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Do long-lasting insecticidal nets retain their efficacy after three years of usage in Afghanistan? Findings from a study on survivorship, physical integrity, insecticidal activity and wash resistance

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Abstract

Background Long-Lasting Insecticidal Nets (LLINs) are effective malaria prevention tools. However, information is limited about their durability and wash resistance in field circumstances, especially in seasonal transmission areas in South Asia. This study comprised a systematic examination of usage, physical integrity and insecticidal activity of LLINs in households in Afghanistan, three years after distribution.

Methods In 2014, 500 households in 5 malaria endemic Afghan provinces (Balkh, Herat, Khost, Kunduz and Nangarhar) that had received LLINs (PermaNet 2.0) three years earlier were randomly selected through cluster sampling. All household heads were interviewed about LLIN survivorship, usage and maintenance. One randomly selected LLIN from each household was rigorously inspected to calculate the proportionate Hole Index (pHI). Four location-specific pieces from 200 randomly selected LLINs (40 per province) underwent cone bioassay testing in the Jalalabad entomology laboratory, to measure mosquito knock-down after 60 min and 24-h mortality. The number and percentage of nets with ≥ 80% mosquito mortality was assessed. Five location-specific pieces from 34 randomly selected LLINs (5–8 per province) were tested for insecticidal content by High-Performance Liquid Chromatography (HPLC).

Results Of the 1190 distributed LLINs, 1045 were still in the household at the time of the survey (survivorship 87.8%) and 1006 of those (96.3%) had been used every night in the past week. 9.1% of the LLINs were used by more than three people.. Physical integrity measurements indicated that 97.0% of the LLINs were in a serviceable condition (pHI 0–642), while 3.0% were 'too torn' (pHI > 642). Functional Net survival was 93.4% (95%CI 91.7–94.8%). However, only 28% of the LLINs met the WHOPES ≥ 80% mortality criterion. Washing of LLINs was associated with a significant reduction in mosquito mortality. Median deltamethrin concentration was 0.12 g/kg netting material (6.7% of the original concentration at production).

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Conclusions LLIN survivorship and functional net survival in this setting was excellent, while only a minority of LLINs retained sufficient insecticidal activity after three years of usage. This study underlines the need for evaluation of real-life LLIN durability in field circumstances. LLIN washing should be avoided, as it lowers insecticide content and LLIN efficacy.

Keywords Malaria, Long lasting insecticide treated nets, Bio-efficacy, Durability, Afghanistan, LLIN, Attrition, Fabric integrity, Functional survival, Chemical durability

Background

In the past 20 years, insecticide-treated nets (ITNs) have played a pivotal role in the reduction of malaria incidence in endemic countries [1]. Next to the physical barrier, bed nets create a chemical barrier against malaria-transmitting *Anopheles* mosquitoes, as the insecticide-treated netting material has a direct 'knock-down' and a rapid killing effect (within 24 h) on the mosquitoes. When bed nets are used on a mass-scale in areas with anthropophilic malaria vectors, they act as a "lure and kill" and can also cause reductions in mosquito densities, thereby reducing malaria transmission—the so-called 'mass effect' [2, 3]. The first ITNs that came on the market in the 1990s needed to be re-impregnated every 6–12 months [4]. By 2003, these were replaced by long-lasting insecticidal nets (LLINs) [5], which are impregnated during production, by incorporating the insecticide either directly into a polyethylene fibre or in a resin-based polymer coating, applied on a multifilament polyester fibre [6].

ITNs are evaluated in longitudinal cohort studies and if they demonstrate efficacy after 3 years they can be classified as LLINs [7]. Even so, a recent systematic review demonstrated that average ITN survival in Africa was 2 years [8]. Understanding the functional life of LLINs is critical in order to plan replenishment campaigns [9]. The number of published studies done in the past 20 years measuring LLIN durability and insecticidal activity in field circumstances remains limited despite the clear link between ITN damage and subsequent loss [10]. The vast majority of studies were done in high-transmission areas in Africa [11–14] with few studies done in seasonal transmission areas in Asia [15–17]. Also, limited data are available on the wash resistance of ITNs beyond the World Health Organization Pesticide Evaluation Scheme (WHOPES) reports [18].

Malaria is one of the major health problems in Afghanistan, with 287,835 confirmed cases in 2022 [1]. The disease is endemic in areas below 2,000 m altitude and highly prevalent in river valleys and areas used for rice cultivation [19]. The 13 malaria endemic provinces are spread throughout the country [20] and 8.2 million people (27% of the total population) are at risk for malaria. *Plasmodium vivax* is responsible for 95% of the cases, and *Plasmodium falciparum* for 5% [1]. Transmission is seasonal from June to November, with negligible transmission occurring between December and May [21].

Afghanistan's national malaria control strategy relies on early diagnosis and treatment and on mass distribution of bed nets among households in endemic provinces [1]. Between 2007 and 2014 more than 9 million LLINs were distributed in malaria endemic provinces in Afghanistan. However, few data are available about the performance of these LLINs in the various climatic zones in the country and about their durability to inform the recommended replenishment interval. The two studies [22, 23] that were done so far used household sampling to collect the LLINs, deriving a sample of bed nets with different brands and ages.

It is important to ascertain the real-life performance of these LLINs and factors influencing performance after three years of usage in Asian countries with seasonal malaria transmission. Therefore, three aspects of LLIN durability in Afghanistan were studied: (1) survivorship (proportion of distributed nets still available for intended used in the households to which they were given), (2) physical integrity (number, size and location of holes in a LLIN) and (3) insecticidal activity (capacity of an LLIN to cause knock-down and/or death of an *Anopheles* mosquito coming into contact with the fabric). The following questions were also assessed: (4) the deltamethrin concentration in the netting material; (5) whether LLIN usage patterns are associated with physical integrity and (6) whether washing practices are associated with insecticidal activity and deltamethrin concentration.

