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Field evaluation of Icon®Life, a long-lasting insecticidal net (LLIN) against *Anopheles culicifacies* and transmission of malaria in District Gautam Budh Nagar (Uttar Pradesh), India

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ABSTRACT

Background & objectives: In the present study, Icon®Life net, a long-lasting polyethylene net, 100 denier and bursting strength of minimum 280 kpa incorporated with deltamethrin @ 65 mg/m^2 was evaluated for its efficacy in reducing the density of malaria vector *Anopheles culicifacies* and impact on malaria prevalence in a malaria endemic area of District Gautam Budh Nagar, India.

Methods: Wash resistance of Icon® Life LLIN was determined up to 20 serial washings using *An. culicifacies* in cone bioassays under field conditions. Efficacy of Icon®Life LLIN was determined in the field in three sets of villages in District Gautam Budh Nagar (Uttar Pradesh), India, selected randomly for the intervention with Icon®Life LLIN, untreated nets and a control without any intervention for the period of August 2008–July 2009. Entomological and malariometric indices in all the three villages were compared during pre- and post-intervention periods for one year against *An. culicifacies*. A survey was also conducted in the village provided with Icon® Life LLIN to assessing the perception of community regarding acceptance of these nets by the community.

Results: In cone bioassays on Icon®Life LLIN with *An. culicifacies,* ≥95% knockdown within 1 h and 100% mortality after 24 h exposure were reported even after 20× serial washings under field conditions. Results of the field study revealed reduced entry rate, resting density and parity rate of *An. culicifacies* in the village with Icon®Life LLIN when compared to no net and untreated net villages. Number of malaria cases reported were less in the Icon®Life LLIN used villages when compared to other two villages. The community compliance and acceptance was high and no adverse health events were reported by the households using these nets.

Conclusions: Icon® Life LLIN is an effective intervention for the control of *An. culicifacies* transmitted malaria in India. Long-term studies are indicated for the duration of effectiveness and to ascertain the epidemiological impact of the use of Icon® Life nets.

Key words *Anopheles culicifacies;* Icon® Life; India; long-lasting insecticidal nets; mosquito density; wash resistance

INTRODUCTION

Recently, long-lasting insecticidal nets (LLINs) have been developed for wash resistance and long-lasting effects. These nets are treated at the manufacturing level with insecticide either coated with wash resistant resin on polyester fibers or incorporated into matrix of polyethylene fibers. The biological activity lasts as long as the useful life of the net itself and estimated to be 3–5 yr. A variety of LLINs, viz. Olyset® Net, incorporated with permethrin, PermaNet® 2.0, 2.5, 3.0 and Yorkool® LN coated with deltamethrin, Interceptor® coated with alpha-cypermethrin, Duranet® incorporated with alpha-cypermethrin and NetProtect® and Icon®Life incorporated with deltamethrin have been developed. All these LLINs have been evaluated by WHOPES[1,2]. Of these only Olyset® Net, PermaNet® 2.0 and Yorkool® LN have been given full recommendations. In India, Olyset® Net, PermaNet® 2.0, DuraNet®, Interceptor Net® and have been evaluated for wash resistance and long-lasting effects[3–8]. Icon®Life net manufactured by Bestnet Europe and supplied by M/s. Syngenta India Ltd. have similar specifications as that of NetProtect® LLIN, which got interim recommendation from the WHOPES for use against malaria vectors[9]. This study was undertaken to test the field efficacy of Icon®Life LLIN against *An. culicifacies* and its impact on malaria prevalence in a village scale trial in the endemic areas of District Gautam Budh Nagar, Uttar Pradesh, India.

