Journal of Infection and Public Health (2016) 9, 298—307

http://www.elsevier.com/locate/jiph

A cross-sectional study assessing the residual bio-efficacy and durability of field-distributed long-lasting insecticidal nets in malaria endemic ethnic communities of Assam, Northeast India

Vas Dev[a,*], **Keshab Barman**[b], **Kamal Khound**[b]

[a] *National Institute of Malaria Research (Field Station), Guwahati 781 022, Assam, India*
[b] *National Vector Borne Disease Control Programme, Government of Assam, Guwahati 781 005, Assam, India*

Received 4 June 2015; received in revised form 23 July 2015; accepted 7 October 2015

KEYWORDS
Malaria;
Long-lasting insecticidal nets;
Residual bio-efficacy;
Vector control;
Net durability;
Northeast India

Summary Long-lasting insecticidal nets (LLINs) are being promoted for malaria vector control in the northeastern Indian state of Assam. A cross-sectional study was conducted to assess the current residual bio-efficacy and durability of both the Olyset® and PermaNet®2.0 LLINs that were distributed earlier in 2009, 2011 and 2013 to help formulate informed policy regarding net procurement, supplies and replacement. The study was undertaken in three different malaria endemic blocks of Assam during the period of June to October of 2014. The residual bio-efficacies were ascertained using the WHO cone-bioassay method for mosquito mortality post-exposure and corroborated with the ring-net assay for the median knockdown times of both types of LLINs in use by these communities. Cross-sectional community surveys were distributed to assess net ownership, utilization, community practices and the physical conditions of the nets in terms of being torn and the numbers of holes per position. Both the Olyset® and PermaNet®2.0 LLINs that were distributed in 2009 (i.e., nearly after five years of community usage) were completely torn, worn out and obsolete. However, the LLINs distributed in 2011 (i.e., three years of community usage) retained their residual bio-efficacies in susceptibility ranges that varied from 57% to 79%. However, for the LLINs that were distributed in 2013, the observed residual efficacy was adequate and resulted in a mosquito mortality rate >80 percent. Of the two types of LLINs inspected, the Olyset®nets were more durable and

* Corresponding author. Tel.: +91 9435345154; fax: +91 3612335256.
E-mail address: mrcassam@hotmail.com (V. Dev).

http://dx.doi.org/10.1016/j.jiph.2015.10.005
1876-0341/© 2015 King Saud Bin Abdulaziz University for Health Sciences. Published by Elsevier Limited. All rights reserved.

robust in terms of being torn less frequently (37.1%, 39/105) compared with the PermaNet®2.0 nets (51.8%, 204/394). Regarding the LLINs that were distributed in 2013, all were physically intact and in good condition. The majority of the distributed LLINs (99.2%, 639/644) were still in the possession of the householders of the surveyed populations. This study revealed that the serviceable life of the nets was slightly less than three years in terms of waning residual bio-efficacy and durability that warranted replacement. The communities were aware of the benefits of the use of mosquito net for personal protection and regularly used the nets; thus, LLIN-based interventions for sustained vector control should be scaled up.
© 2015 King Saud Bin Abdulaziz University for Health Sciences. Published by Elsevier Limited. All rights reserved.

Introduction

Malaria is major public health concern in the north-eastern Indian state of Assam (24°44′—27°45′ N lat.; 89°41′—96°2′ E long.), in which the majority of districts report cases, but the disease distributions and relative risks vary [1,2]. *Plasmodium falciparum* and *P. vivax* are co-endemic, but *P. falciparum* predominantly responsible for infections (>70%) and solely responsible for the substantial increase in cases and attributable deaths. The transmission of the causative parasites is persistent with a seasonal peak from April to September that coincides with the months of heavy rainfall. *Anopheles minimus* and *An. baimaii* have been incriminated by several independent investigators and unequivocally proven to be the major vectors in the state, but a host of other mosquito species, including *An. nivipes* and *An. culicifacies*, are also believed to contribute to the overall disease burden of malaria [3]. Both the *An. minimus* and *An. baimaii* mosquito species are highly susceptible to DDT, which remains the choice insecticide in the control program specific to northeast India. However, despite decades of attempted control, malaria transmission remains uninterrupted largely due to the poor acceptance of indoor spray coverage [4]. To overcome this operational constraint, the advent of long-lasting insecticidal nets (LLINs) has proven to be an evidenced-based intervention for disease vector control and the incorporation of program implementation planning by healthcare services [5—7]. Due to the reductions in transmission that have been demonstrated in village-scale field-based studies, LLINs are now being promoted as the mainstay of vector control for prioritized high-risk population groups [8]. Among the variety of LLINs that are recommended by the WHO Pesticide Evaluation Scheme (WHOPES) for procurement and supply [9], Olyset ®nets (polyethylene netting with incorporated permethrin) and PermaNet®2.0 nets (deltamethrin-coated polyester netting) have been approved by the Central Insecticide Board of the Government of India for use in public healthcare services (http://www.cibrc.nic.in/) in the country. Due to these specific approvals, in the period from 2009 to 2013, more than 1.5 million Olyset® (Sumitomo Chemical Company, Osaka, Japan) and PermaNet®2.0 (Vestergaard Frandsen, Lausanne, Switzerland) nets were distributed gratis in the state of Assam through primary healthcare services including nets distributed by non-governmental organizations in high-risk districts/population groups for the promotion of personal protection against mosquito bites. Given the observed declines in transmission trends and community acceptance across the state [4], the distribution of additional supplies in a phased manner is envisaged, and for this goal, the formulation of an informed policy for net procurement, supply and replacement, including campaigns for behavior-change communication to increase net usage and compliance, was mandated. Included in this report are data on the current residual bio-efficacies of the LLINs distributed in the field in 2009, 2011 and 2013 in villages/population groups of select blocks of the state in terms of malaria transmission/suspected mosquito vector species and observations regarding net durability, community practices, retention and attrition rates and any potential public perceptions of occurrences of adverse events.

