New methodological approaches to monitor long lasting insecticidal nets (LLINs) durability for sustained malaria control

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AIC	Akaike Information Criterion
BRTC	Bagamoyo Research and Training Centre
CI	Confidence Interval
CIPAC	Collaborative International Pesticides Analytical Council.
CRA-W	Walloon Agricultural Research Centre
ERG	Evidence Review Group
GC-FID	Gas Chromatography with Flame Ionisation 252 Detection
GMP	Global Malaria Program
I-ACT	Ifakara Ambient Chamber Test
IHI	Ifakara Health Institute
IRB	Institutional Review Board
LLINs	Long-lasting Insecticidal Treated Nets
LSHTM	London School of Hygiene and Tropical Medicine
NIMR	National Institute for Medical Research
NMCP	National Malaria Control Program
NMCPs	National Malaria Control Program
PCA	Principal Component Analysis
P*f*PR	*Plasmodium falciparum* parasite rate
pHI	proportionate hole index
SAVVY	sample vital registration with verbal autopsy
SNP	School Net Program
SPD	Sentinel Panel of District
TNVS	Tanzania National Voucher Scheme
U5CC	Under 5 coverage campaign
UCC	Universal coverage campaign
URC	Universal Replacement Campaign
WHO	World Health Organisation
WHOPES	World health Organisation Pesticide Evaluation Scheme

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Abstract

Long lasting insecticidal nets (LLINs) are the current primary vector control measure to prevent malaria transmission. They function by inhibiting mosquitoes from blood feeding and also killing mosquitoes and hence provide personal and community protection respectively. With findings from different LLIN distribution programmes in different settings, it is assumed that the effective life of LLINs is 3 years. This is mostly due to wear and tear of the fabric and hence need for the introduction of the guidelines that provide standard methods to monitor the longevity of LLINs. The standard means established to monitor longevity of ITN is through cone bioassays, WHO tunnel tests and experimental hut evaluations. However, all the standard methods for assessing LLIN durability have limitations and the information collected on LLIN durability will only be useful if correctly collected using simple, realistic and reproducible methods. Thus to address this issue of high public health significance, we undertook two projects namely 1) ABCDR (Attrition, Bioefficacy, Chemical residual, Damage and Resistance) and 2) Holed Net project. The ABCDR project aimed at understanding of bednet durability in malaria endemic countries and factors affecting bednet durability with the main focus of using that information in improving the current methodologies by developing simple, realistic methods for assessing bednet efficacy while Holed Net project, aimed at understanding the association of size and location of net damage and interaction with insecticide concentration in order to ensure their continued efficacy and also work with manufacturers to optimize their longevity.

The results showed that, Of 6067 campaign nets reported to have been received between 2009 and 2011, 35 % (2145 nets) were no longer present. In addition, most of those nets had been discarded (84 %) mainly because they were too torn (94 %) and only 39 % of distributed nets remain both present and in serviceable physical condition, a functional survival considerably below WHO assumptions of 50 % survival of a ‘three- year’ net. However, the majority of nets still retained substantial levels of permethrin and could still be bio-chemically useful against mosquitoes if their holes were repaired, adding evidence to the value of net care and repair campaigns.

In conclusion, the findings from this study provided not only a deep insight into many aspects of LLIN durability but also evidence for revising the existing standard methods for LLIN durability. It also served as baseline information that was used to revise; i) the measurement of the standard entomological parameters i.e. mortality and blood feeding inhibition in order to develop a logistically simple, time saving and realistic method for assessing LLIN durability and ii) the measurement of proportional hole index (phi) in order to develop a “location adjusted phi”. Through findings from these studies, new bioassays have been developed to measure bednet durability with high throughput and robust data power. The developed bioassays are simple to use, very cost effective and reproducible for use in multiple countries.

Summary

Malaria is still a public health problem in sub-Saharan Africa and accounts for over 90% of the global burden. Pregnant mothers and children under the age of 5 years are the most vulnerable group. Worldwide, malaria is estimated to cause 216 million cases of malaria and 4450,000 deaths from malaria. In Tanzania, the National Bureau of Statistics estimates that a total of 10-12 million citizens contract the disease with 80,000 die (majority of them being children) each year. The impact of malaria is manifested through loss of working time when people are ill or taking care of family members, through loss of resources that are used to finance treatment, and through disabilities that result from severe malaria.

Long lasting insecticidal nets (LLINs) are the current primary vector control measure to prevent malaria transmission. They function by inhibiting mosquitoes from blood feeding and also killing mosquitoes and hence provide personal and community protection respectively. The effectiveness of LLINs in controlling malaria in many different settings has already been extensively studied and documented. Recent findings from Bhatt and colleagues showed that LLINs were the largest contributor (68%) of all malaria cases averted using all available interventions.

With findings from different LLIN distribution programmes in different settings, it is assumed that the effective life of LLINs is 3 years. This is mostly due to wear and tear of the fabric and hence need for the introduction of the guidelines that provide standard methods to monitor the longevity of LLINs. These guidelines were developed in order to assist the National malaria control programmes in different countries in monitoring the durability (longevity) of insecticidal treated nets under operational conditions. The information obtained from monitoring surveys helps in; i) evidence-based planning the replacement of badly torn nets, ii) making informed decisions on procuring the most durable nets and iii) understanding factors causing wear and tear of nets.