Methods

Study design

This study was a cross-sectional study, consisting of four different elements: (1) Interviews in 500 randomly selected households, that had previously received one or more LLINs; (2) Inspection of the physical integrity of one LLIN randomly selected from each of these households; (3) Insecticidal activity testing through the World Health Organization (WHO) standard cone bioassays of 200 randomly selected LLINs and (4) Insecticidal content testing of 40 randomly selected LLINs.

Study setting

In 2011 a total of 3,352,326 LLINs (PermaNet 2.0; Vestergaard, Lausanne, Switzerland) were distributed in the 14 provinces of Afghanistan where malaria is endemic. For this study five malaria-endemic provinces (Balkh, Herat, Khost, Kunduz and Nangarhar) were selected, which are evenly spread over the country (Fig. 1). Together they represent the different geographical and meteorological zones in Afghanistan.

Study population

In each of these five provinces, 100 households were sampled, using a cluster sampling approach. During the LLIN distribution in 2011 all households in villages located in malaria high-risk and medium-risk districts [24] had received sufficient LLINs for their household size. The original distribution lists, with the names and the population size of these villages, were used to randomly sample 10 villages, using a random starting point and the sampling interval (= cumulative population figure divided by the sample size). On arrival in these villages, the surveyors randomly selected 10 households, by 'twisting a pen' at the village centre and selecting the first/sixth/eleventh/etc. house in that direction. When more households (defined as: a group of people eating together) lived in the same *qala* (group of houses with a common wall), the interviewer randomly selected one of the households. The surveyors continued sampling households in the same manner until they had included 10 households. When the surveyor reached the village border before that, (s)he returned to the village centre and defined a new direction by twisting the pen. The interview was done with the household head or with his/her representative (e.g. spouse, brother or son).

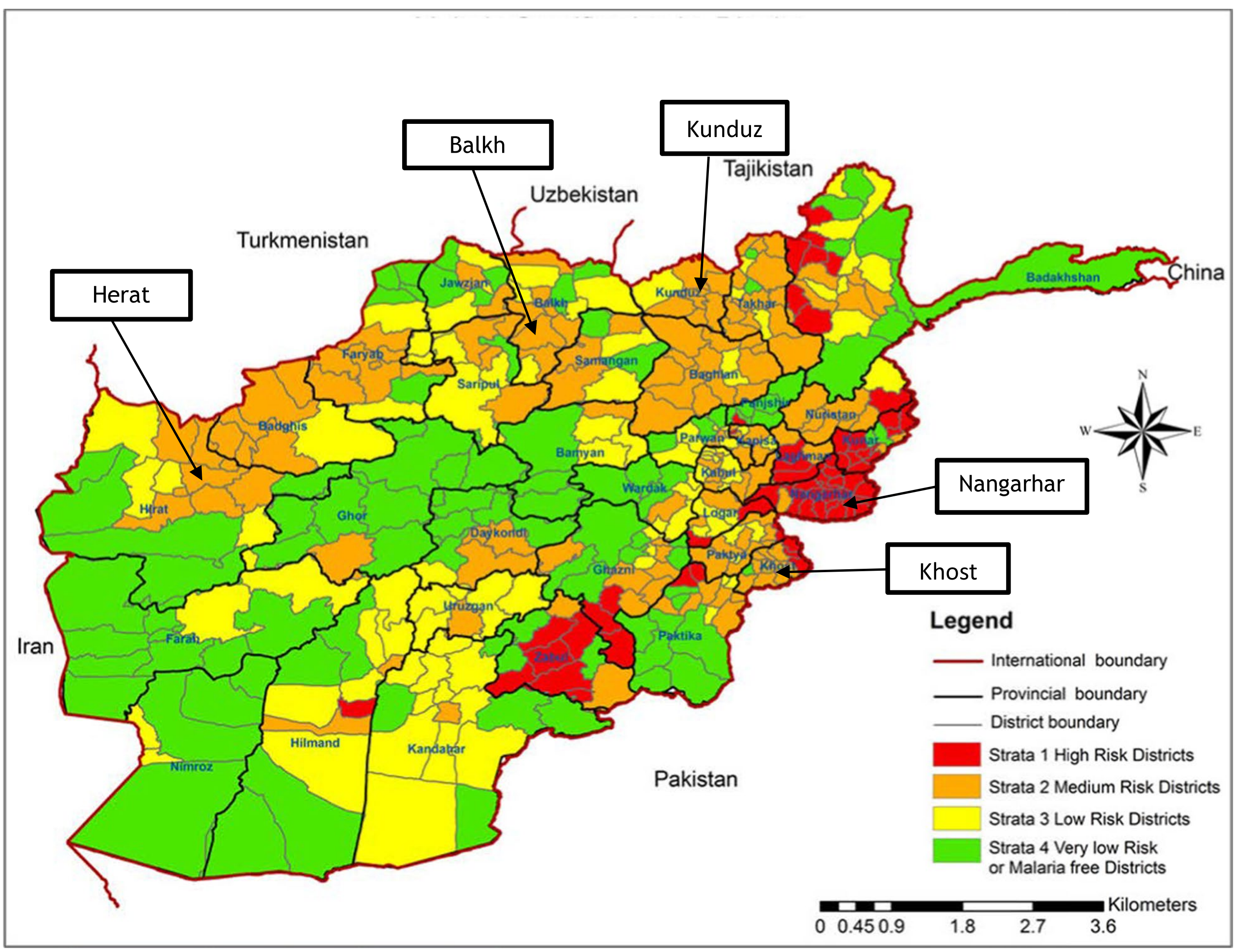

Fig. 1 Location of the five provinces selected for the LLIN durability study. Source of the map: (24) (Figure used with permission from the rights holder)

Each respondent had to give written informed consent prior to the interview. When the respondent declined collaboration or when nobody in the household was available for the interview, the 5th next household was selected instead.