Materials and methods

Topography, climate and study populations

The state of Assam is rich in biodiversity due to the extended monsoons, and the malaria receptivity is estimated to be low-to-moderate [1]. Both the Olyset®net (dimensions,

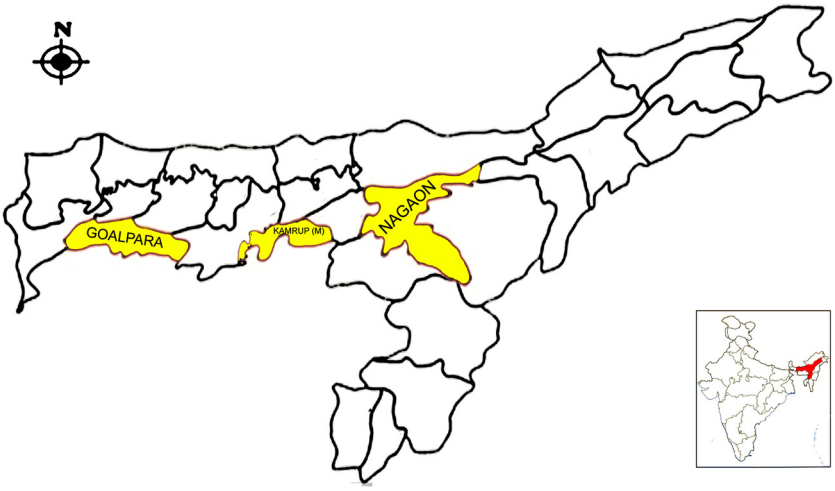

Fig. 1 Map of the state of Assam indicating the study districts in yellow. The inset map of India indicates the geographical location of Assam in red.

130 cm × 180 cm × 150 cm, blue color, 150-denier polyethylene yarn with incorporated 2%, w/w 1 g/m^2 permethrin) and the PermaNet®2.0 net (dimensions, 190 cm × 180 cm × 150 cm, white color, 100-denier polyester yarn coated with 55 mg/m^2 deltamethrin) were distributed in districts reporting strong increases in *P. falciparum* cases and attributable deaths in batches in 2009, 2011 and 2013. In the beneficiary districts/blocks in the state that received LLINs (Olyset®nets, PermaNet®2.0 nets, or both), this study was undertaken from June to October 2014 in three different malaria-endemic blocks in the Goalpara, Kamrup Metro and Nagaon districts (Fig. 1). These districts are considered high-risk for malaria and have large concentration of cases in the forest fringe/foothill villages inhabited by indigenous ethnic tribes living in low socio-economic conditions. Based on the available LLIN distribution records, the investigations were conducted in a few representative beneficiary index villages of each district/block, and a total of 391 households from all three districts were selected randomly. The data were collected as per a protocol for the uniform evaluation of insecticides used in vector control that is approved by the institutional scientific advisory committee [10]. In these villages, the population settlements are scattered (<100 per sq km), and the average household comprises 4—5 members. Typically, houses comprise of two to three mud-plastered rooms made of split bamboo with thatched roofing that frequently have an adjacent cattle shed. Paddy cultivation is the major occupation for subsistence, but other occupations include handlooms, forest produce and meager daily wages. The climate is typically tropical to subtropical with hot and humid summers and receives heavy rainfall (2—3 m) beginning with pre-monsoon activity during March/April, and the maximum precipitation occurs during May—September/October. During this period (i.e., the wet season), temperatures range from 23 °C to 34 °C, and many parts of the state are annually affected by flash floods. Monsoons begin start retreating in October with the concomitant fall in temperatures, and minimum temperatures of 9—10 °C are recorded during December/January (i.e., winter season). The relative humidity (60—80%) throughout the year is conductive to disease vector proliferation and longevity, which permits the active transmission of the causative parasites. Malaria control operations largely consist of the radical treatment of cases and two rounds of indoor residual DDT sprays that are scheduled during the peak transmission times in villages that report high case incidences and deaths. Other intervention measures include the impregnation of community-owned mosquito nets with pyrethroid supplemented by the provision of one or two LLINs per household (averaging one net per two persons) that is restricted to select high-risk districts/population groups.

