

Physical Durability and Insecticidal Efficacy of Interceptor® G2, PermaNet® 3.0, Yahe® and Olyset® Brand Insecticide-Treated Nets between 2020 and 2023, Rwanda

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Abstract

Background

Understanding insecticide-treated net (ITN) durability helps National Malaria Programmes making crucial procurement and deployment decisions. Yahe® LN, Olyset®, Interceptor® G2 and PermaNet® 3.0 ITNs were monitored across four Rwandan districts for 36 months following a 2020 mass campaign. This enabled the calculation of physical integrity, median survival, insecticidal effectiveness, and key factors influencing ITN's physical durability.

Methodology

We deployed a prospective longitudinal methodology on a cohort of ITNs in each district. Each annual survey round, 30 ITNs were withdrawn from the cohort for bio-efficacy and chemical content testing. Determinants of ITN survival were assessed using a household questionnaire and analyzed using Cox proportional hazard models.

Results

At 36 months, the survival of cohort ITNs varied significantly between study sites ($p < 0.001$). The estimated median useful life was 4.0, 4.3, 3.1 and 2.3 years, respectively, for PermaNet® 3.0, Yahe® LN, Interceptor® G2, and Olyset® ITNs. The 24-h mortality of susceptible *An. gambiae* s.s. and reduction of insecticide content compared to the manufacturer's target dose was 54.2% and 77.8% for Yahe®LN, and 57.0% and 13.6% for Olyset® ITNs. The endline 24-h mortality using resistant mosquito strain for PermaNet® 3.0 was 48% on roof panels, which incorporated PBO. The mean roof panel PBO content was reduced by 81% compared to the manufacturer's target dose. For the Interceptor® G2 ITNs, 72-h mortality against resistant mosquitoes was 56.5%, with a 60% chlorfenapyr chemical content reduction. ITN-level factors influencing survival included not having children under 5 years old ($p = 0.012$), older heads of households ($p = 0.041$), drying practices after ITN washings ($p = 0.026$), folding ITN when not

in use ($p=0.020$), exposures to SBC messages ($p = 0.037$), cooking in rooms used for sleeping ($p = 0.010$).

Conclusion

Findings highlight site-specific trends in ITN median useful life and insecticidal efficacy. Risk factors impacting ITN survival may be used to guide SBC message and thus improve physical integrity of ITNs. The only Olyset® was under three years of survival life and replacement of new nets may be scheduled for three years' period in Rwanda.

Keywords: Malaria, Insecticide Treated Nets, Physical Integrity, Piperonyl Butoxide-PBO, Chlorfenapyr, Dual Active Ingredient, Insecticidal effectiveness, Rwanda

Background

Insecticide-treated nets (ITNs) are a crucial intervention in the fight against malaria, a leading cause of morbidity and mortality in sub-Saharan Africa (1), preventing infection through the provision of a physical barrier between humans and mosquitoes and the delivery of lethal chemicals when mosquitoes make contact with the ITN (2–4). ITNs are recommended for large-scale deployment by the World Health Organization (WHO) based on granular data for sub-nationally tailored deployments that prioritize those at most risk (5). Approximately 3.5 billion ITNs have been shipped to sub-Saharan Africa since 2004, contributing to a reduction in malaria deaths from 806,000 in 2004 to 610,000 in 2024 (1,6). Between 2001 and 2015, 78% of the 663 million clinical malaria cases prevented in sub-Saharan Africa have been attributed to the use of ITNs and indoor residual spraying (IRS) (7). An estimated 1.2 billion cases and 3.6 million deaths were averted between 2000 and 2024 through vector control interventions, including ITNs (8). Understanding the physical durability and insecticidal efficacy of ITNs is an essential consideration for national malaria programs (NMPs) to inform ITN procurement and deployment decisions (9–13); as such, ITN durability monitoring is recommended by the WHO (14).

WHO-supervised evaluations found that pyrethroid-only ITNs remain partially effective against malaria even in areas with pyrethroid resistance (15). ITN durability monitoring data for pyrethroid-only and newer ITN types from across 37 monitoring sites across sub-Saharan African show median ITN survival of 2.7 years, though results varied from 1.6 to 6.0 years (16). Durability monitoring data for new ITN types has demonstrated that ITNs may physically and chemically degrade more quickly than the assumed three-year lifespan of a typical ITN, with some studies suggesting that their actual useful lifetime is around two years in “real-world”

conditions (16–19). Further, efforts to repair damaged ITNs were largely ineffective, with many holes and or tears remaining only partially sealed (12,20).

Widespread pyrethroid resistance in sub-Saharan African mosquito vectors has threatened the insecticidal function of ITNs (21–23). In response, manufacturers have developed new ITN types, which combine a pyrethroid active ingredient (AI) with a non-pyrethroid AI or synergist: chlorfenapyr (CFP), piperonyl butoxide (PBO)-synergist, or pyriproxyfen. Since 2023, WHO has recommended CFP ITNs over pyrethroid-only ITNs, and conditionally recommended CFP ITNs over PBO ITNs, in areas with established pyrethroid resistance (24,25).

ITNs have been the primary vector control intervention in Rwanda since 2005. A nationwide study from 2011-2013 revealed rising pyrethroid resistance among Rwanda’s malaria vectors, highlighting the need for expanded monitoring of insecticide resistance and new vector control tools (26). While universal ITN coverage achieved in 2011 led to an 86% (27) decline in malaria cases compared to 2005, new cases increased annually from 2013 to 2016, reaching 308 per 1,000 people. This was attributed to pyrethroid resistance in malaria vectors and the expansion of water bodies from rice cultivation and dams (28). Between 2017 and 2020, Rwanda saw a decline in household ITN ownership and population ITN access, from 84% to 66%, and from 72% to 51%, respectively (29,30). However, in 2023, ITN ownership climbed back to 80%, and access rebounded to 72% (31). The Demographic and Health Survey conducted in 2020 found that 48% of the national survey population had used ITNs the previous night. The use: access ratio, which measures population-level use relative to access, was at 0.94 in 2020, reflecting almost universal use of ITNs among those with access (32). Use increased in 2023 to 66%, which indicates a use: access ratio of 0.92 (31).

In 2020, with support primarily from the U. S. President’s Malaria Initiative (PMI) and Global Fund, the Rwanda Biomedical Centre - Malaria & Other Parasitic Diseases Control Division

(RBC–MOPDCD) distributed approximately 7.5 million ITNs to the Rwandan population. The distributed ITNs included pyrethroid-only Yahe® LN (deltamethrin) and Olyset® (Permethrin) ITNs, as well as PermaNet® 3.0 ITNs (deltamethrin plus PBO-synergist), and Interceptor® G2 ITNs (alpha-cypermethrin plus CFP).

In response to the rise of insecticide resistance, the RBC-MOPDCD and PMI VectorLink (and its follow-on project, PMI Evolve) conducted a three-year assessment of the physical durability and insecticidal effectiveness of the four ITN brands distributed in 2020 under field conditions, measuring levels of ITN attrition, physical integrity, insecticidal activity, and individual attitudes to ITNs and ITN care following WHO guidelines (33,34). The specific objectives were: (1) to assess the physical durability (attrition and physical integrity), estimate median ITN survival, and identify major determinants of durability of PermaNet® 3.0, Yahe® LN, Interceptor® G2 and Olyset® brand ITNs over a three-year period; (2) to examine key behavioral factors related to ITN care and repair and their influence on physical integrity.; (3) to assess the insecticidal effectiveness (through bioassay and chemical content analysis) at prescribed time intervals and after three years of field use. Results will inform decisions about the selection and distribution/replacement of ITNs for future campaigns in Rwanda.

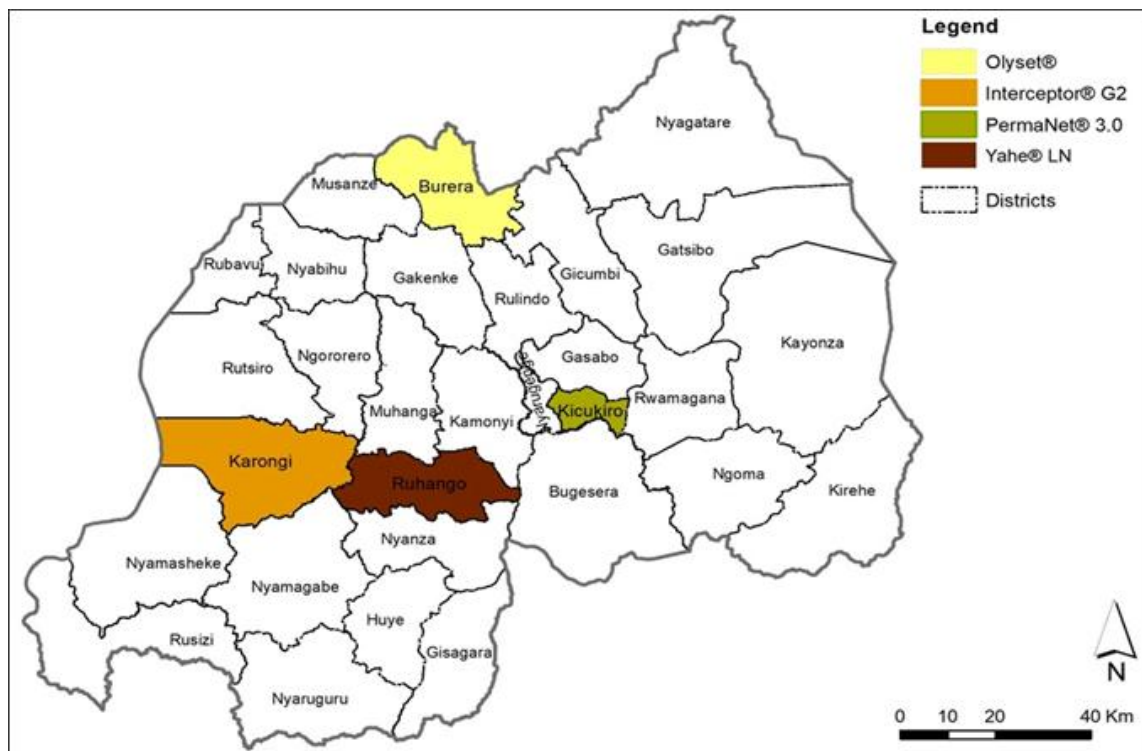
Methodology

Study Sites and ITN Brands

The study evaluated four ITN brands distributed during the 2020 mass campaign in Burera, Karongi, Kicukiro, and Ruhango districts of Rwanda. One rural sector per district was purposefully selected by the RBC–MOPDCD and PMI as study sites based on ITN distribution districts; past ITN durability monitoring and insecticide resistance surveys; proximity to entomology surveillance sentinel sites; stratum of malaria transmission; as well as accessibility and topography (28) (Figure 1). Burera and Karongi districts are rural areas with low malaria

transmission, while Ruhango district experiences a relatively high malaria burden. Kicukiro, situated in Kigali City, is a peri-urban area with moderate malaria transmission. However, only rural areas within Kicukiro were included in the study to ensure comparability across districts with regards to malaria endemicity, ecological factors, ITN access and usage, and household attributes. Farming is the primary socio-economic activity across all sites.

