwell *et al.* (1990) reported that mosquito samples with PKD are likely to yield fewer mosquitoes with infective larvae (L3) of *W. bancrofti* as more lifetime larvae are lost during the feed. This may explain the infectivity status for both cohorts.

Epidemiological surveys on wide coverage of LLIN in Nigeria notwithstanding, poor utilization has undermined efforts in control options. Results from 57 misusing cohorts support poor utilization (Change, 2012) with education status of the family head, wealth, colour and shape of the net, and sleeping arrangement reported as potential factors (Alaii *et al.,* 2003). Studies have found that ownership does not always translate to use (Alaii *et al.,* 2003). Bed nets were found to be misused for purposes such as cover for seedlings and kernels,

Table 6: Challenges of using LLINs in the study areas

Level of LLIN Usage				
Study communities	**No sampled**	**Use (%)**	**Non-use (%)**	
Opuoma	30	13 (43.33)	17 (56.67)	
Etioha	30	9 (30.00)	21 (70.00)	
Umukene	30	17 (56.67)	13 (43.33)	
Total	90	39 (43.33)	51 (56.67)	
Numbers of individuals using LLIN in the households				
Study communities	**Usage**	**Always**	**Sometimes**	
Opuoma	13	5 (38.46)	8 (61.54)	
Etioha	9	4 (44.44)	5 (55.56)	
Umukene	17	7 (41.18)	10 (58.82)	
Total	39	16 (41.03)	23 (58.97)	
Reported reasons for use and non-use of LLIN				
Study communities	**Heat/hot**	**Phobia**	**Ineffective**	**Can't prevent malaria and LF**
Opuoma	7 (41.18)	5 (29.41)	3 (17.65)	2 (11.76)
Etioha	13 (61.90)	3 (14.29)	1 (4.76)	4 (19.05)
Umukene	9 (69.23)	3 (23.08)	1 (7.69)	0 (0.00)
Total	29 (56.86)	11 (21.57)	5 (9.80)	6 (11.76)

room partitions, side walls for outdoor toilets, tobacco and grain sieves etc. These reasons are the prime factors for misuse as have been reported elsewhere (Rissa, 2000; Snow *et al.,* 1999). Other reasons could be attributed to misconceptions and cultural taboos (Alaii *et al.,* 2003). This calls for the evaluation of educational campaigns on net utilization and ownership.

Misuse of net notwithstanding, ownership was 2 (33.33%) and 3 (31.58%) per household respectively. This could reveal good health policy and continuous effect to reduce the adverse effect of malaria towards the attainment of millennium development goals in the country. The number of beds net in a family depends on the number of individuals in the family. Household member's knowledge of the use of net presupposes that its benefits in controlling infection were significant. This may be a good indication of misusing the net even if they had the knowledge of its use as reported in the Imo River Basin of Imo State Nigeria (Chukwuocha *et al.,* 2010) and in Uganda (Okello, 2001). Similar to the study in Butajira District Southern Ethiopia (WHO, 2002b), this study found that household equipment with water in the garden (43.9%) followed by nearby home garden (28.1%) and household dust bins (22.8%) were the most important breeding habitats reported for mosquitoes.

Conclusively, despite no positive malaria vector, the isolation of filarial larvae in the dissected vectors suggested that they are actively engaged in the transmission of these parasites. Thus, an indication of the availability of human sources of blood meals. The apparent human malarial and filarial risk indicators revealed by the results must not be ignored. The misuse of nets due to various reasons poses serious challenges which must be addressed for LLIN to impact malaria and LF control. The comparable vector contact and parous rates are indicators. Educational campaigns about the dangers of misusing the bed nets and the potential benefits of proper use will have an influential impact on the disease control.

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