Experimental design and methods

Mosquito abundance and insecticide susceptibility status of the mosquito disease vectors

To ascertain the prevalences of anopheline mosquito species and their relative abundances, day-resting catches were performed inside human dwellings during the morning hours (0600—0800 h) and in cattle sheds in the evening (1800—2000 h) by

experienced insect collectors aided with mosquito suction tubes and battery torch lights. Among the collected mosquito species, the susceptibility status of *An. nivipes* (the most abundant suspected mosquito vector species) was ascertained using the WHO standard test kit procedures against DDT (4%). Field-collected mixed-aged *An. nivipes* adults were exposed in batches of 10—15 mosquitoes per replicate for 60 min to the given diagnostic concentration, and the mortalities 24 h after the recovery period were recorded. The data were pooled for different replicates while maintaining the appropriate controls.

Monitoring the residual efficacies of long-lasting insecticidal nets

The residual bio-efficacies of the field-distributed LLIN were monitored for insecticidal activities against the candidate test mosquito species *An. nivipes* as detailed in the procedures provided below.

Cone-bioassay. Cone-bioassay tests were performed following the standard WHO method to determine the persistences and bio-availabilities of the insecticides on the netting fibers [10]. For this purpose, three LLINs were drawn randomly in each block with untreated nets as the control. Field-collected adult female *An. nivipes* mosquitoes were exposed to these bio-assays in minimums of three replicates. For each replicate, ten mosquitoes were introduced into the plastic bioassay cone for an exposure to the netting fiber of 3 min, and the numbers of mosquitoes knocked down after the exposure period, and the 24-h recovery period mortality (at the prevailing ambient temperature and humidity) were recorded.

Ring-net bioassay. Ring-net bioassay tests were conducted concurrently with the field-distributed LLINs to substantiate the residual bio-efficacy research findings. Field-collected adult female *An. nivipes* mosquitoes were exposed to these bioassay tests. In each replicate, 11 mosquitoes were introduced into the ring-net, and the times required for the knockdowns of 1st, 6th and 11th mosquito were recorded over a maximum observation period of 1 h. The time required for the knockdown of the 6th mosquito was taken as the median knockdown time.

Community perceptions, practices and net durability

Public perceptions were quantified by interviewing the inhabitants using pre-tested structured questionnaires that were detailed in the institutionally approved study protocol [10]. After receiving informed verbal consent, an adult householder/net user/head of family was interviewed for perceived adverse events, net usage patterns/frequency of use, and method and number of washes, and the nets were inspected for physical condition in terms of being torn and the number of holes and repairs, etc. The number of holes in the torn nets were categorized by size as small (0.5—<2 cm), medium (2—10 cm) and large (>10 cm) and by position as being in the lower half, upper half or the roof of inspected net.

Results

Relative abundances of mosquito vector species and insecticide susceptibility status

During the study period (June—October, 2014), the mosquitoes were collected in different habitats that included inside human dwellings (day-resting) and in cattle sheds (evening biting). In the day-resting mosquito catches from inside human dwellings, only three mosquito species were recorded as prevalent, i.e., *An. annularis, An. culicifacies* and *An. vagus*, and their relative densities per person hour were 1.3, 2.6 and 9.3, respectively (Table 1). Among these species, *An. vagus* was the most abundant, and *An. minimus* (the proven vector species) was virtually absent in all study blocks. Among the cattle biting catches, 11 different mosquito species were recorded of which *An. nivipes* was the most predominant and constituted the major proportions of the collected mosquitoes across the study sites. *Anopheles nivipes* mosquitoes were subjected to insecticide susceptibility test procedures, and 100% (103/103) mortality was recorded in each replicate following 60-min exposures to DDT in the 24-h post-recovery period. Accordingly, due to the scarcity of *An. minimus, An. nivipes* was chosen as the candidate mosquito species for ascertaining the residual efficacies of the field-distributed LLINs at each study site because these species exhibit similar susceptibilities to DDT.

Residual bio-efficacies the field-distributed long-lasting insecticidal nets

Both the Olyset® (permethrin-incorporated polyethylene netting) and PermaNet®2.0 (deltamethrin-coated polyester netting) long-lasting insecticidal nets that were initially distributed in 2009, 2011 and 2013 were subjected to onetime assessments of their residual bio-efficacies in terms of insecticidal activity against *An. nivipes* (the implicated disease vector

Table 1 Relative abundances of anopheline mosquito species in the study blocks/districts of Assam, Northeast India[a].

