Long-Term Insecticidal Activity and Physical Integrity of Olyset Nets in Tafea Province, Vanuatu

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ABSTRACT The long-term efficacy of long-lasting insecticidal nets (LLINs) depends on both the physical condition of the net and the residual activity of the insecticide. This study focused on monitoring these parameters in Olyset nets (Sumitomo Chemical Co., Osaka, Japan) (n = 101) that had been used for 1–3 yr in Tafea Province, Vanuatu. Net usage and frequency of washing was ascertained by questionnaire; the nets were assessed with regards to cleanliness and damage owing to holes. Insecticide efficacy was determined with cone bioassays using *Anopheles farauti* Laveran. Net usage was high and 86.1% (87 of 101) of villages stated that they used the net every night. Washing of nets was low (11.9%, 12 of 101), and most nets (79.2%, 80 of 101) were considered dirty. Most nets were damaged (73.4% had holes), and 22.8% (23 of 101) had large holes (>200 cm^2). The 24-h mortality of *An. farauti* exposed to nets aged 1–2 yr was 79.4%, while the mortality for nets 3 yr of age was significantly lower at 73.7%. There was no difference in the insecticidal activity of clean compared with dirty nets (mean 24-h mortality: Clean = 76.7%, Dirty = 77.1%). Although the majority of nets had holes, the physical condition of 8.9–22.8% of nets was altered so severely to potentially affect efficacy. Although the 3-yr-old nets would still be providing significant levels of insecticidal and personal protection, consideration should be given to replacing nets >3 yr old.

KEY WORDS bednet, Vanuatu, physical integrity, insecticide activity, long-lasting insecticidal net

Long-lasting insecticidal nets (LLINs) are a key vector control intervention used to reduce malaria transmission and recommended to be used by all populations at risk of malaria (World Health Organization 2008). LLINs provide a combined physical and chemical barrier protecting those who sleep under them. In addition, sustained wide-spread LLIN use can provide a community-wide and mass effect where vector longevity, density, and human–vector contact is reduced (Hawley et al. 2003, Lengeler 2004, Killeen et al. 2007). LLINs were specifically developed to overcome the cost and logistical issues of having to retreat bednets with insecticide, as with LLIN the insecticide is impregnated into the filaments of the fabric during manufacture, producing a slow release effect over 3–5 yr (World Health Organization 2005). The long-term efficacy of LLINs depends on both the physical condition and the residual insecticide activity (Briët et al. 2012). These factors should to be monitored under programmatic conditions, as this information will influence policy regarding when replacement is required. This study focused on monitoring LLIN efficacy in Vanuatu, where Olyset nets were distributed.

An effective LLIN, in terms of insecticidal activity, has been defined as causing at least 80% mortality of exposed mosquitoes using standard cone bioassays (World Health Organization 2005). Regarding wash resistance under laboratory conditions, new Olyset nets generally retain adequate mortality (>80%) after being machine or hand washed when assisted through heated regeneration (exposing the net to heat during the drying process; Gimnig et al. 2005, Ansari et al. 2006, Jeyalakshmi et al. 2006, Sharma et al. 2006, Sreehari et al. 2009, Dev et al. 2010). In contrast, the efficacy of a conventionally treated net (ITN) drops to 5% mortality after only five washes (Jeyalakshmi et al. 2006). Regarding the efficacy of Olyset nets after use under field conditions, numerous studies have reported 95–100% efficacy when used for 1 yr or less (Ansari et al. 2006; Sharma et al. 2006, 2009; Dev et al. 2010). A handful of studies have assessed efficacy for longer periods, generally demonstrating a decline in efficacy: after 6 mo to 2 yr of use, Lindblade et al. (2005) reported 40–60% mortality; after 3 yr of use, N'Guessan et al. (2001) showed 55–85% mortality; and

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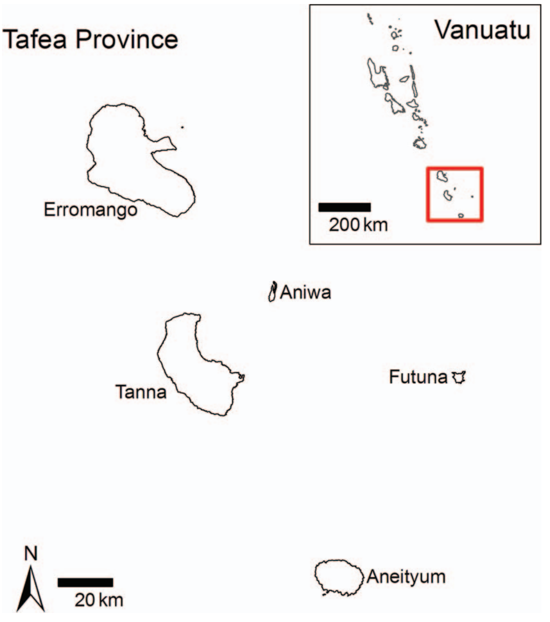

