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Field Durability of *Yorkool®LN* Nets in the Benin Republic

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Abstract

Context: Recent publications on WHO recommended methods for estimating the survival of LLINs are good guidelines for assessing the performance of long-lasting insecticidal nets (LLINs). Thus, this field trial study was undertaken to evaluate the durability of the *Yorkool® LN* mosquito net distributed during the 2017 campaign in Benin. **Methods:** The monitoring of *Yorkool® LN* nets was carried out in two districts (Djougou III and Barienou) in Djougou, department of Donga, northern Benin from October 2017 to March 2019. A representative sample of 250 households that had received the *Yorkool® LN* polyester LLINs during the 2017 campaign was selected in the rural and urban areas of each district and monitored for 6, 12 and 18 months. An evaluation of the survival of *Yorkool® LN* nets was conducted based on the rate of loss and physical condition of the surviving nets as measured by the proportional hole index (pHI). Finally, the chemical efficacy of these LLINs during each period was determined using the WHO cone tests. **Results:** Survival of *Yorkool® LN* nets was similar in both rural and urban areas, although there was a difference in survival between the 6-month (95.3%), 12-month

(89.7%), and 18-month follow-up periods (74.4%). A difference in survival was also observed between the NetCalc model (84%) compared to the *Yorkool® LN* nets of this study (74.4%). The attrition rate was 29.6% for LLINs at 18 months. Surprisingly, the physical integrity of the LLINs was minimally affected in the municipality. Indeed, the proportion of mosquito nets in good condition without a hole was 51.8% compared to 56.8% with a hole after 18 months. Only 7.8% of the LLINs in the two districts were damaged compared to 2.6% which needed to be replaced. The washing frequency, location of the LLINs and the frequency of use are some factors contributing to the appearance of the holes in LLINs. The bio-efficacy results of LLINs based on the cone test were good with mortality rates of 74%, 66%, 72% and 58% respectively after baseline, 6, 12 and 18 months of use. **Conclusions:** The observed differences in the survival of *Yorkool® LN* nets are due to community living conditions and movements and not to the equipment used to manufacture LLINs. However, the estimated median survival has shown that *Yorkool® LN* nets would have an average lifespan of 2 years 8 months despite their fairly good physical condition. These results may be useful to the National Malaria Control Program (NMCP) during the period of replacement of these nets on the field.

Keywords

Durability, *Yorkool® LN*, LLINs, Survival, Physical Integrity, Bio-Efficacy

1. Introduction

Malaria is one of the common diseases affecting humans around the world. It is a pathology that can be fatal. It is caused by parasites transmitted to humans by infective mosquito bites [1]. It impacts developing countries, especially in Africa [1]. Almost half of the world's population is at risk of contracting the disease [2]. In 2017, the estimated number of cases of this disease globally was 219 million, compared to 239 million in 2010 and 217 million in 2016. It is estimated that 11 countries, 10 in sub-Saharan Africa plus India, have recorded close to 70% of malaria cases and related deaths in 2017 worldwide. Only one of these countries, India, reported a decrease in malaria cases in 2017 compared to 2016 [3]. The data for 2015-2017 shows no significant progress towards reducing the number of malaria cases worldwide. The eleventh WHO global report on the disease confirms that the two key objectives of global technical strategy against malaria 2016-2030 aimed at reducing cases and deaths from malaria by at least 40% by 2020, will not be achieved [3]. The African continent is laden with several risk factors for the disease. These factors are related to the existence of the vector that promotes the transmission of the parasite and to this must be added the weak socio-economic conditions and the hot climate that have an impact on the control of the disease [4]. These risk factors contribute to the endemicity of the disease in many countries where it is prevalent.

Benin, a country in West Africa, also experiences a year-long endemicity with its peak transmission during the rainy season. Strengthening control and prevention measures could dramatically reduce the burden of malaria in some countries. Among these measures is the use of insecticide-treated mosquito net [5]. The latter aims to eliminate or limit human-vector contact to prevent infection by parasites [6]. The use of mosquito nets to reduce the transmission of vector-borne disease agents, including malaria, but especially against culicidae caused-problems, is a very old practice [7]. In Benin, malaria is one of the leading causes of morbidity and mortality and also a major reason for consultation in health facilities. The incidence of malaria in the country in 2017 was 15% in the general population and 37.2% in children under five, lethality is 0.8 per thousand, with pregnant women also included as a vulnerable population "according to the NMCP reports" [8]. Morbidity and mortality can be explained by several factors such as uncontrolled construction, unsanitary environment, low participation of the population in vector control activities, ignorance of prevention methods, increasing resistance of plasmodium to antimalarials, and lack of personal protection against mosquitoes [9] [10]. However, the problem related to the durability and good use of the LLINs after the free distribution interventions persists [11] and many authors have approached this issue in one way or the other [12] [13]. The association of mortality reduction with the use of LLIN is well established [14]. The decrease in malaria incidence in the continent in the last 15 years is due in part to LLINs [3] [15]. However, questions remain as to the actual lifespan, *i.e.* the net survival, physical integrity and chemical efficacy of LLINs. Indeed, although more and more studies are looking at the issue, the information deficit on the net lifespan and the variation of the performances between the different LLINs under different conditions of use persists. The physical integrity of the LLINs showed that 45% - 78% of the nets were damaged after one year of use under operational conditions in Uganda [16]. Concerns about the effective lifespan of LLINs and a faster than expected deterioration in biological efficacy have been noted in studies in Kenya and Benin [17] [18]. In Laos, another study found that around 40% of impregnated mosquito nets were physically damaged 2 - 3 years after use [19]. In Rwanda and Benin, studies have revealed lower life expectancies as against 3 years normally indicated by the manufacturer for both polyethylene and polyester LLINs [11] [20] [21].

In addition, a survey conducted in Nigeria showed that a communication campaign for behavioral change significantly improved household attitudes towards maintenance and reparation [17]. In Mozambique, a first study compared the lifespan of two types of LLINs between 2008 and 2011 in Nampula [13]. The results showed a better performance of 100 denier polyester LLINs compared to 150 denier polyethylene LLINs, and higher efficacy of nets in out-of-shore homes than at the coast.

