Large-scale (Phase III) evaluation of the efficacy, fabric integrity and community acceptability of the PermaNet® 3.0 long-lasting insecticidal nets compared with the PermaNet® 2.0 in Western Kenya

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Introduction:

Long-lasting insecticidal nets are one of the primary tools for the prevention of malaria. LLINs are defined as retaining their insecticidal activity through 20 standard washes in laboratory and experimental hut studies and over three years in large scale field studies. The PermaNet 3.0 was tested in western Kenya to assess net attrition, physical durability and whether it would retain insecticidal activity for three years under field conditions.

Methods

Nets were distributed in 11 villages in Bungoma County, western Kenya as part of a cohort study to assess net attrition and physical durability or a cross-sectional study to assess the insecticidal activity of nets over time. PermaNet 3.0 were distributed along with PermaNet 2.0 nets to provide a measure of the validity of the study. Nets in the cohort study were visited every 6 months to assess whether attrition of nets and the physical integrity of nets remaining in the household. Nets in the cross-sectional study were also visited every 6 months and a random sample nets were removed and replaced to assess biological activity of the nets by WHO cone tests. At 36 months, nets that did not pass by the cone test were subjected to the tunnel test. Chemical analysis of sampled nets was done at baseline, 12, 24 and 36 months.

Results

Attrition increased gradually in the cohort study. After 36 months, 64.4% of PermaNet 2.0 and 70.1% of PermaNet 3.0 nets were lost. In the first two years, most nets were lost due to people giving away their nets or to unknown reasons. At 36 months, 44.5% of PermaNet 2.0 and 49.9% of PermaNet 3.0 were lost due to damage. The physical integrity of the nets

assessed in the cohort study also declined gradually over time. By 36 months after distribution, 18.7% of PermaNet 2.0 and 21.3% of PermaNet 3.0 examined had substantial damage and were in need of replacement. There were no differences in damage between the two nets. In nets sampled for bioassays and chemical analysis, the deltamethrin content declined by 66.7% for the PermaNet 2.0, 57.9% for the PermaNet 3.0 side panels and 42.6% for the PermaNet 3.0 top panels. The piperonyl butoxide content declined by 79.7%. Despite the loss of insecticide, at least 85% of PermaNet 3.0 side and top panels met the criteria for a long-lasting net through 36 months based on the WHO cone test. When tunnel tests were included 96% of side panels and 94% of the top panels met the criteria for a long-lasting insecticidal net after 36 months of use in the field.

Conclusions

The PermaNet 3.0 met the criteria for an LLIN as bioefficacy was retained for 36 months of use in the field. Rates of attrition and loss of physical integrity were similar to the PermaNet 2.0, an LLIN that had already been evaluated under field conditions. The PermaNet 3.0 can therefore be recommended for use in malaria control programs that implement LLINs.

Introduction/Background:

With the scale up of proven interventions since 2000, the burden of malaria has decreased substantially. Through 2015, malaria prevalence was estimated to have declined by half and the incidence of clinical malaria by 40%. Much of the decline was attributed to the scale up of vector control interventions, particularly insecticide treated nets (ITNs) which were estimated to have contributed to 68% of the decline [1]. However, progress has stalled since 2015 and in some areas, malaria has increased [2]. The reasons for this decline are multifactorial but likely include stagnating investment by the global donor community, biological limits of the effectiveness of current vector control interventions, and the rise and spread of insecticide resistance.

The bulk of ITNs distributed as part of the scale up of malaria control efforts have been long-lasting insecticidal nets (LLINs). LLINs are ITNs where the insecticidal activity is retained through 20 standardized washings and after 3 years of normal use. The insecticide on LLINs is either mixed with the fabric polymer before fibre extrusion or is bound to the net within a resin coating to reduce insecticide loss through washing and regular use. Through 2017, LLINs were evaluated by the WHO Pesticide Evaluation Scheme in three phases. Phase I included laboratory studies to estimate regeneration of insecticidal activity after washing and wash resistance using standardized wash procedures and WHO cone bioassays and/or tunnel tests. LLINs that met phase I criteria were then tested in semi-field experimental huts to assess the activity of washed and unwashed candidate LLINs against wild, free-flying anopheline mosquitoes. Candidate LLINs that met the phase I and phase II criteria were provided an interim recommendation by WHOPES and were then tested in field studies over 3 years to assess attrition, physical

integrity and insecticidal activity under normal use conditions. LLINs that met the phase III criteria were provided a full recommendation by WHOPES [3].

Initially, LLINs were treated only with pyrethroid insecticides such as permethrin, deltamethrin or alphacypermethrin as these insecticides were extremely effective against mosquitoes at low doses, had minimal adverse effects on humans and had chemical characteristics such as low water solubility and low vapor pressure that made them ideal for long residual treatment of ITNs. However, pyrethroid resistance has become widespread throughout sub-Saharan Africa [4] threatening the efficacy of LLINs. In response, several net manufacturers have incorporated piperonyl-butoxide (PBO) into LLINs. PBO is a synergist that inhibits oxidase enzymes that are often implicated as mechanisms of pyrethroid resistance [5]. LLINs with PBO have been demonstrated to have increased activity against pyrethroid resistant mosquitoes in cone bioassays [6-8] and in experimental huts [9-14]. More recently, a cluster randomized trial of PBO LLINs in Tanzania demonstrated that the odds of malaria infection was 60% lower among users of PBO LLINs compared to standard LLINs after 21 months of use [15].

One PBO LLIN is the PermaNet® 3.0 which is manufactured by M/s Vestergaard Frandsen, Switzerland. The PermaNet® 3.0 is constructed of multifilament polyester yarn on the side panels and is available in either 75 or 100 denier. The 75 denier version has a fabric weight of 30 g/m^2 although the lower portion of the side panels is reinforced, resulting in a fabric weight of 40 g/m^2. The side panels are treated with deltamethrin at a target dose of 2.8 g AI/kg, corresponding to 85 mg AI /m^2 for the upper panels and 115 mg AI/m^2 for the lower panels. The 100 denier version is treated at a target dose of 2.1 g/kg which corresponds to 85 mg/m2. The top panel of the PermaNet 3.0 is constructed from a

monofilament polyethylene fibre with deltamethrin incorporated at 4.0 g/kg and PBO incorporated at 25 g/kg. The PermaNet 3.0 has been evaluated in phase I and phase II studies and currently has an interim recommendation from WHOPES. Furthermore, the PermaNet 3.0 has been shown to have increased efficacy against pyrethroid resistant mosquitoes in WHO cone bioassays and in experimental hut studies.

However, the PermaNet 3.0 has never been evaluated in phase III studies to assess the efficacy of this product over 3 years under normal use conditions. We tested the PermaNet 3.0 in Bungoma County in western Kenya to evaluate its insecticidal efficacy, fabric integrity and acceptability in comparison to the PermaNet 2.0, a standard pyrethroid only LLIN.

Objectives

The main objective of this study was to determine the duration of efficacy of the PermaNet 3.0 in comparison to the PermaNet 2.0, a product that has been extensively tested under field conditions in several countries endemic with malaria.

Additional objectives:

1. To determine and compare the insecticidal activity and fabric integrity of PermaNet 3.0 LNs with PermaNet 2.0 over three years of use by households under field conditions
2. To assess washing mode and washing habits of LNs by the householders, and
3. To assess the community acceptability of the two LNs over three years of use under field conditions.

Materials and Methods

Description of LLINs

Under WHOPES guidelines, the PermaNet® 3.0 LLIN was compared to PermaNet® 2.0 LLIN (Vestergaard-Frandsen) in terms of their insecticidal efficacy, physical integrity and acceptability. The PermaNet 3.0 LLINs tested were constructed of 100 denier polyester on the side panels with no reinforced border. The top was made of monofilament polyethylene with deltamethrin incorporated at 4 g/kg and PBO incorporated at 25 g/kg. The PermaNet 2.0 LLINs were made of multifilament 100 denier polyester and treated with deltamethrin at a target dose of 1.4 g/kg which corresponds to 55 mg/m^2. The PermaNet 2.0 has been evaluated in phase III studies and has received a full recommendation from WHOPES.

Study site and population.

The study site was in Sirisia sub-County of Bungoma County on the border of Uganda. It lies on the northern tip of western province of Kenya borders Mt. Elgon to the northwest. Sirisia receives bimodal type of rainfall with an average annual rainfall ranging from 1200mm to 1800mm per annum. Most of the rainfall is experienced in the months of April-May and July-August. Temperatures range from minimum of between 15 – 20^{0}C to a maximum of between 22–30^{0}C. The coldest months are July, August and September. The malaria burden in Bungoma County is among the highest in Kenya [16]. The main malaria vectors in these areas are *Anopheles gambiae* Giles, and *Anopheles funestus* Giles.

The residents of this area are predominantly of the Bukusu ethnic group of Luhya tribe. The local residents depend upon farming and herding for subsistence. Homesteads (or

compounds) are distributed broadly across a rolling landscape intersected with small streams and rivers. Eleven villages with similar topographical and epidemiological situations were selected for implementation of the LLINs evaluation and all persons living in the selected villages were eligible to be enrolled in the study.

Enumeration and Net Distribution

Before the study started, initial approval was obtained from the chief and/or traditional authority in each village. After obtaining approval from the chief, the study was explained to the head of each household and a list of all households was generated. After obtaining consent from the head of each household or other responsible adult, a survey was conducted of all households in the target villages in June 2015 to assess baseline demographic and net ownership before the distribution. During the baseline survey, all households were mapped to assist in tracing nets during follow up and the numbers of sleeping spaces were also assessed. A total of 1229 eligible households were enrolled and randomly assigned to either a cohort study to assess attrition and physical integrity of the LLINs or a cross-sectional study to assess insecticidal activity and content of the LLINs. Within each study, the households were then randomized to receive either PermaNet 2.0 or PermaNet 3.0 nets. The household head was then provided a voucher with the number and type of nets his household was to receive and instructed to come to a central location on a specific date for net collection. Nets were distributed from a central location after it was confirmed that the consent had been obtained and a baseline interview had been conducted. A mop up of households not interviewed during the baseline survey or who did not come to receive their nets was done by visiting the households in the villages and was completed in early July 2015. Of the 1229 households, 447 were enrolled in the cohort

study while 782 were enrolled in the cross-sectional study. Within the cohort study, 234 households were randomized to the PermaNet 2.0 arm and received 604 LLINs while 213 were randomized to the PermaNet 3.0 arm and received 547 LLINs. Within the cross-sectional study, 392 households were randomized to the PermaNet 2.0 arm and received 1,003 LLINs while 390 were randomized to the PermaNet 3.0 arm and received 961 LLINs (Figure 1).