Household interviews

The number of LLINs received three years earlier in the sampled households was derived from the original LLIN distribution lists. A slightly adapted version of the standard WHO questionnaire [7], translated in Dari and Pashto (local languages in Afghanistan), was used for the household interviews. This questionnaire focused on LLIN survivorship (comparing the number of LLINs originally received with the number still present), attrition, usage, handling and maintenance, and also enquired about size and composition of household, and about the type of floor, walls, and roof (Supplement 1). The questionnaire was pilot tested in the field and adapted. In each province, the surveyors were locally recruited and were supervised by a team supervisor. In Herat and Balkh one of the data collectors was female, while in the other three provinces all data collectors were male. All five team supervisors followed a three-day training in Jalalabad, focusing on the study purpose, the sampling procedure, the questionnaire, interview techniques and arrangements for supervision and quality control. The data collectors followed a similar training in their province, which also included a practical training day.

The data collectors conducted their interviews in Dari or Pashto and recorded the answers on the interview form. At the end of the interview the data collectors inspected all LLINs in the household for their physical integrity, following the procedure explained in the next section. This measurement was only used to calculate the Functional Net Survival [25]. While still in the village, the team supervisor checked all filled questionnaires for completeness, clarity and consistency. If any information was unclear or missing, the surveyor was asked to clarify or complete it, if needed by re-visiting the household. At the end of the study period the team supervisor sent all completed questionnaires to the HealthNet TPO (HNTPO) office in Jalalabad, where they were further processed. Two data entry officers entered all paper-based questionnaires in a computer database, using double data entry, after which the databases were compared for inconsistencies. Next to that, the databases were checked by the principal investigator for missing values, errors, outliers and inconsistencies.

Physical integrity testing

Next to the fabric integrity testing on the spot of all available LLINs in the sampled households, one LLIN per household was randomly selected for a more thorough inspection. In total 500 LLINs (100 per province) were collected and replaced by a new LLIN (PermaNet 2.0). All collected LLINs were labelled and transported in a non-transparent plastic bag, avoiding excessive heat and direct sunlight, to the HNTPO insectary in Jalalabad. Here the physical integrity of all collected 500 LLINs was inspected, by hanging them in a separate room or in the shadow under a tree and by recording all holes larger than 0.5 cm, distinguishing their size, location and nature. The WHO classification (13) was used to categorize the four sizes of holes: category 1 (0.5–2 cm), category 2 (2–10 cm), category 3 (10–25 cm) and category 4 (> 25 cm). Specific types of holes (holes caused by burns or by animal gnawing, holes in the seams or at the hanging points, horizontal tears at the bottom and missing sections) and evidence of repairs (knots, patches or stitches) were also recorded. All inspections were done by two trained staff members (one entomologist and one entomology assistant), following a standard procedure.

Cone bioassay testing

Cone bioassay tests were done on a sub-set of 200 LLINs (40 LLINs per province), selected from the 500 collected LLINs through a stratified simple random sampling procedure. In addition, five negative control nets (without insecticides) and two positive controls nets (unused PermaNets 2.0, from the same batch as distributed in 2011) were also used. Four pieces, each sized 25 × 25 cm, were cut from pre-defined positions 2, 3, 4 and 5 (See Supplementary Figure S1 (in Additional file 1) and [7]) of these LLINs. These pieces were then labelled, wrapped in aluminium foil and separate plastic bags, avoiding cross-contamination, and stored at 4 °C.

WHO guidelines [7] were followed to perform the cone bio-assay tests. Two hard boards were set up: one was solely used for tests on the 200 study LLINs, while the other was used for the positive and negative control tests. Two standard WHO cones were fixed with a plastic manifold onto each of the four netting pieces cut from one LLIN.

Susceptible *Anopheles stephensi* mosquitoes were reared in the insectary in Jalalabad where this species was colonized since 2006. The mosquito larvae were given a mix of 50% fish food and 50% Cerelac powder (baby food), while adult mosquitoes were fed on rabbit blood for egg production and maintained on 10% glucose solution ad libitum. Rearing, cone test exposure and post exposure holding conditions were controlled for temperature (25 ± 2 °C) and humidity (75 ± 10%), by using an air conditioner or a heater in combination with a bowl of water. Under each cone 10–12 lab-reared 3-day old *Anopheles stephensi* mosquitoes were exposed to the netting material for a period of three minutes. Subsequently the mosquitoes were transferred to a labelled paper cup, where they were held for 24 h. One-hour post-exposure a cotton soaked with 10% sugar solution was put on the top of each paper cup. Knock-down was recorded one hour after exposure, while mortality was measured after 24 h.

On each LLIN netting position, 10 bioassay tests were done. Position-specific knock-down and mortality results were calculated by averaging results from these 10 tests. Negative control tests were performed throughout the testing day, to ensure bioassay quality (i.e., to detect excess mosquito mortality due to poor handling). Also, positive control tests were performed throughout the testing day, to check bioassay validity, as a new LLIN is expected to perform above the WHOPES recommended mosquito knock-down (≥ 95%) or mortality (≥ 80%) thresholds [7]. Although more recent publications [26] regard mortality as a more valid bio-efficacy measure than knock-down, in this study both measures are reported. All cone bioassay tests were done by three trained staff members (two entomologists and one entomology assistant), following standard procedures. One of the three staff members was solely responsible for the negative control tests, to avoid cross-contamination between the different testing locations in the laboratory.

Insecticidal content testing

Insecticidal content was measured in a sub-selection of 34 LLINs (5–8 LLINs per province), which were selected from the 200 LLINs earlier selected for the cone bioassays, using a stratified simple random sampling procedure. Pieces, sized 30 × 30 cm, were cut from positions 1, 2, 3, 4 and 5 of each selected LLIN from the positive control nets. These pieces were then labelled, wrapped in aluminium foil, packed in separate bags and shipped to an external laboratory (Vimta Labs, Hyderabad, India). Normal-phase High-Performance Liquid Chromatography (HPLC), following CIP333/LN procedures [7, 27–29], was used to assess chemical concentrations of deltamethrin, expressed as g/kg netting material.

Effect of washing practices

Information about washing practices was available for 195 of the 200 LLINs undergoing cone bioassay testing and for 33 of the 34 LLINs undergoing insecticidal content testing.

This enabled us to assess associations between LLIN washing practices and both the insecticidal activity and insecticidal content.