Figure 1: Map of Study Districts



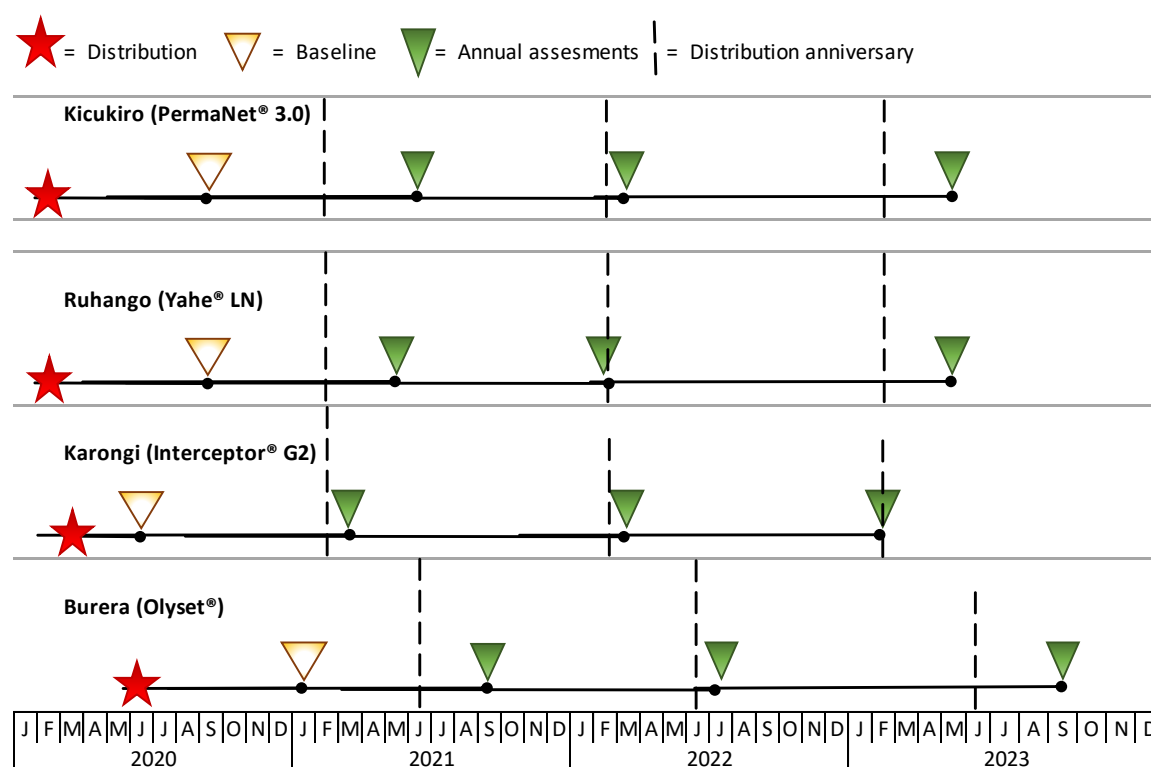
PermaNet® 3.0, manufactured by Vestergaard (<https://vestergaard.com/products/public-health/permanet-3-0/>) is a rectangular PBO-synergist ITN with polyester sides and a polyethylene roof, containing deltamethrin (2.8 g/kg on the sides and 4.0 g/kg on the roof) and PBO (25 g/kg on the roof). It has a denier of 75 on all sides and 100 denier on the roof. Yahe® LN ITNs, manufactured by Fujian Yamei Industry and Trade Co. Ltd. (yaheln.com), are rectangular, polyester 130 denier ITNs that contain 1.4g/kg of deltamethrin on all sides. Interceptor® G2, manufactured by BASF (agriculture.basf.com/global/en/business-areas/public-health/products/interceptor-g2), is a dual-AI polyester ITN with a denier of 100.

It is rectangular in shape and contains alpha-cypermethrin (2.4g/kg) and CFP (4.8g/kg) on all sides. Olyset®, manufactured by Sumitomo Chemical Co., Ltd. (sumitomo-chem-envirohealth.com), are 150 denier, rectangular polyethylene ITNs containing 20 g/kg of permethrin on all sides. All studied ITN brands are included in WHO's list of pre-qualified ITNs (35).

Study Design

This was a standard durability monitoring prospective longitudinal study on an ITN cohort to assess their durability from their distribution in 2020 until 2023 (34). The baseline round was conducted between September 1 and October 10, 2020 (three to seven months post-campaign). Subsequent data collection rounds included the 12-month survey from May 24 to July 3, 2021 (12 to 16 months post--campaign); the 24-month survey from March 8 to July 13, 2022 (24 to 25 months post-campaign); and the 36-month survey (also referred to as endline) from May 2 to July 27, 2023 (35 to 39 months post-campaign).

Figure 2: Durability Monitoring Timeline



During each round, data on ITN status, ITN durability risk factors, respondent knowledge, ITN attitudes, and ITN handling practices were recorded for cohort and non-cohort ITNs. A hole assessment was conducted on cohort ITNs to assess physical integrity. Thirty ITNs per site per round were taken from neighboring non-cohort households to test insecticidal efficacy, after confirming they were the required brand and were from the 2020 campaign. Withdrawn ITNs were replaced with identical replacements.

Sample Size and Sampling

This study employed standard PMI guidance for sample size calculations (36), assuming a design effect of 2.0 due to its clustered design with power of 80% and a buffer of 5% for loss-to-follow-up. ITNs from all households receiving an ITN during the mass campaign in selected study clusters were enlisted into the study. A total of 2,130 ITNs were reportedly received by 891 households during the mass campaign across the four districts. Some 116 ITNs suffered attrition between campaign distribution and the baseline survey round, leaving 2,024 ITNs from 883 households to be enrolled in the cohort. The sampling strategy was designed to detect a minimum 7.1 percentage-point difference in median ITN survival time across the study sites, assuming the median survival is three years. The sample size was not adjusted for multiple comparison testing. During the campaign distribution process, households were geo-located to facilitate subsequent visits. Methods for collecting ITNs for laboratory evaluation, were consistent with approaches documented in prior studies (37,38).

Training and Fieldwork Procedures

The RBC-MOPDCD, with support from PMI VectorLink and later, PMI Evolve, planned and conducted this study. Prior to each survey round, PMI project staff gave a one-day training of trainers and a three-day data collector training. During data collection, groups of seven members, including a supervisor, were deployed across districts. Each district had three data field teams, comprising a notetaker and an interviewer. Data collectors obtained necessary

approvals at the local level and enlisted local guides to facilitate the data collection process. Training and fieldwork procedures aligned with the existing literature (36–38).

Laboratory Procedures

During each survey round, 30 ITNs were collected from each site for bioassays and chemical analysis, following WHO guidelines (39). Bioassays were conducted by the RBC-MOPDCD for Olyset®, Yahe®, and PermaNet® 3.0 ITNs, employing standard WHO cone tests with an insectary-reared pyrethroid-susceptible strain (*Anopheles [An] gambiae* s.s.). Tests were also performed on PBO ITNs using wild-collected pyrethroid-resistant mosquitoes (*An. gambiae* s.l.). Both strains underwent characterization through WHO susceptibility tests, including deltamethrin 0.05% (the pyrethroid in Yahe® and PermaNet® 3.0), permethrin 0.75% (the pyrethroid in Olyset®), and alpha-cypermethrin 0.05% (the pyrethroid in Interceptor® G2). ITN samples were taken by cutting five 30x30 cm pieces per ITN: one from the roof panel, four from the side panels; for PermaNet® 3.0, three pieces from the roof for WHO cones tests. These samples were stored at 4°C until testing. The primary test used was the WHO cone assay, involving insectary-reared, susceptible two-to five-day-old, unfed female *An.gambiae* s.s. (Kisumu strain). Two WHO cones were applied to each piece of ITN sample for a three-minute exposure, with five mosquitoes per cone test, using a total of 50 mosquitoes per ITN. Post-exposure, mosquitoes were held in 200 ml plastic cups under controlled conditions (28°C ± 2°C, 80% ± 10% relative humidity), knock-down was recorded at 60 minutes (KD60), and sugar solution was provided afterward. A control group of untreated ITNs was included with two sets of five cones that had five mosquitoes per cone, using a total of 50 mosquitoes in the control for each day. Mortality rates were adjusted using Abbott's formula, accounting for results from negative controls, and percentage mortality was assessed 24 hours post-exposure, following WHO guidelines (39). Cone bioassays were also used to test the PermaNet® 3.0 ITN brand containing PBO using pyrethroid-resistant wild *An. gambiae* s.l. Five mosquitoes per

replicate, with three replicates per ITN piece (three pieces from roof panel), with a total of nine cone tests with 45 pyrethroid-resistant wild *An. gambiae* s.l. per ITN sample. In each case, KD60 and 24-hour mortality were measured. Additionally, both positive and negative controls control ITNs were tested alongside samples taken from ITNs collected from study communities. Each day, three new control nets were tested (an untreated net, a new pyrethroid + PBO ITN, and a new pyrethroid-only ITN). The new, untreated net served as negative control, the new PermaNet® 3.0 and new pyrethroid-only ITNs served as positive controls. Each of the three control nets was tested using one piece with five mosquitoes per cone and three replicates (nine cone tests with 45 mosquitoes per net per day in total). KD60 and 24-hour mortality for the controls was recorded and compared to the results from the field collected ITNs.

Tunnel tests were used for Interceptor® G2, using pyrethroid-resistant wild *An. gambiae* s.l. to assess the rate of mortality and blood-feeding success in host-seeking mosquitoes. A 60-cm glass tunnel with each end fitted with a 25-centimeter square cage covered by polyester netting was used for the test. A disposable cardboard frame held the ITN sample, positioned one-third of the way down the tunnel. The netting surface of the randomly selected ITN piece, with an area of 400 cm², contained nine single centimeter diameter holes: one central hole and eight equidistant holes near the edges. A restrained guinea pig was placed in the shorter section of the tunnel as bait. One hundred, five to eight-day old mosquitoes, that had been sugar-starved for six hours were released at the opposite end at 6:00 p.m., and the lights were switched off until 6:00 a.m. the following day. The mosquitoes had to make contact with the netting and find the holes to reach the bait. Post-feeding, mosquitoes could return to the cage to rest. An untreated netting tunnel served as the negative control, whereas a new Interceptor® G2 ITN and a new alpha-cypermethrin-only ITN were used as positive control. At 6:00 a.m., mosquitoes were collected from the tunnel, and the team noted the compartment where the

mosquitoes were collected (initial compartment/animal compartment), the blood feeding status (fed/unfed), and mortality (living/dead). Living mosquitoes were placed into cups covered with untreated netting and provided with sugar solution. Mortality was recorded from 24 to 72 hours post-exposure, and blood feeding inhibition (BFI) was determined.

The 30 cm x 30 cm pieces cut adjacent to those of bioassays samples were sent to the U.S. Centers for Disease Control and Prevention (CDC) in Atlanta for chemical residue analysis using procedures described elsewhere (38). The AIs were extracted using a standardized method (40), and the pooled mean and median AI content across the samples were reported and compared to the manufacturer's target dose.

Data Management

The study utilized digital data collection methods, including adapting questionnaires to the Rwandan context and using Survey CTO software (www.surveyccto.com) for electronic data capture. The data was uploaded daily and monitored remotely by the research team. Datasets were generated after each round and prepared for longitudinal analysis using Stata v15.1 (Stata, Texas, USA) software.

Definition of Outcomes and Explanatory Variables

Specific durability monitoring outcomes, associated explanatory variables and definitions, are discussed in depth in prior research studies (10,13,37,38,41,42). In summary, the primary outcome was the proportion of cohort ITNs that remained in serviceable condition over time. This was measured by tracking ITN attrition (ITNs no longer in households) and physical integrity, which categorized ITNs as "torn" or "serviceable." The term 'ITN attrition' refers to the loss of ITNs from the study cohort, often due to wear and tear, which may include ITNs that were discarded, destroyed, or repurposed. Conversely, unknown outcomes encompassed ITNs reportedly given to relatives or others, sold, lost, or classified as stolen. For the purposes of estimating ITN survival, ITNs with unknown outcomes were excluded from the analysis.

The proportion of cohort ITNs surviving in serviceable condition was plotted against hypothetical survival curves to determine the median survival, extrapolated from the final survey. The study collected various factors at the household and ITN levels to assess risks for ITN durability, including ITN handling, sleeping space environment, and exposure to ITN messaging and attitudes. The study utilized Likert scale questions to evaluate respondents' attitudes towards ITNs and their associated care and repair practices. Respondents indicated their level of agreement with a set of standardized statements, which were then numerically recorded on a scale ranging from -2 for 'strongly disagree' to +2 for 'strongly agree.' An overall attitude score was calculated by summing the values for each response and dividing by the total number of statements. A positive score greater than 0 signified a favorable attitude towards the topic under assessment. Additionally, the physical condition of the remaining cohort ITNs was evaluated by data collectors using a standardized hole assessment tool to enumerate and categorize holes by size.

The measured laboratory outcomes for the PBO ITN (PermaNet® 3.0) included the number of mosquitoes exposed, KD60, and mortality at 24 hours. For standard Yahe® and Olyset® ITNs, the primary outcome was optimal effectiveness, which is defined by a KD60 of at least 95% and a 24-hour mortality rate of 80% or higher. Outcomes for Interceptor® G2 ITNs included 24-, 48-, and 72-hour mortality rates, as well as blood-feeding and BFI rates.