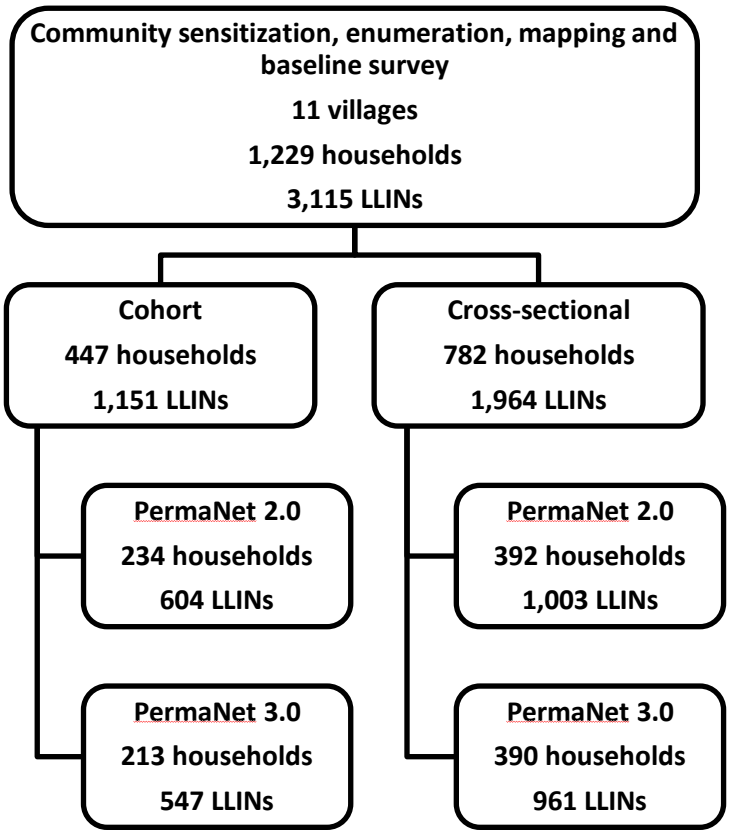

Figure1: Study Profile, Enumeration and Net Distribution

Adverse events

In October of 2015, all households enrolled in the study were visited to assess any adverse events that had occurred. Household owners or a responsible adult was asked about specific adverse events associated with pyrethroid exposure including itching or tingling of

the skin (paresthesia), burning of the skin or mucous membranes, itching or watery eyes, headache and nausea. Respondents were also asked whether anyone sought medical attention for their symptoms.

Follow up of the nets

Nets in both the cohort study and the cross-sectional study were visit every 6 months to assess whether they were present and available for use. If not, the household owner was asked about the reason for net loss. For nets in the cohort study that remained in the household, a questionnaire was administered to determine behaviors around net use, care and repair. In addition, all nets were assessed in the field for the presence of holes. Holes were counted by category as small (smaller than a thumb), medium (larger than a thumb but smaller than a fist), large (larger than a fist but smaller than a head) or extra-large (larger than a head).

Sampling of LLINs for physical integrity evaluation, chemical analysis and bioassays assays

Thirty nets were randomly sampled at baseline and every 6 months from the cross sectional arm for assessment of holes, bioassays and chemical analysis, except at month 36 when 50 nets were randomly sampled. The randomly selected LLINs were withdrawn from household and replaced with a new net of the same brand to avoid confounding in the study. All sampled nets were assessed both in the field by a community health volunteer (CHV) prior to the sampling and in the laboratory with trained technicians for physical integrity. In the lab, nets were draped over a frame made of PVC pipes and holes were measured at the largest width and, for holes on the side of the net, the distance from the bottom of the net was measured.

Bioassays were done at baseline, 6, 12, 18, 24, 30 and 36 months while chemical assays were done at baseline, 12, 24 and 36 months. Pieces of net that were 30 cm x 30 cm in size were cut from each net as per WHO LLIN guidelines [3]. From each sampled PermaNet 2.0 nets, five pieces were cut while for each sampled PermaNet 3.0, four pieces were taken from the side panels of the net and three pieces were removed from the roof panels.

Separate panels were removed for bioassays and chemical assays. All pieces were labeled with the net code of the sampled net and the location of the piece on the net. All five net pieces from each PermaNet 2.0 LLIN were placed together, wrapped in aluminum foil and labeled with the net code. For the PermaNet 3.0, the four side pieces from each net were combined and wrapped in aluminum foil while the three top pieces from each net were combined and wrapped in aluminum foil. All net pieces were stored at room temperature until they could be processed for bioassays or shipped for chemical analysis.

WHO cone bioassays

WHO Cone bioassays were conducted at a room temperature and relative humidity of 27 ± 2 °C and 80 ± 5 %, respectively with a susceptible colony of *An. gambiae* s.s. (Kisumu strain) 2–5 day old sugar-fed females following standard WHO procedures [3]. After the start of the study, the colony was found to have been contaminated with the kdr-East allele (L1014S). However, WHO tube assays indicated full susceptibility to deltamethrin. Individual net pieces were placed on a 30 cm x 30 cm piece of Teflon backing and then four cones were placed on the net piece. A plastic frame with four holes the size of the base of the WHO cones was then placed over the cones and binder clips were used to hold the apparatus in place. The apparatus was placed at a 45° angle and then 5 mosquitoes were

introduced into each cone. The mosquitoes were exposed to the net pieces for 3 minutes after which they were removed and transferred to paper cups and provided access to 10% sugar solution. Mosquito knockdown (KD) was recorded at 60 min post-exposure and mortality (MT) was assessed after 24 hours. Each day, an mosquitoes were exposed to an untreated control net to assess mortality due to handling or other factors unrelated to the test nets. The mortality rate was corrected using Abbott's formula when mortality in the controls was 5–10 %. If mortality rate in the controls was >10 %, the bioassay round was discarded and a new test was conducted.

Tunnel tests

According to WHOPES guidelines, an individual LLIN is considered to meet the efficacy criteria in a cone test if knockdown is ≥95% or mortality is ≥80%. Nets that did not meet the efficacy criteria for the cone test at 36 months were subjected to a tunnel test. The net piece with the median mortality of each net was selected for testing. Nine holes that were 1 cm in diameter we cut in the net and the net was then placed in a 60cm tunnel (25 cm x 25 cm) approximately 20cm from one end. A guinea pig was restrained on the short side of the tunnel while 100 mosquitoes were released at 6pm in the long end of the tunnel. The mosquitoes were collected the following morning approximately 15 hours after the start of the test. Mosquitoes were recorded as dead or alive, fed or unfed and their location in the tunnel. An untreated control net was run the same night as each test net. LLINs were considered to meet the efficacy criteria if mortality was ≥80% and/or blood-feeding inhibition relative to the control was ≥90%.

Chemical analysis for insecticide contents

Samples of LLINs were subjected to chemical residue analysis at the WHO Collaborating Centre at the Walloon Agricultural Research Centre (Gembloux, Belgium). A circular 100cm^2 piece was cut from one piece of each net and weighed. Then a quarter of each piece was cut and the quarters from each net were pooled for chemical extraction with extraction done separately for the sides and top of each PermaNet 3.0. The pieces were cut to small sizes (5-10cm^2) for homogenization. For the PermaNet 2.0 and the sides of the PermaNet 3.0, deltamethrin was extracted by sonication and shaking in isooctane/dioxane (80/20, v/v) (baseline samples only) or heptane in presence of dipropyl phthalate (baseline samples only) or dicyclohexyl phthalate as internal standards. Deltamethrin content was determined by normal phase High Performance Liquid Chromatography with UV Diode Array Detection (HPLC-DAD) (CIPAC 333/LN/(M)/3). For the top panels of the PermaNet 3.0, deltamethrin was extracted by refluxing for 30 minutes in xylene with dibutyl phthalate (baseline samples only) or dicyclohexyl phthalate as internal standards, solvent exchange to the mobile phase (baseline samples only) or hexane and determination of deltamethrin content was by normal phase High Performance Liquid Chromatography with UV Diode Array Detection (HPLC-DAD). For estimate of PBO content in the top panels of the PermaNet 3.0, extraction was done by refluxing for 30 minutes with xylene in presence of octadecane (baseline samples only) or dicyclohexyl phthalate as internal standards and determination was by Gas Chromatography using Flame Ionisation Detection (GC-FID).

Data analysis

Data analysis was done by study (cohort vs. cross-sectional) as well as by follow up. For binary variables (specific adverse events, presence/absence of a net, presence/absence of holes, use and washing of nets, mortality/knockdown of mosquitoes), outcomes were expressed as percentages calculated using the SURVEYFREQ procedure in SAS. Confidence limits were calculated while adjusting for clustering at the household level and comparisons between net types were done using c2 tests. For continuous variables such as the number of holes on nets, the total area damaged and the chemical content on nets, mean and median values were calculated along with 95% confidence limits and interquartile ranges using the MEANS procedure in SAS.

Results

Monitoring data

To ensure that the nets were present and hung in the households and being used by the residents, a household survey was conducted approximately 1 month after distribution. The nets were checked and assistance was provided if residents required help in hanging the nets. A total of 3,115 nets were distributed in June 2015. Overall, 2962 (95.1%) of the nets were reported present in the household. Of the 2962 nets present in the household 91.4% were hanging over the bed/sleeping places. The main reasons given for why the 254 nets that were not hanging were: the household already had nets hanging (n=130, 51.2% of nets not hanging), the nets were too difficult to hang (n=11, 4.3% of nets not hanging), or the owners don't like to use nets (n=7, 2.8%)of nets not hanging. In addition, for 105 nets (41.3% of those not hanging), no specific reason was given.

Adverse effects

Nets users or their guardians were visited in October 2015 to assess the frequency of adverse events associated with the different net types. Nearly 30% of respondents reported that they had experienced at least 1 adverse event since receiving their nets. The most commonly reported adverse effects included paresthesia (skin tingling), burning sensations around the face, eye irritation or watery eyes and sneezing or runny nose. A smaller percentage of people reported headaches or nausea. Net users also reported a bad smell associated with the nets. However, there were no statistically significant differences between the PermaNet 2.0 and the PermaNet 3.0 in the frequency of any of the reported adverse events except for bad smell which was reported more frequently by users of the PermaNet 2.0 (12.1% vs. 9.2%, χ^2=4.002, p=0.045). Although there were few differences in the frequency of adverse events, users of the PermaNet 3.0 were more likely to seek treatment for the adverse events than users of the PermaNet 2.0 (3.3% vs. 1.7%, χ^2=5.762, p=0.017). However, there were no differences in the frequency of net presence, net hanging, net use or plans to continue using their nets among users of the different net types (Table 1).

Table 1. Adverse events by net type reported by users at 4 months post-distribution. Percent of respondents reporting each symptom are provided with 95% confidence limits in parentheses.

Parameter	**PermaNet 2.0 N=1,607**	**PermaNet 3.0 N=1,508**	χ^2	***P***
Net present	95.3% (94.1-96.6)	94.8% (93.5-96.1)	0.312	0.577
Net hanging	87.8% (85.8-89.8)	86% (83.8-88.2)	1.432	0.232
Net used last night	95.0% (93.7-96.3)	94.0% (92.7-95.4)	0.973	0.324
Any adverse event	29.8% (26.9-32.7)	28.2% (25.4-31)	0.637	0.425
Parasthesia	21.1% (18.3-23.9)	18% (15.5-20.6)	2.605	0.107
Facial burning	17.5% (15.0-20.0)	16.8% (14.3-19.2)	0.162	0.688
Eye irritation	14.9% (12.6-17.1)	13.0% (10.9-15.1)	1.418	0.234
Tears from eyes	10.2% (8.1-12.3)	8.8% (6.8-10.7)	0.992	0.319
Runny nose	10.1% (7.9-12.2)	8.2% (6.2-10.1)	1.727	0.189
Sneezing	14.3% (11.9-16.6)	14.2% (11.8-16.5)	0.001	0.972
Headache	10.1% (8-12.1)	8.4% (6.5-10.2)	1.512	0.219
Nausea	4.6% (3.4-5.8)	4.8% (3.6-6.1)	0.070	0.792
Bad smell	12.1% (9.9-14.2)	9.2% (7.2-11.1)	**4.002**	**0.045**
Other symptoms	0.0% (0-0)	0.1% (0-0.2)	---	---
Sought medical treatment	1.7% (1-2.5)	3.3% (2.2-4.4)	**5.726**	**0.017**
Plan to use net	97.9% (97.2-98.7)	97.7% (96.9-98.6)	0.122	0.727

Net Attrition-Cohort

Overall attrition rates in the cohort were 18.4% for the PermaNet 2.0 and 16.5% for the PermaNet 3.0 after just 6 months. Attrition rose gradually to 64.4% for the PermaNet 2.0 and 70.1% for the PermaNet 3.0 after 36 months (Figure 2, Table 2). At 12 months, the rate of attrition of the PermaNet 2.0 was significantly higher than the rate of attrition of the PermaNet 3.0 (31.0% vs. 24.5%, χ^2=3.951, p=0.047). However, there were no differences in the rate of attrition at any other follow up visit.