Sample size

The sample size is based on WHO guidelines for LLIN field studies [7], which recommend a minimum sample size of 150 and 30 LLINs for fabric integrity testing and insecticidal activity testing, respectively. In order toto compare outcomes between the five provinces, the aforementioned sample sizes were increased to 500 and 200 LLINs. Furthermore, the study budget allowed for 40 LLINs to undergo insecticidal content testing.

Definitions

Information about the number and size of the holes was used to calculate the proportionate Hole Index (pHI). This measure for the ‘total hole surface’ was calculated using WHO guidelines [7], estimating the average sizes of the four categories of holes at 1, 23, 196 and 576 cm^2 respectively. Based on its pHI value, the LLIN was then grouped in one of the three WHO-defined categories ‘good’ (pHI 0–64), ‘damaged’ (pHI 65–642) or ‘too torn’ (pHI > 642). LLINs with a pHI value in the first or second category (pHI ≤ 642) are considered to be in a ‘serviceable condition’ [30].

The following formula was used to calculate ‘(functional) net survival’ [25]:

$$\text{(Functional) Net Survival} = \frac{N_{serv}}{N_{orign\ distr} - N_{lost}} * 100\%$$

where N_{serv}= Number of LLINs in a ‘serviceable condition’ (pHI ≤ 642), $N_{origndistr}$= Number of LLINs originally distributed to the households, N_{lost}= Number of LLINs lost due to use at a different location than the recipient household (e.g. given away, stolen, sold).

The results of all negative control cone bioassay tests done throughout one testing day were averaged. If this mortality figure was higher than 10%, then the adjusted mortality was calculated, using Abbott’s formula [31]:

$$\text{Adjusted mortality} = \frac{(Mortality_{Experimental\ test} - Mortality_{Control\ test})}{(1 - Mortality_{Control\ test})} * 100\%$$

Statistical analysis

Characteristics of households were provided, and compared between provinces, using chi-squared tests for categorical variables and Kruskal–Wallis tests for non-normally distributed continuous variables. The pHI was summarized as median (interquartile range (IQR)), overall and per province; pHIs were compared between provinces by Wilcoxon rank-sum tests. Proportions of nets in good, damaged or 'too torn' state were reported, and compared between provinces with chi-squared test. These results were provided for all nets (based on assessment by field data collectors) and for the selected nets (based on assessments by insectary staff in Jalalabad). Net survival is reported overall and by province and compared between provinces using Chi-squared tests. The net survival was calculated based on the physical integrity assessment in the field. Cone bioassay knock-down rate (kdr) and mortality results per LLIN were calculated, by taking median values of the four position-specific measurements. The proportion of nets meeting the ≥ 95% kdr was calculated, as was the proportion of nets having ≥ 80% mosquito mortality. Also, proportion of LLINS meeting the WHOPES criterion of ≥ 95% kdr or ≥ 80% mosquito mortality was calculated and compared between provinces. The median deltamethrin concentration of tested nets was calculated with 95%CIs, overall and by province, and results were compared between provinces using the Kruskal–Wallis test.

Negative binomial regression analysis was used to test the associations between LLIN usage (all dichotomized) and the pHI. Logistic regression analysis was used to assess associations between washing practices and cone bioassay mosquito mortality ≥ 80%. Linear regression analysis was used to assess the association between washing practices and deltamethrin concentration of the nets. Because of the non-normal distribution of the deltamethrin concentration values, the values were normalized by taking the natural log of the concentration plus 1 (because of zero values) and used the logged variable in the linear regression. The level of significance was set at < 0.05. All data were analysed using STATA (version 15.1).

Results

Overview of the sample

In total 500 households (100 per province), with a median household size of 7 (IQR 5–9), were included in the sample (See Table 1). The median male/female ratio of the household members was 1.0 (IQR 0.7–1.5). One third of the household heads did not follow any formal education, while the percentages following religious schooling, primary schooling, secondary schooling and higher education were 16.6%, 22.0%, 19.2% and 7.0%, respectively. The interviews were mostly (64.4%) done with the (male or female) household head, and sometimes with the wife of the male household head (22.2%) or another adult household member (13.4%). Table 1 also presents the percentages of households with (un)improved types of roofs, walls and floors.

Survivorship and usage of LLINs

The 500 sampled households had received 1,190 LLINs three years earlier, of which 1,045 LLINs (87.8%) were still in the household. A total of 145 LLINs (12.2%) were lost by attrition, due to being given away to others (69 LLINs, 47.6%), thrown away after being damaged (52 LLINs, 35.9%), used for other purposes (11 LLINs, 7.6%), used at another location (7 LLINs, 4.8%) or were stolen (6 LLINs, 4.1%). All available LLINs were inspected for their fabric integrity on the spot. Median pHI was 17 (IQR 1–84), and this varied significantly by province. 70.5% of nets were in good condition, 31.8% were damaged, and 3.0% were too torn (Table 2, section B). Figure 2 shows a traffic light plot with these categories, overall and by province. Attrition was variable, being highest (22.8%) in Khost province and lowest (0.4%) in Nangarhar province.

Functional net survival was calculated at 93.4% [95% confidence interval (95% CI) 91.7–94.8%] (See Supplementary Table S1 in Additional file 2). This figure was highest in Herat province [97.9% (95% CI: 94.7–99.4%)] and lowest in Nangarhar province [89.8% (95% CI: 85.1–93.4%); $P < 0.001$].

Of the remaining 1,006 LLINs, 1,045 (96.3%) had been used every night in the past week. Only 2.4% of the still available LLINs were not used at all. Most (97.5%) of the LLINs were only used in the hot season (April–October) in which most of the malaria transmission takes place, while 2.1% were used year-round. In Nangarhar province the proportion of LLINs used year-round is slightly higher (6.2%) than in the other four provinces combined (1.0%) ($P < 0.001$).

90.7% of the available LLINs were used by a maximum of three household members to sleep under, while 9.3% of the LLINs were used by more than three people.