Statistical Analyses

Bivariate analysis with significance testing was conducted to identify differences between groups. Bivariate analysis utilized statistical tests for trend to assess significance across study sites; no direct pairwise comparisons were performed except for selected outcomes, such as survival analysis, in which pairwise log-rank comparisons were done between study sites. The analysis accounted for the clustered survey design when calculating standard errors and 95% confidence intervals (CI). Continuous variables were analyzed using medians and means, while

285 one-way analysis of variance was employed to compare results across more than two groups
286 for both non-parametric and normally distributed data. Statistical analyses using chi-square
287 tests and contingency tables were conducted to compare proportions across the study sites.
288 Determinants of survival were explored using Cox proportional hazard models that accounted
289 for the clustering of ITNs within households. An ITN was considered to have failed if it was
290 classified as either "torn" or had undergone attrition due to wear and tear. Failure time for
291 attrition was directly calculated based on respondent recall of how long the ITN was in the
292 household. In the absence of valid recall data, and for ITNs failing due to physical integrity,
293 the failure time was taken as the midpoint between the last two survey rounds. Two models
294 were fitted, following distinct survival analysis approaches. The first was a household-level
295 model that considered household-level determinants invariant for ITNs within a given
296 household (e.g., study district, household size, household head characteristics, household
297 wealth tertile, presence of children in the household, exposure to social and behavior change
298 [SBC] messaging, cooking practices, attitudes to ITNs), with failure risk starting at the
299 distribution date. The second was an ITN-level model that examined both household-level and
300 ITN-level determinants, such as, ITN folding, washing, and drying, with failure risk, starting
301 at the date the ITN was first observed hanging. Bivariate analysis identified significant survival
302 factors, and variables with p-values below 0.3 were included in an initial multivariate model.
303 The models were then refined through a stepwise process, dropping variables exceeding the
304 $p=0.1$ level and comparing model specifications using the likelihood-ratio test. The $p=0.1$
305 threshold for stepwise selection was conservative relative to automated approaches, and all
306 reported alphas were significant at the typical 5% level.

307 A sensitivity analysis was conducted post-hoc, in which physical integrity categories and
308 serviceability status were compared between ITNs subsequently reported stolen and those not

reported stolen using chi-square tests. Continuous proportionate hole index (pHI) values were compared using the Wilcoxon rank-sum test.

Results

Household and Cohort ITNs

In Rwanda, campaign ITNs were uniquely labeled or tagged with identification numbers before distribution. A total of 3,353 tagged ITNs were distributed in the four study sites during the 2020 mass distribution campaign. At baseline, 883 households were enrolled with 2,024 campaign ITNs assigned for follow-up. Follow-up status is summarized in Table 1, with round-specific details available in the supplementary materials [see *Additional Files: A and B*].

After three years, most households in the study sites maintained possession of one or more cohort ITNs (Kicukiro: 81%, Ruhango: 85%, Karongi: 72%, and Burera: 85%). The most common reasons for households being lost to follow-up were relocation in Kicukiro (10%) and Ruhango (8%), and the almost complete loss of cohort ITNs in Karongi (13%) and Burera (8%). Known outcomes were determined for 58% of cohort ITNs in Kicukiro, 67% in Ruhango, 51% in Karongi, and 62% in Burera. Across all study sites, unknown outcomes were commonly due to ITNs being given away or taken for use elsewhere (Kicukiro: 27%; Ruhango: 20%; Karongi: 29%; Burera: 28%).

Table 1. Follow-up status of households and cohort ITNs at 36 months

Variable	Kicukiro (PermaNet® 3.0) % (95% CI)	Ruhango (Yahe®) % (95% CI)	Karongi (Interceptor® G2) % (95% CI)	Burera (Olyset®) % (95% CI)
Households	N=213	N=215	N=235	N=220
Still has any cohort ITN	80.8 (72.6-86.9)	85.1 (80.2-89.0)	71.9 (65.8-77.3)	85.0 (80.4-88.7)
Lost all their cohort ITNs	5.6 (3.4-9.1)	6.0 (3.7-9.8)	12.8 (9.1-17.6)	8.2 (5.4-12.2)
Moved away	10.3 (6.2-16.7)	7.9 (5.2-11.8)	8.5 (5.3-13.3)	5.0 (2.6-9.2)
Refused	0.5 (0.1-3.4)	0.0 (--)	2.6 (1.1-5.7)	0.0 (--)
Nobody home at survey or not reached	2.8 (1.1-7.2)	0.9 (0.2-3.6)	4.3 (2.6-6.9)	1.8 (0.6-5.7)
Labelled cohort ITNs	N=496	N=504	N=536	N=488
Known outcome	58.1 (47.2-68.2)	66.5 (55.8-75.7)	51.3 (40.7-61.8)	62.1 (51.7-71.5)
Unknown outcome	41.9 (31.8-52.8)	33.5 (24.3-44.2)	48.7 (38.2-59.3)	37.9 (28.5-48.3)

Household moved away or refused participation	8.5 (4.1-16.8)	6.9 (3.3-14.0)	9.3 (4.7-17.5)	3.9 (1.6-9.0)
ITNs given away or used elsewhere	27.4 (22.3-33.2)	20.0 (14.2-27.5)	28.7 (22.7-35.6)	27.5 (20.6-35.5)
Fate of ITN unknown	6.0 (3.5-10.3)	6.5 (4.5-9.5)	10.6 (8.1-13.8)	6.6 (4.3-9.8)

327 **Household Characteristics**

328 Some key demographic and household characteristics varied significantly across study
329 districts. Female-headed households were most common in Karongi (42%) and least common
330 in Kicukiro (21%) ($p = 0.0001$, test for trend). The percentage of households with non-literate
331 male household heads was highest in Karongi (29%) and lowest in Kicukiro (15%) ($p=0.014$).
332 The percentage of households with non-literate female household heads was Karongi (61%)
333 and Burera (57%) ($p=0.006$). Significant disparities were observed in infrastructure and assets:
334 improved wall materials were highest in Burera (76%) and lowest in Kicukiro (37%) ($p <$
335 0.001), while improved flooring was most common in Kicukiro (50%) and least in Karongi
336 and Burera (18-19%) ($p < 0.001$). Access to safe water was highest in Ruhango (98%) ($p =$
337 0.0034), while access to latrine averaged 99% across study district ($p = 0.1505$). Ownership of
338 transport, livestock, radios, and mobile phones varied widely, with Kicukiro leading in most
339 assets, including smartphone ownership (p -values < 0.05) (Table 2).

340 *[Table 2. Baseline characteristics of households enrolled in the Rwanda 2020-2023 ITN*
341 *durability monitoring evaluation]*

342 **Risk Factors for ITN Durability**

343 Household-level ITN durability risk factors differed between evaluation districts (Table 3).
344 Across all districts, most households reported "always" observing rodents in the past six
345 months, with percentages ranging from 78% to 84% ($p = 0.2757$). A notably small portion of
346 households reported "always" storing food in sleeping rooms (Kicukiro: 9%; Ruhango: 11%;
347 Karongi: 12%; Burera: 3%, $p<0.001$).

Cooking in sleeping rooms was most prevalent in Ruhango (30%) and Karongi (25%), with households reporting they do so "at times", while almost all households in Kicukiro (93%) and Burera (92%) reported "never" cooking in sleeping rooms ($p < 0.001$). A significant portion of households in Kicukiro (70%) had never been exposed to ITN use or care messages across survey rounds, while in Ruhango, Karongi, and Burera, only 40%, 25% and 38%, respectively, reported no exposure ($p < 0.001$).

Based on responses across the four survey rounds, most households in Kicukiro (85%) and Karongi (84%) were classified as having a very positive attitude towards ITN use (score > 1.0) in at least two survey rounds, compared to Ruhango (67%) and Burera (37%) ($p < 0.001$). Most households in Ruhango (94%) and Karongi (87%) had a very positive attitude towards ITN care, while Burera had the lowest percentage (56%) with significant differences between districts ($p < 0.001$).

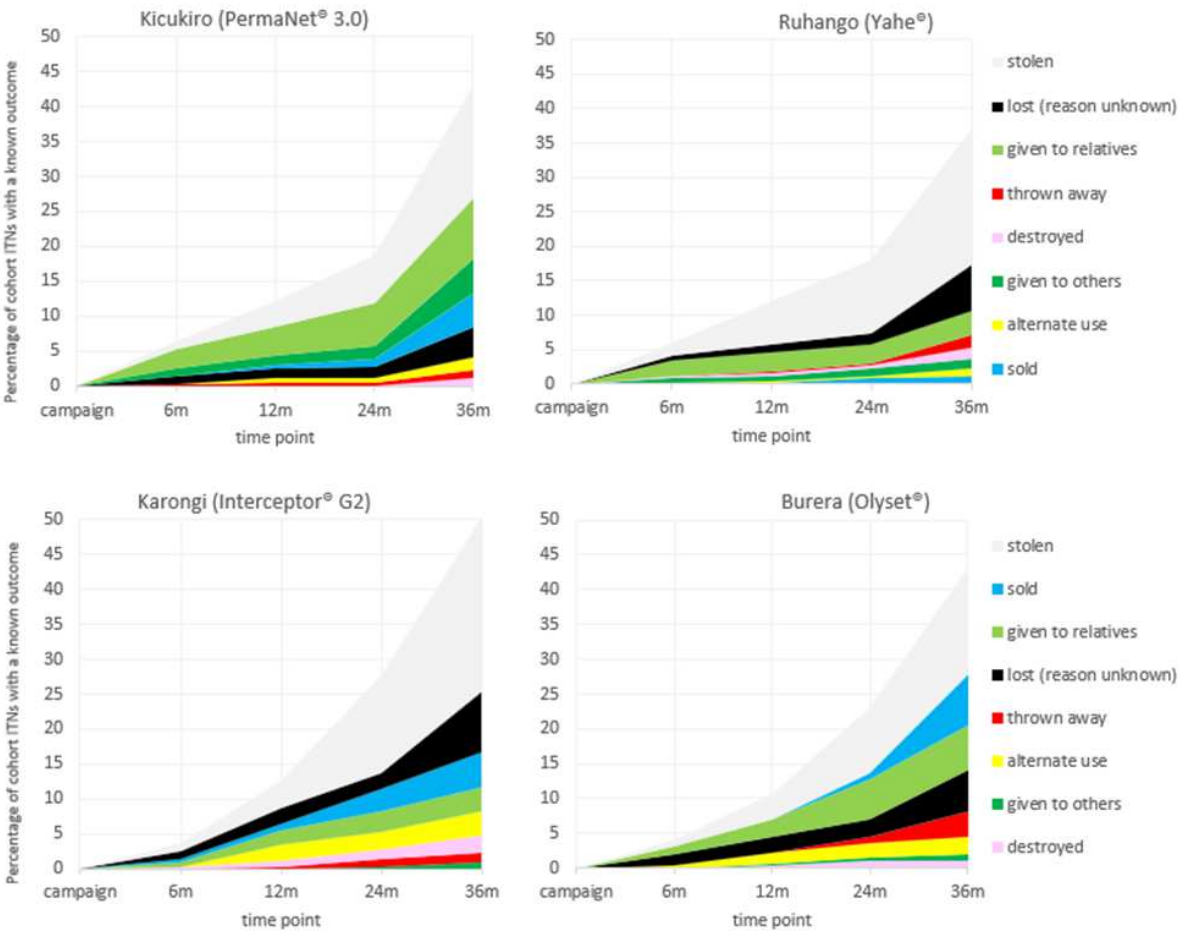
[Table 3. Household-level risk factors across survey rounds]

[Table 4. ITN-level risk factors across all survey rounds]

Attrition

At endline, total cohort ITN attrition was similar across study sites (Kicukiro: 43%; Ruhango: 37%; Karongi: 50%; Burera: 43%) ($p = 0.3819$). Across all districts, the most frequently reported reason for ITN attrition was nets classified as "stolen" (Kicukiro: 16%; Ruhango: 20%; Karongi: 25%; Burera: 15%), based on respondent self-reporting; however, these reports were not independently verified. Attrition due to wear and tear ranged from 4% in Kicukiro to 7% in Karongi [see Additional File C].