With the increasing demand for LLINs to cover populations living in endemic areas, critics are protesting against the unsatisfactory quality of certain mosquito net products of WHOPES approved brands. The present study proposed to evaluate the survival, physical durability and biological effectiveness of *Yorkool® LN* in community use under real conditions in Benin. The aim is to collect relevant data on the performance of these nets in field conditions to guide the NMCP in decision-making.

2. Methods

2.1. Study Site

It is recalled that the campaign of free mosquito net distribution started in 2007 in Benin with the distribution of 1,500,000 mosquito nets to children under 5 years. In 2011 the campaign targeted nearly 4,674,800 households nationwide. Few years later in 2014, nearly 6,050,212 mosquito nets had been distributed. This continued and by October 2017 free distribution of long-lasting insecticide-treated mosquito nets to households in Benin totaled 6,695,750 households [8]. This distribution covered the whole extent of the territory. As in 2011 and 2014, the NMCP with support from USAID and PMI partners, follows the physical durability and efficacy of LLINs distributed to the population in certain parts of the country. The community of Djougou was chosen from the list of communities in Benin (using numerical codes) to carry out the study. Two districts were chosen at random in the community: one in the central area of the community (Djougou III) and the other in the peripheral zone (Barienou). The municipality was selected using the Random Number Generator (http://randomnumbergenerator.intemodino.com/en/). The choice of the community of Djougou is motivated by the fact that the *Yorkool® LN* mosquito net in study is distributed only in this region of the country. In addition, this municipality is characterized by a large rainy season (June to November) which compels the population to sleep under mosquito nets during this period to protect themselves from mosquito bites. It should also be noted that the Donga River flows through the area, thus providing a permanent breeding site to support the proliferation of malaria vectors all year round. **Figure 1** shows the selected study areas.

2.2. Household Selection and Tagging of Mosquitos Nets

Two teams of two technicians and one village health worker visited and selected the households where the *Yorkool® LN* nets were used in each zone. Five hundred (500) LLINs were selected, marked and enrolled in the study, *i.e.*, 250 LLINs from the central (urban) zone and 250 LLINs from the peripheral (rural) zone. A subsample of 50 MIILD (varying at each pass) representing 10% of the total sample was randomly selected to measure insecticidal activity at each monitoring period (every 6 months) in both areas.

2.3. Reference Survey and Questionnaire

A questionnaire, recommended by the WHO [22] and adapted to the needs of

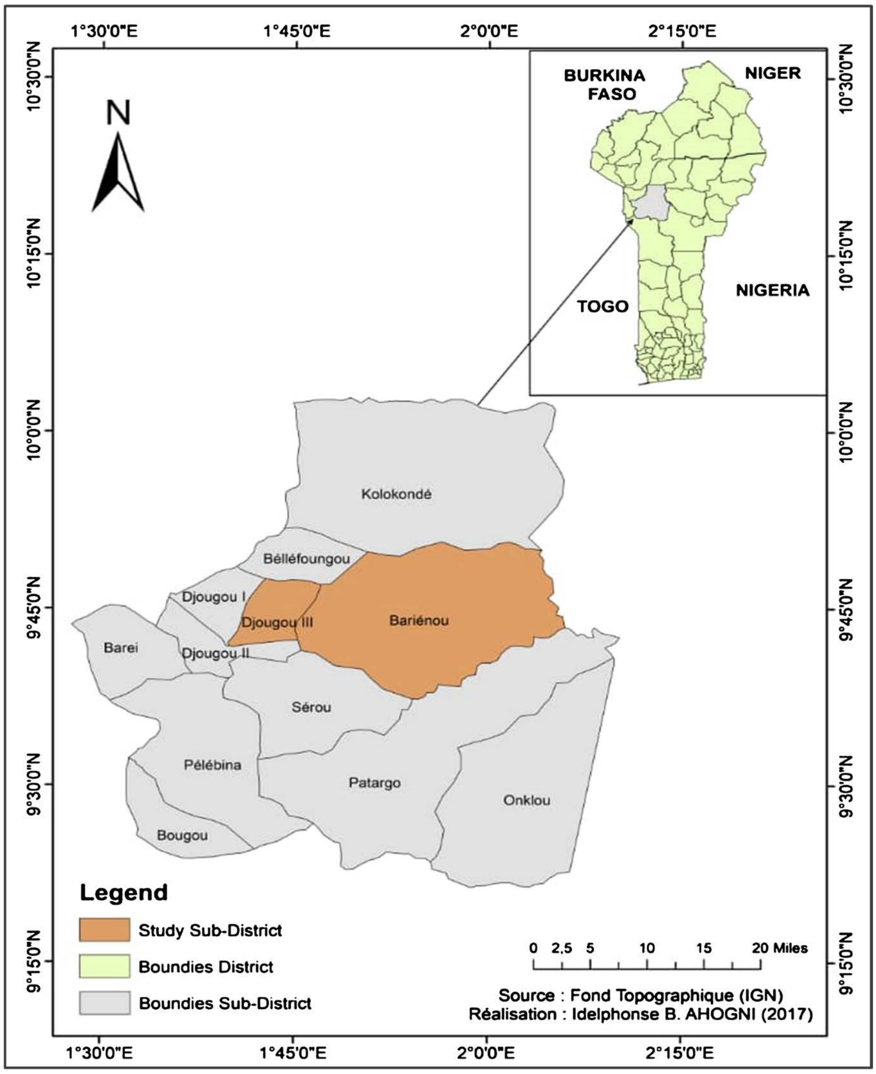


Figure 1. Map of Benin showing the study site.

our study, was used to identify the basic characteristics of the households, sleeping patterns, the level of education, the presence or the absence of the LLINs, reasons for absence, number and size of holes. The tools used for data collection were Samsung Galaxy Tab 10.1 tablets. An electronic survey form was created on these tablets using the Open Data Kit (ODK) data collection software. This technique allowed the recording and instantaneous measurement of data. All interviews were conducted at 6, 12 and 18 months using these tablets to record the responses.