MATERIAL & METHODS

Icon®Life LLIN mosquito nets supplied by M/s. Syngenta Crop Protection (P) Ltd., India, were used in this study. Icon®Life net (LLIN) is made up of 100% poly-

ethylene and is incorporated with deltamethrin (2 g a.i./kg fiber) @ 65 mg/m^2 having 100 denier, monofilament and bursting strength of minimum 280 kpa with a mesh size of 136, wrap knitted. The size of the net is 160×180×150 cm and colour is white. Untreated polyethylene nets of same specifications of thickness and bursting strength as that of Icon®Life net were used as controls supplied by the M/s. Syngenta Crop Protection (P) Ltd., India. The study was carried out as per common protocol for evaluation of insecticides with some modifications[10]. Wash resistance of Icon®Life LLIN was determined up to 20 serial washings using *An. culicifacies* in cone bioassays[11] under field conditions. Icon®Life LLIN were washed at weekly intervals. Surf Excel soap powder (Manufactured by M/s. Hindustan Lever, Mumbai, India) was used for washing. Five grams (one tea spoon) of the detergent was dissolved in 5 litres of water and Icon®Life net/untreated net was dipped in the soap solution for 10 min. Subsequently, the net was washed with hand and thoroughly rinsed with tap water thrice. The washed nets were dried in shade. Wild caught fully-fed female *An. culicifacies* were used in cone bioassays. The impact of washing on residual bioefficacy of insecticide on Icon®Life LLIN washed up to 20 times was determined by cone bioassays under field conditions. The cone bioassays on unwashed, washed, and untreated nets were performed as per standard WHO procedure. Ten mosquitoes were released in each cone with the help of suction tube and exposed to Icon®Life LLIN for 3 min. After 3 min exposure, the mosquitoes were transferred to holding tubes and observed for knockdown after 1 h and delayed mortality was scored after 24 h recovery period. Mosquitoes were provided with cotton swab soaked in 10% glucose solution during recovery period. Four replicates were used. The untreated nets were taken as control.

Field evaluation

Field evaluation was carried out at Dadri Community Health Centre (CHC) of Gautam Budh Nagar district in western Uttar Pradesh during August 2008–July 2009. *Anopheles culicifacies* is the primary malaria vector species in this area, which breeds in irrigation channels, ponds, pools and rice-fields. Agriculture is the major occupation in these villages and the land is irrigated through the main upper Ganga canal and its tributaries. Man to cattle ratio is 1: 2 and the population mainly relies on agriculture for their livelihood. Though, there are no regular vector control measures undertaken in this area by the state health programme, people on their own use various mosquito control measures including use of mosquito nets[12].

Three villages with almost similar topography and malaria endemicity were selected for this trial. Gulawati-Khurd village having population of 1381 was selected for the distribution of 1233 Icon®Life nets in 226 houses; Nangla-Chamru village having a population of 1840 was selected for distribution of 1600 untreated nets in 244 houses and Nangla-Nainsukh was selected as control where nets were not used. Community group meetings were organized in the study villages and inhabitants were educated on proper and regular use of nets and importance of the study. The written consent of each household was obtained for their willingness to participate in the study before the distribution of nets. The distribution of nets as per sleeping pattern survey was carried out in the month of August 2008 and number of nets distributed to each household was recorded and signatures of the recipients were obtained. Villagers were asked to wash the nets during study period, if required. The proper use and maintenance of mosquito nets in the study village was supervised by the staff of NIMR in consultation with the community leaders. One net was given to each individual of >10 yr of age and one net was shared between two children aged <10 yr. The entomological and parasitological data in the study villages were collected during the pre-intervention period in May–July 2008 and after distribution of Icon®Life LLIN, from August 2008 to July 2009.

Entomological evaluation

Baseline insecticide susceptibility status of wild caught malaria vector *An. culicifacies* against DDT (4%), malathion (5%) and deltamethrin (0.05%) was determined as per standard procedure[13]. The field-collected mosquitoes were exposed for one hour to DDT, malathion and deltamethrin-impregnated papers, using WHO adult susceptibility kit (University Sans Malaysia, Malaysia). The mosquitoes were kept in the recovery tubes for 24 h and mortality was recorded.