The standard means established to monitor longevity of ITN is through cone bioassays, WHO tunnel tests and experimental hut evaluations. The cone test is a contact assay where mosquitoes are held in proximity to the ITN and mosquito knockdown (KD60) and 24 h mortality are recorded after 60 min and 24 h respectively. The tunnel test uses a live animal as a bait (rabbit or guinea pig), so mosquitoes are able to exercise host-seeking behaviour, and ITN efficacy is assessed

by measuring mosquito mortality and blood feeding inhibition. Experimental huts are small-scale field (phase II) testing assays used to evaluate ITNs that meet laboratory (phase I) testing criteria. Huts are built in areas with high densities of target mosquito species and are designed to resemble small local housing but have features to retain mosquitoes that enter huts such as window traps and baffles. Volunteers sleep underneath the ITNs and wild mosquitoes attempt to feed and interact with the ITNs in the same way as they would in local homes. Both mortality and feeding inhibition are key outcome parameters, which translate not only to personal and community protection from malaria but also in i) planning the replacement of badly torn nets, ii) making decision to procure the suitable and longer lasting LLINs and iii) understand the factors that affect the LLINs not to last longer.

However, all the standard methods for assessing LLIN durability have limitations and the information collected on LLIN durability will only be useful if correctly collected using simple, realistic and reproducible methods. Thus to address this issue of high public health significance, we undertook two projects namely 1) ABCDR (Attrition, Bioefficacy, Chemical residual, Damage and Resistance) and 2) Holed Net project. The ABCDR project aimed at understanding of bednet durability in malaria endemic countries and factors affecting bednet durability with the main focus of using that information in improving the current methodologies by developing simple, realistic methods for assessing bednet efficacy. The project had 2 main components:

i) To understand durability of LLINs and reasons that can affect durability using the retrospective study survey of Olyset campaign nets that were distributed between 2009-2010 (under five campaigns-U5CC) and between 2010-2011 for universal coverage campaign (UCC) in 8 districts in mainland Tanzania. This involved household questionnaires that were delivered to get information of attrition and reasons for attrition. All bednets remaining in households were collected, transported to insectary of the Ifakara Health Institute in Bagamoyo and sorted for campaign nets through different available records. A sub-sample of 198 Olyset campaign nets were randomly selected and examined for bio-efficacy (against Anopheles gambiae s.s. mosquitoes), chemical residual (amount of permethrin content remaining) and physical integrity (number, location and size of holes) using standard WHO methods.

ii) To explore the standard WHO methods used to monitor the durability of LLINs using a prospective follow-up study to determine the useful life of three different LLIN products (Olyset, PermaNet 2.0 and Netprotect) that were randomly distributed in 2013 to 3420 houses across 8 districts in mainland Tanzania and followed yearly for 3 years and information on attrition (net loss and reasons) and physical damage were taken. In addition, a sub-sample of representative "wild" nets (from the three brands) were randomly selected and assessed further for physical integrity (number, size and location of damage-holes), biological efficacy (%mortality and % bloodfeeding) and chemical residual (amount of active ingredient remaining in each sampled net).

The second project, Holed Net, aimed at understanding the association of size and location of net damage and interaction with insecticide concentration in order to ensure their continued efficacy and also work with manufacturers to optimize their longevity. Under this study, a series of laboratory experiments were conducted with "deliberately holed" nets in order to assess the effect of (a) degree and magnitude of net damage and (b) effect of tucking on net efficacy in terms of personal protection to the user through reduction in mosquito feeding and community protection through mosquito mortality.

The core of this thesis and the main objective was to explore the standard methods used to monitor durability of LLINs and to use that information to improve the understanding and devise new methods to assess the LLIN durability.

We took the opportunity of the LLINs distributed through the under five campaigns-U5CC and the universal coverage campaign (UCC) between 2009 and 2010, to perform the retrospective study survey in 2013 to understand the durability of the Olyset campaign nets and factors that could affect their durability. The results showed that, Of 6067 campaign nets reported to have been received between 2009 and 2011, 35 % (2145 nets) were no longer present. In addition, most of those nets had been discarded (84 %) mainly because they were too torn (94 %) and only 39 % of distributed nets remain both present and in serviceable physical condition, a functional survival considerably below WHO assumptions of 50 % survival of a 'three- year' net. However, the majority of nets still retained substantial levels of permethrin and could still be bio-chemically useful against mosquitoes if their holes were repaired, adding evidence to the value of net care and repair campaigns.