Table 1 Background characteristics of 500 participating households across five provinces in Afghanistan, 2015

	All provinces (N = 500)	Balkh province (N = 100)	Herat province (N = 100)	Khost province (N = 100)	Kunduz province (N = 100)	Nangarhar province (N = 100)	P value
Median household size (IQR)	7 (5–9)	6 (4–7)	6 (4–8.5)	9 (7–11)	8 (6–9)	5 (3–9)	0.0001
Median household size per age group (IQR)							
< 5 years	0 (0–2)	0 (0–1)	0 (0–1.5)	1 (0–3)	1 (0–2)	0 (0–0.5)	0.0001
5–15 years	2 (1–3)	2 (1–3)	1 (0–3)	3 (2–4)	2 (1–3)	2 (1–3)	0.0001
> 15 years	4 (2–5)	3 (2–4.5)	3.5 (2–5)	4 (3–5)	4 (2–6)	2 (2–4.5)	0.0001
Median male: female ratio of household members (IQR)	1.0 (0.7–1.5)	1.0 (0.8–2.0)	1.0 (0.7–1.5)	0.8 (0.7–1.3)	1.0 (0.7–1.5)	1.0 (0.7–1.5)	0.0238
Highest level of education of the household head, n (%)							
None	176 (35.2%)	33 (33.0%)	64 (64.0%)	17 (17.0%)	44 (44.0%)	18 (18.0%)	< 0.001
Religious school	83 (16.6%)	8 (8.0%)	4 (4.0%)	26 (26.0%)	26 (26.0%)	19 (19.0%)	
Primary school	110 (22.0%)	19 (19.0%)	14 (14.0%)	32 (32.0%)	14 (14.0%)	31 (31.0%)	
Secondary school	96 (19.2%)	28 (28.0%)	15 (15.0%)	12 (12.0%)	13 (13.0%)	28 (28.0%)	
Higher education	35 (7.0%)	12 (12.0%)	3 (3.0%)	13 (13.0%)	3 (3.0%)	4 (4.0%)	
Family position of the respondent, n (%)							
Household head (male/female)	322 (64.4%)	11 (11.0%)	50 (50.0%)	85 (85.0%)	77 (77.0%)	99 (99.0%)	< 0.001
Wife of male household head	111 (22.2%)	74 (74.0%)	35 (35.0%)	0	2 (2.0%)	0	
Other adult in the household	67 (13.4%)	15 (15.0%)	15 (15.0%)	15 (15.0%)	21 (21.0%)	1 (1.0%)	
Type of floor, n (%)							
Unimproved type of floor*	175 (44.1%)	42 (48.3%)	2 (2.4%)	55 (61.8%)	22 (30.1%)	54 (83.1%)	< 0.001
Improved type of floor*	218 (54.9%)	41 (47.1%)	81 (97.6%)	34 (38.2%)	51 (69.9%)	11 (16.9%)	
Other types of floors*	4 (1.0%)	4 (4.6%)	0	0	0	0	
Missing	103	13	17	11	27	35	
Type of wall, n (%)							
Unimproved type of wall†	295 (71.4%)	41 (41.4%)	66 (76.4%)	70 (81.4%)	63 (85.1%)	55 (80.9%)	< 0.001
Improved type of wall†	109 (26.4%)	54 (54.6%)	20 (23.3%)	12 (14.0%)	10 (13.5%)	13 (19.1%)	
Other types of walls†	9 (2.2%)	4 (4.0%)	0	4 (4.7%)	1 (1.4%)	0	
Missing	87	1	14	14	26	32	
Type of roof, n (%)							
Unimproved type of roof‡	200 (56.3%)	6 (8.7%)	20 (34.5%)	62 (72.1%)	53 (71.6%)	59 (86.8%)	< 0.001
Improved type of roof‡	138 (38.9%)	46 (66.7%)	38 (65.5%)	24 (27.9%)	21 (28.4%)	9 (13.2%)	
Other types of roofs‡	17 (4.8%)	17 (24.6%)	0	0	0	0	
Missing	145	31	42	14	26	32	

IQR = interquartile range

*Unimproved type of floor: soil, sand, wood, palm or bamboo; Improved type of floor: carpet or cement; Other type of floor: used outside or type of floor unknown

† Unimproved type of wall: mud brick, mud with wood frame, wood, bamboo or straw; Improved type of wall: concrete or lime-plastered wall; Other type of wall: bricks (unspecified), used outside or unknown

‡ Unimproved type of roof: wood, grass thatch or reeds mats; Improved type of roof: concrete, corrugated iron or tiles; Other type of roof: used outside or unknown

Physical integrity of LLINs

Physical integrity testing of one LLIN per household was done in the insectary in Jalalabad (N = 500). The median pHI was 39 (IQR: 11–98) (See Table 2, section A). After three years, 65.2% of the LLINs were still in a good condition (pHI 0–64), while 31.8% were damaged (pHI 65–642) and 3.0% were too torn (pHI > 642). Holes at the hanging points were relatively common, with 29.8% of the LLINs having one or more of these types of holes. Also, 16.8% of the LLINs had one or more horizontal tears at the bottom and 8.6% had other types of damage (open seam, burn hole, hole from rodents or missing section).

Insecticidal activity of LLINs

In the 1,174 negative control tests performed throughout the testing period, average mortality per testing day was < 10%, with no significant differences within and between testing days. Therefore, the Abbott's formula was not used to calculate the adjusted mortality. Also

Table 2 Physical durability of inspected LLINs three years after distribution in five provinces in Afghanistan, 2015