2.4. Tracking the LLINs

Net monitoring was conducted by visiting door-to-door households to record the physical presence/absence and integrity of the net tissue in order to estimate the periodic attrition rate, in addition to information on the perceptions and practices of mosquitos nets. Populations as mentioned above. Through these home visits, presence or absence of mosquito nets were confirmed and recorded.

When a net was no longer present in a household, homeowners were interviewed to determine the reason for its loss or absence.

Overall, the survival rate of LLINs was evaluated by the following formula:

$$\frac{\text{Number of LLINs marked present and in serviceable condition in the household}}{\text{Total number of LLINs selected at}\left(T_0\right)-\text{LLINs displaced or given out}}\times 100$$

Households unavailable during an assessment visit were censored using the non-parametric Kaplan-Meier survival method [22]. The survival of the nets over time was compared to NetCalc's 2 and 3-year life expectancy models (http://www.networksmalaria.org) recommended by Roll Back Malaria.

The equations used to calculate the loss rates associated with the different reasons that labeled nets are no longer present in households are as follows:

2.4.1. LLIN Attrition

LLIN attrition represents the proportion of LLINs initially enrolled in the study and which have been lost due to different reasons. Attrition can be associated with different reasons why labelled nets are no longer present and used in households. These reasons include: destroyed, so torn and worn; stolen, given, moved; and used for other purposes.

2.4.2. Median Survival of Mosquito Nets

The median survival of the nets in this study was estimated from at least two time points, the lowest being less than 85% according to the following formula:

$$t_m = t_1 + \frac{\left(t_2 - t_1\right) * \left(p_1 - 50\right)}{p_1 - p_2}$$

where t_m = median survival, t_1 and t_2 are the first and second time points respectively and p_1 and p_2 denote the percentage

2.4.3. Integrity of the LLINs

At each follow-up visit (6, 12 and 18 months), all mosquito nets were inspected for physical integrity (hole size and distribution) by draping them on a frame and counting the number of holes of different sizes and location on the LLIN (top, middle, bottom).

For each of the selected LLINs, the number of holes (including tears in the LLINs and seam openings) according to their location on the LLIN and their size were evaluated. The observed holes were categorized into 4 types:

- Hole size T1 (smaller than one inch: 0.5 - 2 cm).
- Hole size T2 (larger than one inch, but smaller than a fist: 2 - 10 cm).
- Hole size T3 (larger than a fist but smaller than a head: 10 - 25 cm).
- Hole size T4: (bigger than a head: >25 cm).

Thus, the integrity of each type of LLIN was assessed through the determination of two indicators:

- the proportion of mosquito nets with any hole: (%) surviving the first and second time points.

$$(\%)\text{surviving} = \frac{\text{Number of LLIN with holes}}{\text{Total number of LLIN in the household}} \times 100$$

- the proportionale Hole Index (pHI) in accordance with WHO guidelines [23]. This index is measured according to the formula: pHI = 1 × #T1 + 23 × #T2 + 196 × #T3 + 576 × #T4 (#T = number of holes of the size). It estimates the approximate value of the area occupied by the holes on each type of LLIN.

Depending on the pHI obtained, each net was classified according to the following three categories [24].

- in good condition: (pHI ≤ 64),
- Damaged: (65 ≤ pHI ≤ 642) where
- Too torn: (pHI > 642) [24] [25].
- Serviceable: (LLIN in good condition + damaged LLIN).

To assess the influence of user-related factors on the physical integrity of LLINs, we used Negative Binomial regression [26] taking the pHI parameter as a variable of interest. A Type III variance analysis of the regression [27] allowed to assess the influence of each factor on the pHI.

2.5. Biological Effectiveness of Mosquito Nets

The collected mosquito nets were tagged and stored in separate plastic bags that were transported to the laboratory of the Cotonou Entomological Research Center (CREC) for biological testing (cone testing). During this period, usually within one week, the owners receive new mosquito nets to replace those taken in order not to render them unprotected while waiting for the return of their mosquito net. Cone tests were carried out on 200 nets (50 at each biannual follow-up) in accordance with the WHO protocol [26]. Two standard cones were attached to each of the five faces of each mosquito net. Ten susceptible unfed female *Anopheles gambiae* (Kisumu) mosquito strains aged 2 - 5 days were introduced and exposed for 3 minutes into each cone. After 3 minutes of exposure, the Knock-down effect is first measured every 5 min for 60 minutes before the mortality recording 24 hours' post-exposure. Two controls (one positive on fresh LLINs exiting directly from packaging at the start of the study) and one negative control (untreated mosquito net) were included in each cone test series. Mosquitoes were kept under observation for 24 hours in cups supplied with 10% glucose solution. The mortality in the negative control was less than 1%, thus the application of the corrected mortality with Abbott's formula to the data was not necessary. The average of the bioassay results for the 5 faces of each LLIN sampled was calculated to determine if the LLIN met the WHO efficacy criteria of ≥80% mortality on average and/or ≥95% Knock-down. The biological tests were carried out at a temperature of 28˚C ± 2˚C and a humidity of 80% ± 10%. The mosquito nets tested were returned to the owners at the end of the tests for the continuation of their follow-up and the new ones which had been given to them were retrieved.

2.6. Statistical Analysis

The binomial test was used to calculate survival and loss rates and their confidence intervals [28]. Comparisons of proportions were made using the chi^2 test. The proportional hole index (pHI) for factors that may affect physical integrity was determined through the poisson regression on LLINs. Wald's tests of the significance of the regression coefficients and the likelihood ratio were used to evaluate the influence of population habits on the physical integrity of LLINs. Data analysis was done using R Core Team software version 3.5.1 (2018).