Figure 3: Reported causes of ITN attrition by study site and ITN brand



371

372 **Physical Integrity**

373 By evaluation endline, the proportion of ITNs with any holes varied significantly among study
374 sites, with Burera (93%) having the highest proportion, followed by Ruhango (92%), Karongi
375 (88%), and Kicukiro (82%) ($p=0.005$). The highest proportion of ITNs classified as “too torn”
376 was in Burera (59%, 36% in Karongi, 31% in Kicukiro, 26% in Ruhango; $p<0.001$). Levels of
377 repair varied significantly across districts, from 48% in Ruhango, to 34% in Kicukiro, 20% in
378 Karongi and 12% in Burera ($p<0.001$).

379 *[Table 5. Physical integrity of observed cohort ITNs]*

380 **Survival**

381 At endline, the proportion of all cohort ITNs surviving in serviceable condition varied
382 significantly between sites and was 33% and lowest in Burera, 53% in Karongi, 61% in

Kicukiro, 66% in Ruhango; $p < 0.001$) (Table 6). Olyset® ITNs from Burera consistently exhibited significantly lower survival across all survey rounds. Pairwise comparisons (Table 6a) showed a significantly higher proportion of ITNs surviving in serviceable condition in Kicukiro than Burera ($p < 0.001$), in Ruhango than Karongi ($p = 0.026$), in Ruhango than Burera ($p < 0.001$), and in Karongi than Burera ($p = 0.006$) (Table 6a). Estimated median survival, extrapolated from physical survival data (Figure 4), was 4.3 (95% CI: 4.0-4.7) years in Ruhango (Yahe® LN), 4.0 (95% CI: 3.6-4.4) years in Kicukiro (PermaNet® 3.0), 3.1 (95% CI: 2.7-3.8) years for ITNs in Karongi (Interceptor® G2), and 2.3 (95% CI: 2.0-2.7) years for ITNs in Burera (Olyset®).

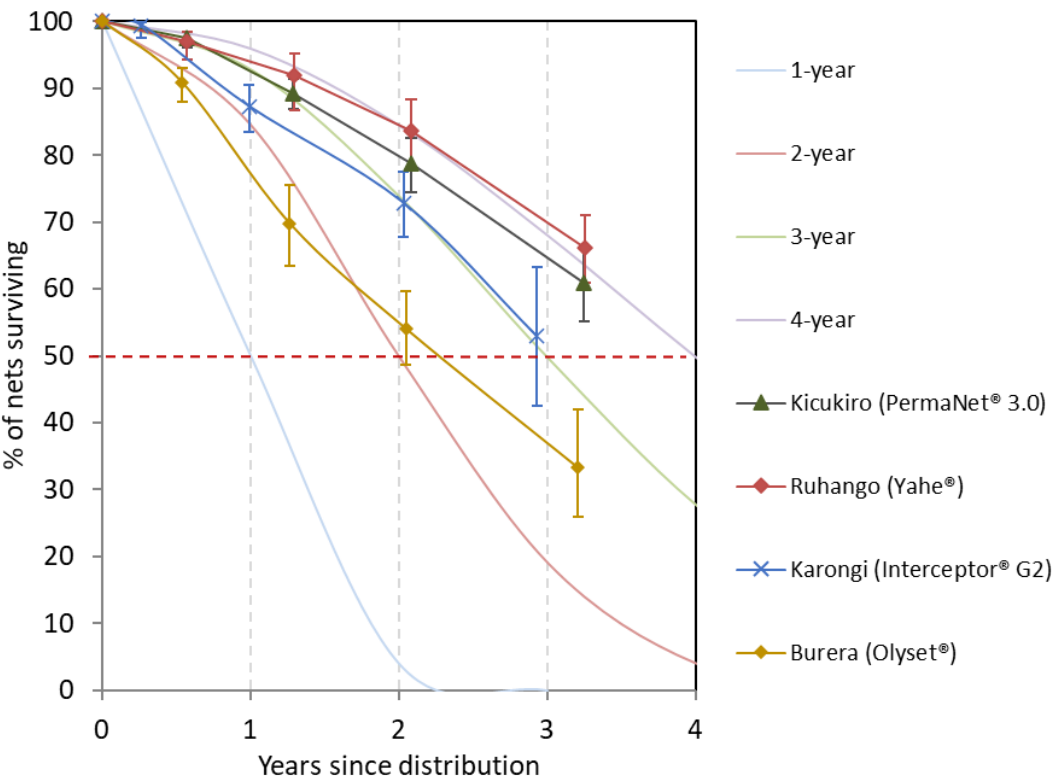
Table 6. Cohort ITNs surviving in serviceable condition and estimated median survival in years

	Second round 12 months % (95% CI)	Third round 24 months % (95% CI)	Fourth round 36 months % (95% CI)
Kicukiro (PermaNet® 3.0)			
Percentage surviving in serviceable condition	89.3 (86.4-91.7)	78.8 (74.3-82.7)	60.9 (55.1-66.4)
Median survival in years			
Graphical interpretation from Figure 4		3.4	3.8
Calculated from last two data points			4.0 (3.6-4.4)
Ruhango (Yahe®)			
Percentage surviving in serviceable condition	91.9 (87.8-94.7)	83.7 (78.2-88.1)	66.2 (60.9-71.1)
Median survival in years			
Graphical interpretation from Figure 4		4.0	4.2
Calculated from last two data points			4.3 (4.0-4.7)
Karongi (Interceptor® G2)			
Percentage surviving in serviceable condition	87.3 (82.2-91.2)	72.9 (66.4-78.6)	53.0 (42.4-63.3)
Median survival in years			
Graphical interpretation from Figure 4		3.0	3.1
Calculated from last two data points			3.1 (2.7-3.8)
Burera (Olyset®)			
Percentage surviving in serviceable condition	69.8 (63.6-75.4)	54.1 (48.3-59.9)	33.4 (25.8-42.0)
Median survival in years			
Graphical interpretation from Figure 4		2.4	2.6
Calculated from last two data points			2.3 (2.0-2.7)

Table 6a. Pairwise comparisons p-values for the proportion of cohort ITNs surviving in serviceable conditions at 36 months

Pairwise p-values	Kicukiro	Ruhango	Karongi	Burera
Kicukiro	--	0.173	0.191	<0.001
Ruhango		--	0.026	<0.001
Karongi			--	0.006
Burera				--

Figure 4. Physical survival



Survival differed significantly across the four study sites using either time from distribution or time from first hang as the time origin (overall log-rank test, $p<0.001$ for both). Kaplan–Meier survival curves are shown in Figure 5 and pairwise comparisons are shown in Table 6b. Pairwise comparisons showed that survival was significantly higher in Ruhango (Yahe®) than in Karongi (Interceptor® G2) and Burera (Olyset®) for both time origins (both $p<0.001$). When time from distribution was used, survival in Ruhango was not significantly different from Kicukiro (PermaNet® 3.0) ($p=0.137$), but survival was significantly higher when time from first hang was used as the time origin ($p=0.040$).

Figure 5. Kaplan-Meier curves for ITNs surviving in serviceable condition

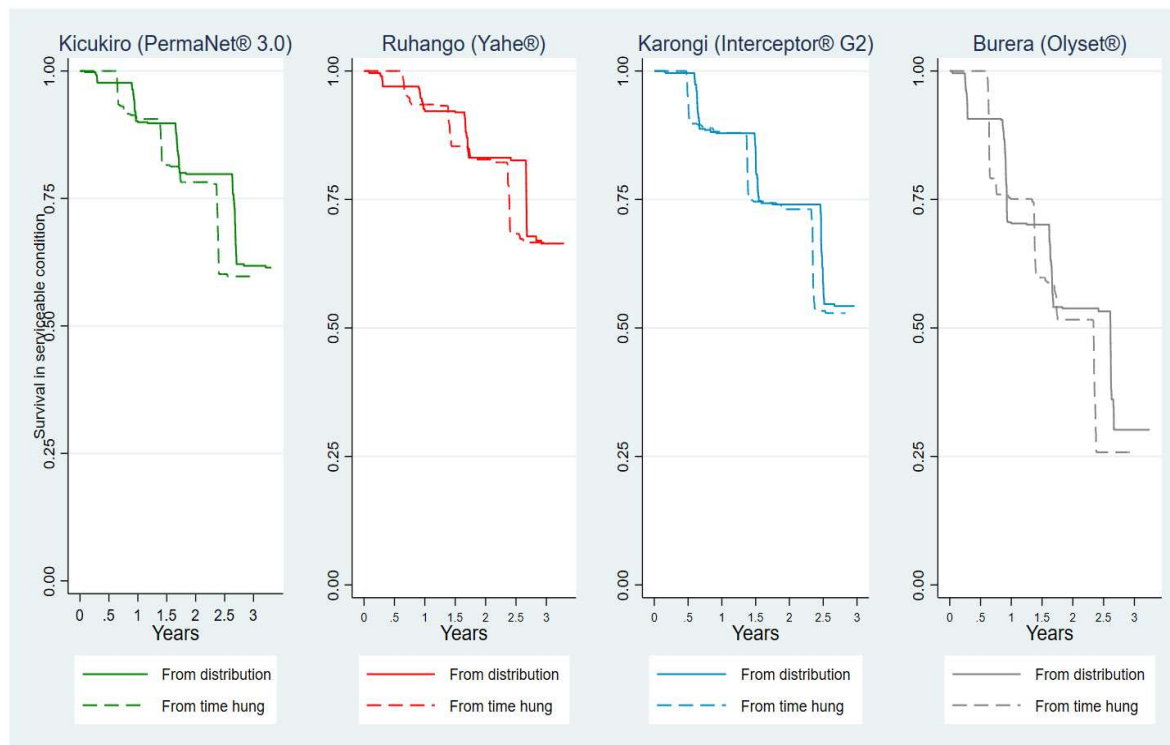


Table 6b. Pairwise comparison p-values for Kaplan-Meier survival curves by study district using time from distribution and time from first hang as the time origin

Pairwise comparison	Time from distribution (p-value)	Time from first hang (p-value)
Kicukiro vs Ruhango	0.137	0.040
Kicukiro vs Karongi	0.003	0.011
Kicukiro vs Burera	<0.001	<0.001
Ruhango vs Karongi	<0.001	<0.001
Ruhango vs Burera	<0.001	<0.001
Karongi vs Burera	<0.001	<0.001

Determinants of Survival

Cox proportional hazard models at the household and ITN levels were developed to identify factors influencing physical ITN durability (Table 7). The initial model for the Cox proportional hazards analysis included the following household-level covariates: location (district), wealth tertile, age and sex of the household head, presence of children under five (CU5), education level of the household head, storing food in the sleeping room, cooking in sleeping rooms, net attitudes across surveys, net care attitudes across surveys, SBC messaging, and reports of seeing rodents. For the net-level model, additional covariates were included:

418 folding the net, type of sleeping place, type of net users, net ever washed, number of washes in
419 the last six months, use of detergent during washing, and methods of drying the net (on a bush-
420 fence or outside). The final household-level regression model included the study site, socio-
421 economic status tertiles (derived from the household wealth index), presence of CU5, age of
422 the household head, ITN care attitudes, exposure to SBC messages, cooking in sleeping rooms,
423 and rodent observations.

424 Interceptor® G2 ITNs in Karongi demonstrated a 39% reduced risk of failure compared to
425 Olyset® in Burera (adjusted hazard ratio [aHR] = 0.61, 95% CI: 0.45-0.83, $p = 0.001$).
426 PermaNet® 3.0 ITNs in Kicukiro had an even lower risk of failure compared to Olyset®, with
427 a 57% reduction (aHR = 0.43, 95% CI: 0.32-0.57, $p = 0.000$). Yahe® LN ITNs in Ruhango
428 exhibited the lowest risk of failure compared to Olyset®, with an aHR of 0.31 (95% CI: 0.23-
429 0.41, $p < 0.001$).

430 ITNs in households from the highest socio-economic status tertile had a 24% lower risk of
431 failure than those in households in the lowest tertile (aHR = 0.76, 95% CI: 0.59-0.98, $p =$
432 0.036).