The reasons for net loss are shown for the PermaNet 2.0 and the PermaNet 3.0 in Figures 3 and 4, respectively. For both the PermaNet 2.0 and the PermaNet 3.0, the primary reasons for nets loss through 18 months were unrelated to the quality of the net. Most nets that were lost were due to the nets being given away or for unknown reasons, generally because the household was locked and no information could be obtained on the status of the nets. Beginning at the 24 month follow up, an increasing proportion of nets were lost due to damage. For the PermaNet 2.0, 13.2%, 33.3% and 44.5% of nets were reported lost or discarded due to damage after 24, 30 and 36 months, respectively. For the PermaNet 3.0, these figures were 13.7%, 33.2%, and 49.9%

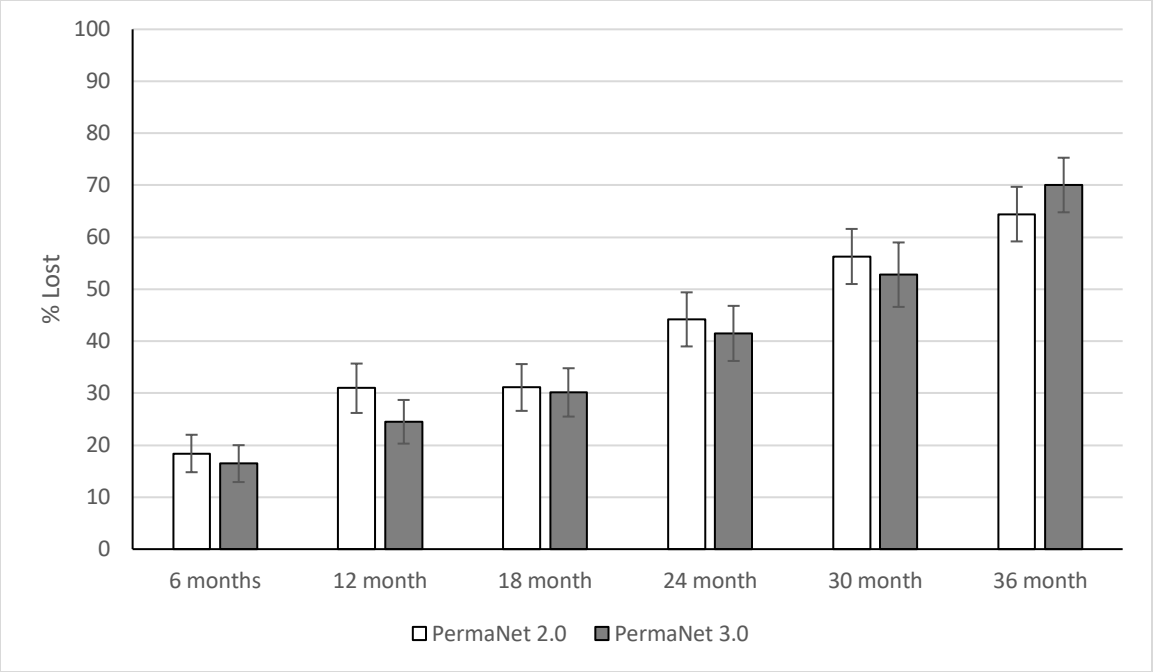

Figure 2. Overall attrition by follow up for the PermaNet 2.0 and the PermaNet 3.0 in the cross-sectional study.

Table 2. Percent overall net attrition (95% confidence limits) in the cohort study.

Follow Up	**PermaNet 2.0 N=604**	**PermaNet 3.0 N=547**	χ^2	***P***
6 months	18.4% (14.8-22)	16.5% (12.9-20)	0.568	0.451
12 month	31.0% (26.2-35.7)	24.5% (20.3-28.7)	**3.951**	**0.047**
18 month	31.1% (26.6-35.6)	30.2% (25.5-34.8)	0.086	0.760
24 month	44.2% (39-49.4)	41.5% (36.2-46.8)	0.521	0.470
30 month	56.3% (51-61.6)	52.8% (46.6-59)	0.711	0.399
36 month	64.4% (59.2-69.7)	70.1% (64.8-75.3)	2.292	0.130

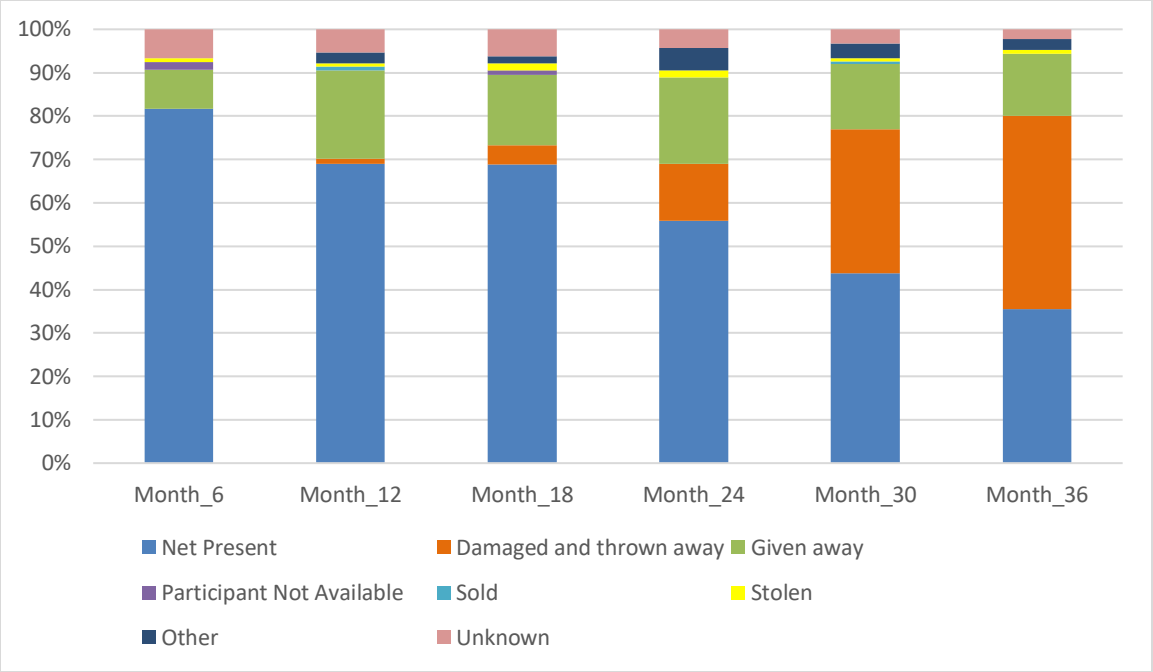

Figure 3. Reasons for PermaNet 2.0 attrition in the cohort study.

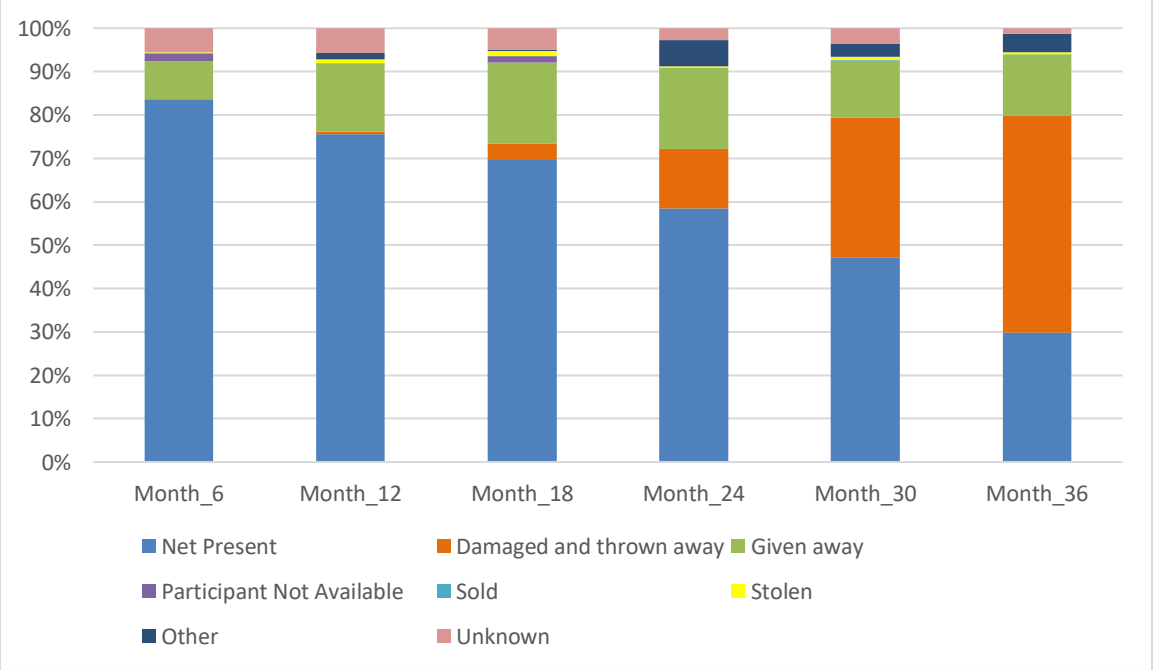

Figure 4. Reasons for PermaNet 3.0 attrition in the cohort study.

Net Attrition-Cross Sectional

Patterns of net loss in the cross-sectional arms of the study were similar to those observed in the cohort. The nets in this arm of the study did not represent a true cohort as nets were removed for bioassays and chemical assays at each time point. However, the net loss was estimated by adjusting the denominator at each time point to account for the sampled nets. The overall attrition for the PermaNet 2.0 rose from 15.1% after 6 months to 56.7% after 36 months (Figure 5, Table 3). For the PermaNet 3.0, overall attrition rose from 19.6% after 6 months to 68.3% after 36 months. The rate of attrition was generally higher for the PermaNet 3.0 with significant differences observed at the 6 month, 24 month, 30 month and 36 month follow ups.

Similar to the cohort study, the primary reasons for the loss of nets was largely unrelated to the physical qualities of the nets through the 18 month follow up with the primary reasons again being that nets were given away or the owners were not available to assess the status of the nets. Loss due to damage became more apparent at the 24 month follow up with 10.8%, 24.2%, 41.9% lost at 24, 30 and 36 months, respectively (Figure 6). For the PermaNet 3.0, these figures were 12.5%, 30.0%, and 48.5% (Figure 7).

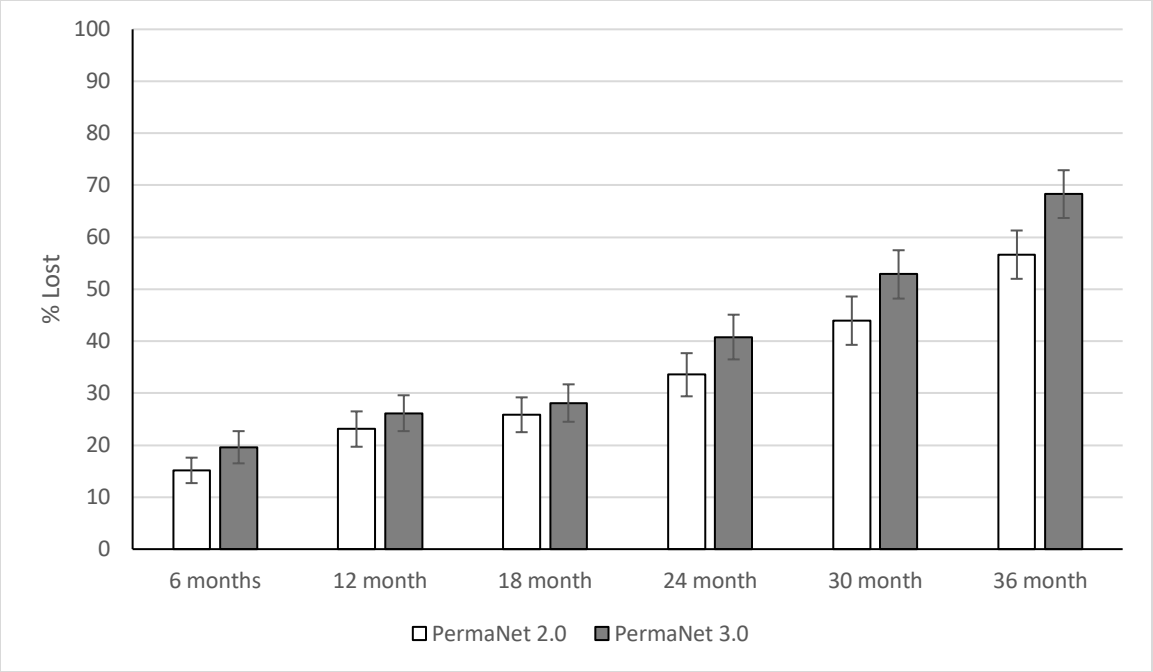

Figure 5. Overall attrition by follow up for the PermaNet 2.0 and the PermaNet 3.0 in the cross-sectional study.