Anopheles (*An.*) mosquito species	Kathiatoli/Nagaon		Rangjuli/Goalpara		Sonapur/Kamrup Metro	
	No. of mosquitoes collected inside human dwellings (person hour density)[b]	No. of mosquitoes collected in cattle sheds (person hour density)	No. of mosquitoes collected inside human dwellings (person hour density)	No. of mosquitoes collected in cattle sheds (person hour density)	No. of mosquitoes collected inside human dwellings (person hour density)	No. of mosquitoes collected in cattle sheds (person hour density)
An. annularis	12 (1.3)	12 (1.0)	9 (0.9)	8 (0.8)	3 (0.3)	14 (1.2)
An. barbirostris	0	3 (0.3)	0	8 (0.8)	0	5 (0.4)
An. culicifacies	23 (2.6)	15 (1.3)	5 (0.5)	4 (0.4)	2 (0.2)	4 (0.3)
An. jamesii	0	5 (0.4)	0	7 (0.7)	0	12 (1.0)
An. jeyporiensis	0	0	0	2 (0.2)	0	3 (0.3)
An. kochi	0	11 (0.9)	0	10 (1.0)	0	14 (1.2)
An. maculatus	0	2 (0.2)	0	2 (0.2)	0	3 (0.3)
An. nigerrimus	0	26 (2.2)	0	22 (2.2)	0	28 (2.3)
An. nivipes	0	141 (11.8)	0	180 (18.0)	0	154 (12.8)
An. vagus	84 (9.3)	29 (2.4)	60 (6.0)	25 (2.5)	31 (3.4)	23 (1.9)
An. varuna	0	2 (0.2)	0	2 (0.2)	0	3 (0.3)

[a] Study period: June—October, 2014.
[b] Number of mosquitoes collected per person hour.

of malaria) from June to October of 2014, i.e., following approximately one to five years of community usage.

The minimal residual bio-efficacy was observed for the LLINs distributed in 2009 (after nearly five years of community usage) as evidenced by mosquito mortalities of 22% and 27% for the Olyset® and PermaNet®2.0 nets, respectively (Table 2). Regarding the LLINs distributed in 2011 (after nearly three years of community usage), the mosquito mortalities after 24 h of exposure were similar for both the Olyset® and PermaNet®2.0 LLIN at all study locations and varied from 57 to 61% at Kathiatoli/Nagaon to 79% in Rangjuli/Goalpara ($P > 0.05$), but the mortalities varied significantly between locations ($P < 0.05$). In Sonapur/Kamrup Metro, the mosquito mortality after 24 h of exposure to the PermaNet®2.0 LLIN was 60%, which was similar to the mortality observed in Kathiatoli/Nagaon but significantly different from that observed in Rangjuli/Goalpara ($P < 0.05$). However, for LLINs distributed in 2013, the observed mosquito vector species mortality was 99% and optimum. Similarly, 3 min post-exposure, the mosquito knockdown percentage was lowest and varied from 0 to 3% for the LLINs distributed in 2009, and for the LLINs distributed in 2011, the knockdown percentages varied from 12 to 14% in Kathiatoli/Nagaon and Sonapur/Kamrup Metro, and 21—23% in Rangjuli/Goalpara, respectively.

The decrease in the residual efficacy was further substantiated by significant increases ($P < 0.05$) in the knockdown times of the 1st, 6th and 11th mosquitoes in the ring-net bioassays of the LLINs distributed in 2009 and 2011 compared with the values that were observed for the LLINs recently distributed in 2013, which suggests the depletion of the available residual insecticides on netting fibers (Table 3). The median knockdown times for the 6th mosquito for the LLINs distributed in 2009 were $27.2 \pm 7.7$for the Olyset®nets and 35.7 ± 4.0 for the PermaNet®2.0 nets. Regarding the LLINs distributed in 2011, the median knockdown times of both types of LLINs were similar and ranged from 15.7 ± 0.7 to 19.7 ± 1.6 between locations.

Durability and retention of long-lasting insecticidal nets

Among all of the Olyset® and PermaNet®2.0 LLINs distributed in 2009, all of the inspected nets (49/49) were torn in large parts and assessed as unusable. Among the Olyset® and PermaNet®2.0 LLINs distributed in 2011, 48.7% (243/499) were torn with holes that included small — (0.5—<2 cm), medium — (2—10 cm) and large-diameter holes

Table 2 Residual bio-efficacies of the field-distributed long-lasting insecticidal nets used by householders expressed in terms of percent knockdown of *Anopheles nivipes* mosquitoes after 3 min of exposure and the mortalities after 24 h of recovery according to the results of the cone-bioassay test method in Assam, Northeast India[a].