433 ITNs from households without CU5 in all survey rounds had significantly lower (29%) risk of
434 failure compared to those with CU5 (aHR = 0.71, 95% CI: 0.57-0.87, $p = 0.001$).

435 Household head age data was categorized into two groups based on the median value of 47
436 years: those at or below the median, and those above the median. ITNs from households with
437 older heads had significantly lower risk of failure compared to younger heads (aHR = 0.76,
438 95% CI: 0.61-0.94, $p = 0.010$). ITNs from households with positive attitudes towards ITN care
439 and repair in two or more survey rounds were associated with significantly lower risk of failure
440 than those without (aHR = 0.72, 95% CI: 0.56-0.93, $p = 0.013$). Exposure to SBC two or more
441 times significantly reduced the risk of ITN failure compared to no SBC exposure (aHR = 0.68,
442 95% CI: 0.53-0.88, $p = 0.004$). Households that never cooked in the room used for sleeping

had significantly lower risk of ITN failure than those who had done so (aHR = 0.74, 95% CI: 0.58-0.94, $p = 0.016$).

At the ITN level, the variables included in the final model were: study site, socio-economic status tertiles (based on household wealth index), presence of CU5 in the household, age of head of household, exposure to SBC messages, cooking in rooms used for sleeping, and ITN care attitudes, frequency of ITN washing, use of detergents, ITN drying practices after washing, ITN folding.

As with the household-level model, at the ITN-level, ITN from Ruhango had the least likelihood of failure compared to ITNs from Burera (aHR = 0.28, 95% CI: 0.2-0.38, $p < 0.001$). ITNs from households without CU5 had significantly lower (25%) risk of failure compared to those with CU5 (aHR = 0.75, 95% CI: 0.59-0.94, $p = 0.012$). ITNs from households with older heads had a significantly lower risk of failure compared to younger heads (aHR = 0.79, 95% CI: 0.62-0.99, $p = 0.041$). ITNs never dried on bushes or fences had a 21% lower risk of failure than ITNs dried on bushes or fences after washing (aHR = 0.79 95% CI: 0.64-0.97, $p = 0.026$). ITNs that were always folded when not in use had a 27% lower risk of failure than ITNs that were never folded when not in use (aHR = 0.73 95% CI: 0.56-0.95, $p = 0.020$). Exposure to SBC two or more times significantly reduced the risk of ITN failure compared to no SBC exposure (aHR = 0.75, 95% CI: 0.58-0.98, $p = 0.037$). Households that never cooked in the room used for sleeping had 29% lower risk of ITN failure than those who had done so (aHR = 0.71, 95% CI: 0.55-0.92, $p = 0.010$).

[Table 7. Modelled determinants of physical durability from Cox proportional hazard models]

Insecticidal Efficacy

In pyrethroid-only PermaNet® 3.0 ITN side samples, 24-hour mortality against susceptible mosquitoes ranged from 99.8% (95% CI: 99.6-100.1) at baseline to 56.7% (95% CI: 51.8-61.7) by endline. Roof samples incorporating PBO-synergist demonstrated 100% (95% CI: 100.0-

100.0) 24-hour mortality against susceptible mosquitoes at baseline, decreasing to 70.3% (95% CI: 67.2-73.4) by endline.

Against pyrethroid-resistant wild mosquitoes, 24-hour roof sample mortality reduced from 98.9% (95% CI: 98.4-99.4) at baseline to 48.0% (95% CI: 44.9-51.2) at endline. Mean side panel deltamethrin content decreased from 1.5 g/kg (95% CI: 1.0-2.1) at baseline to 0.4 g/kg (95% CI: 0.0-0.8) at endline. Mean roof panel deltamethrin content remained similar, ranging from 3.3 g/kg (95% CI: 3.1-3.6) at baseline to 3.7 g/kg (95% CI: 3.2-4.2) at endline. Mean roof PBO content decreased from 14.9 g/kg (95% CI: 13.0-16.7) at baseline to 4.8 g/kg (95% CI: 3.1-6.5) by the endline.

KD60 in Yahe® ITNs declined from 98.7% (95% CI: 98.1-99.2) at baseline to 54.2% (95% CI: 48.3-60.1) at endline against susceptible mosquitoes. Mortality at 24 hours declined from 100.0% (95% CI: 100.0-100.0) at baseline to 53.3% (95% CI: 47.3-59.4) at endline. These results corresponded to a decline of both optimal and minimal effectiveness to 0% and 1%, respectively. Mean deltamethrin concentration declined from 0.9 g/kg (95% CI: 0.7-1.2) at baseline to 0.2 g/kg (95% CI: 0.2-0.3) at endline.

In tunnel tests conducted on Interceptor® G2 ITN samples using pyrethroid-resistant wild mosquitoes, 72-hour mortality declined from 81.8% (95% CI: 76.7-86.9) to 56.5% (95% CI: 52.7-60.2). Throughout the study, BFI did not follow a consistent pattern, starting at 55.0% (95% CI: 41.7-68.3) at baseline, peaking at 81.3% (95% CI: 71.0-91.6) in the third round, and then declining to 44.7% (95% CI: 38.0-51.4) by the endline. Mean alpha-cypermethrin declined from 2.6 g/kg (95% CI: 2.3-2.8) to 1.3 g/kg (95% CI: 1.0-1.6) at endline. Mean CFP content declined from 4.4 g/kg (95% CI: 3.9-5.0) to 1.9 g/kg (95% CI: 1.2-2.5) at endline.

Against susceptible mosquitoes, KD60 declined for Olyset® ITN samples from 69.7% (95% CI: 63.5-75.9) at baseline to 51.7% (95% CI: 46.3-57.1) at endline. Baseline 24-hour mortality was 75.8% (95% CI: 69.9-81.7), which decreased to 52.2% (95% CI: 47.4-57.0) at endline.

This resulted in both optimal and minimal effectiveness, declining to 0% and 1%, respectively.

Mean permethrin content declined, although not linearly, from 22.0 g/kg (95% CI: 21.5-22.4) at baseline to 19.0 g/kg (95% CI: 18.0-20.0) at endline.

[Table 8. Insecticide effectiveness and chemical content results of campaign ITNs of campaign ITNs: PermaNet® 3.0]

Table 9. Insecticide effectiveness and chemical content results of campaign ITNs: Yahe®

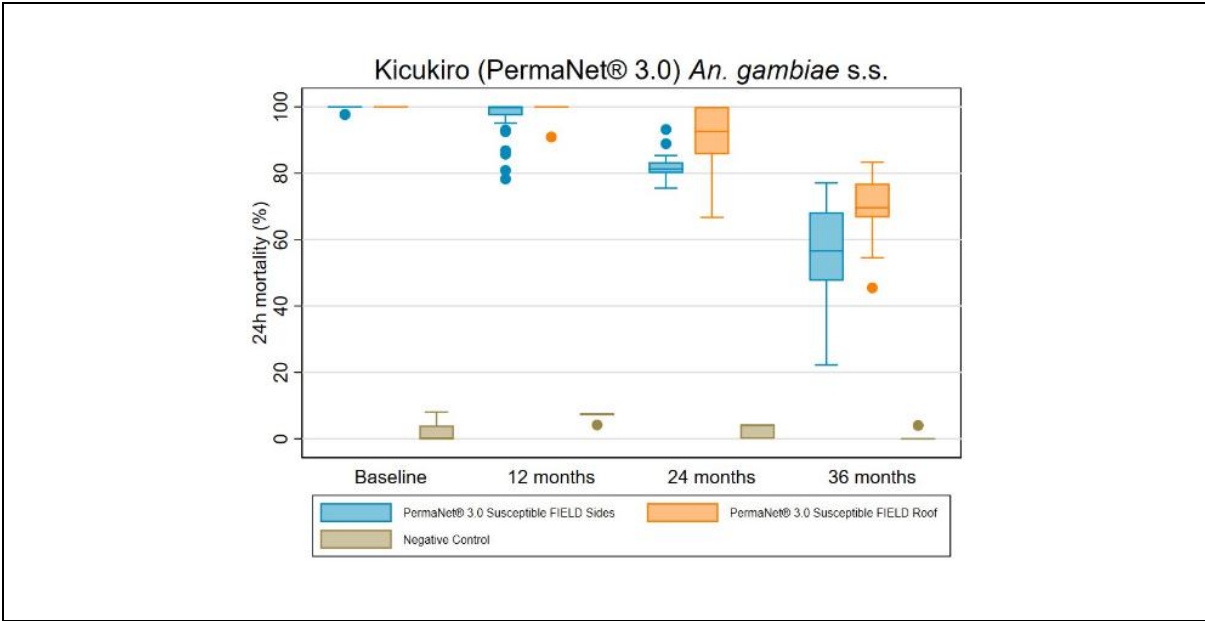
	Baseline % (95% CI)		Second round % (95 % CI)		Third round % (95 % CI)		Endline % (95 % CI)	
Yahe®	N=30		N=30		N=30		N=30	
Mean months since campaign	6.9		15.5		25		39	
Cone bioassays (Susceptible strain) KD60								
Mean	98.7	(98.1-99.2)	83.1	(78.8-87.5)	85.9	(82.6-89.2)	54.2	(48.3-60.1)
Median [IQR]	99.1	(98.0-100.0)	85.9	(78.0-90.7)	86.9	(77.4-93.2)	53.0	(42.6-68.0)
Mortality 24 hours								
Mean	100.0	(100.0-100.0)	85.1	(81.9-88.2)	81.3	(77.4-85.2)	53.3	(47.3-59.4)
Median [IQR]	100.0	(100.0-100.0)	85.6	(80.3-90.6)	81.7	(73.6-90.0)	54.1	(36.5-68.6)
Chemical Content	N=30		N=30		N=30		N=30	
Pyrethroid (Deltamethrin)								
Mean	0.9	(0.7-1.2)	0.5	(0.3-0.8)	0.4	(0.3-0.6)	0.2	(0.2-0.3)
Median [IQR]	0.9	(0.7-1.3)	0.4	(0.2-0.9)	0.4	(0.2-0.7)	0.2	(0.1-0.3)

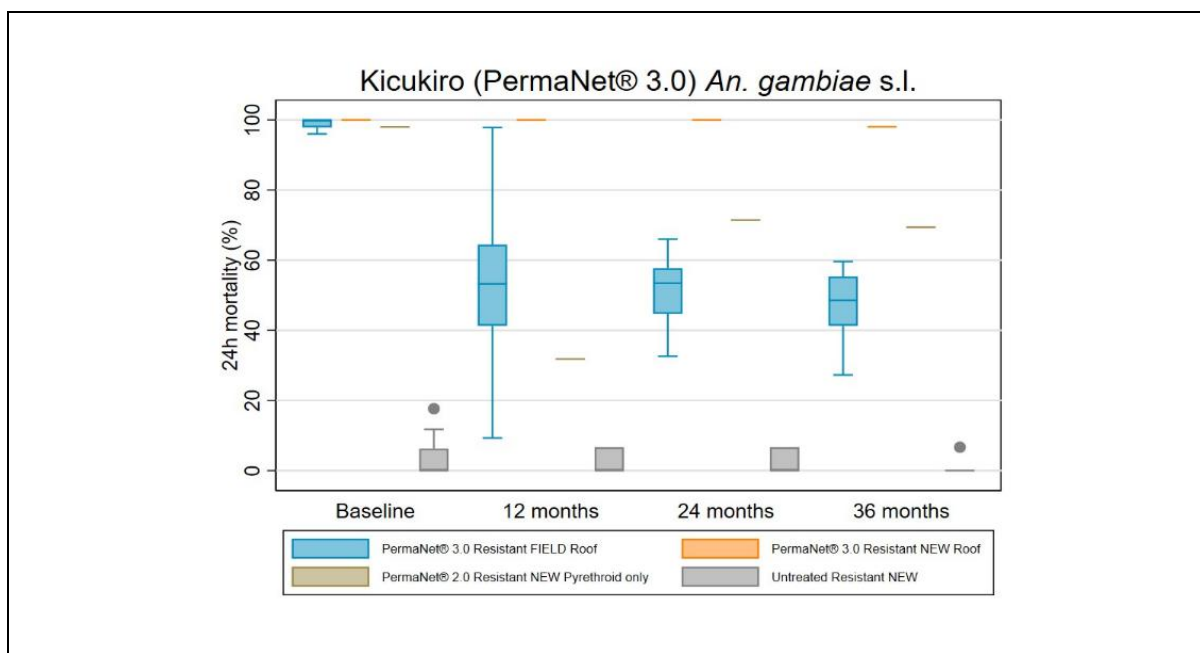
[Table 10. Insecticide effectiveness and chemical content results of campaign ITNs of campaign ITNs: Interceptor ® G2]