Entry and excito-repellency of *An. culicifacies* were determined by collecting indoor resting adult mosquitoes fortnightly in four fixed houses and four houses selected randomly each in trial villages with Icon®Life, untreated net and no net control villages by hand catch in the morning hours. Mosquitoes were collected in the fixed and random houses for 15 min in each dwelling with the help of suction tubes using flashlights. In addition to hand catches, floor sheet collection and exit-trap collection methods were also used to determine the entry rate and excito-repellancy of *An. culicifacies* in four fixed structures in each study village. These collections were made in the early morning during the transmission months from August to October 2008. For floor sheet collection, in the evening, white cloth

sheets were spread over the entire floor of the houses before the occupants retired to bed. Next morning, dead and morbid mosquitoes lying on the floor sheets were picked up, identified to species and their abdominal condition was recorded. For exit-trap collection, one rectangular exit-trap of size 12" × 15" having conical cone of plastic material with an orifice of 1 cm^2 were fixed in the mud walls of four houses for entomological monitoring. Trap cloth cages were fixed in each exit-trap before the sunset and next morning all the mosquitoes from the exit-traps were collected. The live mosquitoes were transferred to a cage and brought to the laboratory to check the mortality after 24 h under optimal conditions. All the mosquitoes collected from exit-traps were identified to species and their abdominal conditions were recorded. The total number of *An. culicifacies* mosquitoes collected by all these collection methods were pooled and denoted as entry rate. The number of mosquitoes collected from exit-traps was recorded and the percentage of these mosquitoes out of total entered was denoted as percent excito-repellency. Insect collectors were deployed in each room. One was assigned to collect the mosquitoes resting on walls and the other to collect the mosquitoes landing on the net. The total number of mosquitoes landing on the nets was considered as landing rate. The mosquitoes were identified as fed or unfed and feeding percentage was calculated. The mean monthly density of indoor-resting mosquitoes was calculated as per man hour density.

The unfed mosquitoes collected through different sampling techniques were dissected for ovaries as per WHO technique based on distended tracheolar skeins and were categorized as parous and nulliparous. Parity rate was calculated as percentage of mosquitoes with parous ovaries from the total mosquitoes dissected.

Epidemiological evaluation

Malaria prevalence in the study villages was measured through cross-sectional survey and fortnightly active fever surveys. Mass survey was undertaken in every fourth house of the study villages during pre- and post-intervention periods. Blood smears were collected from households of every fourth house by finger-prick method. Active surveys for fever cases were undertaken after the visit of each household once a fortnight and collected finger-prick blood from all individuals reporting fever and prepared thick and thin smears from finger-prick blood. All the slides were brought to the Laboratory at NIMR Delhi, for detection and identification of malaria parasites. Blood slides were stained with JSB stain and examined under oil immersion microscopy for malaria parasite. All the slide-positive cases were provided antimalarial treatment as per guidelines of the National Vector Borne Disease Control Programme (NVBDCP), Government of India.

Compliance rate of net usage, side effects and collateral benefits

The compliance rate of the net usage in the trial and control villages was ascertained through random checking of houses and recording of people sleeping pattern under mosquito nets. Cross-sectional surveys were conducted among Icon®Life users using a structured questionnaire for assessing their perception about the net usage, side-effects and collateral benefits.

Ethical clearance

The study was approved by the Human Ethics Committee of the National Institute of Malaria Research vide PH/NIMR/EC/2008/113.

Data analysis

Mean and standard deviations were calculated wherever required. Chi-square test and analysis of variance (ANOVA) were performed using Epi-Info software freely downloaded from Centres of Disease Control and Prevention, Atlanta, USA for testing the significance among Icon®Life, untreated net and control villages. *P*-values <0.05 were considered statistically significant at 5% level of significance.

RESULTS

Insecticide susceptibility tests performed on adult wild caught female *An. culicifacies* showed 20% mortality to DDT (4%) but this spp was completely susceptible to malathion (5%) and deltamethrin (0.05%). Cone bioassays on serially washed Icon®Life LLINs revealed 95% knockdown and 100% mortality even after 20 washings under field conditions (Table 1).