Table 3. Percent overall net attrition (95% confidence limits) in the cross-sectional study.

Follow Up	PermaNet 2.0	PermaNet 3.0	χ^2	*P*
6 months	15.1% (12.7-17.6) N=1,004	19.6% (16.5-22.7) N=960	**5.199**	**0.023**
12 month	23.1% (19.7-26.5) N=974	26.1% (22.7-29.6) N=930	1.553	0.213
18 month	25.8% (22.5-29.2) N=944	28.1% (24.5-31.7) N=900	0.811	0.368
24 month	33.6% (29.4-37.7) N=915	40.8% (36.5-45.1) N=870	**5.597**	**0.018**
30 month	44% (39.3-48.6) N=885	52.9% (48.2-57.5) N=840	**7.124**	**0.008**
36 month	56.7% (52-61.3) N=861	68.3% (63.7-72.9) N=802	**12.432**	**<0.001**

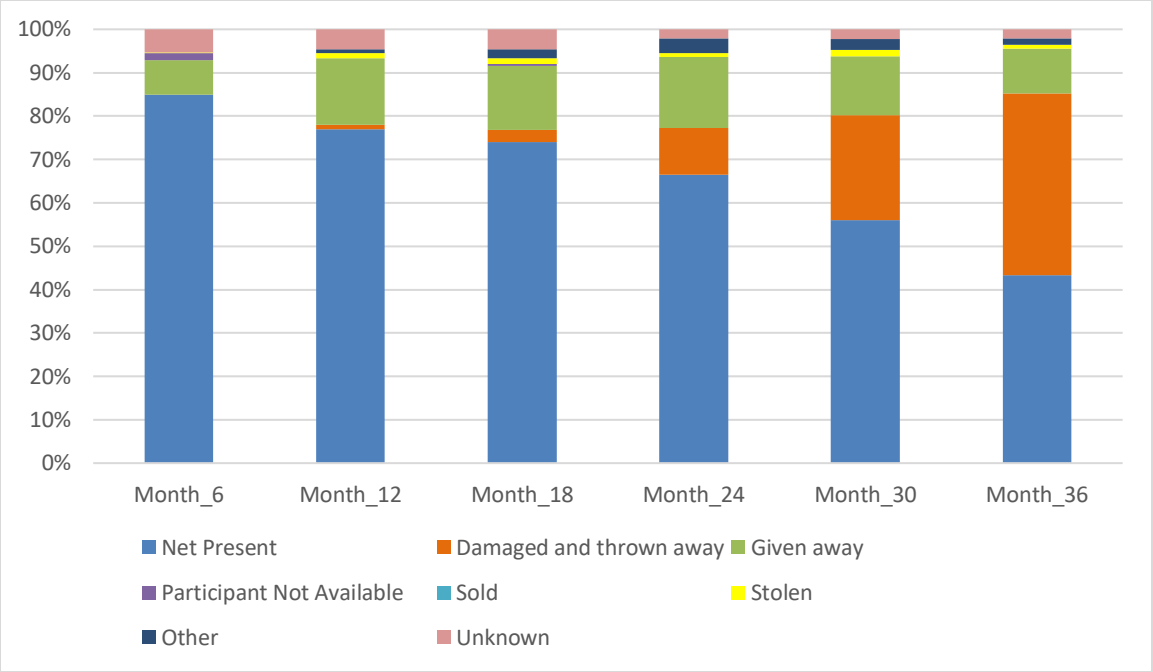

Figure 6. Reasons for PermaNet 2.0 attrition in the cohort study.

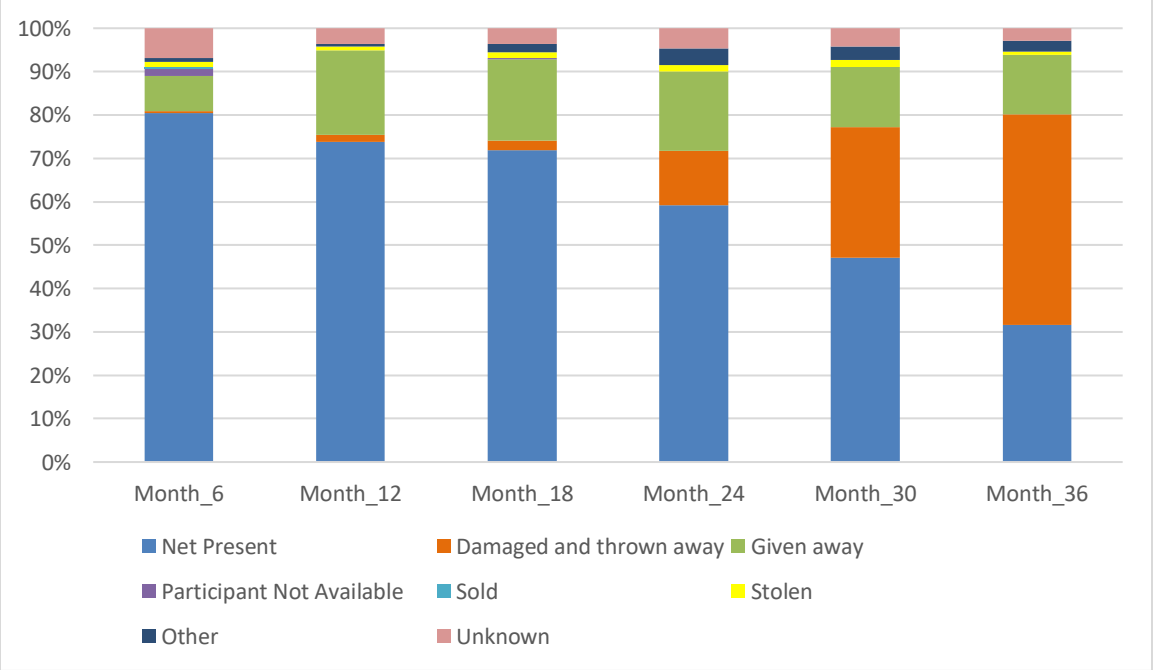

Figure 7. Reasons for PermaNet 3.0 attrition in the cohort study.

Physical integrity-Cohort

The physical integrity of the nets in the cohort study were monitored by staff in the field at the time of each follow up. A summary of net condition is provided in Table 4. The proportion of PermaNet 2.0 nets with at least one hole rose from 8.5% after 6 months to 74.0% at 36 months. For the PermaNet 3.0, 7.2% of nets had at least one hole at 6 months while 74.4% had at least one hole after 36 months (Figure 8). Overall damage was variable with most nets having no or few holes while a few nets had extensive damage. The median area of damage was zero through the 18 month follow up for the PermaNet 3.0 and through the 24 month follow up for the PermaNet 2.0. At 36 months, the median area of damage was 84.8 cm^2 for the PermaNet 2.0 and 74.4 cm^2 for the PermaNet 3.0 (Figure 9). The percentage of PermaNet 2.0 nets needing replacement based on WHO guidelines (pHI>642) was 0% at 6 months but rose to 27.9% at 36 months. The percentage of PermaNet 3.0 nets in need of replacement was 1.2% at 6 months and rose to 31.9% at 36 months (Figure 10). The percentage of nets with visible repairs was generally low (<10%) although at 36 months, 18.7% of PermaNet 2.0 nets and 21.3% of PermaNet 3.0 nets had visible repairs (Figure 11).

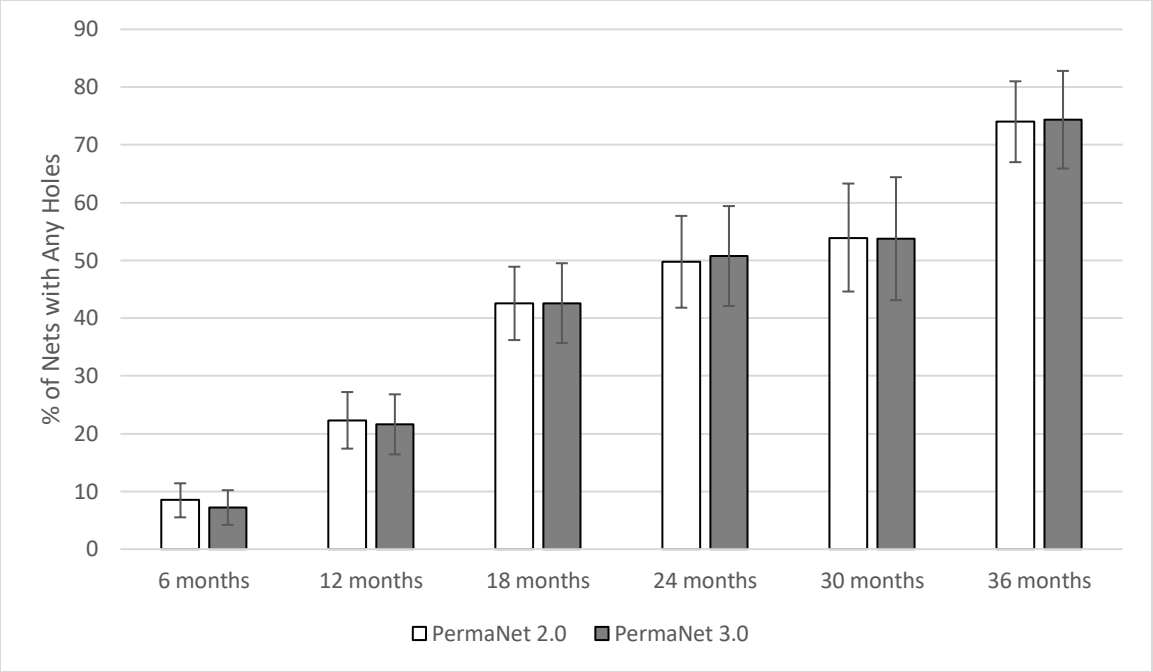

Figure 8. Percent of nets with at least one hole.