502 Table 11. Insecticide effectiveness and chemical content results of campaign ITNs of
503 campaign ITNs: Olyset®

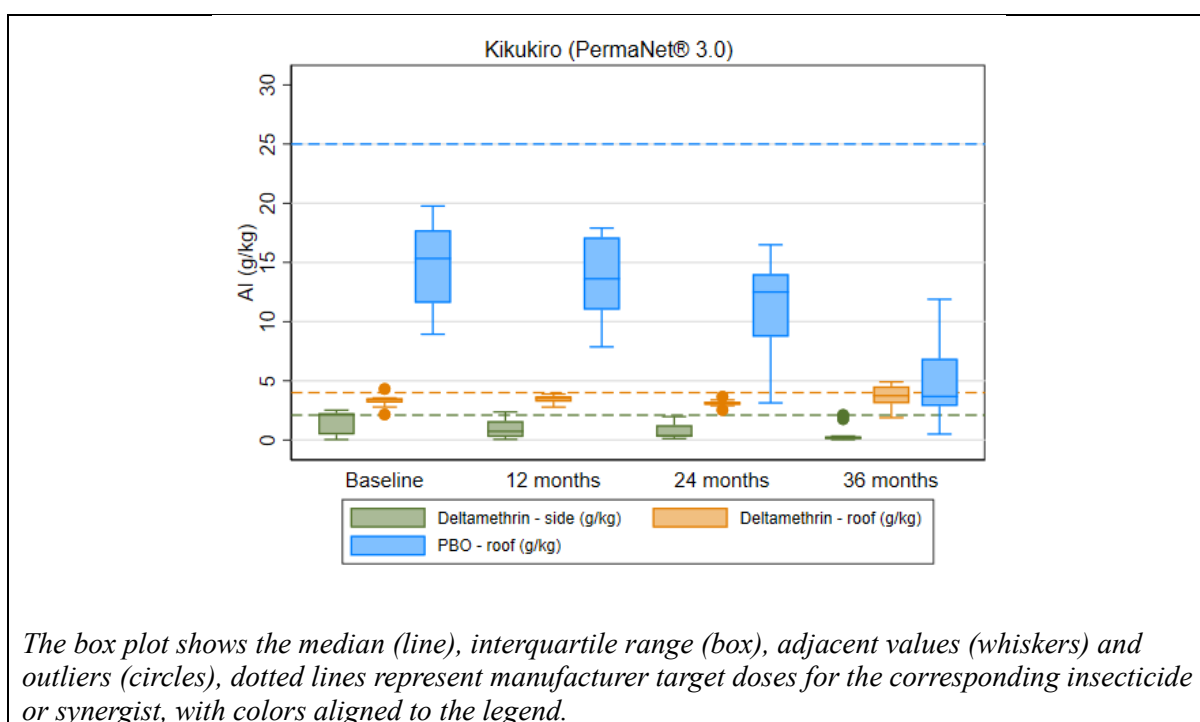
	Baseline % (95% CI)		Second round % (95 % CI)		Third round % (95 % CI)		Endline % (95 % CI)	
Olyset®	N=30		N=30		N=30		N=30	
Mean months since campaign	6.9		15.5		25		39	
Cone bioassays (Susceptible strain) KD60								
	Mean	69.7 (63.5-75.9)	85.0 (78.7-91.3)	67.5 (64.1-70.9)	51.7 (46.3-57.1)			
	Median [IQR]	69.9 (59.3-82.7)	89.7 (79.2-98.4)	68.5 (63.6-73.1)	55.2 (38.9-63.3)			
	Mortality 24 hours							
	Mean	75.8 (69.9-81.7)	66.2 (55.9-76.6)	62.6 (59.5-65.7)	52.2 (47.4-57.0)			
	Median [IQR]	78.1 (63.0-87.0)	73.8 (41.4-88.7)	64.9 (58.5-69.7)	55.2 (41.2-63.0)			
Chemical Content	N=30		N=30		N=30		N=30	
Pyrethroid (Permethrin)								
Mean	22.0 (21.5-22.4)	20.0 (19.6-20.5)	17.7 (17.0-18.5)	19.0 (18.0-20.0)				
Median [IQR]	21.9 (21.5-22.8)	20.3 (19.3-20.5)	17.5 (16.7-19.0)	19.5 (18.9-20.1)				

504 Figure 6. Box plot of PermaNet® 3.0 cone bioassay results for residual efficacy of Pyrethroid

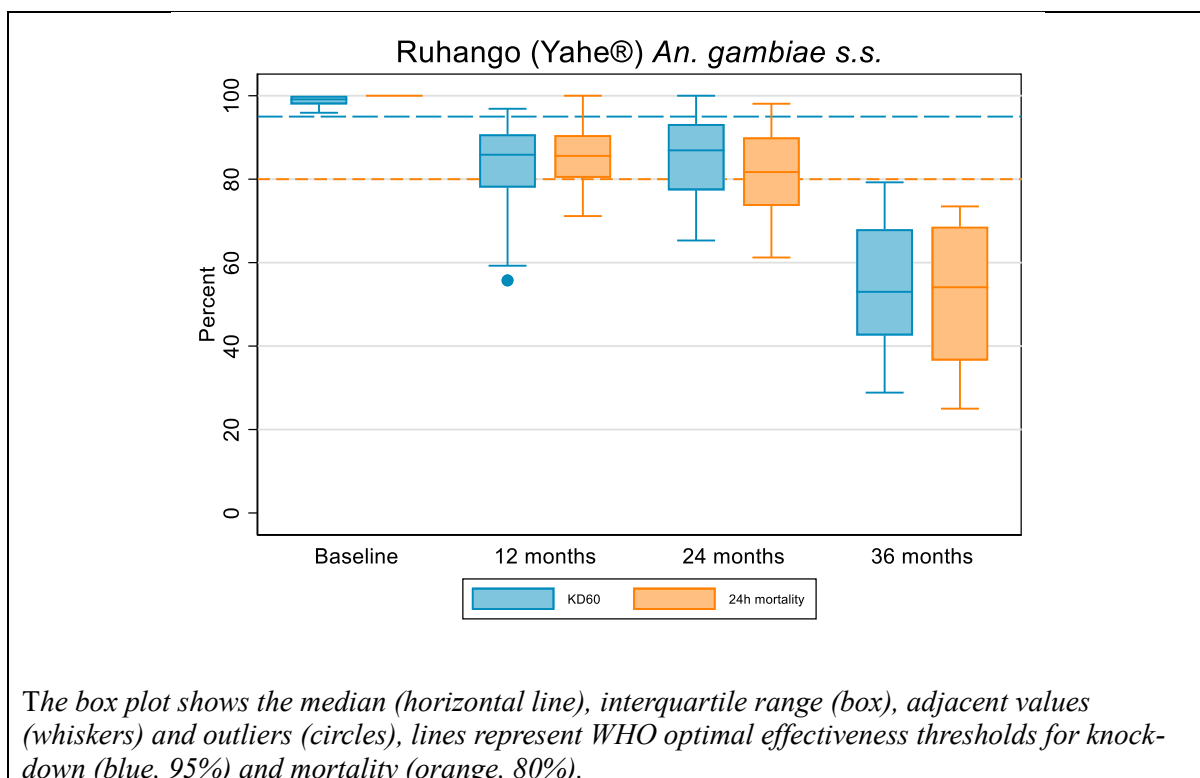




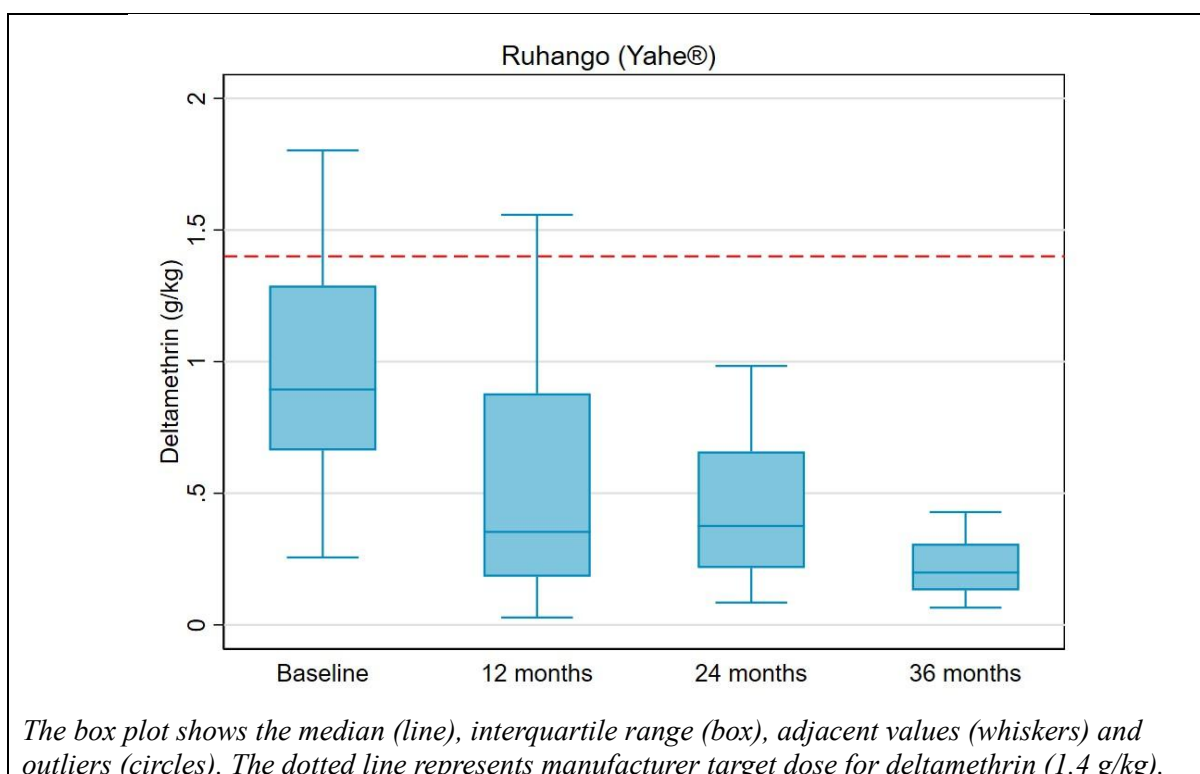
505 Figure 7. Box plot of PermaNet® 3.0 chemical content results of Pyrethroid and PBO



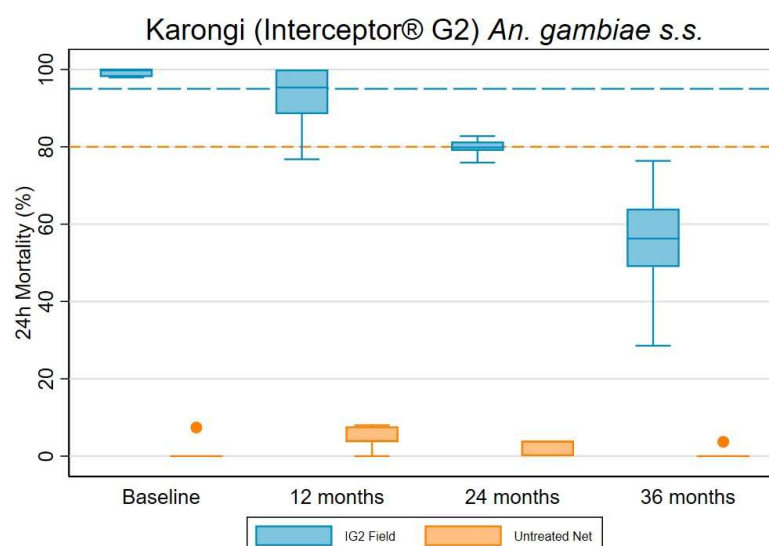
506 Figure 8. Box plot of Yahe® cone bioassay results for residual efficacy and chemical content