SECTION A: One randomly selected LLIN per sampled household, examined in the insectary in Jalalabad	**All provinces (N = 500)**	**Balkh province (N = 100)**	**Herat province (N = 100)**	**Khost province (N = 100)**	**Kunduz province (N = 100)**	**Nangarhar province (N = 100)**	**P value**
Median proportionate Hole Index (IQR)	39 (11–98)	16.5 (7–54.5)	34 (17.5–90)	41 (10.5–111.5)	32 (8–83.5)	74 (33.5–300.5)	0.0001
Proportionate Hole Index (pHI), n (%) categorized							
Good (pHI 0–64)	326 (65.2%)	82 (82.0%)	65 (65.0%)	64 (64.0%)	71 (71.0%)	44 (44.0%)	< 0.001
Damaged (pHI: 65–642)	159 (31.8%)	15 (15.0%)	33 (33.0%)	33 (33.0%)	27 (27.0%)	51 (51.0%)	
Too torn (pHI > 642)	15 (3.0%)	3 (3.0%)	2 (2.0%)	3 (3.0%)	2 (2.0%)	5 (5.0%)	
No. (%) of LLINs with ≥ 1 horizontal tear at the bottom	84 (16.8%)	19 (19.0%)	19 (19.0%)	3 (3.0%)	24 (24.0%)	19 (19.0%)	0.001
No. (%) of LLINs with ≥ 1 hole at hanging points	149 (29.8%)	43 (43.0%)	30 (30.0%)	12 (12.0%)	37 (37.0%)	27 (27.0%)	< 0.001
No. (%) of LLINs with ≥ 1 damage of another type (open seam, burn hole, hole from rodents or missing section)	43 (8.6%)	13 (13.0%)	8 (8.0%)	4 (4.0%)	10 (10.0%)	8 (8.0%)	0.240
No. (%) of LLINs with ≥ 1 repair (knotted, patched or stitched hole)	254 (50.8%)	32 (32.0%)	39 (39.0%)	61 (61.0%)	51 (51.0%)	71 (71.0%)	< 0.001
SECTION B: All LLINs in the sampled 500 households, examined during field work	**(N = 1045)**	**(N = 175)**	**(N = 190)**	**(N = 234)**	**(N = 221)**	**(N = 225)**	
Median proportionate Hole Index (IQR)	17 (1–84)	5 (0–200)	7 (0–47)	11 (3–59)	13 (1–28)	63 (9–246)	0.0001
Proportionate Hole Index (pHI), n (%) categorized							
Good (pHI 0–64)	737 (70.5%)	106 (60.6%)	150 (79.0%)	176 (75.2%)	191 (86.4%)	114 (50.7%)	< 0.001
Damaged (pHI: 65–642)	239 (22.9%)	44 (25.1%)	36 (19.0%)	46 (19.7%)	25 (11.3%)	88 (39.1%)	
Too torn (pHI > 642)	69 (6.6%)	25 (14.3%)	4 (2.1%)	12 (5.1%)	5 (2.3%)	23 (10.2%)	

IQR = interquartile range; LLIN = Long-lasting Insecticidal Nets; pHI = proportionate Hole Index

183 positive control tests were done on four positions of two new PermaNets 2.0. The median mosquito kdr in these tests was 100% (IQR: 100–100%), while the median mosquito mortality was 100% (IQR:100–100%). Neither of these results differed significantly between the four positions and the two nets.

Cone bio-assay results for the 200 LLINs are shown in Table 3. Median mosquito kdr was 92% (IQR: 84–97%), while median mosquito mortality was 58% (IQR: 36–84%). Kdr and mortality results showed significant differences between the five provinces. Overall, 37% of the examined LLINs had a median knock-down ≥ 95%, while 28% had a median mosquito mortality ≥ 80%; overall only 44% of the examined LLINs met one of these 2013 WHOPES criteria and can thus be regarded as 'sufficiently insecticidal'.

Insecticidal content

PermaNet 2.0 product specifications [32] indicate that the deltamethrin concentration should be in the following range: 1.8 ± 25% g/kg netting material. This was the case for 9 of the 10 positive control LLIN pieces (one piece had a higher concentration (2.28)), with a median value of 1.71 (IQR: 1.38–2.07). Median deltamethrin concentration in the 34 LLINs examined after three years of use, measured through HPLC, was 0.12 g/kg netting material (IQR: 0.03–0.27), which is only 6.7% of the median concentration at production (Table 3).

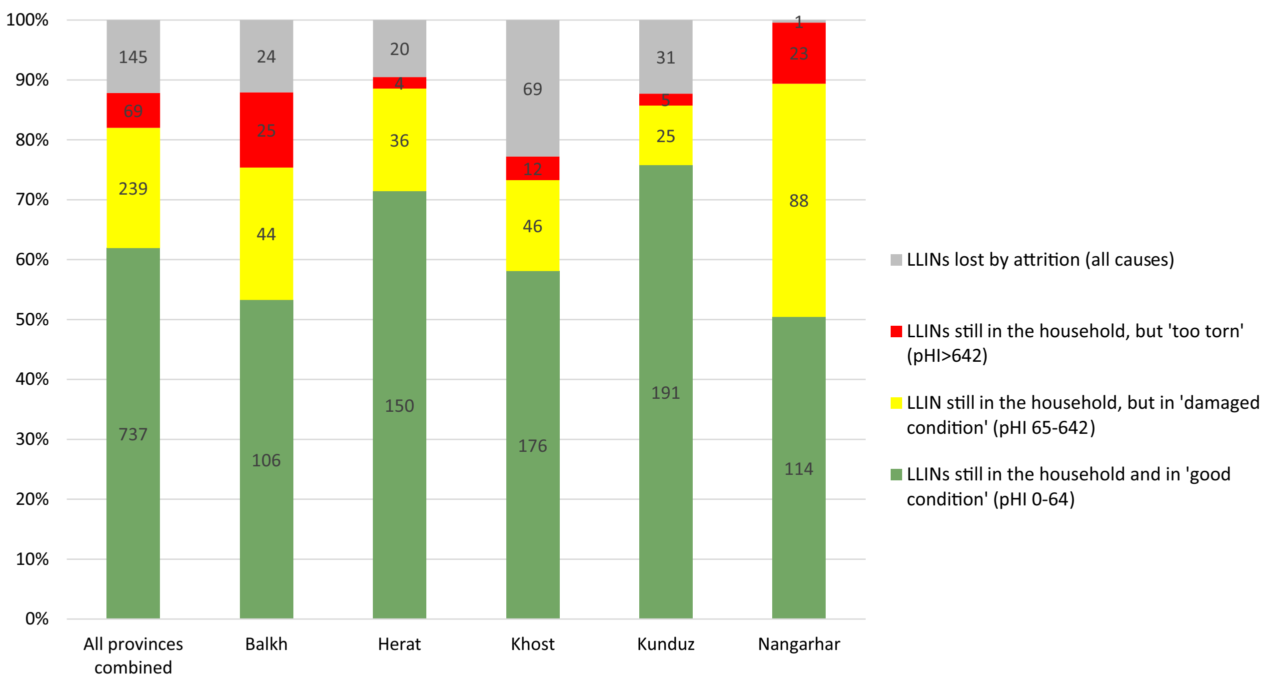

Fig. 2 'Traffic light plot' on LLIN survivorship in five Afghan provinces (2015), overall and per province