Type of LLIN	Manufacturing year (month of distribution)	Study location (block/district)					
		(Kathiatoli/Nagaon)		(Rangjuli/Goalpara)		(Sonapur/Kamrup Metro)	
		No. and (%) of mosquitoes knockdown after 3 min of exposure/total exposed[b]	No. and (%) mosquitoes dead 24 h post exposure/total exposed[c]	No. and (%) of mosquitoes knockdown after 3 min of exposure/total exposed[b]	No. and (%) mosquitoes dead 24 h post exposure/total exposed[c]	No. and (%) of mosquitoes knockdown after 3 min of exposure/total exposed[b]	No. and (%) of mosquitoes dead at 24 h post exposure/total exposed[c]
Olyset®	2009 (Oct., 2009)	2/60 (3)	13/60 (22)	N/A	N/A	N/A	N/A
PermaNet®2.0	2009 (Oct., 2009)	0/30 (0)	8/30 (27)	N/A	N/A	N/A	N/A
Olyset®	March 2011 (Sept., 2011)	17/120 (14)	73/120 (61)	21/90 (23)	71/90 (79)	N/A	N/A
PermaNet®2.0	March 2011 (July 2011)	7/60 (12)	34/60 (57)	19/90 (21)	71/90 (79)	12/90 (13)	54/90 (60)
PermaNet®2.0	Dec, 2012 (March 2013)	N/A	N/A	N/A	N/A	168/90 (18)	89/90 (99)

[a] Study period: July—October, 2014.
[b] The data are based on the exposures of 10 *Anopheles nivipes* mosquitoes per cone-bioassay. N/A denotes that the given type of LLIN was not provided.
[c] Mosquito mortality <80% denotes depletion of residual insecticide on net fiber than requisite optimum.

Table 3 Ring-net bioassay results for the field-distributed long-lasting insecticidal nets in use by the householders in Assam, Northeast India[a].

Type of LLIN	Manufacture date (month of distribution)	Study location (block/district)								
		(Kathiatoli/Nagaon) Mosquito knock-down time in minutes (mean ± SD)[b]			(Rangjuli/Goalpara) Mosquito knock-down time in minutes (mean ± SD)[b]			(Sonapur/Kamrup Metro) Mosquito knock-down time in minutes (mean ± SD)[b]		
		1st	6th[c]	11th	1st	6th[c]	11th	1st	6th[c]	11th
Olyset®	2009 (Oct.)	11.8 ± 2.1	27.2 ± 7.7	>60	N/A	N/A	N/A	N/A	N/A	N/A
PermaNet®2.0	2009 (Oct.)	9.4 ± 0.5	35.7 ± 4.0	>60	N/A	N/A	N/A	N/A	N/A	N/A
Olyset®	2011 (Sept.)	7.0 ± 0.2	15.7 ± 0.7	25.6 ± 0.8	7.5 ± 0.6	15.7 ± 0.7	26.3 ± 1.1	N/A	N/A	N/A
PermaNet®2.0	2011 (July)	7.3 ± 0.4	16.8 ± 1.2	26.9 ± 0.8	8.6 ± 0.6	17.3 ± 0.8	29.4 ± 1.2	9.5 ± 0.9	19.7 ± 1.6	34.7 ± 1.1
PermaNet®2.0	Dec, 2012 (March 2013)	N/A	N/A	N/A	N/A	N/A	N/A	5.9 ± 0.7	10.3 ± 1.7	17.6 ± 1.5

[a] Study period: June—October 2014.
[b] The data are based on the exposures of 11 *Anopheles nivipes* mosquitoes per ring-net bioassay.
[c] A median knockdown time for the 6th mosquito of >10 min denotes a significant depletion of the residual insecticide on net fiber; N/A denotes that the given type of LLIN was not provided.

(>10 cm in diameter; Table 4). However, the small holes were more frequent with a cumulative average of 3.4 (832/243) holes per LLIN. The majority of the holes in the torn LLINs, however, were concentrated in the lower half of the net, which had an overall average of 3.3 holes per (806/243) per LLIN. Some of the community users (80/243, 33%) had repaired the torn net by stitching, which appeared to be the common practice rather than tying knots and applying patches. Among all of the nets that were inspected for physical appearance, 55% (274/499) were clean, 29% (146/499) were slightly dirty, and the remaining nets were dirty to very dirty. Of the LLINs that were distributed in 2013, all (134/134) of the inspected nets were physically intact and in good condition.

The Olyset®nets were more durable and robust in terms of less frequent tears (37.1%, 39/105) than the PermaNet®2.0 nets (51.8%, 204/394), and the Olyset®nets had fewer holes 2.4 (92/39) per net than the PermaNet®2.0 nets (740/204). Additionally, the Olyset® nets were more dirt-repellent; the proportion of clean Olyset® (79%, 83/105) nets was greater than that of the PermaNet®2.0 nets (48%, 191/394). The majority of the total of 391households that were inspected for net retention (99.2%, 639/644) still possessed their nets irrespective of the present physical condition of the nets.