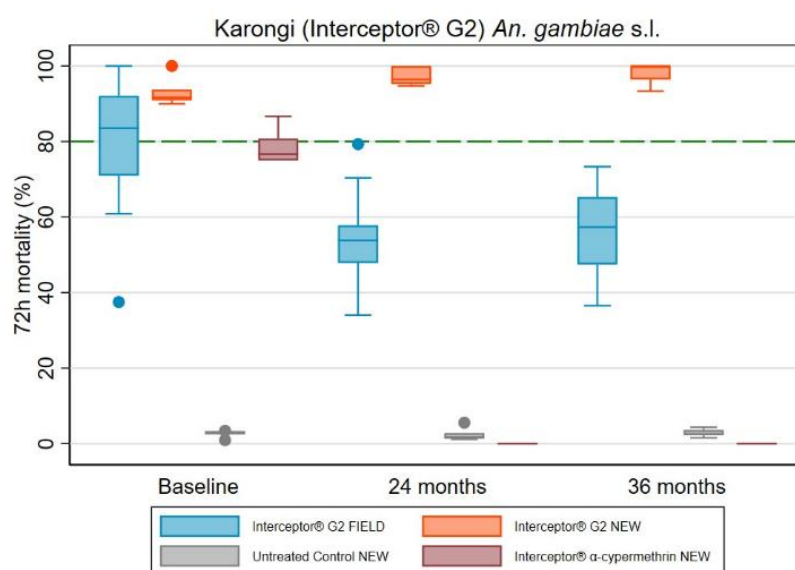
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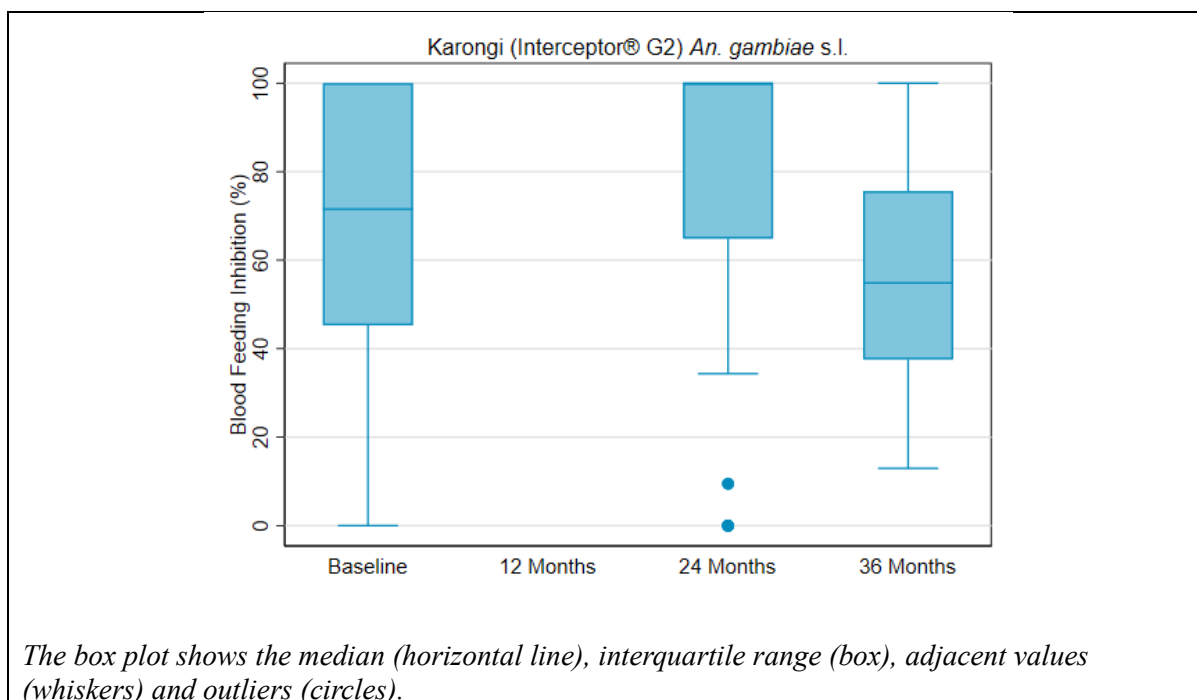
508 *Figure 9. Box plot of Interceptor® G2 bioassay results for residual efficacy*



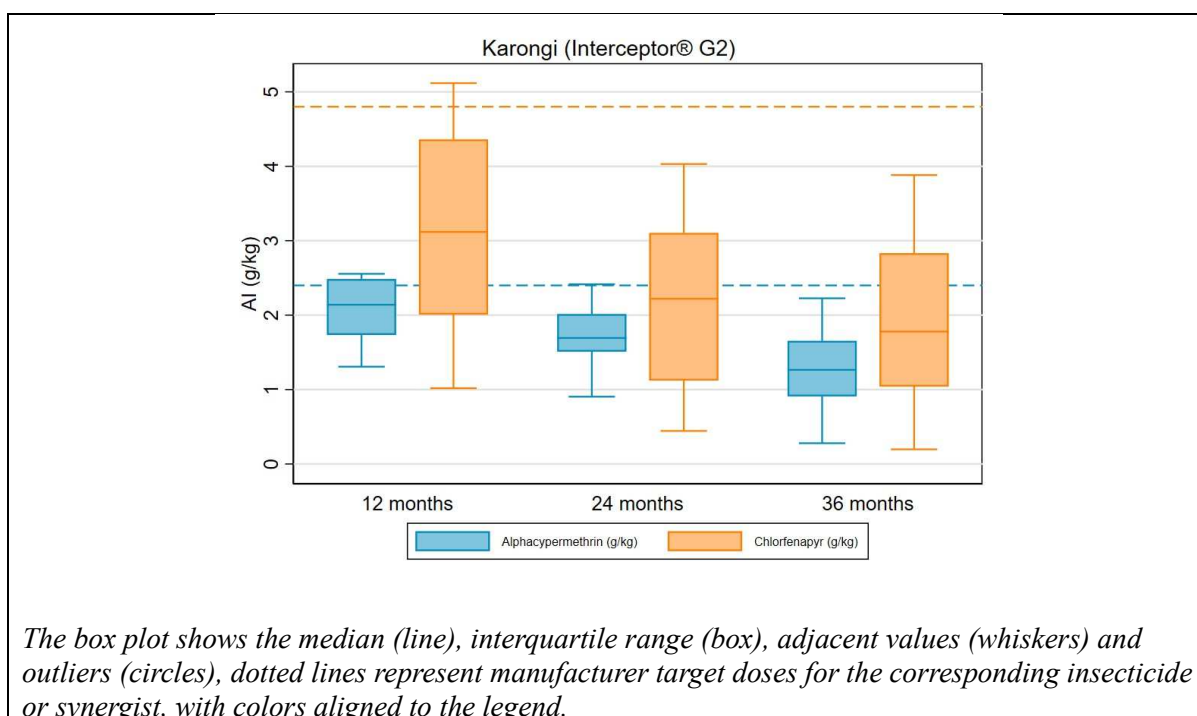
The box plot shows the median (horizontal line), interquartile range (box), adjacent values (whiskers) and outliers (circles), lines represent WHO optimal effectiveness thresholds for knock-down (blue, 95%) and mortality (orange, 80%).



The box plot shows the median (horizontal line), interquartile range (box), adjacent values (whiskers) and outliers (circles), lines represent WHO thresholds for 72-mortality (green, 80%).

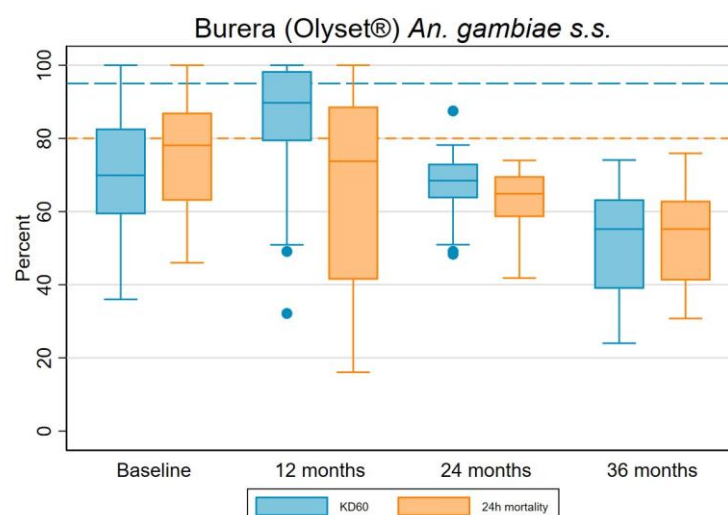


509 Figure 7. Box plot of Interceptor® G2 chemical content results

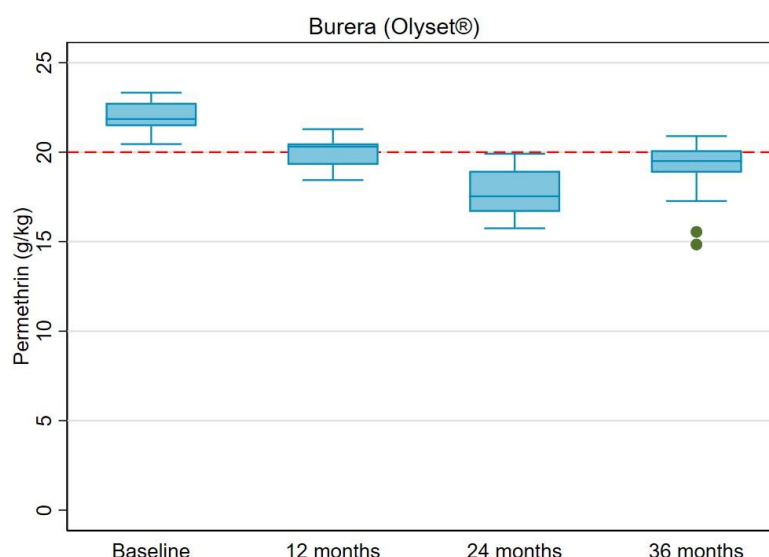


510 Figure 9. Box plot of Olyset® bioassay results for residual efficacy and chemical content

511 results



The box plot shows the median (horizontal line), interquartile range (box), adjacent values (whiskers) and outliers (circles), lines represent WHO optimal effectiveness thresholds for knock-down (blue, 95%) and mortality (orange, 80%).



The box plot shows the median (line), interquartile range (box), adjacent values (whiskers) and outliers (circles). The dotted line represents manufacturer target dose for permethrin (20 g/kg).

Discussion

This study assessed the physical durability and insecticidal effectiveness of Interceptor® G2, PermaNet® 3.0, Olyset®, and Yahe® ITNs under field conditions over three years in Rwanda following a national ITN distribution campaign performed in 2020.

ITN Physical Durability and Insecticidal effectiveness

Total levels of attrition for cohort ITNs were comparable across the four study districts. Attrition due to wear and tear was minimal in all districts. Theft emerged as the primary driver of ITN attrition, being reported for 17.3% of cohort ITNs. Unknown ITN outcomes, including those classified as stolen, were based on respondent self-report. Across eight recent PMI VectorLink and PMI Evolve standard durability monitoring studies (34) (Burkina Faso, Cote d'Ivoire, Ghana, Liberia, Madagascar, Niger, Sierra Leone and Zambia), the mean proportion of nets reported stolen was 3.3% (range: 0.1% - 6.8%). Rwanda's outlier status triggered a sensitivity analysis (see *Additional File D*). At least one household in each of the 60 study clusters reported one or more ITNs stolen, indicating that this phenomenon was not clustered. ITNs reported as stolen had poorer physical integrity and higher median pHI scores measured during the previous round than nets that were not. Heads of households reporting at least one ITN stolen were more likely to come from the lowest wealth tertile. Taken together, it is likely that some households reporting an ITN as stolen did so to meet social desirability standards that would discourage the perceived premature damaging or throwing away of an ITN that was expected to last for three years. Results from the sensitivity analysis demonstrate that expected median lifespan was reduced across study sites and more closely aligned with those values reported in a durability monitoring study conducted in Rwanda in 2014 (43).