Table 3 Bio-assay results for 200 LLINs and deltamethrin concentration of 40 selected LLINs three years after distribution in five provinces in Afghanistan, 2015

	All provinces	Balkh province	Herat province	Khost province	Kunduz province	Nangarhar province	P value
Bio-assay results (on 200 LLINs)							
Median mosquito knock-down rate*, % (IQR)	92 (84–97)	90 (76–97)	90 (81–96)	94 (90–98)	91 (85–97)	91 (81–97)	< 0.0001
Median mosquito mortality*, % (IQR)*	58 (36–84)	50 (18–79)	55 (43–82)	69 (41–86)	71 (48–86)	55 (27–80)	< 0.0001
Percentage of LLINs meeting the ≥ 95% mosquito knock-down criterion*	37	35	31	48	35	35	0.654
Percentage of LLINs meeting the ≥ 80% mosquito mortality criterion*	28	25	21	35	38	23	0.307
Percentage of LLINs meeting the WHOPES criteria (≥ 95% mosquito knock-down or ≥ 80% mosquito mortality)*	44	40	35	55	48	43	0.437
Deltamethrin measurement results (on 34 LLINs)							
Median Deltamethrin concentration*,† (g/kg netting material) (IQR)	0.12 (0.03–0.27)	0.09 (0.03–0.31)	0.11 (0.08–0.23)	0.23 (0.02–0.87)	0.14 (0.02–0.34)	0.17 (0.03–0.26)	0.99

IQR = interquartile range; kdr = knock-down rate; LLIN = Long-lasting Insecticidal Nets; WHOPES = World Health Organization Pesticide Evaluation Scheme

*Calculation based on the average of the four position-specific measurements per LLIN

† Analysis done on a random sub-selection of 34 LLINs (5–8 per province)

Table 4 Association between washing practices and cone bioassay mosquito mortality ≥ 80% (results of univariable logistic regression, Section A) and log deltamethrin concentration (in g/kg netting material, by univariable linear regression, Section B)

	SECTION A: outcome: cone bioassay mosquito mortality ≥ 80%		**SECTION B: outcome: log deltamethrin concentration (in g/kg netting material)**		
	n/N (%)	**Odds ratio (95% CI)**	**n**	**Median (IQR)**	**Regression coefficient (95% CI)**
Washing method					
LLIN not washed[†]	24/56 (42.9%)	Ref	8	0.22 (0.11–0.62)	Ref
LLIN washed by hand or with wooden bat in tub or in washing machine	30/139 (21.6%)	0.37 (0.19–0.72)	25	0.09 (0.03–0.21)	− 0.20 (− 0.43 to 0.03)
P value	0.003	0.0032		0.1928	0.0886
Detergent use					
LLIN washed, without soap or with non-detergent soap	14/52 (26.9%)	Ref	11	0.10 (0.03–0.24)	Ref
LLIN washed with (harsh) detergent	15/81 (18.5%)	0.62 (0.27–1.42)	13	0.09 (0.04–0.19)	0.03 (− 0.11 to 0.17)
P value	0.252	0.2561		0.4496	0.6911
Type of water used					
Tap water	9/41 (22.0%)	Ref	8	0.07 (0.03–0.17)	Ref
Water from pump or well or open water source	21/97 (21.6%)	0.98 (0.40–2.38)	17	0.10 (0.03–0.21)	0.04 (− 0.09 to 0.17)
P value	0.969	0.9688		0.4130	0.5518
Soaking of the LLIN					
LLIN washed, but not soaked	9/41 (22.0%)	Ref	7	0.06 (0.03–0.10)	Ref
LLIN soaked (< 1 h)	19/85 (22.4%)	1.02 (0.42–2.52)	15	0.11 (0.02–0.21)	0.05 (− 0.04 to 0.14)
P value	0.959	0.9596		0.2847	0.2736
Drying in the sun					
Inside or outside in the shade	13/63 (20.6%)	Ref	10	0.20 (0.03–0.35)	Ref
Outside in the sun	16/71 (22.5%)	1.12 (0.49–2.56)	13	0.08 (0.02–0.11)	− 0.14 (− 0.30 to 0.01)
P value	0.790	0.7906		0.1360	0.0710

LLIN = Long-lasting Insecticidal Nets

*Adjusted for clustering at village level (through robust Standard Errors method)

[†] To avoid collinearity, the group with unwashed nets was only included in the 'washing method' analysis, not in the other analyses

Deltamethrin concentrations did not differ significantly between LLINs from the five provinces.

Effects of usage, washing and drying

In the negative binomial regression analysis, the association between pHI and usage factors was examined (See Supplementary Table S2 in Additional file 3). No significant association was found with the type of bed being used and with overcrowding (more than three people sleeping under the LLIN). Also, there was no significant association with period of usage of the LLIN (throughout the year or seasonal) and with the presence of an open flame in the room where the LLIN was used. The pHI was significantly (P < 0.0001) associated with the province, with the highest median pHI value in Nangarhar province [74 (IQR: 33.5–300.5)] and the lowest median pHI value in Balkh province [16.5 (IQR: 7–54.5)].

The proportion of LLINs with a cone bioassay median mosquito mortality ≥ 80% was significantly higher (P = 0.0032) in the nets that were reported to have been unwashed (42.9%) than those reported to have been washed (21.6%) (Table 4). Deltamethrin concentration was also higher in the unwashed nets [0.25 g/kg (IQR 0.12–0.87)] than in the washed nets [0.10 g/kg (IQR − 0.03 to 0.23)], but this difference was not significant (P = 0.0957). Within the group of washed nets cone bioassay mosquito mortality and deltamethrin concentration were not significantly lower in nets washed with detergent soap or with non-tap water and in nets being soaked or dried in the sun during the washing process, although nets dried in the sun tended to have lower deltamethrin concentrations (p = 0.0847).