Community perceptions, acceptance and practices

Based on the retrospective cross-sectional questionnaire-based verbal surveys of the different ethnic communities, the householders were fully aware of the benefits of the use of mosquito net for personal protection and regularly used the nets (100%, 391/391). However, a few respondents reported skin-related adverse events that included itching (13.5%, 53/391) and facial burning (6.6%, 26/391) that lasted for a few hours on the first few days of initial usage. Other reported perceptions included a foul smell (4.3%, 17/391), body rashes (0.5%, 2/391) and headaches (0.2%, 1/391), but all of these adverse events were reported to be transitory. The majority of the beneficiaries (92.7%, 407/439) used the LLIN year-round and hung the mosquito nets over their sleeping cots. All respondents (100%, 439/439) stated that they had benefited from the use of LLINs in terms of decreased nuisance mosquito bites. A good proportion of these community users washed the net at quarterly intervals (73.3%, 322/439) with commonly available detergent powder by soaking

Table 4 Comparative assessment of the physical integrities of the long-lasting insecticidal nets (LLINs) that were initially distributed in 2011 in the ethnic communities of Assam, Northeast India[a].

S. no.	Question	Physical condition of LLIN		Any $N = 499$
		Olyset® $N = 105$	PermaNet®2.0 $N = 394$	
1.	No. of torn nets with holes (% of nets inspected)	39 (37.1)	204 (51.8)	243 (48.7)
2.	Total numbers of holes (number of holes/no. of torn net)			
	Small (0.5—<2 cm diameter)	92 (2.4)	740 (3.6)	832 (3.4)
	Medium (2—10 cm diameter)	22 (0.6)	250 (1.2)	272 (1.1)
	Large (>10 cm diameter)	10 (0.3)	109 (0.5)	119 (0.5)
3.	Total numbers of holes by position (number of holes/no. of torn net)			
	Lower half	97 (2.5)	709 (3.5)	806 (3.3)
	Upper half	21 (0.5)	287 (1.4)	308 (1.3)
	Roof	5 (0.1)	103 (0.5)	108 (0.4)
4.	Physical aspects of the nets (percentage of nets inspected)			
	Clean	83 (79)	191 (48)	274 (55)
	Slightly dirty	13 (12)	133 (34)	146 (29)
	Dirty	6 (6)	44 (11)	50 (10)
	Very dirty	3 (3)	26 (7)	29 (6)

[a] Study period: June—October 2014.

and dipping following by drying in open broad daylight (88.4%, 388/439).

Discussion

Vector control is an integral component of the containment of malaria, and other than indoor residual spraying, long-lasting insecticidal nets are the only appropriate technologies that are being provided to high-risk states/districts with marginalized population groups [11,12]. Although at present, the distribution of LLINs is patchy, these nets are increasingly popular, and community compliance and acceptance are high. In accordance with the regional malaria control strategy in the Southeast region, the control program aims to scale up the distribution to achieve maximal utilization by populations living all areas of malaria risk [13]. However, understanding public perceptions, net survival, attrition, and retention and monitoring of the residual bio-efficacies and previously field-distributed LLINs in local transmission areas are crucial to support the national control program for the management of procurement and net replacement [14,15].

As per the WHOPES criteria, any given LLIN should maintain its effectiveness (i.e., a mosquito mortality $\geq$80% by bio-assay) against mosquito vector species following three years of continuous use in susceptible communities [16]. Based on the present cross-sectional study performed in the beneficiary population groups, *An. minimus*, which is the most efficient mosquito vector, was not prevalent in any of the study locations (Table 1). Although the residual bio-efficacies of the LLINs distributed in 2009 had waned and those distributed in 2011 retained mosquito mortalities of <80% (Table 2), the mere retention of an LLIN seemed to deter the entry of *An. minimus* into human dwellings. There is body of evidence that suggests that *An. minimus* populations are diminishing in areas of prior distribution and being replaced by *An. culicifacies*, which is rapidly spreading in northeast India [17]. These data suggest that, given the climatic conditions and community practices of the northeast India, the serviceable life of a net is slightly less than three years before replacement is required. Similar observations regarding the serviceable lives of nets have been reported in many other malaria-endemic countries with different transmission settings, although, these studies have reported serviceable lives slightly closer to 2 years [18—22].

For both types of LLINs, i.e., the Olyset® and PermaNet®2.0 nets, which were in 2011, the residual bio-efficacies varied significantly between locations ($P < 0.05$) but were similar within sites, which suggests that the community practices for net care and maintenance varied (Table 2). The Olyset®LLINs (polyethylene netting fiber) were observed to be more durable and robust than the PermaNet®2.0 LLINs (polyester netting fiber) in terms of the numbers of tears and holes (Table 4). Because the majorities of the holes in both types of LLINs were in the lower halves of the nets, this area should be reinforced for greater durability. Similar observations regarding the lower halves of

nets have been reported in other malaria-ridden countries around the world [23,24]. Additionally, the Olyset® LLINs were more dirt-repellent, and a greater proportion of these nets were clean (79%) compared with the PermaNet®2.0 nets (48%).