Burera district had the highest percentage of ITNs with holes, followed by Ruhango, Karongi, and Kicukiro. Burera also had the highest proportion of ITNs classified as "too torn" at 59%, compared to 36% in Karongi, 31% in Kicukiro, and 26% in Ruhango. Conversely, Ruhango had the highest proportion of ITNs deemed "serviceable."

The study found significant differences in ITN survival at 36 months across the four districts, with the highest survival in Ruhango, followed by Kicukiro, Karongi, and Burera. The estimated median useful life of ITNs ranged from 2.3 years in Burera to 4.3 years in Ruhango.

541 The WHO has recently recommended prioritizing the procurement of ITNs containing
542 pyrethroid-CFP or pyrethroid-PBO over pyrethroid-only ITNs in areas with pyrethroid-
543 resistant mosquitoes (44). Stakeholders should adhere to these recommendations when making
544 ITN procurement and distribution decisions. It is noteworthy that the 24-hour mortality rates
545 for the two pyrethroid-only ITNs evaluated in this study were between 52% and 53% against
546 susceptible mosquitoes at the end of the study period. Despite experiencing reductions in
547 chemical content and corresponding decreases in mortality, PermaNet® 3.0 and Interceptor®
548 G2 still outperformed the pyrethroid-only ITNs in terms of mortality against susceptible
549 mosquitoes at the study's conclusion (24-hour susceptible strain mortality for PermaNet® ITNs:
550 70.3% and Interceptor® G2 ITNs: 56.5%, as no 72-hour mortality was recorded against the
551 susceptible strain). Furthermore, PermaNet® 3.0 and Interceptor® G2 ITNs demonstrated the
552 ability to kill 48% and 57% of resistant mosquitoes. Finally, both PermaNet® 3.0 and
553 Interceptor® G2 ITNs exhibited median survival estimates exceeding three years.

554 At the end of the study, the median survival duration of the Yahe® LN ITNs from Ruhango
555 was 4.3 years. The Yahe® LN exhibited the lowest proportion of damaged ITNs, leading to the
556 longest estimated median survival time among the ITN types assessed. However, by endline,
557 optimal and minimal insecticidal effectiveness had declined to 0% and 1%, respectively, and
558 the mean deltamethrin content had decreased by 86% compared to the manufacturer's target
559 dose. Similar evidence regarding the optimal effectiveness of Yahe® LN ITNs in ITN
560 durability monitoring studies indicates concerns about their insecticidal effectiveness over
561 time. Additionally, another study recently highlighted that Yahe® ITNs exhibited a notably
562 short-lived insecticidal efficacy, failing to meet the WHO's recommended performance criteria
563 after three years of use (45).

564 Olyset® ITNs in Burera failed to achieve the median expected lifespan of three years, primarily
565 due to a high incidence of physical damage, evidenced by a significant number of ITNs with

holes. This deterioration may have been compounded by notably lower levels of repair compared to other study sites (10,46). The 2.3-year median survival estimate for Olyset® ITNs from Burera was comparable to 1.5 - 1.9 year estimates in Olyset® ITNs in Benin (47). Furthermore, the insecticidal effectiveness of the Olyset® ITNs diminished markedly, with optimal and minimal efficacy dropping to 0% and 1%, respectively, indicating a severe loss of chemical content against malaria vectors (46,48). Despite declines in bio-efficacy, the mean permethrin content decreased by only 5% relative to the manufacturer's target dose, suggesting that the chemical retention was not the primary issue affecting ITN performance. This discrepancy highlights the need for further utilization of dual-AI or PBO-synergist ITNs in areas where resistance has been detected.

The estimated median lifespan of PermaNet® 3.0 ITNs in Kicukiro was found to be 4.0 years, which surpasses the 3.2-year and 3.0-year median survival estimates recorded for the same ITN type in Orodara, Burkina Faso, and Bo, Sierra Leone, respectively (37,38). This extended survival period suggests that the environmental and usage conditions in Kicukiro, a district in the City of Kigali with greater access to social amenities, including education, may be more favorable for the longevity of these ITNs. However, despite these robust physical survival outcomes, the 24-hour mortality rate against pyrethroid-resistant mosquitoes at endline was 48%, indicating a decline in insecticidal effectiveness over time. Furthermore, mean PBO content dropped by 81% compared to the manufacturer's target dose, raising concerns about the ITNs' ability to provide adequate chemical protection in areas with high levels of insecticide resistance over their target lifespan.

The estimated median survival of Interceptor® G2 ITNs from Karongi was found to be 3.1 years, which is longer than the 2.6-year median lifespan reported for the same ITN type in Banfora, Burkina Faso (37). However, this survival time is concerning when considered alongside the observed reductions in both BFI and 72-hour mortality rates, which were

recorded at 45% and 57%, respectively. These declines correspond with reductions in the mean content of the AIs, alpha-cypermethrin and CFP, which decreased by 46% and 60%, respectively, compared to the manufacturer's target dose by endline. The gradual degradation of these ITNs compromises their ability to effectively kill pyrethroid-resistant mosquitoes, highlighting a critical issue in the long-term efficacy of dual-AI ITNs (49).

These findings underscore the importance of selecting appropriate ITN types based on their durability, as well as the need for continuous monitoring of ITN performance in varying environmental conditions to ensure effective malaria control strategies (50).

Risk factors and SBC

Recent work refining resistance-to-damage scores (51) and developing an ITN risk index composite metric (52) has demonstrated that ITN survivorship is dependent upon both brand-level physical durability characteristics and exposure to environmental, handling and care/repair risk factors. The study findings highlight several household-level factors that influenced the durability of ITNs. ITNs distributed in the Ruhango district exhibited the lowest risk of failure, followed by those in Karongi, Kicukiro, and Burera. This site and brand variability in ITN durability underscores the importance of localized strategies in malaria prevention efforts. Consistent with previous research, households with higher socioeconomic status were less likely to experience ITN failure (9). This suggests that wealthier households may have greater resources and better practices for maintaining ITNs, thereby extending their effectiveness.

The presence of CU5 was associated with increased ITN failure, likely due to more frequent handling and strain as noted in other durability monitoring studies (38). Conversely, households with older heads had lower failure risk. Positive attitudes towards ITN care and exposure to behavior change messages reduced failure risk, consistent with other studies (10,37,38). The assessment found that households that did not cook in sleeping areas had a reduced risk of ITN

failure, consistent with established evidence that cooking in sleeping spaces is a known risk factor for ITN durability (9).

The present study's analysis of ITN-level factors revealed critical determinants of ITN durability. Household-level characteristics also impacted the risk of ITN failure at the ITN-level analysis. Households with higher socioeconomic status and older heads were less likely to experience failure. Conversely, the presence of young children was associated with increased failure risk, possibly due to more frequent and rigorous ITN use.

The study also highlighted the crucial role of ITN care practices in determining ITN durability. ITNs that were consistently folded and never dried on bushes or fences exhibited a lower risk of failure, as found in other studies (9,10,38,53). This point suggests that promoting proper ITN care and handling practices can extend the lifespan of ITNs, emphasizing the need for sustained education within communities on the correct maintenance of these malaria prevention tools.

Additionally, SBC messaging exposure was associated with reduced ITN failure risk in line with existing literature, highlighting the effectiveness of SBC interventions in reinforcing positive ITN care behaviors (10,37,38). Risk factor analysis suggests that a multifaceted approach, addressing ITN type and household- and ITN-level factors, is essential for improving ITN durability and strengthening malaria prevention.

Limitations

Adhering to COVID-19 prevention protocols, data collectors did not enter inside homes. Instead, household members themselves reported the household's ITN care and repair habits, a practice which may have caused recall bias among participants. Additionally, household members may have been influenced by the presence and involvement of data collectors, which could indicate an incidence of the Hawthorne effect (54). However, there is no reason that differing effects should be expected across the four districts evaluated. The study could not be

designed to assess four ITN brands across a single district, as the NMP distributed each ITN brand to a different district – potentially introducing confounding results due to inter-district variability (28). To lessen potential disparities, rural sectors with comparable malaria endemicity, ecological factors, ITN access and usage, and household attributes were selected in the four districts. In Ruhango District, malaria prevention efforts included IRS, which could impact vector density and behavior, directly influencing malaria transmission and epidemiology and potentially contributing to variability between Ruhango and other sites. Like other durability monitoring studies (besides endline evaluations), the ITNs analyzed were from neighboring households, and not cohort households. This sampling method could cause doubt about the ITNs' usage duration, subsequently affecting the assessment of the insecticidal efficacy. Examining ITN survival, bio-efficacy, and chemical content in conjunction with malaria prevalence data could reveal whether there is a correlation between ITN survival and malaria incidence rates. However, this was not part of the scope of this study.

Conclusion

This study provides a comprehensive evaluation of the physical durability and insecticidal efficacy of four ITN brands (Interceptor® G2, PermaNet® 3.0, Olyset®, and Yahe®) over a three-year period in four districts across Rwanda. Estimated median survival of Yahe® LN, PermaNet® 3.0, and Interceptor® G2 brands was above the commonly assumed three-year lifespan. After a sensitivity analysis related to the classification of ITNs reported as stolen, only PermaNet® 3.0 and Yahe® brands achieved an estimated median survival above the three-year mark. Socioeconomic status, household characteristics, and ITN care practices were critical determinants of ITN durability, emphasizing the need for targeted SBC messaging, education, and community engagement to enhance ITN maintenance. Decreases in insecticidal efficacy across all ITN types throughout the assessment were associated with the degradation of AIs. While mortality was low by endline, new ITN brands offered at least some protection against

pyrethroid-resistant mosquitoes. Continued post-market ITN surveillance across sub-Saharan Africa will provide insights on ITN physical durability and insecticidal effectiveness as human behavior and insecticidal resistance evolve.

Disclaimer

The findings and conclusions expressed herein are those of the authors and do not necessarily represent the official position of the United States Agency for International Development (USAID), CDC, nor PMI.

List of Abbreviations

aHR	Adjusted Hazard Ratio
AI	Active Ingredient
An	Anopheles
BFI	Blood-Feeding Inhibition
CDC	U.S. Centers for Disease Control and Prevention
CFP	Chlorfenapyr
CI	Confidence Interval
CU5	Children Under Five
KD60	Knock-down rate at 60 minutes
IQR	Interquartile Range
IRS	Indoor Residual Spraying
ITN	Insecticide-Treated Net
NMP	National Malaria Program
PBO	Piperonyl Butoxide
pHI	Proportionate Hole Index
PMI	U.S. President's Malaria Initiative

RBC-MOPDCD Rwanda Biomedical Centre - Malaria & Other Parasitic Diseases
Control Division

SBC Social and Behavior Change

USAID United States Agency for International Development

WHO World Health Organization

Declarations

Ethics Approval and Consent to Participate

The evaluation, initiated in 2020, was a collaborative effort between researchers from RBC-MOPDCD, PMI, CDC-Atlanta, Abt Global-, Population Services International. Ethical approval was secured from the Republic of Rwanda National Ethics Committee on July 23, 2019, under reference number 517/RNEC/2019. Additionally, clearance was obtained from PMI, the Research Ethics Board of PSI, Washington, DC, USA. Prior to each evaluation round, all participants gave informed consent.

Consent for Publication

Not applicable

Availability of Data and Materials

The data sets and syntax used for this analysis are available from the corresponding author on request. Clean anonymized data from the evaluation are available at www.durabilitymonitoring.org.

Competing Interests

The authors declare that they have no competing interests.

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Author's Contributions

Design of study and tools: BC, EN, SP, AM; EH; implementation and supervision: BC, RS, KE, GR, JN, XM, EN, YY, DM, TH, IS, CM, AM, PM, AM, YM, EA, SP, EH; bio-assay: BC, RS, KE, GR, JN, XM, PM, EN, JR, YY, TH, IS, CM, AM, SP, EH; Insecticide content analysis: IS, BC, EN, CM, EH; analysis of data for publication: JR; interpretation: RS, BC, KE, GR, JN, EN, JR, YY, TH, IS, CM, AM, SP, EH; drafting and review manuscript: all. All authors read and approved the final manuscript.

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