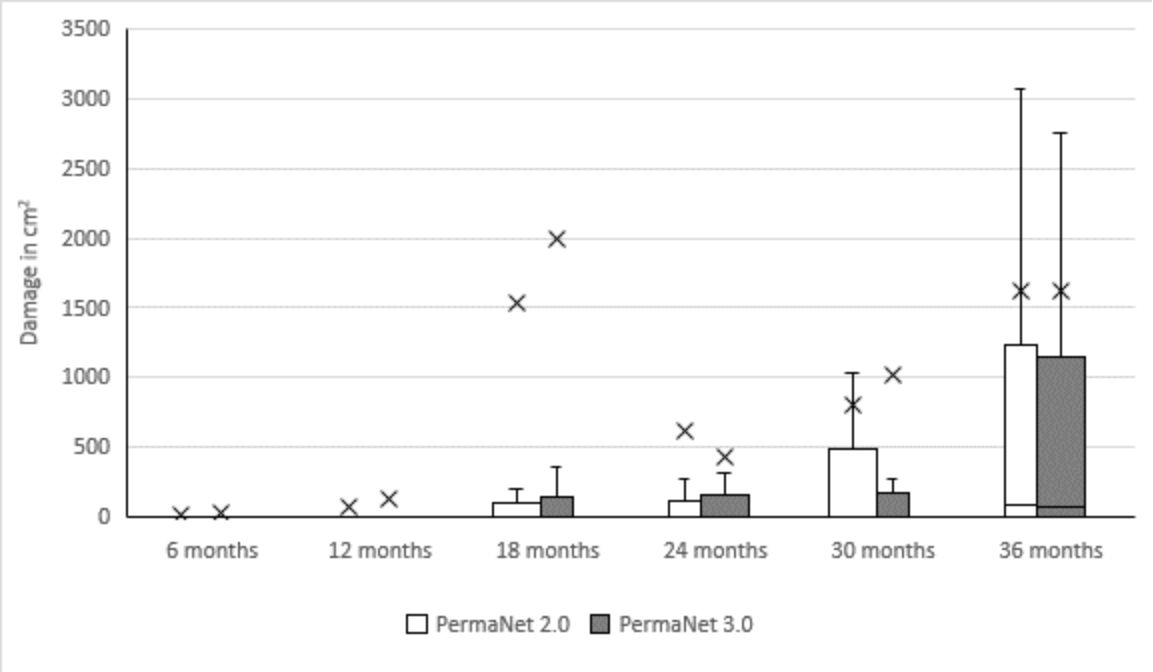

Figure 9. Whisker plot showing mean, median and interquartile estimates of damage on nets in the cohort study in square centimeters.

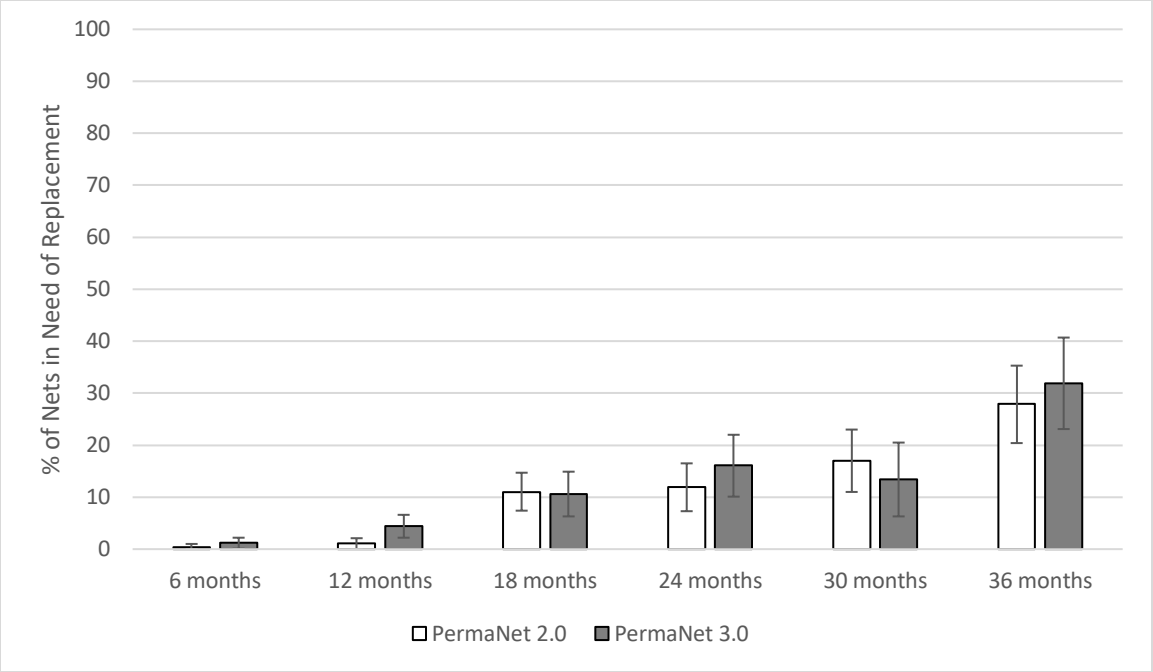

Figure 10. Percent of nets in the cohort study in need of replacement based on observed physical damage (pHI>642).

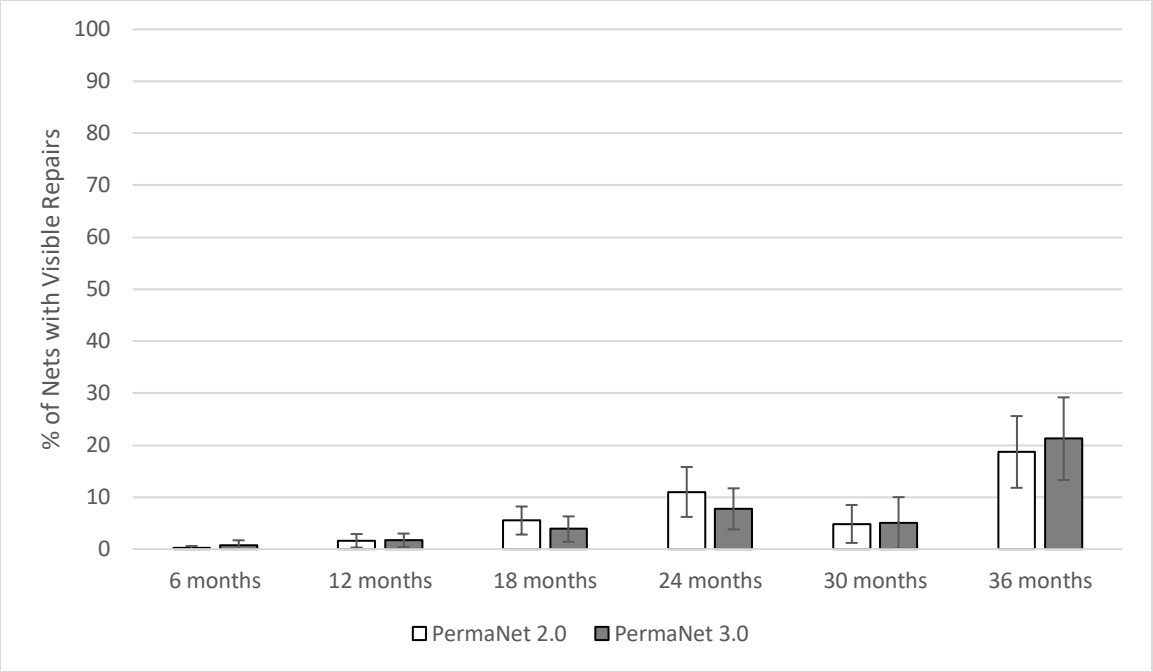

Figure 11. Percent of nets in the cohort study with visible repairs.

Table 4. Summary of net conditions in the cohort study. Figures in brackets indicate 95% confidence limits.

Follow Up	Variable	PermaNet 2.0	PermaNet 3.0
6 months	Any holes	8.5% (5.5-11.4)	7.2% (4.2-10.2)
	Mean damage, cm^2	14.5 (4.4-24.6)	23.3 (7-39.7)
	Median damage, cm^2	0 (0-0)	0 (0-0)
	Condition, Good	97% (95.4-98.5)	97% (95.3-98.7)
	Condition, Acceptable	2.6% (1.2-4)	1.9% (0.6-3.1)
	Condition, Replace	0.4% (0-1)	1.2% (0.1-2.2)
	Repairs	0.2% (0-0.6)	0.7% (0-1.7)
12 months	Any holes	22.3% (17.4-27.2)	21.6% (16.4-26.8)
	Mean damage, cm^2	67 (25.5-108.4)	129.8 (68.5-191)
	Median damage, cm^2	0 (0-0)	0 (0-0)
	Condition, Good	90.4% (87-93.9)	87.8% (84-91.6)
	Condition, Acceptable	8.5% (5.2-11.8)	7.8% (4.7-10.8)
	Condition, Replace	1.1% (0-2.1)	4.4% (2.2-6.6)
	Repairs	1.6% (0.3-2.9)	1.7% (0.4-3)
18 months	Any holes	42.5% (36.2-48.9)	42.6% (35.7-49.5)
	Mean damage, cm^2	1538.9 (182.9-2894.9)	1990.3 (-558.5-4539.2)
	Median damage, cm^2	0 (0-92.2)	0 (0-143.9)
	Condition, Good	74.3% (68.7-80)	72.9% (67.1-78.7)
	Condition, Acceptable	14.7% (10.3-19.1)	16.5% (12-21.1)
	Condition, Replace	11% (7.4-14.7)	10.6% (6.3-14.9)
	Repairs	5.5% (2.8-8.2)	3.9% (1.4-6.3)
24 months	Any holes	49.8% (41.8-57.7)	50.8% (42.1-59.4)
	Mean damage, cm^2	621.6 (201.2-1042)	423.5 (264.9-582.1)
	Median damage, cm^2	0 (0-114.4)	1.2 (0-147.6)
	Condition, Good	73.1% (66.5-79.8)	70.5% (62.5-78.4)
	Condition, Acceptable	15% (9.6-20.3)	13.5% (8.4-18.5)
	Condition, Replace	11.9% (7.3-16.5)	16.1% (10.1-22)
	Repairs	11% (6.2-15.8)	7.8% (3.8-11.7)
30 months	Any holes	53.9% (44.6-63.3)	53.8% (43.1-64.4)
	Mean damage, cm^2	797 (400.6-1193.4)	1016.9 (74.6-1959.2)
	Median damage, cm^2	2.5 (0-483.6)	2.5 (0-173.4)
	Condition, Good	63% (54.6-71.4)	71.4% (62-80.9)
	Condition, Acceptable	20% (13.5-26.5)	15.1% (8.3-21.9)
	Condition, Replace	17% (11-23)	13.4% (6.3-20.5)
	Repairs	4.8% (1.2-8.5)	5% (0.1-10)
36 months	Any holes	74% (67-81)	74.4% (65.9-82.8)
	Mean damage, cm^2	1621.2 (1057-2185.4)	1616.4 (981.6-2251.2)
	Median damage, cm^2	84.8 (0-1235.7)	74.4 (0-1140.8)
	Condition, Good	49.3% (41-57.6)	50% (40.7-59.3)
	Condition, Acceptable	22.8% (16.9-28.7)	18.1% (11.5-24.7)
	Condition, Replace	27.9% (20.4-35.3)	31.9% (23.1-40.7)
	Repairs	18.7% (11.8-25.6)	21.3% (13.3-29.2)

Bioassays

Average adjusted mortality and knockdown of mosquitoes in cone bioassays against the PermaNet 2.0, and the side and top panels of the PermaNet 3.0 are provided in Figures 12 and 13, respectively. Average mortality was equal to or greater than the WHOPES recommended threshold of 80% through the 30 month follow up for both the PermaNet 2.0 and the side and top panels of the PermaNet 3.0. At the 36 month follow up, only the top panel of the PermaNet 3.0 achieved an average mortality above 80%. For the PermaNet 3.0, average knockdown on the side and top panels was above the WHOPES recommended threshold of 95% through 36 months with the exception of the 24 month follow up when average knockdown on the side panels was 94.0%. Average knockdown on the PermaNet 2.0 nets was below 95% for the 24, 30 and 36 month follow ups. However, the lowest average knockdown was 89.9% at 36 months.