Fig. 1. Map of Tafea Province, Vanuatu. The used Olyset nets were collected from Tanna and Aniwa Islands. (Online figure in color.)

after 7 yr of use, Tami et al. (2004) found only 34% mortality, while Malima et al. (2008) reported 89% mortality after 7 yr of use.

Effective LLINs, in terms of physical condition, need to remain intact. The physical condition and insecticidal activity of nets interact and when nets are adequately treated the ability of mosquitoes to enter through holes is reduced. For treated nets, it has been observed mosquitoes are unlikely to pass through small (50 cm^2) holes and obtain a bloodmeal; however, larger holes (200 cm^2), can facilitate the entry of 44% mosquitoes with 25% obtaining a bloodmeal (Hossain and Curtis 1989).

Vanuatu has embarked on a country-wide intensive malaria control program with elimination being attempted in Tafea Province (The Ministries of Health of Vanuatu and Solomon Islands et al. 2010). Olyset LLINs (Sumitomo Chemical Co., Osaka, Japan) were provided to the population through the years 2007–2010, and are still in use. However, there is no information regarding the long-term efficacy of Olyset nets in Vanuatu. Therefore, the purpose of this study was to estimate the long-term insecticidal activity of LLINs over a 1- to 3-yr period using cone bioassays, and to estimate the long-term physical quality of LLINs through the use of a physical examination. Net efficacy was tested against *Anopheles farauti* Laveran, which is the only vector of malaria in Vanuatu.

Materials and Methods

Bednet Collection. Bednets were collected from randomly selected houses from two islands (Tanna and Aniwa) in Tafea Province (Fig. 1). The nets collected were Olyset nets, which were treated with permethrin at 2% wt:wt and have a fiber thickness of >150 denier (Sumitomo Chemical 2013). During each household visit, a new LLIN (in line with the Ministry of Health guidelines) was offered in exchange for the household's current bed net(s). The age of the net was recorded at the time of collection—the date of distribution had been written on the tags. Each net was examined for cleanliness and for holes. The number of holes on each net was counted and the sizes of the holes were separated into three categories: 1) thumb (<5 cm^2); 2) fist (<200 cm^2); and 3) larger than a fist (>200 cm^2; World Health Organization 2005). Three swatches, each 30 by 30 cm, were cut from the sides of each net. Each swatch was cut from a different side and at a different height (bottom, middle, and top). The net samples were individually wrapped and sealed in aluminum foil and labeled with the house, net number, and sample number. During the net collection period, owners of the nets were asked how often they used their nets and how often they washed their net. The nets were collected in the field between May 2010 and May 2011.

Mosquito Rearing and Maintenance. Female mosquitoes that were used in this study were from a colony of permethrin-susceptible *An. farauti.* The colony was maintained at 26°C and 70–75% humidity under a photoperiod of 12:12 (L:D) h. Larvae were fed powdered goldfish food ad lib, and adults were given continuous access to 30% sugar water. Adult females used in the bioassays were 4–7 d old. The tests were conducted at room temperature (23°C).

Bioassay Procedures. Cone bioassays were performed according to World Health Organization guidelines (World Health Organization 2005). For each LLIN, three swatches from the sides were tested. To test each sample, three replicates containing five mosquitoes were exposed to the swatch for a period of 3 min. Owing to the large quantity of nets, testing was conducted over a series of days, and on each day of testing, three replicate controls containing five mosquitoes were each exposed to untreated netting for a period of 3 min using a plastic cone. The mosquitoes, from both the test and control cones, were then transferred to holding cups for a period of 24 h. Knockdown was recorded at 60 min, while mortality was recorded at 24 h. Knockdown was defined as mosquitoes that were in a state of partial paralysis and unable to sustain flight. During the holding period, each cup was covered with moist cotton impregnated with sugar solution.