Although community-based verbal surveys have limitations, the householders were fully aware of the benefits of using mosquito net for personal protection during all seasons. The other limitations of this study include the lack of data regarding the proportionate hole index (pHI), which is used to track net serviceable life, the lack of data about the available insecticide residues on the net fiber, and the lack of data regarding the inhibition of the feeding of the disease vectors, which is used to determine the end of the useful life of a net [25]. Moreover, because the LLINs were only distributed to a subset of the population, assessments of the epidemiological impacts of damaged nets on malaria transmission were not attempted. This was merely a cross-sectional study, and prospective study would have yielded more information about the control program. Most importantly the presented data are related only to the ethnic communities of Assam; thus, inferences derived from these data may not be applicable to other parts of the country with varied disease epidemiologies and contextual determinants [26].

In the northeastern region of India, due to the wide community acceptance and compliance, a net-based intervention appears to be appropriate for increasing net ownership and utilization practices [27,28]. Nevertheless, the demand for universal coverage is enormous, and there is a window of opportunity to mobilize resources and develop innovative strategies for net distribution and replacement. Presently, the LLIN distribution represents only a miniscule fraction of that needed for universal coverage for at-risk communities. Future research priorities should include the development of LLINs that are more robust and more potent in terms of insecticides, and innovative strategies for the mass distribution of LLINs are needed for sustained malaria control efforts [29—31].

Conclusions

Based on the presented data, it is apparent that LLIN-based intervention technology is appropriate for the control of malaria-transmitting mosquitoes that are specific to northeast India and for greater community acceptance, compliance and retention. However, net replacement is necessary every three years due to the waning residual efficacy and physical integrity. Strengthened fibers in the lower halves of the LLINs are needed for prolonged net serviceable lives. There is body of evidence that suggests that *An. culicifacies*is fast spreading throughout northeast India; thus, monitoring of the residual efficacy against this vector species is needed to develop LLINs that are more robust and potent against multiple insecticide-resistant insects. The promotion of this intervention is strongly advocated to ensure greater population coverage for the continued success of malaria control operations.

Funding

National Health Mission, Government of Assam, Guwahati — 781005, India.

Competing interests

None declared.

Ethical approval

Not required.

Acknowledgments

The investigators wish to thank the State Programme Officer and the district health authorities for informal consultations and local logistical support. Thanks are also due to the village communities for continued cooperation and the project staffs for technical expertise. This study protocol was approval of the Institutional Scientific Advisory Committee and bears the project ID No. NIMR/IDVC/2013/25, and the household heads/subjects provided informed verbal consent. This study project was financed by the National Health Mission, State Health Directorate, National Vector Borne Disease Control Programme, Government of Assam (document Number NVBDCP/Bio-efficacy/LLINs/2013/23719 dated 21-12-2013).

References

[1] Dev V, Phookan S, Sharma VP, Anand SP. Physiographic and entomologic risk factors of malaria in Assam, India. Am J Trop Med Hyg 2004;71:451—6.
[2] Dev V, Dash AP, Khound K. High-risk areas of malaria and prioritizing interventions in Assam. Curr Sci 2006;90:32—6.
[3] Dev V, Sharma VP. The dominant mosquito vectors of human malaria in India. In: Manguin S, editor. Anopheles