There was a significant difference in the entry rates of *An. culicifacies* mosquitoes in structures provided with Icon®Life when compared to untreated net (p =0.037) and no net structures (0.013). No significant difference in the entry rate of mosquitoes was observed between untreated net structures and no net structures (p =0.24) (Table 2). Analysis of variance showed significant difference in entry rate of mosquitoes in all the three types of structures (p <0.05). Lower entry of mosquitoes was observed in structures provided with Icon®Life LLIN than in structures with untreated nets. The excito-repellent action on female *An. culicifacies* also showed significant difference between LLIN and untreated net. The percentage of feed-

Table 1. Wash resistance and efficacy of Icon® Life LLIN against *An. culicifacies* in the laboratory and after one year of use in study village in cone bioassays*

Type of LLIN	Icon®Life LLIN		Untreated net	
	Knockdown in 1 h	% mortality after 24 h	Knockdown in 1 h	% mortality after 24 h
Unused fresh	100	100	0	10
Washed × 1	100	100	0	5
Washed × 5	100	100	0	10
Washed × 10	100	100	0	5
Washed × 15	95	100	0	5
Washed × 20	95	100	0	0
Field used (after one year)	96	100	0	15

*Four replicates of 10 mosquitoes each were used in cone bioassays on each net.

ing success was much lower in the case of Icon®Life net village as compared to untreated net ($p = 0.031$) and without village ($p = 0.005$). Resting density of malaria vector *An. culicifacies* and other mosquitoes was monitored in houses from Icon®Life, untreated net, and no net study villages. The average per man hour density (MHD) of *An. culicifacies* during pre-intervention period (May–July 2008), in Icon®Life , untreated net and no net villages was 20.8, 26 and 28.5, respectively which showed no statistically significant difference between all the villages (Table 3). The average density after the distribution of nets declined to 3.6, 10.2 and 20.2 respectively. Statistical tests using ANOVA showed highly significant difference (August 2008–July 2009) in the density of *An. culicifacies* in the LLIN used village as compared to the other two villages (Table 3). The resting density of *An. culicifacies* showed an increasing trend in all the villages during the monsoon and post-monsoon period of August to November 2008, but the build-up of *An. culicifacies* density was much higher in the control villages as compared to LLIN used village. The parity rate of *An.culicifacies* during pre-distribution period showed similar parity rate which was not statistically different among them ($p > 0.05$) as compared to post-distribution period which showed highly significant difference between all the villages (Table 4).

The impact of Icon®Life on malaria prevalence was measured through fortnightly active surveillance in comparison to untreated net and without net villages. The para-

Table 2. Total entry, excito-repellency and percentage of feeding success of *An. culicifacies* in houses with Icon®Life net, untreated net and no net (during August–October 2008) intervention period in study villages of District Gautam Budh Nagar, U.P.

Intervention*	Total entry of female mosquitoes (resting+floor sheet+windowtrap)	No of mosquitoes in exit-trap	Excito-repellency (%)	Feeding success (%)
Icon®Life LLIN	8.33 ± 1.15	2.66 ± 0.57	10.56 ± 2.7	15.8 ± 5.72
Untreated net	26.6 ± 13.27	0.33 ± 0.57	1.13 ± 1.96	26.63 ± 0.75
No net	39.33 ± 12.66	0 ± 0	0 ± 0	34.7 ± 1.24

*$p < 0.05$ significant at 5% level of significance.

Table 3. Efficacy of Icon®Life LLIN on indoor resting density of malaria vector *An. culicifacies* and other mosquitoes in the study villages in Dadri PHC, District Gautam Budh Nagar, U.P.