3. Results

3.1. Net Survivorship

There were 500 mosquito nets (*Yorkool® LN*) enrolled at beginning of the study. During follow-up visits of 6 (T6), 12 (T12) and 18 (T18) months respectively 469, 420 and 352 LLINs were available for inspection. The average survival rate was 95.3% (95% CI: 93 - 96.9), 89.7% (95% CI: 86.6 - 92.3) and 74.4% (95% CI: 69.7 - 78.6) after 6, 12 and 18 months of use respectively with a significant difference observed from one period to another ($p < 0.05$) (**Table 2**). In contrast, the survival rate was similar in both rural and urban areas ($p > 0.05$) regardless of the follow-up period (**Table 1**). The observed survivals were compared to those of the NetCalc model that predicts the shelf life of LLINs (**Figure 2**). According to the model, at 18 months, a survival rate of 84% should be observed for mosquito nets with a shelf life of 3 years against 68% for those with a life of 2 years. However, the average survival rate observed for *Yorkool® LN* nets in our 18-month study was 74.4%. This rate is closer to that expected for LLINs with a shelf life of two years (**Figure 2**). The determination of the median survival at 18 months (t_m = 2 years 7 months) confirms this result.

3.2. Median Survival of Mosquito Nets

Twelve and eighteen months after the use of LLINs, we calculated the median survival to estimate their median lifespan (t_m), $-t_1$ = T6 (Follow-up at 6 months), $-t_2$ = T18 (Follow-up at 18 months).

- At 12 months

$$t_m = 6 \text{ months} + \frac{(12 \text{ months} - 6 \text{ months}) * (95.3 - 50)}{95.3 - 89.7}$$

$$t_m = 55 \text{ months}$$

The median time for the *Yorkool® LN* nets in the study at 12 months was 55 months, which is an average of 4 years 7 months of life.

- At 18 months

$$t_m = 6 \text{ months} + \frac{(18 \text{ months} - 6 \text{ months}) * (95.3 - 50)}{95.3 - 74.4}$$

$$t_m = 32 \text{ months}$$

Table 1. *Yorkool® LN* nets survivorship by assessment area at 6, 12, and 18 months after distribution.

Periods	Areas		Households enrolled	LLIN found	Serviceable LLIN	Sum of removed LLINs at 6, 12 and 18 months	Survivorship (%)
6th month	Urban area	N	250	233	232	8	_
		%	100.0	-	92.8	3.2	96
		CI-95%	-	-	88.9 - 95.7	1.4 - 6.2	32.3 - 44.7
	Rural area	N	250	236	234	3	_
		%	100.0	-	93.6	1.2	95
		CI-95%	-	-	89.8 - 96.3	0.2 - 3.5	32.0 - 44.3
12th month	Urban area	N	250	215	214	6	_
		%	100.0	-	85.6	2.4	91[a]
		CI-95%	-	-	80.6 - 89.7	0.9 - 5.2	30.4 - 42.7
	Rural area	N	250	205	204	17	_
		%	1.0	-	81.6	6.8	89[a]
		CI-95%	-	-	76.2 - 86.2	4.0 - 10.7	29.7 - 41.9
18th month	Urban area	N	250	191	145	39	_
		%	100.0	-	99.3	15.6	68.7[a]
		CI-95%	-	-	74.9 - 85.1	11.3 - 20.7	61.9 - 74.9
	Rural area	N	250	161	145	71	_
		%	100.0	-	97.3	28.4	81a
		CI-95%	_	-	60.6	22.9 - 34.4	74.5 - 86.5
					Overall survivorship by period		
6th month		N	500	469	466	11	_
		%	100.0	-	93.2	2.2	95.3*
		CI-95%	-	-	90.6 - 95.2	1.1 - 3.9	93 - 96.9
12th month		N	500	420	418	34	_
		%	100.0	-	83.6	6.8	89.7*
		CI-95%	-	-	80.1 - 86.7	4.8 - 9.4	86.6 - 92.3
18th month		N	500	352	290	110	_
		%	1.0	-	58	22	74.4*
		CI-95%	-	-	53.5 - 62.3	18.4 - 25.9	69.7 - 78.6

LLINs = Long-Lasting Insecticidal Nets, CI = Confidence interval, N = Number, % = Percentage, [a]$p > 0.05$; *$p < 0.05$.

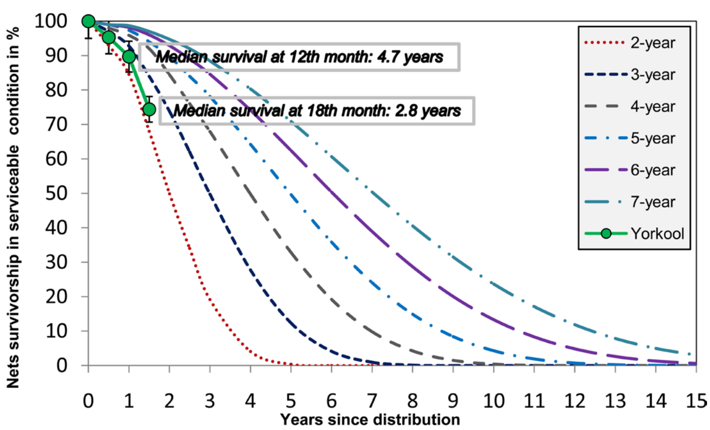


Figure 2. Estimated LLIN survivorship in serviceable condition at 6, 12 and 18 months in community plotted against hypothetical survival curves (NetCalc model of curves of nets loss over 2, 3, 4,5, 6 and 7 years) with defined median survival.

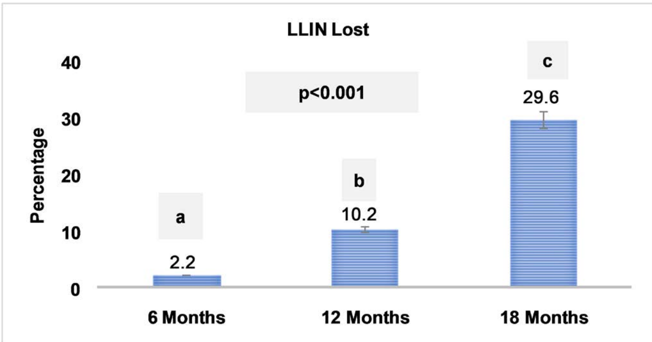
The median time for *Yorkool® LN* nets in study at 18 months was 32 months, which is an average of 2 years 8 months of life.