Discussion

This is the first study in Afghanistan in which survivorship, usage, physical integrity and insecticidal activity of LLINs three years after distribution was systematically examined. Survivorship (87.8%) and usage (96.3%) of the LLINs were found to be high, while 97.0% of the surviving LLINs were still in a serviceable condition. However, in the bioassay tests median mosquito mortality [58% (IQR: 36–84%)] was well below the 80% threshold, and the median deltamethrin concentration was only 0.12 g/kg netting material (7% of the original concentration at production).

Apart from the national mass LLIN distribution campaigns, local Afghans have few opportunities to purchase or receive bed nets. This may explain why the large majority of LLINs distributed three years earlier was still in the households and was actively used. In comparable studies in Africa, LLIN survivorship after three years of usage was much lower, with values ranging between 12.9% and 91.2% [14, 33–35]. Information on LLIN survivorship in Asia is limited: in a study in high malaria endemic districts in Eastern India this figure was found to be 74.8% after 2.5 years of usage [17]. The high proportion of LLINs in a serviceable condition in this study can partially be explained by the fact that almost all users (97.5%) only used their LLIN in the malaria season.

In contrast, insecticidal activity of most examined PermaNet 2.0 LLINs did not meet the WHOPES 80% mosquito mortality criterium [median mortality 58% (IQR: 36–84%)]. In other studies with three-year-used PermaNets 2.0 (produced before 2013 [36]) higher mean mortality figures (83% in Senegal [13], 88.5% in Uganda [5]) were found, although in a study at two locations in India [15] mortality figures ranged between 57 and 79%. Studies in Zambia [37] and Guatemala [38] with similar LLINs found even lower mortality figures (geometric mean mortality 31.9% at 24 months and mean mortality 45% at 32 months respectively).

Since mosquito mortality in the washed LLINs was significantly lower than in the unwashed LLINs, it seems likely that LLIN washing may have contributed to insufficient performance. WHO guidelines recommend that approved LLINs have to withstand 20 washes with a palm-based soap (Savon de Marseille) [7]. As part of the approval procedure, all new LLIN brands have to go through a rigorous testing procedure at an independent testing facility. On the other hand, since product performance was insufficient both among washed and unwashed LLINs, other factors that lead to deltamethrin loss may also have played a role; this includes evaporation and abrasion [6], aggravated by the fact that most LLINs in the sample were extensively used and survived the 3-year follow-up period. It is for this reason that long-term community studies are also important to evaluate community acceptability of LLINs that influences their longevity alongside the influence of environment on the true LLIN performance [39].

This study has some limitations. First, LLIN durability was only measured at one point in time. It would have been informative to follow LLIN performance at different time points after distribution, allowing estimation of functional LLIN survival over time. Also, no information was collected about the number of washes that each net underwent. Last, due to the time elapsed since the actual LLIN loss, respondents may occasionally have been mistaken about the reason of the loss. A strength of the study is its large sample size and its use of multiple methods, allowing us to compare results between LLIN usage and handling and its effect on the durability. Although this study was performed ten years ago and in the meantime the binder of PermaNet 2.0 was changed [40], this does not affect the study conclusions.

Conclusions

In conclusion, this study underlines the importance of independent research monitoring the quality of vector control products in field circumstances. Unfortunately, these types of studies are not routinely conducted by national malaria control programs and are insufficiently funded, especially in fragile countries like Afghanistan. However, information from these studies is essential for Ministries of Health to ascertain if products still work in field circumstances or if they need to be replaced by another product. These types of studies can be done at relatively low costs and given the high costs of LLIN procurement, they seem a rational and wise investment. For this reason, LLIN durability testing in field circumstances should become an integral part of the malaria vector control programme in endemic countries to enable selection of the most cost-effective, best-performing products for the setting [11, 41]. Secondly this study shows that LLIN washing has a negative effect on their insecticidal activity, which has also been shown in other studies. Therefore, health authorities in endemic countries should educate LLIN recipients to care for their bed nets, to reduce the number of washes and dry them out of the sun.

Abbreviations

ANPHI	Afghanistan National Public Health Institute
GFATM	Global fund to fight AIDS, tuberculosis and malaria
HNTPO	HealthNet TPO
HPLC	High-performance liquid chromatography
IQR	Interquartile range
ITN	Insecticide-treated net
Kdr	Knock-down rate
LLIN	Long-lasting insecticidal net
NMLCP	National Malaria and Leishmaniasis Control Program
pHI	Proportionate hole index
WHO	World Health Organization
WHOPES	World Health Organization Pesticide Evaluation Scheme

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12936-025-05346-1.

Additional file 1. WHO recommended positions from which netting pieces should be cut for bio-assay testing

Additional file 2. LLIN survivorship and attrition for 1,190 distributed LLINs three years after distribution in 500 sampled households across five provinces in Afghanistan, 2015

Additional file 3. Association between types of usage and proportionate hole index through negative binomial regression

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Author contributions

M.V., M.A., M.F.S., M.S.N., A.M.S. and M.N. collaborated on the study conception. M.V. and M.A. jointly designed the study protocol. A.M.S. and M.N. were responsible for funding acquisition and for project administration. Under the supervision of M.F.S. and with the support of M.S.N., M.A. was responsible for data acquisition, including the household interviews, the physical integrity testing and the entomological tests. M.V. was responsible for the analyses and interpretation of the data, under the supervision of S.M. and M.SvdL. and with support from H.D.S. and J.B.. M.V. drafted the manuscript, while M.A., N.A., H.D.S., S.M. and M.SvdL. substantially revised it. All authors reviewed the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Ethical approval for this study was obtained from the Institutional Review Board of the Afghanistan National Public Health Institute (ANPHI) of the Ministry of Public Health.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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