Period	Average per man hour density		
	Gulawati-Khurd Icon Life LLIN	Nangla-Chamru untreated net	Nangla-Nainsukh no net
Pre-intervention (May–July 2008)			
An. culicifacies	20.8 ± 6.6	26 ± 27.3	28.5 ± 18.6
Total anophelines	138.8 ± 125.6	203 ± 249	110 ± 104
All mosquitoes	269.8 ± 84.1	323 ± 212.5	377.3 ± 30.3
Post-intervention (August 2008–July 2009)			
An. culicifacies	3.6 ± 5.14*	10.2 ± 9.12*	20.2 ± 20.4*
Total anophelines	81.7 ± 74.2	95 ± 153.3	128.4 ± 171
All mosquitoes	134.4 ± 96.4	194 ± 149.8	325 ± 147.8

*$p < 0.05$ significant at 5% level of significance.

Table 4. Parity rate of *An. culicifacies* in study villages provided with Icon®Life LLIN, untreated net and no net (during May 2008–July 2009) in Dadri PHC District Gautam Budh Nagar, U.P.

Month/Year	No. disseceted (% parous)		
	Icon®Life nets	Untreated net	No net
Pre-intervention (May–July 2008)	30 (66.6)	35 (60)	49 (63.2)
Post-intervention (August–July 2009)	91 (36.2)	83 (63.8)	121 (62.8)

site index (PI) or number of cases per 1000 population in the LLIN used village was 7.96 as compared to 3.80 in untreated net village and 5.23 in no net village during pre-intervention period of July 2008 which are statistically insignificant (p >0.05) (Table 5). During post-intervention phase there was reduction in number of malaria cases in Icon® Life used village (4.34) as compared to untreated net and no net villages which showed statistically significant difference (p <0.05 (Table 5 & Fig. 1). The month-wise data on SPR revealed almost complete interruption of malaria transmission in the Icon®Life used village.

There was >90% compliance rate of net usage in the study population during the transmission months of August–October 2008. The community perceptions on adverse effects and collateral benefits of Icon®Life net was assessed by conducting cross-sectional survey among the users (n = 100) of Icon®Life (Table 6). Almost every respondent asserted that they were sleeping under the treated net. There were minimal complaints of skin irritation (2%) and eye irritation (5%). However, these effects were only transitory in nature for few hours on the first usage. Majority of the respondents enthusiastically reported that Icon®Life net provided them relief not only from mosquitoes but also from other household pests such as head lice, bed bugs, cockroaches, ants, and houseflies.

DISCUSSION

Wash resistant long-lasting insecticidal nets treated with synthetic pyrethroids are viewed as an important tool in the field of malaria prevention that would ease the problems associated with conventional insecticide treated nets, which lacks wash resistance of insecticide[14]. Icon® Life LLIN, has similar specifications as that of NetProtect® LLIN, which has been given interim recommendation by WHOPES. Studies in the past showed that the deltamethrin treated nets were found effective against malaria transmitted by *An. minimus*[15]. Other trials on nets treated with deltamethrin or lambdacyhalothrin EC against *An.*

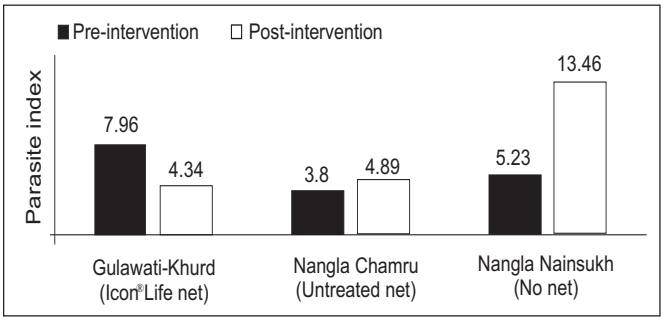

Fig. 1: Malaria incidence in the study villages during pre- and post-intervention with Icon Life net, untreated net and no net.

Table 5. Efficacy of Icon®Life LLIN on malaria prevalence in the study villages recorded through fortnightly active surveillance in the study villages in Dadri PHC, District Gautam Budh Nagar, U.P.