Statistical Analysis. To analyze the differences in the physical condition of nets over time, the overall number of holes per net was compared using a generalized linear model (GLM) with a negative binomial distribution, as this data were not normally distributed. In addition, to provide a standard method for comparing net damage, the proportional hole index (pHI) was calculated. This calculation was published in the World Health Organization guidelines for monitoring LLIN durability in 2011 (World Health Organization 2011) and has been used in recent publica-

Table 1. Cone bioassay knockdown at 60 min and mortality after 24 h for *An. farauti* exposed to Olyset nets that have been used for different periods of time

Net age	n	Knockdown (%) at 60 min (95% CI)	Mortality (%) at 24 h (95% CI)	Abbott's adjusted mortality (%) at 24 h[a]
Controls	21	0.3 (−0.2–0.9)	5.4 (3.3–7.6)	
1–2 yr old	59	76.7 (75.3–78.1)	79.4 (78.0–80.7)	78.19
3 yr old	42	59.9 (57.6–62.1)	73.7 (71.6–75.7)	72.16

[a] See (Abbott 1925).

tions (Kilian et al. 2011, Allan et al. 2012, Batisso et al. 2012, Mutuku et al. 2013). An average surface area for each hole size category was estimated to be—thumb = 4 cm^2, fist = 36 cm^2, and larger than a fist = 225 cm^2 (Kilian et al. 2011). The ratio of these surface areas was used as the weights to calculate pHI using the following formula:

$$pHI = size\ 1\ holes + (size\ 2\ holes \times 9) + (size\ 3\ holes \times 56)$$

As such, one unit of the resulting pHI represents 4 cm^2 of hole surface. The pHI was used to categorize net condition as—good 0–24, fair 25–174, poor 175–299, and very poor >300. Here a good net is classified as one with <100 cm^2 of total hole surface and a fair net has 100–700 cm^2 of total hole surface.

The insecticidal efficacy of the nets, as determined by cone bioassays, was compared using GLMs with a binomial distribution. The data from nets aged 1 and 2 yr old were compared with nets 3 yr of age. This comparison was made because the current WHOPES guidelines state that LLINs should remain effective (95% knockdown after 60 min or 80% mortality after 24 h) for a minimum of 3 yr under field use (World Health Organization 2005). The effect of net age on mortality was compared between each net age group and the untreated controls, and also to compare all treated nets in the study with the untreated control nets. Cleanliness (clean nets vs. dirty nets) and mortality were also compared using a GLM with a binomial distribution. The mean mortality was corrected for control mortality using Abbott's formula (Abbott 1925). All analyses were conducted using the raw datasets with R statistical software (version 2.13.1). The statistical significance used for the GLM testing was determined at a level of $\alpha = 0.05$.

Ethics. Ethical approval for conducting the surveys and testing was obtained from the University of Queensland Medical Ethics Research Committee (Approval number: 2010000412) and the Vanuatu Health Ethics Committee (PH gt/07/04).

Results

In total, 101 Olyset nets were collected. All nets were more than 1 yr old at the time of collection and within a range of 1–3 yr, with 5, 54, and 42 nets aged 1, 2, and 3 yr, respectively. Reported net usage was relatively high, out of the 101 households surveyed, 87 (86.13%) households reported using the nets every night, 1 used the net occasionally, 2 used the nets seasonally, and 11 used the nets seasonally and occasionally.

Physical Integrity of Nets. The number of households who reported washing their nets was low. Only 12 out of the 101 (11.9%) households surveyed reported washing their nets and of these, 6 washed the nets once a month, 3 washed the nets every 2 mo, 1 washed the nets once per year, and 2 households washed the nets only once since they received the net.

Regarding the cleanliness of the net when collected, 20.8% (21 of 101) were considered clean (obviously used but in near new condition with no stains, dust, or dirt on the net) and 79.2% (80 of 101) were considered dirty. There was a 3% increase in the number of dirty nets in the 3-yr-old nets compared with the 1- to 2-yr-old nets.