3.3. Reason for Loss of LLINs

In total, 148/500 or 29.6% of LLINs were lost 18 months after distribution with a significant difference from one period to another ($p < 0.001$) (**Figure 3**). Displacement remains the main reason for this loss (25.2%). There was a significant difference between rural and urban areas for displaced LLINs ($p < 0.05$) (25.2% vs. 14.4% respectively) or used for other purposes ($p < 0.05$) (1.2% vs. 6.4% respectively). About 6% of lost *Yorkool® LN* nets were due to physical deterioration in both rural and urban areas (**Figure 4**).

3.4. LLIN Fabric Integrity

At 6, 12 and 18 months of use of LLINs, no significant difference between integrity losses was observed in rural areas compared to urban areas ($p < 0.05$). Proportional Hole Indices (pHI) were similar for all three LLIN categories except the observed differences (rural vs urban) at 12 and 18 months for the LLIN category "to be replaced" ($p < 0.05$) (**Table 2**). The proportion of LLIN in good condition and without any hole was 51.8% (for both study areas) versus 56.8% with hole (for both study areas) after 18 months of use (**Figure 5**). Only 7.8% of the LLINs were damaged, compared to 2.6% that was too torn and would need



Legend: Same letter = No significant difference ($p > 0.05$), different letter = Significant difference ($p < 0.05$).

Figure 3. Loss rate of *Yorkool® LN* LLINs after 6, 12 and 18 months of use in both areas.

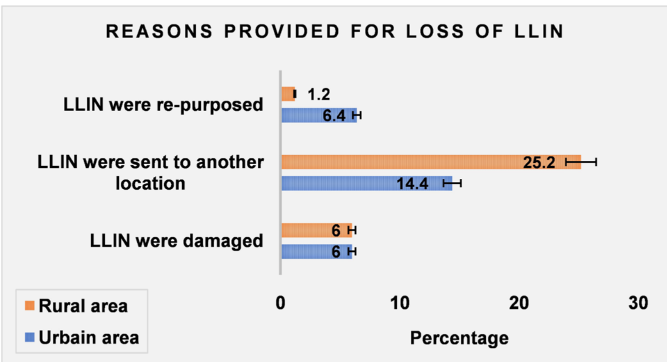


Figure 4. Reasons for net loss (attrition) after 18 months use.

Table 2. LLIN Fabric integrity (pHI) after 6, 12 and 18 months.

			Households enrolled	Tagged LLIN found	Nets found with any hole (s)	Nets in pHI = 0	Nets in pHI ≤ 64	Nets in 64 < pHI ≤ 642	Nets in pHI > 642
6th month	Urban area	N	250	231	11	220	227	3	1
		%	100.00	92.4ª	4.8ª	95.2ª	98.2ª	1.3ª	0.4ª
		CI	_	88.4 - 95.36	2.40 - 8.36	91.64 - 97.6	95.6 - 99.5	0.30 - 3.75	0. - 2.4
	Rural area	N	250	229	14	215	221	8	0
		%	100.00	91.6ª	6.1ª	93.9ª	96.5ª	2.5ª	0ª
		CI	_	87.45 - 94.72	3.38 - 10.05	93.2 - 98.5	93.2 - 98.5	1.9 - 3.1	0 - 1.6
12th month	Urban area	N	250	191	21	170	179	15	1
		%	100.00	76.4ª	10.99ª	89.0ª	93.7ª	7.9ª	0.4ª
		CI	_	70.64 - 81.52	6.94 - 16.31	83.69 - 93.06	89.3 - 96.7	4.5 - 12.6	0 - 2.4
	Rural area	N	250	161	37	124	145	16	8
		%	100.00	64.4ª	22.98ª	77.02ª	90.1ª	9.9ª	4.9ᵇ
		CI	_	58.1 - 70.3	16.7 - 30.3	69.7 - 83.3	84.4 - 94.2	5.8 - 15.6	2.2 - 9.5
18th month	Urban area	N	250	147	124	124	143	18	2
		%	100.00	58.8ª	84.4ª	84.4ª	97.3ª	12.2ª	0.7ª
		CI	_	52.4 - 0.65	77.4 - 89.8	77.4 - 89.8	93.2 - 99.3	7.4 - 18.7	0 - 0.04
	Rural area	N	250	149	135	135	141	21	11
		%	100.00	59.6ª	90.6ª	90.6ª	94.6ª	14.1ª	7.4ᵇ
		CI	_	53.2 - 65.7	84.7 - 94.7	84.7 - 94.7	89.7 - 97.7	8.9 - 20.7	3.7 - 12.8

N = Number, CI = Confidence interval, pHI = proportionate hole index, % = percentage, Same letter = No significant difference ($p > 0.05$), different letter = Significant difference ($p < 0.05$).

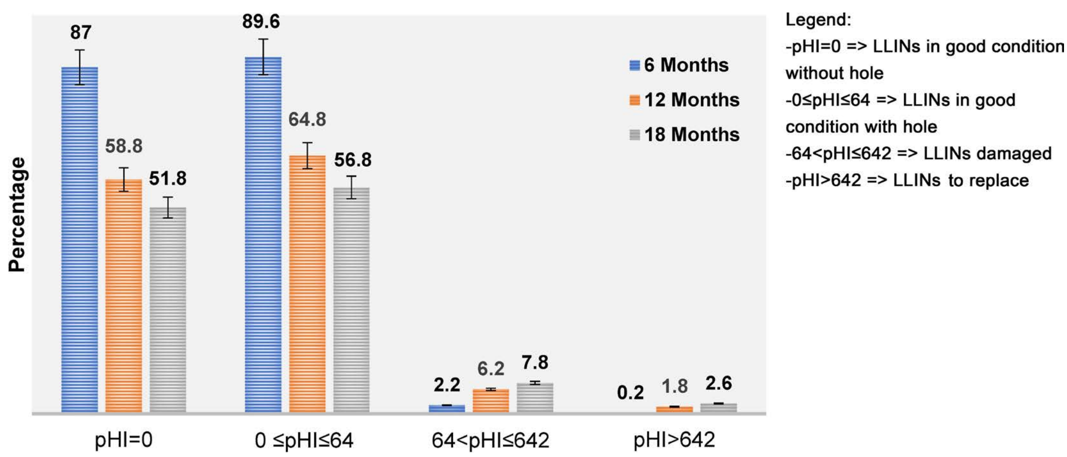


Figure 5. Physical integrity of *Yorkool® LN* LLIN after 6, 12 and 18 months of use in the two areas.

to be replaced after 18 months of use for all of the two study areas (Figure 5).