Village/Type of intervention	Pop.	Study period	BSE	Total malaria (+)ve cases	SPR (% reduction)	SfR	Parasite index (% reduction)
Gulawati-Khurd/Icon® Life- LLIN	1381	Pre-intervention (May–July 2008)	44	11	25	0	7.96
		Post intervention (August 2008–July 2009)	133	6	4.5	0	4.34 (–45.4)
Nangla-Chamru/ Untreated net	1840	Pre-intervention (May–July 2008)	34	7	20.6	0	3.80
		Post-intervention (August 2008–July 2009)	128	9	7	0	4.89 (+28.68)
Nangla-Nainsukh/ No net	1337	Pre-intervention (May–July2008)	42	7	16.6	0	5.23
		Post-intervention (August 2008–July 2009)	100	18	18	1	13.46 (+157)

Table 6. Cross-sectional surveys among Icon®Life net users in experimental village of District Gautam Budh Nagar U.P. for assessment of community perceptions on adverse effects and collateral benefits of long-lasting insecticide net

S. No.	Questions	% Users (Total N=100)
1.	Do you know why mosquito nets are used?	100
2.	Do you or your family members use mosquito nets for personal protection of your family members?	15
3.	Do you use any indigenous methods for mosquito control?	40
4.	Do you sleep inside the insecticide-treated Icon®Life for personal protection or of your family members?	98
5.	Perceived side-effects Do you suffer from any of the following:	
	—Skin irritation	2
	—Nausea	0
	—Vommiting	0
	—Itching	2
	—Headache	0
	—Drowsiness	0
	—Eye irritation	5
	—Difficulty in breathing	0
	—Any other	0
6.	Do you feel suffocation while sleeping inside net?	0
7.	Do you fear of poisoning for using Icon® Life net?	0
8.	Perception about collateral benefits	
	—Reduction in mosquito bites	92
	—Reduction in nuisance of house fly, cockroaches, etc.	80
9.	Do you recommend use of Icon® Life net in future?	100

culicifacies were successful in reducing high malaria morbidity[16–18]. In the past, various workers have reported high efficacy of Net Protect® (Icon®Life net) against *An. gambiae*[9]. Recently, a study reported that more *An. gambiae* succeeded in feeding through Olyset® net which got full recommendations from WHOPES as compared to NetProtect® which presently has interim recommendation. This study showed better performance of NetProtect® against *An. gambiae*[19].

The results of present study have clearly demonstrated wash resistance of Icon®Life LLIN under field conditions. There was significant difference in the total entry rate and excito-repellency rate between structures provided with Icon®Life net, untreated net and no net. Significant difference in reduction was observed in average per man hour density of *An. culicifacies* and total anophelines in Icon® Life net village as compared to untreated and no net villages. There was significant reduction in percentege of parous female *An. culicifacies* in Icon®Life village as compared to other villages after intervention. Cross-sectional surveys clearly documented the significant reduction in malaria incidences in Gulawati-Khurd village where Icon® Life net was distributed. The parasite index (number of malaria cases/1000 of population in Icon®Life village was significantly lower than the untreated net and no net villages. There was drastic reduction in PI rate of Icon® Life village as compared to other villages.

Nearly, all the respondents asserted the efficacy of this net as it showed effect on malarial vectors. The Icon® Life nets were found to be safe to humans as no adverse effect was recorded among the net users that can be attributed to the use of Icon®Life net use. There are no safety concerns in using synthetic pyrethroids because the recommended concentration of the insecticide for the treatment of mosquito nets is quite safe[20].

Due to the perceived benefits, cent percent villagers, expressed their willingness to use Icon®Life net. There is a prime need of awareness and building up positive attitude in the community members regarding the use of LLINs[21]. A recent study also reported that LLINs were widely acceptable in the user community and safest method of vector control in India[12].

Icon® Life proved to be a good method of vector control as these nets are safer and no complaints were reported in area of use. These results indicated that Icon® Life nets undoubtedly proved to be a good tool in malaria vector control but further studies are required to assess its bioefficacy for longer periods.

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