Out of the 101 nets collected, 74 (73.3%) had holes. Most holes were found along the bottom portion of the net. The majority of the holes were small: 65 nets had holes <5 cm^2 and 42 nets had holes 5–200 cm^2. However, 23 (22.8%) had holes >200 cm^2. These large (>200 cm^2) holes were found in both 2- and 3-yr-old nets. Overall, the mean number of holes per net was 6.76 ± 0.83, with a range of 0–44 holes. By age class, the mean number of holes per net was 2.6 ± 1.89 for 1-yr-old, 5.54 ± 0.97 for 2-yr-old, and 8.83 ± 1.51 for 3-yr-old nets. The frequency of holes increased with net age (odds ratio [OR] = 1.68; 95% CI = 1.06–2.65; $P = 0.0261$). The condition of the nets was also compared with the pHI and the overall mean was 61.03. By age class, the mean pHI was 36.00 for 1-yr-old, 58.25 for 2-yr-old and 68.23 for 3-yr-old nets. The majority, 63.37% ($n = 64$), of the nets were considered to be in good condition, having a pHI of 0–24 and a maximum total hole surface area of 100 cm^2. The remaining nets were classified as fair: 27.72% ($n = 28$), poor: 6.93% ($n = 7$), and very poor: 1.98% ($n = 2$). The poor and very poor nets, equating to 8.91% of nets, were both 2 yr ($n = 4$) and 3 yr ($n = 5$) of age.

Insecticidal Activity of All Nets. Overall results regarding knockdown after 60 min and mortality after 24 h from the cone bioassays against *An. farauti* separated by net age are presented in Table 1. Mortality for the nets aged 1–2 yr was 79.4%, while the mortality for nets 3 yr of age was 73.7%. The GLM analysis shows that the risk of mosquito death increased significantly when mosquitoes were exposed to all used Olyset nets compared with untreated control nets (OR = 57.89; 95% CI = 35.25–95.07; $P \leq 0.0001$). The risk of death for mosquitoes exposed to 3-yr-old nets was 1.37 times

Table 2. Cone bioassay knockdown at 60 min and mortality at 24 h for *An. farauti* exposed to used Olyset nets with different levels of cleanliness

Net cleanliness	*n*	Knockdown (%) at 60 min (95% CI)	Mortality (%) at 24 h (95% CI)	Abbott's adjusted mortality (%) at 24 h[a]
Untreated controls	21	0.3 (−0.2–0.9)	5.5 (3.3–7.6)	
Clean nets	21	77.3 (59.4–95.2)	76.7 (58.6–94.8)	75.34
Dirty nets	80	67.7 (57.5–78.0)	77.0 (67.9–86.3)	75.66

[a] See (Abbott 1925).

lower than 1- to 2-yr-old nets (OR = 1.37; 95% CI = 1.15–1.62; $P = 0.0003$).

Insecticidal Activity of Clean and Dirty Nets. The cone bioassay dataset was also analyzed to compare the efficacy of clean and dirty nets. Though knockdown was less in the dirty nets when compared with the clean nets, there was little difference in the mortality results between these two categorizations (Table 2). This is supported by the GLM analysis, where no significant difference in mortality between the clean and dirty Olyset nets was found (OR = 2.15; 95% CI = 1.75–2.64; $P = 0.467$).

Discussion

Net Usage. Net usage appeared to be high, with 86.1% (87 of 101) of villages reporting that they used a net every night. Although the rate of usage is higher than other published literature (Alaii et al. 2003, Atkinson et al. 2009b, Baume et al. 2009), there is currently a large push for malaria elimination within Tafea Province, which may have contributed to the high rate of net usage. Though elicited by questionnaire, this level of usage is probably realistic, as during the warm wet season, there are a high numbers of pest mosquitoes (container breeding species: *Aedes hebrideus* and *Culex quinquefasciatus*) to warrant use of the net, and during the dry season, when mosquito numbers are low, the nights are cold and the net would provide some additional warmth.

Physical Integrity of Nets. In this study, the number of bed nets that were washed was low (11.9%, 12 of 101), similar to other observations in other countries (Solomon Islands and Papua New Guinea) in the region (Atkinson et al. 2009a, Katusele et al. 2013). With the low wash rate found in this study, the majority of nets (79.2%, 80 of 101) were dirty. However, the presence of dirt did not significantly affect the bioavailability of the permethrin nor its efficacy, with no difference in the mortality of mosquitoes exposed to dirty net as compared with clean nets. This is reassuring considering that N'Guessan et al. (2001) indicated that the insecticidal activity of dirty Olyset nets may be reduced compared with clean nets.