As summary of the percentage of nets meeting WHOPES criteria for the cone test is provided in Table 5. For the PermaNet 2.0, >95% of the nets met the WHOPES criteria for the cone test through 18 months. At the 24 and 30 month follow ups, >85% of the nets still met the criteria. However, at the 36 month follow up, only 70% of PermaNet 2.0 nets met the WHOPES criteria for the cone test. The nets that failed by the cone test were subjected to tunnel tests and an additional 20% passed according to the tunnel test for an overall pass rate of 90%. For the side panels of the PermaNet 3.0, 100% passed by the cone test through 30 months with the exception of the 24 month follow up when the pass rate by the cone test was 86.7%. At the 36 month follow up, 90% passed by the cone test. An additional 6% passed by the tunnel test for an overall pass rate of 96%. However, it should be noted that one side panel was not subjected to the tunnel test so the overall pass rate

would be either 96% or 98%. For the top panel of the PermaNet 2.0, the pass rate by the cone test alone was 100% through the 12 month follow up and was ≥90% through the remaining follow ups. At 36 months, the pass rate by the cone test was 90%. An additional 2 nets passed by the tunnel test resulting in a total pass rate of 94%. However, two nets were not tested in the tunnel test which means the overall pass rate was between 94% and 98%.

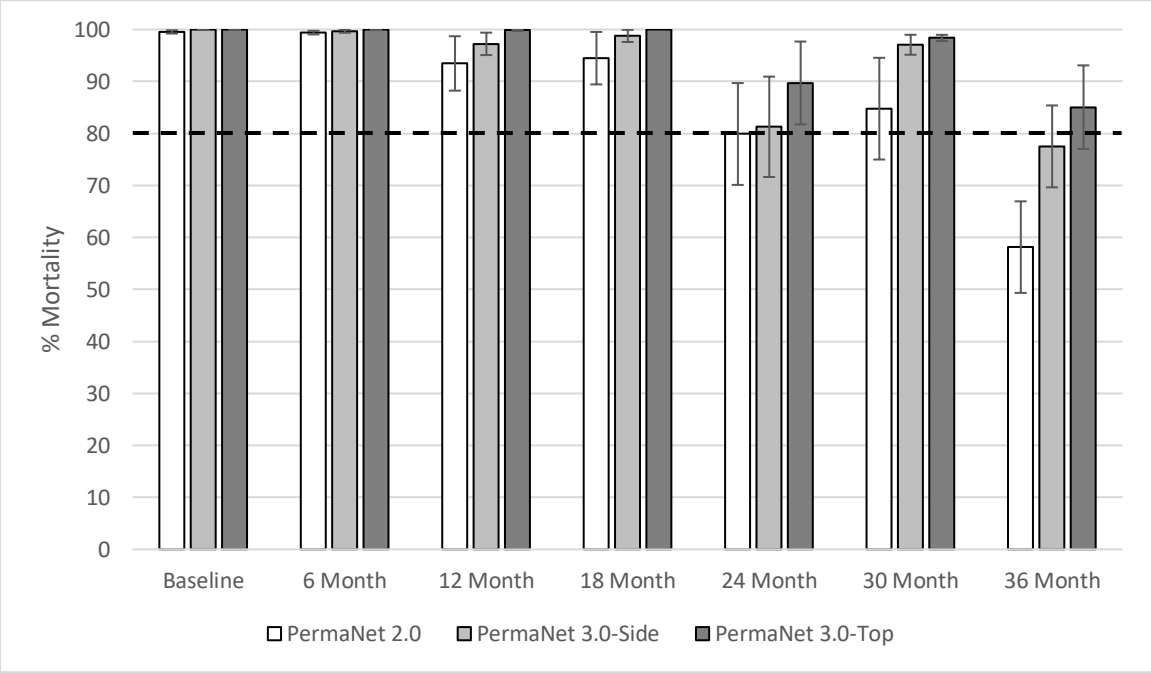

Figure 12. Adjusted mortality of *An. gambiae* Kisumu strain in cone bioassays. Dashed line indicates minimum efficacy criteria for mortality in the cone test.

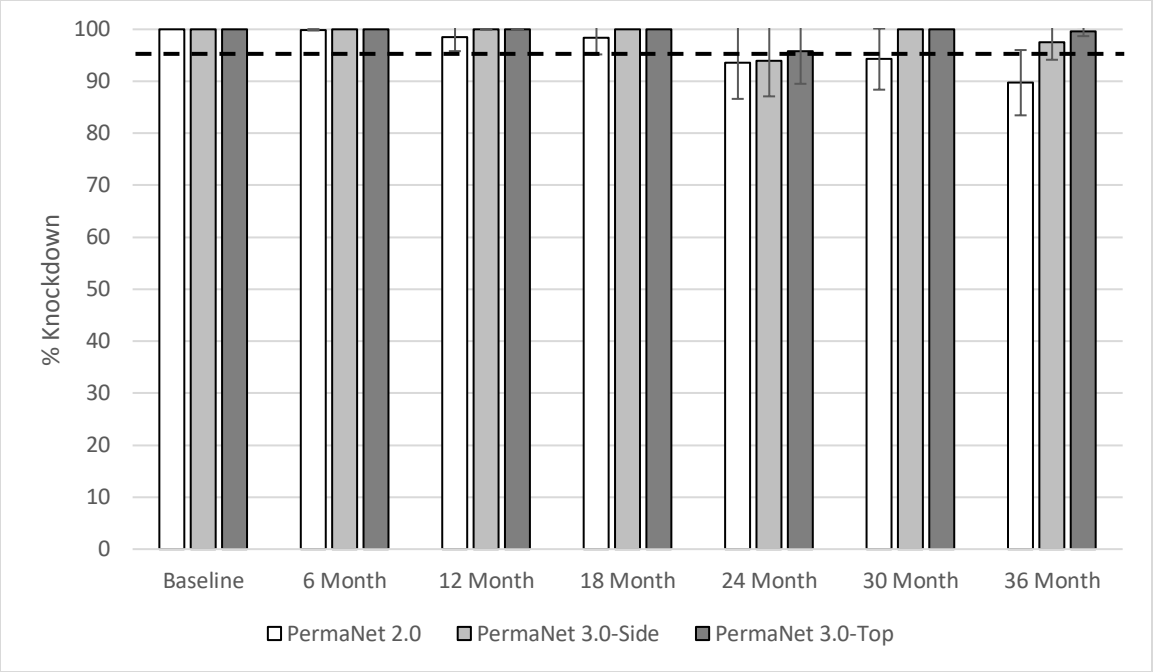

Figure 13. Adjusted knockdown of *An. gambiae* Kisumu strain in cone bioassays. Dashed line indicates minimum efficacy criteria for mortality in the cone test.

Table 5. Summary of bioassay results including the number of nets tested, the number meeting the criteria for the cone test and the number meeting the criteria for the tunnel test as well as the overall combined pass rate.

Net type	Parameter	Baseline	6 months	12 months	18 months	24 months	30 months	36 months
PermaNet 2.0	N	30	30	30	30	30	29	50
	Cone	30	30	29	29	26	25	35
	Tunnel	--	--	--	--	--	--	10
	% Passing	100	100	97	97	87	86	90
PermaNet 3.0	N	30	30	30	29	30	32	50
Side Panels	Cone	30	30	30	29	26	32	45
	Tunnel	--	--	--	--	--	--	3*
	% Passing	100	100	100	100	87	100	96
PermaNet 3.0	N	30	30	29	29	30	31	50
Top Panel	Cone	30	30	29	28	28	30	45
	Tunnel	--	--	--	--	--	--	2*
	% Passing	100	100	100	97	93	97	94

*Note that one side panel and two top panels from nets that did not pass the cone test were not available for the tunnel test.

Chemical assays

The chemical content of the PermaNet 2.0 and the side and top panels of the PermaNet 3.0 are provided in Figures 14 to 17 and in Table 6. At baseline, all nets were within specification for deltamethrin content and PBO content (PermaNet 3.0 top panels). For the PermaNet 2.0, average deltamethrin content declined steadily from 1.2 g/kg at baseline to 0.4 g/kg at 36 months. Similar loss of deltamethrin was observed for the side panels of the PermaNet 3.0 where 1.9 g/kg was observed at baseline, declining to 0.8 g/kg after 36 months. For the top panels, the deltamethrin content was 4.7 g/kg at baseline and 2.7 g/kg after 36 months. The PBO content on the top panel of the PermaNet 3.0 was 26.1 g/kg at baseline but this declined rapidly to 11.0 g/kg after 12 months. The loss of PBO then declined to 5.3 g/kg by 36 months.

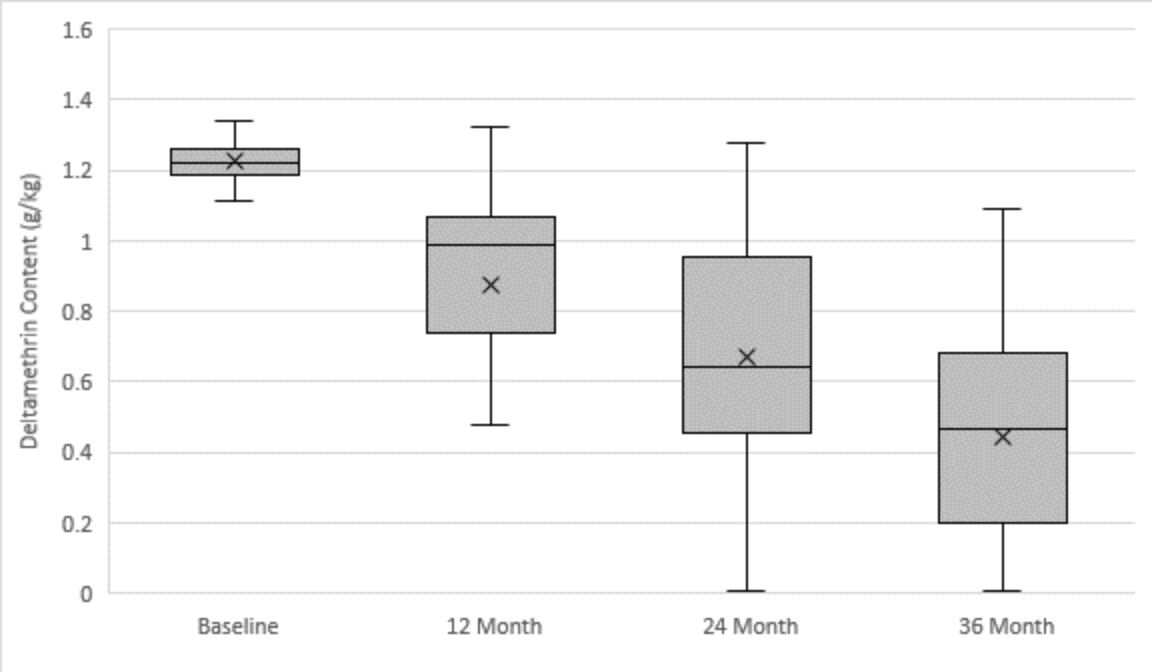

Figure 14. Deltamethrin content on PermaNet 2.0 nets. Target dose = 1.4 g/kg ± 25%.