mosquitoes — new insights into malaria vectors. InTech publications; 2013., ISBN 978-953-51-1188-7 p. 239—71, http://dx.doi.org/10.5772/55215.
[4] Dev V, Sharma VP, Hojai D. Malaria transmission and disease burden in Assam: challenges and opportunities. J Parasit Dis 2009;33:13—22.
[5] Dev V, Raghavendra K, Barman K, Phookan S, Dash AP. Wash-resistance and field efficacy of Olyset® net, a permethrin incorporated long-lasting insecticidal netting against *Anopheles minimus* transmitted malaria in Assam, Northeastern India. Vector-Borne Zoonotic Dis 2010;10:403—10.
[6] Sharma SK, Upadhyay AK, Haque MA, Tyagi PK, Raghavendra K, Dash AP. Wash-resistance and field evaluation of alphacypermethrin treated long-lasting insecticidal net (Interceptor) against malaria vectors, *Anopheles culicfacies* and *Anopheles fluviatilis* in tribal area of Orissa, India. Acta Trop 2010;116:24—30.
[7] Bhatt RM, Sharma SN, Uragayala S, Dash AP, Kamaraju R. Effectiveness and durability of Interceptor long-lasting insecticidal nets in a malaria endemic area of central India. Malar J 2012;11:189.
[8] National Vector Borne Disease Control Programme, Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India. Action plan for scaling up long lasting insecticidal nets for malaria control in India. http://www.nvbdcp.gov.in/Doc/LLIN-Action-Plan-2009.
[9] World Health Organization. WHO recommended long-lasting insecticidal mosquito nets; 2013. http://www.who.int/whopes/recommendations/wgm/en/updated.
[10] National Institute of Malaria Research. Indian Council of Medical Research. Protocols for uniform evaluation of insecticides for use in vector control. http://www.mrcindia.org/com_pro/comm_prot.htm.
[11] National Vector Borne Disease Control Programme, Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India. Malaria control strategies. http://www.nvbdcp.gov.in/malaria.
[12] National Vector Borne Disease Control Programme, Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India. Guidelines for supply, distribution and communication on long lasting insecticidal nets. http://www.nvbdcp.gov,in/Doc/Guideliens-forITNS-LLINS.
[13] Dhiman S, Veer V. Culminating anti-malaria efforts at long lasting insecticidal net. J Infect Public Health 2014;7:457—64.
[14] Hii J, Thakur GD, Marasini BR, Pokhrel YR, Upadhyay MP, Rijal KR, et al. Monitoring the durability of long-lasting insecticidal nets in field conditions in Nepal. WHO South-East Asia J Public Health 2014;3:81—4.
[15] Lorenz LM, Overgaard HJ, Massue DJ, Mageni ZD, Bradley J, Moore JD, et al. Investigation mosquito net durability for malaria control in Tanzania — attrition, bioefficacy, chemistry, degradation and insecticide resistance (ABCDR): study protocol. BMC Public Health 2014;14:1266.
[16] World Health Organization. Communicable Disease Control, Prevention and Eradication, WHO Pesticide Evaluation Scheme (WHOPES). Guidelines for laboratory and field testing of long-lasting insecticidal mosquito nets. WHO/CDS/WHOPES/GCDPP/2005.11.
[17] Sexena R, Nagpal BN, Singh VP, Srivastava A, Dev V, Sharma MC, et al. Impact of deforestation on known malaria vectors in Sonitpur district of Assam, India. J Vector Borne Dis 2014;51:211—5.
[18] Batisso E, Habte T, Tesfaye G, Getachew D, Tekalenge A, Kilian A, et al. A stitch in time: a cross-sectional survey looking at long lasting insecticide-treated bed net ownership, utilization and attrition in SNNPR, Ethiopia. Malar J 2012;11:183.
[19] Wills AB, Smith SC, Anshebo GY, Graves PM, Endeshaw T, Shargie EB, et al. Physical durability of PermaNet®2.0 long-lasting insecticidal nets over three to 32 months of use in Ethiopia. Malar J 2013;12:242.
[20] Hakizimana E, Cyubahiro B, Rukundo A, Kabayiza A, Mutabazi A, Beach R, et al. Monitoring long-lasting insecticidal net (LLIN) durability to validate net serviceable life assumptions, in Rwanda. Malar J 2014;13:344.
[21] Gnanguenon V, Azondekon R, Oke-Agbo F, Beach R, Akogbeto M. Durability assessment results suggest a serviceable life of two, rather than three years, for current long-lasting insecticidal (mosquito) net (LLIN) intervention in Benin. BMC Infect Dis 2014;14:69.
[22] Dutta SN, Amon J, Iata H, Cooper RD, Russell TL. Long-term insecticidal activity and physical integrity of Olyset® nets in Tafea Province, Vanuatu. J Med Entomol 2014;51:164—9.
[23] Smith SC, Joshi UB, Grabowsky M, Selanikio J, Nobiva T, Aapore T. Evaluation of bednets after 38 months of household use in northwest Ghana. Am J Trop Med Hyg 2007;77:243—8.
[24] Allan R, O'Reilly L, Giibos V, Kilian A. An observational study of material durability of three World Health Organization — recommended long-lasting insecticidal nets in eastern Chad. Am J Med Hyg 2012;87:407—11.
[25] World Health Organization. Guidelines for monitoring the durability of long-lasting insecticidal mosquito nets under operational conditions. WHO/HTM/NTD/WHOPES/2011.5. Geneva: World Health Organization.
[26] Das A, Anvikar AR, Cator LJ, Dhiman RC, Eapen A, Mishra N, et al. Malaria in India: the centre for the study of complex malaria in India. Acta Trop 2012;121:267—73.
[27] Dev V. Operational aspects of insecticide treated nets for malaria control in Assam. J Commun Dis 2001;33:147—50.
[28] Prakash A, Bhattacharyya DR, Mohapatra PK, Goswami BK, Mahanta J. Community practices of using bed nets, acceptance and prospects of scaling up insecticide-treated nets in north-east India. Indian J Med Res 2008;128:623—9.
[29] Pennetier C, Bouraima A, Chandre F, Piameu M, Etang J, Rossignol M, et al. Efficacy of Olyset® Plus, a new long-lasting insecticidal net incorporating Permethrin and Piperonil Butoxide against multi-resistant malaria vectors. PLOS ONE 2013;8(10):e75134.
[30] Corbel V, Chabi J, Dabire RD, Etang J, Nwane P, Pigeon O, et al. Field efficacy of a new mosaic long lasting net (PermaNet®3.0) against pyrethroid resistant malaria vectors: a multi centre study in Western and Central Africa. Malar J 2010;9:113.
[31] Sharma VP. Battling malaria iceberg incorporating strategic reforms in achieving millennium development goals and malaria elimination in India. Indian J Med Res 2012;136:907—25.

Available online at www.sciencedirect.com
ScienceDirect