3.5. Causes of Damage

Mechanical lesions in the form of tears (77%) and open seams (13%) were the most frequent, followed by injuries caused by burns (6%). It is interesting to note that very little burn damage has been reported. The tears caused by rodents were 4% (**Figure 6**).

3.6. Factors Associated with the Physical Deterioration of LLINs

In sum, data collected over the last 6 months showed the washing frequency, the frequency of use of the LLINs, their location, their maintenance, the type of user, the coating material and the cooking medium used were significantly associated with loss of tissue integrity ($p < 0.001$).

The proportional hole ratio (pHI) is three times higher when the LLINs are washed 2 - 5 times (pHI: 67.3) compared to those washed once (pHI: 18.8) and at least 13 times compared to those that are not washed at all (pHI: 5.8). This means the higher the number of washing of LLINs, the greater the appearance of tears, hence the high rate of pHI. As a result, high pHI (too much torn) LLINs are not used at all for sleeping (pHI: 374.5), unlike those used as often (pHI: 4) or nightly (pHI: 33.5) for sleeping, which is very little torn. This explains why folded (pHI: 123.6) or ranged (pHI: 75.1) MIILDs have higher pHI than those that are suspended (pHI: 10.6) because they can be used every night for protection (**Table 3**).

The pHI is 8 times higher for dirty LLINs compared to clean ones. LLINs used by children alone are much more torn (pHI: 348.4) than those used by children and adults (pHI: 23) as well as adults only (pHI: 2.5). This denotes the impact of children on the appearance of holes in the LLIN. It is the same for LLINs used on the mat as on the bed. Households where wood is used as a cooking medium has a pH-value 16 times higher than those using coal (**Table 3**).

3.7. Assessment of the Bio-Efficacy of the LLINs (WHO Cone Test)

Table 4 shows the averages of shock effects and mortality observed with the WHO cone tests. Of the 200 LLINs tested (50 per period), 31, 30, 29 and 10 had shock effect that means mosquitoes fell on the back (knock-down) ≥ 95% respectively after 0, 6, 12 and 18 months of use. The average mortality observed

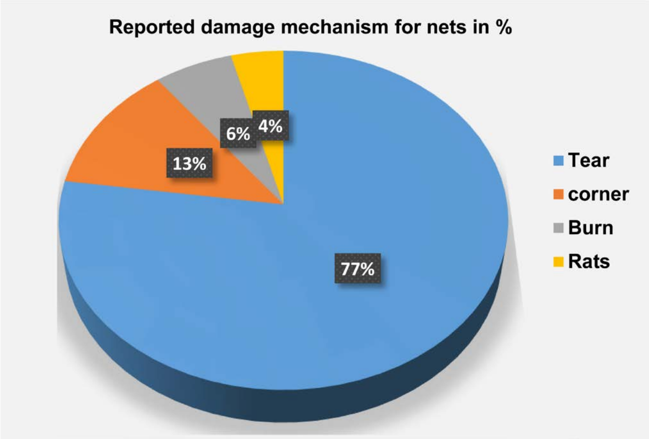


Figure 6. Main reported causes of damage of LLIN after 18 months.

Table 3. Factors associated with the physical deterioration of LLIN after 18 months*.

Factors	Modalities	Mean of pHI	OR (CI-95%)	P (Wald's test)	P (LR-test)
Whashing frequency	Once	18.8	1		
	2 - 5 times	67.3	3.57 (3.4 - 3.76)	<0.001	<0.001
	None	5.8	0.31 (0.28 - 0.34)	<0.001	
Frequency of LLIN use	Not at all	374.5	1		
	Often	4	0.01 (0.01 - 0.01)	<0.001	<0.001
	Every night	33.5	0.09 (0.08 - 0.1)	<0.001	
LLIN Emplacement	Hanging	10.6	1		
	Folded	123.6	11.66 (11.13 - 12.22)	<0.001	<0.001
	Ranged	75.1	7.08 (6.53 - 7.68)	<0.001	
LLIN maintenance	Clean	6.5			<0.001
	Dirty	50.7	7.8 (7.27 - 8.37)	<0.001	
LLIN Users	Adults only	2.5			
	Adults & Children	23	9.35 (8.27 - 10.57)	<0.001	<0.001
	Children only	348.4	141.4 (125.12 - 159.79)	<0.001	
Sleeping material	Bed	18.8			<0.001
	Matt	65.3	3.47 (3.33 - 3.62)	<0.001	
Cooking fire	Wood	46.3			<0.001
	Charcoal	2.8	0.06 (0.05 - 0.07)	<0.001	

*Poisson regression predicting pHI, pHI = proportionate Hole Index, OR = Odds Ratio, CI = Confidence interval.

Table 4. KD and Mean mortality observed with WHO cone tests per periods

	LLIN Total tested		N mosquito tested	Mean KD ≥ 95%	CI-95%	Mean Mortality ≥ 80%	CI-95%	Loss rate (%)
Baseline	*Yorkool® LN*	N = 50	5112	62	47.2 - 75.3	74	59.7 - 85.4	26 (N = 13)
6th month	*Yorkool® LN*	N = 50	5510	60	45.2 - 73.6	66	52.2 - 78.8	34% (N = 17)
12th month	*Yorkool® LN*	N = 50	5457	58	43.2 - 71.8	72	57.5 - 83.8	28% (N = 14)
18th month	*Yorkool® LN*	N = 50	5531	20	10 - 33.7	58	43.2 - 71.8	42% (N = 21)

LLIN = Long-Lasting Insecticidal Nets, N = Number, KD = Knock-down, CI = Confidence interval.

was 74%, 66%, 72% and 58% respectively at 0, 6, 12 and 18 months. Of the 200 LLINs tested, 65 did not reach the WHO threshold of effectiveness (mortality ≥ 80%) after 24 hours. Figure 7 and Figure 8 illustrate the knock-down effects and observed mortalities for each of the 50 nets tested at each monitoring period.