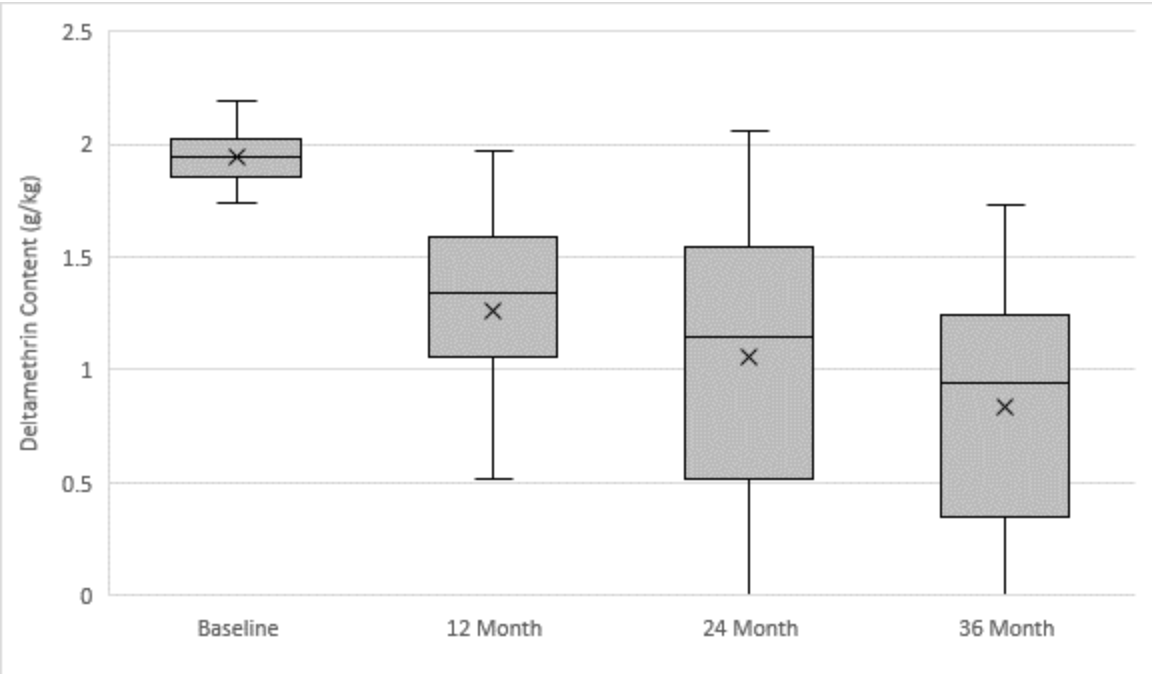

Figure 15. Deltamethrin content on the side panels of PermaNet 3.0 nets. Target dose = 2.1 g/kg ± 25%.

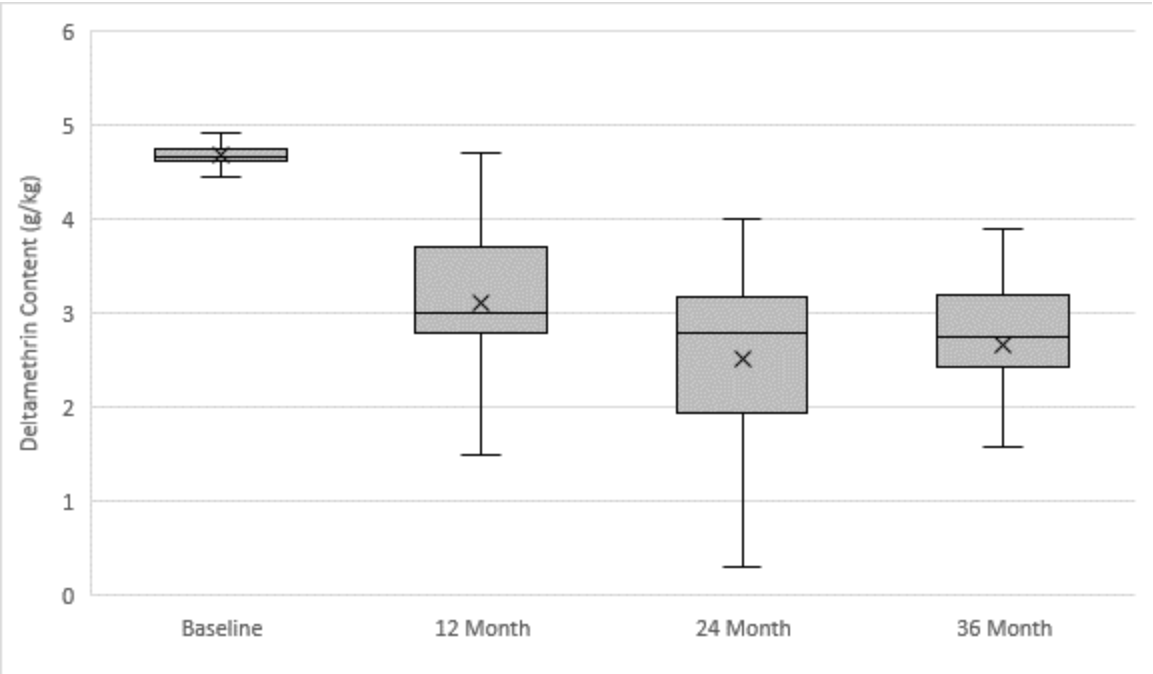

Figure 16. Deltamethrin content on the top panels of PermaNet 3.0 nets. Target dose = 4.0 g/kg ± 25%.

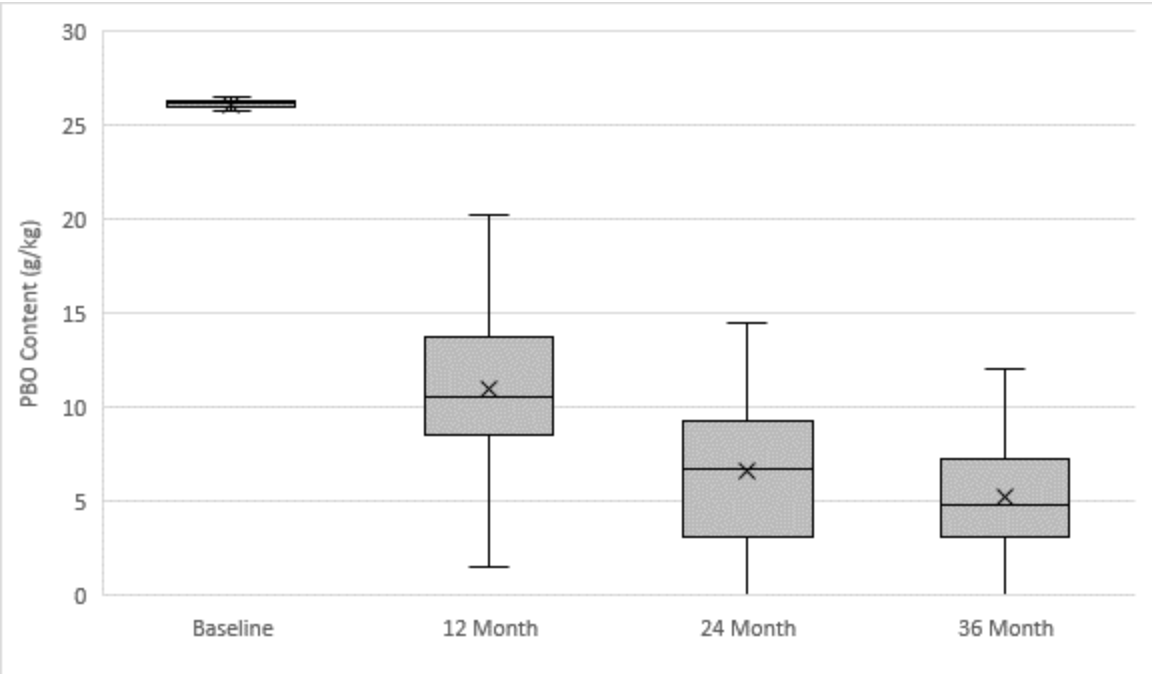

Figure 17. PBO content on the top panels of PermaNet 3.0 nets. Target dose = 25.0 g/kg ± 25%.

Table 6. Mean (95% CI) and median (IQR) deltamethrin and PBO content on PermaNet 2.0 and PermaNet 3.0 by follow up.

FollowUp	Variable	PermaNet 2.0 Deltamethrin	PermaNet 3.0, Sides Deltamethrin	PermaNet 3.0, Top Deltamethrin	PermaNet 3.0, Top PBO
Baseline	Mean content, g/kg	1.2(1.2-1.3)	1.9(1.9-2)	4.7(4.6-4.7)	26.1(26-26.2)
	Median content, g/kg	1.2(1.2-1.3)	1.9(1.9-2)	4.7(4.6-4.8)	26.1(26-26.3)
	Mean content, mg/m^2	55.1(53.9-56.4)	85.2(82.4-88)	182.1(179.4-184.9)	1016.4(1002.7-1030)
	Median content, mg/m^2	54.6(52.5-57)	85.3(80.8-88.7)	181.6(178-187.1)	1007.7(993.4-1040.9)
12 Month	Mean content, g/kg	0.9(0.8-1)	1.3(1.1-1.4)	3.1(2.8-3.4)	11(9.2-12.7)
	Median content, g/kg	1(0.7-1.1)	1.3(1.1-1.6)	3(2.8-3.7)	10.6(8.7-13.6)
	Mean content, mg/m^2	42(36.7-47.4)	58.7(50.5-67)	132.9(122.5-143.4)	465.5(392.3-538.7)
	Median content, mg/m^2	45.5(37-49.5)	63(50.2-72.4)	131.5(117-155)	455(352-521)
24 Month	Mean content, g/kg	0.7(0.5-0.8)	1.1(0.8-1.3)	2.5(2.1-2.9)	6.6(5-8.2)
	Median content, g/kg	0.6(0.5-0.9)	1.1(0.5-1.5)	2.8(2-3.1)	6.8(3.1-9.2)
	Mean content, mg/m^2	32(26.5-37.4)	51.3(40.2-62.3)	114(98.8-129.2)	293.3(222.2-364.3)
	Median content, mg/m^2	29.5(24.2-43.8)	54(26.7-77)	122.5(95-146)	316.5(145-391)
36 Month	Mean content, g/kg	0.4(0.4-0.5)	0.8(0.7-1)	2.7(2.4-2.9)	5.3(4.2-6.3)
	Median content, g/kg	0.5(0.2-0.7)	0.9(0.4-1.2)	2.7(2.5-3.2)	4.8(3.2-7.1)
	Mean content, mg/m^2	21.4(17.2-25.7)	39.2(32.2-46.2)	118.6(108.9-128.3)	229.7(183.9-275.5)
	Median content, mg/m^2	22.8(10.5-33.8)	45.4(18.2-57.3)	120(110-140)	211(148-328)

Net use and care

The percentage of net owners in the cohort study who reported that they used their nets the previous night are shown in Figure 18 and Table 7. Net use was >90% for the 6 and 12 month follow ups and was >80% for the 18 and 24 month follow ups. However, net use declined at the 30 month when it was 60.6% and 58.2% for the PermaNet 2.0 and the PermaNet 3.0, respectively. At the 36 month follow up, these figures were 50.2% and 43.1%. However, there were no significant differences in net use among the two net types at any follow up.

Most net owners reported the reason a net was not used was because they either slept in a different location the previous night or they slept under another net. However, the number reporting that they did not use a net the previous night increased at later rounds with 30.7% of respondents reporting that a net was not used at least in part because it was too torn or too old at the 36 month follow up.