A high number of the used nets had holes (73.3%), which is in line with previously published observations (Curtis et al. 2006, Shirayama et al. 2007, Smith et al. 2007, Haji et al. 2013). It is not straightforward to assess the meaning of this observation, as there is no agreed consensus surrounding what level of net damage will negate the protective efficacy or insecticidal effect of the net. There is some evidence from experimental hut trials with *Culex quinquefasciatus* indicating that the feeding success of mosquitoes increases as the number of holes increases (Irish et al. 2008). Furthermore, the size of the holes is important, with the feeding success of *An. gambiae* increasing as the hole size increases in untreated nets (Port and Boreham 1982). Impregnating nets with permethrin has been shown to inhibit the entry of 93% (2 of 29) of *An. gambiae* if the holes were <50 cm^2, while holes 200 cm^2 allowed 44% (16 of 36) to enter the net, with 25% gaining a bloodmeal (Hossain and Curtis 1989). In this study, 22.8% (23 of 101) of nets had holes >200 cm^2, which could compromise the efficacy of these nets. However, attempts have recently been made to standardize comparisons of the physical condition of nets by calculating the pHI (Kilian et al. 2011, World Health Organization 2011, Allan et al. 2012, Batisso et al. 2012, Mutuku et al. 2013). It has been suggested that a poor or very poor net would have a total hole surface area >700 cm^2 (Allan et al. 2012). Using this pHI standard, 8.91% of the nets tested in the current study were considered to be in poor or very poor condition. This indicates that, the physical condition of between 8.91 (calculated from pHI) and 22.8% (the percentage of nets with holes larger than a fist) of the nets in use had been altered with potential to affect efficacy.

Insecticidal Activity of Nets. There seems to be agreement that Olyset nets, which have been subjected to field use for 6–12 mo, exhibit excellent insecticidal properties, with cone bioassay mortalities of 90–100% (Ansari et al. 2006; Sharma et al. 2006, 2009; Dev et al. 2010). With time insecticidal efficacy, as measured by cone bioassays, falls off and after 2 yr mortality has been reported as 45–60% (Lindblade et al. 2005) and after 3 yr as $<40\%$ (Haji et al. 2013) and 55–85% (N'Guessan et al. 2001). The results reported here, although indicating higher insecticidal activity (79.4% mortality after 1–2 yr and 73.7% after 3 yr), show a similar trend in declining efficacy when nets are >1 yr old. The discrepancy between results seems to widen when nets are used for longer periods, with one report of 89% mortality after 7 yr (Malima et al. 2008) and another of 34% after a similar period (Tami et al. 2004). The wider range of mortalities produced by nets >1 yr probably reflects difference in human-related behavior with regard to net usage: general handling, amount of use, storage, washing, and attempts at insecticide regeneration. These contrasts have only been made with permethrin-treated Olyset nets. However, in nearby Papua New Guinea, Katusele et al. (2013) found that the efficacy of deltamethrin-treated PermaNets was retained at >95% mortality when used for 5 yr.

The mortality and knockdown results presented here indicate that the efficacy of Olyset nets in Vanuatu begins to decrease during the third year of use under programmatic conditions; even though the nets were designed to last for 3–5 yr (Sumitomo Chemical 2013). Although the 3-yr-old nets would still be providing significant levels of insecticidal protection, the impact is reduced when compared with 1- to 2-yr-old nets. Further, the insecticidal efficacy of 3-yr-old nets was below 80% indicating an increased risk of anopheline exposure to sub-lethal dosages of insecticide. As such, the risk of insecticide resistance developing in *An. farauti* is higher; thus, the program within Tafea Province should be diligent with resistance monitoring.

This was a retrospective study of the insecticidal activity and physical integrity of used Olyset nets; although it would have been constructive to estimate the average useful life of the nets, this was not possible with our dataset. The recent guidelines on monitoring the durability of LLINs (World Health Organization 2011) define the useful life of nets as a combination of three factors: insecticide activity, physical integrity, and attrition. Estimating the attrition was not possible with the retrospective sampling design.

In conclusion, owing to the uncertainties surrounding the length of net efficacy, the Ministry of Health has planned to redistribute nets every 3 yr in Vanuatu, and this decision is supported by the data presented here. It is recommended that the Ministry of Health, Vanuatu, monitors the insecticide activity and physical integrity of nets during actual field use, especially after 2 yr of use, and also estimates the attrition of nets. Such studies will provide invaluable information to program managers who need to make real-time decisions to ensure that vector control is being implemented in the most effective manner possible. The data collected over the course of this study indicate that Olyset nets that have been used 3 yr in Tafea Province, Vanuatu, are not as effective (insecticide activity or physically) when compared with nets aged 1–2 yr.

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