4. Discussion

Malaria control is mainly based on long-lasting insecticide-treated mosquito nets (LLINs). The effectiveness of this method in reducing deaths and diseases related to malaria has been shown by several studies. The advent of LLINs is precisely a response to the relatively low rate of re-treatment of conventionally

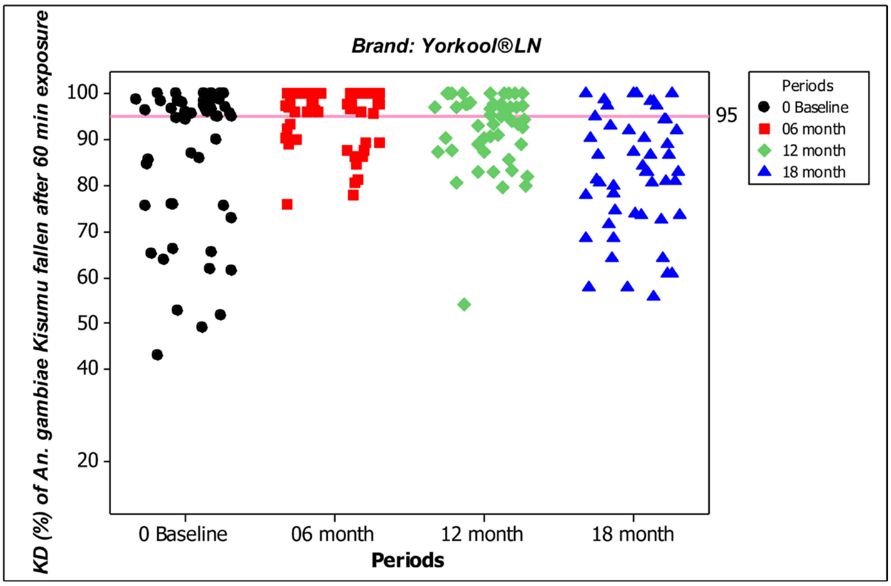


Figure 7. Percentage of *An. gambiae* Kisumu fell on the back (knock-down "KD") after 60 min of exposure to the different mosquito nets.

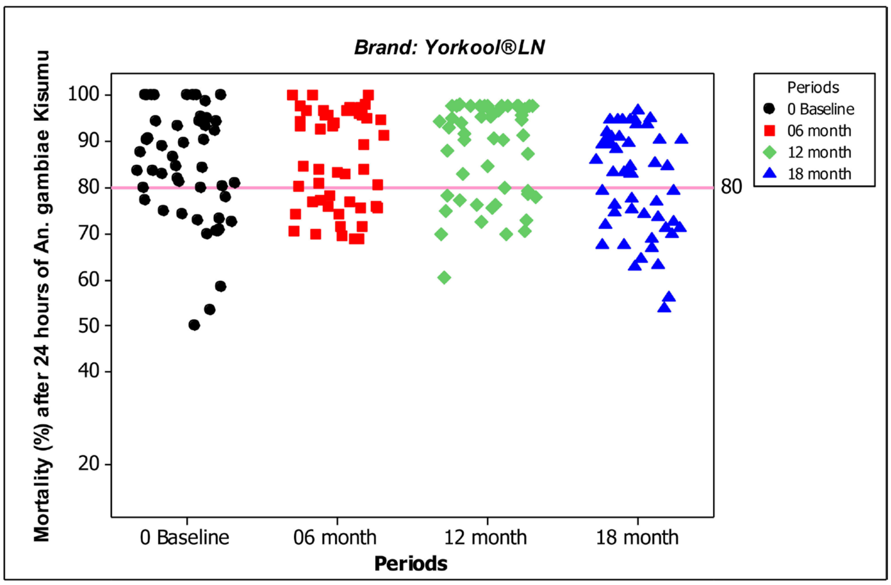


Figure 8. Percentage mortality of *An. gambiae* Kisumu on the different nets after 24 hours of observation.

treated mosquito nets, which should necessarily be reprocessed after six months of use in the past [24]. The distribution and use of these LLINs have become a major priority for national malaria control programs in view of the enormous benefits they offer [22]. However, several extrinsic elements (conditions of use, maintenance and age) and intrinsic (brand) are factors likely to affect the physical integrity and chemical efficacy of LLINs used on a large scale.

A mark of nets (*Yorkool® LN*), was identified during our study in the 2 districts of the municipality followed. Distributed free of charge during the July-October 2017 mass campaign, this LLIN was mainly found in the households surveyed. Eighteen months of use after distribution, just over two-thirds of the *Yorkool® LN* nets distributed (352/500) functionally survived, given both attrition and physical durability. There was a difference in functional longevity between the monitoring periods. The monitoring of the 18-month LLINs revealed a lower survival than expected at that time (74%: *Yorkool® LN* versus 84%: Net-Calc model). This result is associated with the greater than expected net loss and the physical state of the fabric during use. However, the proportion of the remaining nets that needed replacement after 18 months was not as important to suggest that the intervention would lose its impact in the second or third year of the distribution-replacement cycle [11] [25] [29] [30].

The attrition (loss rate) observed in our study (29.3%) after 18 months of monitoring is greater than that observed by Morgan *et al.* in his study after 3 years of monitoring [13]. The loss rate was mainly caused by displacement (25.2% in rural areas compared to 14.4% in urban areas). Rural field work in rural areas would be the basis of this movement of LLINs to the fields, especially during the rainy season. Relatively few LLINs were found used for other purposes (6.4% in rural areas versus 1.2% in urban areas). Only 6% of LLINs lost in our study were due to physical deterioration in both rural and urban areas.

In this evaluation, approximately 51.8% of the nets were still in good physical condition and without a hole after 18 months of use. There is a total of 56.8% of LLINs with a pH of less than or equal to 64 (LLINs in the category good status according to WHO criteria). This result runs in connection to observations made by Sanjiarizaha Randriamaherijaona *et al.* in his study after 12 months in Madagascar. Among the LLINs still present and assessed during the 18-month monitoring in our study, 56.8%, 7.8%, and 2.6% were found to be "in good condition," "damaged," and "too torn," respectively, by one year and half-use. This result contrasts with the 15%, 42.5% and 42.5% respectively "in good condition", "damaged" and "too torn", obtained after two years of use in Madagascar [31]. In Zambia, 9.6% of LLINs (polyester and polyethylene) were classified as "too torn" 12 months after field use [32], which is a relatively high proportion compared to the observations in this study where only 2.6% of the *Yorkool® LN* polyester LLINs were "too torn".