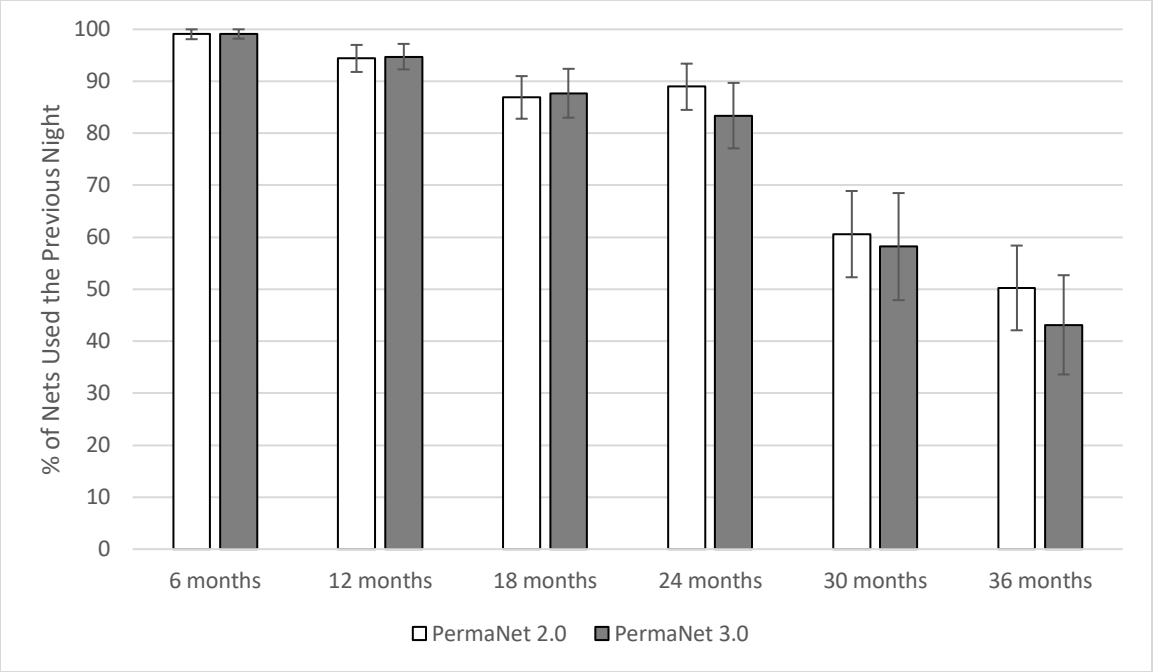

Figure 18. Percentage of nets used in the night before each follow up in the cohort study.

Table 7. Net use the previous night in the cohort study.

Follow Up	PermaNet 2.0	PermaNet 3.0	χ^2	*P*
6 months	99.1% (98.1-100)	99.1% (98.2-100)	0.008	0.927
12 month	94.4% (91.8-97)	94.7% (92.3-97.2)	0.032	0.859
18 month	86.9% (82.8-91)	87.7% (83-92.4)	0.062	0.803
24 month	89% (84.5-93.4)	83.4% (77.1-89.7)	2.134	0.144
30 month	60.6% (52.3-68.9)	58.2% (47.9-68.5)	0.129	0.719
36 month	50.2% (42.1-58.4)	43.1% (33.6-52.7)	1.389	0.239

Net washing

The percentage of nets that had ever been washed is presented in Figure 19 and Table 8. Washing rates generally increased over the 36 month follow up period but were generally relatively low with only 63.0% of PermaNet 2.0 nets and 66.9% of PermaNet 3.0 nets reported having ever been washed, even after 36 months of use. The reported rate of washing of PermaNet 3.0 nets was significantly higher at the 12 month follow up but no other differences in the reported rates of net washing were observed between the PermaNet 2.0 and the PermaNet 3.0.

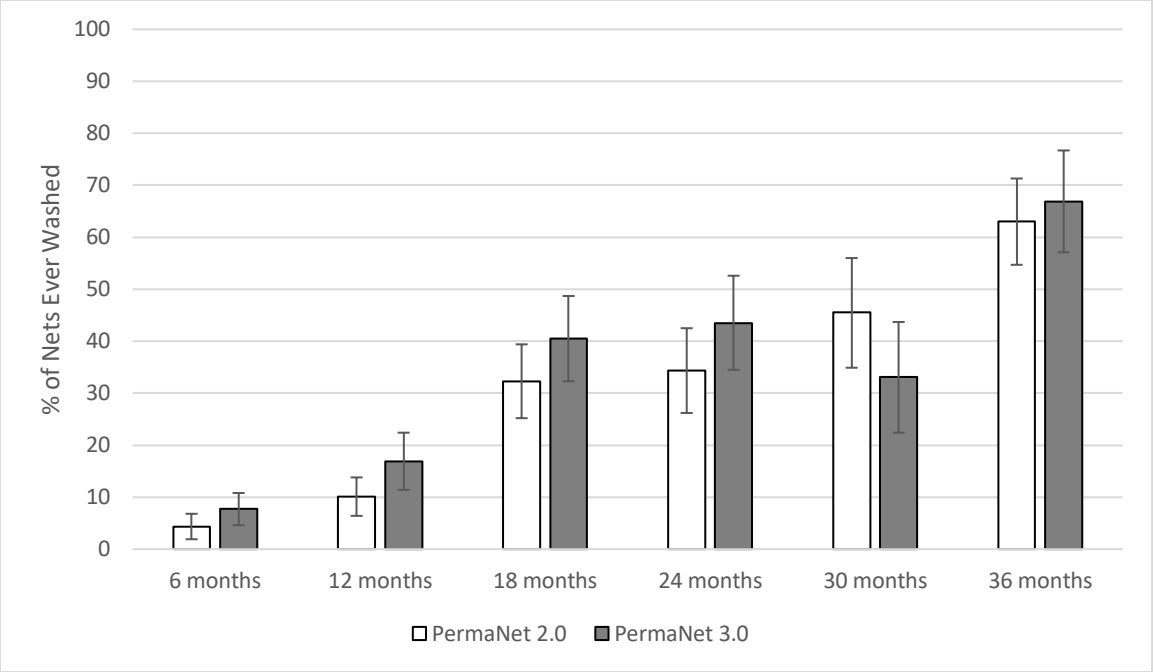

Figure 19. Percentage of nets ever washed by follow up in the cohort study.

Table 8. Frequency of reported net washing in the cohort study.

Follow Up	PermaNet 2.0	PermaNet 3.0	χ^2	*P*
6 months	4.3% (1.9-6.8)	7.7% (4.6-10.8)	3.490	0.175
12 month	10.1% (6.4-13.8)	16.9% (11.4-22.4)	**4.526**	**0.033**
18 month	32.3% (25.2-39.4)	40.5% (32.3-48.7)	2.131	0.144
24 month	34.4% (26.2-42.5)	43.5% (34.5-52.6)	2.518	0.113
30 month	45.5% (34.9-56)	33.1% (22.4-43.7)	3.546	0.170
36 month	63.0% (54.7-71.3)	66.9% (57.1-76.7)	0.470	0.493

Discussion

The PermaNet 3.0 was evaluated in a phase III study following WHOPES guidelines to assess the insecticidal activity, attrition, physical integrity and community acceptance under field conditions in western Kenya. Both the side and top panels of the PermaNet 3.0 meet the criteria for an LLIN based upon the cone test alone as more than 80% of nets had sufficient bioefficacy in the cone test at 36 months after distribution. Furthermore, several nets that did not meet the efficacy criteria for the cone test passed by the tunnel test. Overall, >90% of the PermaNet 3.0 met the WHOPES efficacy criteria for an LLIN after 36 months of use.

The PermaNet 3.0 has been promoted as a potential tool to address pyrethroid resistance {Gleave, 2018 #583} which is widespread in multiple Anopheles species in Africa {Ranson, 2016 #574}. This study was not designed to address the efficacy of the PermaNet 3.0 against pyrethroid resistant mosquitoes as a susceptible strain was used in this study. However, the strain was subsequently found to be contaminated with the kdr allele (L1014S) which confers low levels on insecticide resistance. Furthermore, in comparison to the PermaNet 2.0, the PermaNet 3.0 generally had higher levels of insecticidal activity in the cone bioassays, particularly after the 24 month follow up. PBO acts as a synergist by inhibiting the activity of oxidase enzymes that are implicated in pyrethroid resistance. In this study, the efficacy of the side panels was similar to that of the top panels suggesting that the higher levels of deltamethrin on the PermaNet 3.0 may account for any increased biological efficacy compared to the PermaNet 2.0. The WHO Vector Control Advisory Group has provided support for the claim of increased bioefficacy of the PermaNet 3.0 and WHO has now provided guidance on the deployment of PBO LLINs such as the PermaNet 3.0. Further research, including local studies to assess resistance mechanisms and the efficacy of the PermaNet 3.0 against different strains of *Anopheles* vectors of malaria, are necessary to guide the deployment of this LLIN.

In addition to the bioefficacy criteria, the PermaNet 3.0 was also evaluated for attrition and physical durability as well as community acceptability. In a survey conducted approximately 4 months after distribution, the reported rates of adverse events among users of the PermaNet 3.0 were similar to those of the PermaNet 2.0. The only statistically significant difference observed was in the rates of seeking medical care. Users of the PermaNet 3.0 sought medical care for adverse events more frequently than users of the PermaNet 2.0. However, there was no difference in the percentage of respondents who indicated they planned to continue using their nets.

In the cohort study, overall attrition was significantly higher at the 12 month follow up. No other differences were observed in the overall attrition at other time points in the cohort study. However, attrition was generally higher among the PermaNet 3.0 nets in the cross-sectional study. It is not clear why there were differences among the two net types. Reasons for net loss were generally similar although 44.5% versus 49.9% of PermaNet 3.0 nets were reported lost due to damage after 36 months in the cohort study. The difference was not statistically significant. In the cross-sectional study, 41.9% of the PermaNet 2.0 and 48.5% of the PermaNet 3.0 were lost due to damage at 36 months. Again, the differences were not statistically significant. These analyses suggest the differences were due to chance alone.

Indicators of physical condition of the LLINs in the cohort study were not significantly different between the PermaNet 2.0 and the PermaNet 3.0. The percentage of nets with at least one hole gradually increased in both net types from <10% at 6 months to over 70% after 36 months. There were no significant differences between the PermaNet 2.0 and the PermaNet 3.0 at any time point. Similarly, the proportion of nets that were determined to be in need of replacement gradually increased from 0.4% and 1.2% for the PermaNet 2.0 and the PermaNet 3.0 respectively at 6 months after distribution to 27.9% and 31.9% after 36 months. The differences in the proportion of nets in good, acceptable or replace were not statistically different at any time point except for the 12 month follow up.

Chemical analysis data indicated that both the PermaNet 2.0 and the PermaNet 3.0 nets were within specifications at baseline before net distribution. Annual testing of chemical content showed a gradual decline in deltamethrin content on both nets. After 3 years, 66.7% of deltamethrin was lost from the PermaNet 2.0 while approximately 57.9% of the deltamethrin was lost from the side panels of the PermaNet 3.0 and 42.5% was lost from the top panels. In contrast, 79.7% of the PBO had been lost from the top panels. Furthermore, much of the lost occurred in the first year when the PBO content declined from 26.1 g/kg to 11.0 g/kg. The effect of the high loss of PBO is cannot be assessed as susceptible mosquitoes were used in the cone bioassays. Additional studies should be conducted to assess the longevity of the PermaNet 3.0 against pyrethroid resistant strains to determine the effect of the high loss of PBO.

Finally, net use and washing were assessed to determine if these might have affected the outcome of the study. Net use steadily declined through 24 months after distribution but remained above 80%. After 24 months, net use dropped more rapidly. This may have been due to increasing rates of attrition over time. However, it may also be partly explained by the distribution of LLINs by the Kenya National Malaria Control Programme in early 2017. While net use declined throughout the study for both the PermaNet 2.0 and the PermaNet 3.0, no differences were observed in these outcomes with the exception of a higher washing rate among PermaNet 3.0 nets after 12 months. These results suggest that the handling of the PermaNet 3.0 was not substantially different from other nets and that this should not have affected the outcome of the study.

Conclusion

In summary, the PermaNet 3.0 meets the phase III criteria for evaluation of a long-lasting net based on bioassays with a susceptible strain of *An. gambiae*. Furthermore, the overall attrition, the attrition due to damage and the physical integrity of the PermaNet 3.0 were similar to that of an LLIN with a full recommendation (i.e. the PermaNet 2.0). Therefore, the PermaNet 3.0 can be

expected to provide effective protection for 3 years under regular use conditions. The study did not evaluate the potential for the PermaNet 3.0 to be a tool for mitigation of insecticide resistance but PBO LLINs are now recommended for areas with pyrethroid resistance that is mediated by oxidase based mechanisms. Increased monitoring of pyrethroid resistance, including the effect of PBO pre-exposure is recommended to guide the deployment of PBO nets such as the PermaNet 3.0.

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