A year and a half after the distribution campaign, the proportion of LLINs in good working order was 94.6% in rural areas against 97.3% in urban areas with no significant difference. On the other hand, LLINs in the category "too much torn" were 7.6% in rural areas compared to 0.7% in urban areas with a significant difference. As expected, the pHI as well as the proportion of nets in the "too torn" category have increased over time, as has been the case in several studies [25] [29]. In our study, the rural area would likely have an impact on the appearance of holes in LLINs compared to the urban area. It has been shown that at the household level, mosquito nets can prevent blood supply even when they are damaged (64 <pHI ≤ 642). This finding is partly due to the repellent effect of the pyrethroids incorporated on the fiber [33]. Nevertheless, a high pHI value

(≥643) would easily allow a mosquito to enter an LLIN to bite a sleeping human and then rest outside the net.

LLINs found with larger holes were among those washed between two to five times. The number of washings seems to be at the origin of the tears, accelerating the loss of integrity of the fiber. The significant difference between the excessively torn LLINs (not used for sleeping) and the low tears (used every night), underline the importance of properly maintaining LLINs to maximize their efficient use for the sole purpose of protecting the population against the malaria vector. For this purpose, the suspension of the LLINs at the location of the sleeping material seems essential and cannot be explained by the low tear rate observed for its last ones. These factors, as well as the maintenance of LLINs, the user (adults or children), sleeping equipment and cooking medium, played an important role in the losses associated with the integrity of LLINs. Losses associated with the integrity of LLINs increase the probability of human-mosquito contact and malaria transmission [26] [34] [35]. In contrast to the study conducted in Benin in 2011 where the tissue integrity of more than half of the LLINs was compromised in the six months following distribution [17], our study found that the LLINs were in good physical condition in areas monitored with rates of at least 90%. This could be explained by the good behavior of people with regard to LLINs acquired over time to address the problem of physical damage by improving the education proposed by several authors already [36] to be able to hang, maintain and repair correctly the LLINs [37].

The evaluation of the biological efficacy revealed that the *Yorkool® LN* brand studied had a good residual efficacy, since it induces a rate (more than half of the LLINs tested) of KD after 60 minutes and of mortality after 24 hours. After 18 months, 58% of LLINs on average still met WHO quality standards. Our results corroborate most of the reports reported by some national programs that LLINs keep their insecticidal activity for at least three years [22]. But they go against those already obtained in Madagascar, which show that more than 75% of new *Yorkool® LN* mosquito nets did not meet the limit value set by WHO [31] at the beginning of the study.

Overall, our results showed a very good physical integrity of the LLINs after 18 months of use. However, LLINs found with holes were due to several factors including the frequency of washing in the different areas monitored which was closely related to frequency of use and maintenance. This washing frequency could be incriminated in the low lethality caused by LLINs over time, but which, however, keep a mortality of more than 50%. This is all the more important as the repellent and lethal effect prevents the mosquito from seeking to penetrate the net even when the mosquito net is torn. Survival, however, is below what is expected at this monitoring period. This result is much more attributable to the displacement of the population and to the lacerated and abandoned LLINs. To solve the problem, it may be necessary to focus on less frequent washing of nets and more care during the suspension and use of LLINs to extend LLINs in the field, as the national replacement of LLINs by a schedule of less than three, by programming, perfect highly improbable.

Limitations

This study includes some deficiencies in that among households that reported discarding LLINs (that means those lost sight of monitoring), this would have been due to damage. As a result, the durability of these LLINs may be underestimated by our results. And for good reason, we have not been able to assess the damage that would have been the basis of their loss. On the other hand, keeping LLINs too torn apart by some households beyond their "effective life" would expose household members to malaria risk even though they own and sleep under a LLIN.

Regarding LLINs of our study, repair of tears was not common, but it could affect the longevity of nets and should be investigated further before repair can be recommended as a way to extend the lifespan of LLINs.

Note that there is no standard definition of survival that incorporates a combination of variables that takes into account the three important indicators of attrition, physical integrity, and biological effectiveness of nets. Using current but limited evidence, WHO suggested calculating net survival using both physical integrity and attrition [7], as was the case in this study. However, integrating insecticide efficacy into a measure of the net sustainability of LLINs remains a challenge. A field test, allowing prospective studies of the efficacy of insecticides on a larger sample of nets, should be developed.

5. Conclusion

The observed differences in the survival of *Yorkool® LN* nets are due to community living conditions and movement, not to the equipment used to manufacture LLIN. However, the estimated median survival has shown that *Yorkool® LN* nets would have an average life of 2 years 8 months despite their relatively good physical condition. These results may help the National Malaria Control Program with the period of replacement of these nets in the field.

Ethical Consideration and Consent to Participate

This study received ethical approval (favorable ethical opinion n˚05 of 07 November 2017) from the CREC Institutional Ethics Committee (IECC). All heads of households aged 18 years or older who have received the *Yorkool® LN* nets under evaluation are voluntarily included in the study after signing the consent form and can withdraw if they wish without fear of retaliation.

Authors' Contributions

MCA and IBA designed the study. IBA, RHA, CZK, HS, CK, BA, carried out the surveys. IBA and BA analyzed the data. IBA realised the map. IBA drafted the manuscript. RYA, RAO, JFD, RG, AA, RA, VG, FT, GGP and MCA critically revised the manuscript. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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https://doi.org/10.1186/1475-2875-8-175

List of Abbreviations

CI: Confidence Interval
CREC: Center for Research in Entomology of Cotonou
KD: Knock-down
LLIN: Long-Lasting Insecticidal Net
LLIN: Long-Lasting Insecticidal Nets
NMCP: National Malaria Control Program
pHI: proportional hole index; WHO: World Health Organization
WHOPES: World Health Organization Pesticide Evaluation Scheme