In conclusion, the findings from the retrospective study survey provided not only a deep insight into many aspects of LLIN durability but also evidence for revising the existing standard methods for LLIN durability. It also served as baseline information that was used to revise; i) the measurement of the standard entomological parameters i.e. mortality and blood feeding inhibition in order to develop a logistically simple, time saving and realistic method for assessing LLIN durability and ii) the measurement of proportional hole index (phi) in order to develop a "location adjusted phi". Through findings from these studies, new bioassays have been developed to measure bednet durability with high throughput and robust data power. The developed bioassays are simple to use, very cost effective and reproducible for use in multiple countries. They include the following;

1) Ifakara Ambient Chamber Test (I-ACT)- is an improved method for evaluating bioefficacy of LLINs compared to standard WHO bioassays (cone and tunnel tests). This is a 50m long, 3m wide and 2.1m high steel tube frame construction (Fig. 1a) covered by durable UV resistant polyurethane coated netting with an overlaid polyurethane sheet to minimize wind so that bioassays are conducted in still air (as would occur in a house). It consists of 10 compartments each with a white-netted chamber 5 m long, 2 m wide, and 2 m high that seals with a zip, in which the ITN is hung from a frame with a human volunteer sleeping underneath. This allows whole ITNs to be tested in a controlled ambient chamber test with a human host sleeping beneath to measure the protective efficacy (both personal protection measured by feeding inhibition and community protection measured by mosquito mortality) under user conditions. The design of the chambers allows 100% recovery of released mosquitoes that improves precision of the data, and experiments can be conducted year-round. The advantage of this assay is that it can provide useful additional information compared to standard WHO bioassays and hence act as a link between lab tests and semi field experiments. I-ACT has a potential to be used for product equivalency testing and durability studies because it measures composite bioefficacy and physical integrity with both mortality and feeding inhibition endpoints, using fewer mosquitoes than standard WHO bioassays (cone and tunnel tests). In addition, I-ACT is also suitable for net products, whose mode of action involves either toxicity or feeding inhibition and has potential to be used for novel compounds that are being developed.

2) Location Adjusted Hole Surface Area (LaHoSA)- This is a small modification of the current proportion hole index (pHI). LaHoSA is a simple, time saving and realistic method of assessing physical net integrity compared to standard phi method. It was developed following the information to understand the relative contribution of hole size, hole location and insecticide residue in penetrability and mosquito killing effect of LLIN. The advantage of LaHoSA is that, you can be able to know when the net is in badly torn right in the field and more houses can be done more rapidly and cheaply to give a wider and more representative population sample.

3) Alternative tunnel test using human as bait. This is an alternative method for assessing bio-efficacy of LLINs using a preferred bait (unlike the standard WHO tunnel test that uses rabbits/guinea pigs as bait). This method, in comparison to the standard tunnel test method has got several advantages including the following the new method is simple, takes few mosquitoes (20 compared with 100 in standard WHO tunnel test) with short time of exposure (I hour unlike 12-15 hours in standard WHO tunnel test), and uses preferred bait (human unlike rabbit/guinea pigs in standard tunnel test) hence time saving, cost-effective with 100% sensitivity and 100% specificity.

In addition to above, through findings from this thesis, a new entomological parameter is proposed for use when assessing the efficacy of a bednet. The standard WHO entomological parameter of "*proportion of blood fed mosquitoes*" means number of mosquitoes that are fully blood-fed including the dead ones that were already sorted and counted when scoring for "*mortality*" parameter and hence duplication of data. Interestingly, experimental results from this thesis found that, most of blood-fed mosquitoes did not survive after 24 h post exposure.

Given this fact, we think "*survival of feeding*" parameter may be used as useful alternative composite metrics as it captures the information of only those mosquitoes that managed to enter in the treated bednet, blood fed and leave without being killed.

Findings reported in this thesis have generated important knowledge that can be integrated in bednet durability studies. This thesis demonstrated that the most of distributed nets are still protective and retained substantial levels of active ingredients even when in badly torn meaning, if the holes could be repaired, the nets could still be bio-chemically useful. Therefore, this study recommends that a badly torn treated net should never be thrown away unless replaced with a new net.

In addition, this thesis found that, most of damage occurred in the bottom part of the net, but interestingly, this part is usually tucked in under the mattress, and from laboratory findings, it had little effect on mosquito entry and feeding. This is very useful finding and can be incorporated into existing malaria SBCC platforms to improve net condition and hence lasting longer, providing more protection leading to reduction in malaria transmission. A "care is better than repair" slogan can also be used.

1. General Introduction

1.1. Global burden of Malaria

Malaria is still a major public health problem globally. The 2017 World Malaria report has shown that in 2016 there was an increase of 5 million in the total malaria cases compared to the previous year i.e. from 217 million in 2015 to 211 million in 2016 [1]. Additionally, the mortality rate has reached 445,000 deaths, about the same as in the 2015 report, meaning that much efforts are still needed to combat this disease especially in Africa which account for about 90% of malaria cases and deaths. In Tanzania, the National Bureau of Statistics estimates that a total of 10-12 million citizens contract the disease with 80,000 die (most of them being children) each year [2]. Women of childbearing age and children under the age of five years are particularly at high risk, especially those living in remote rural areas without adequate access to formal healthcare.

1.2. Malaria Parasites

Malaria is caused by Plasmodium parasites. The parasites are transmitted to people through the bites of infected Anopheles mosquitoes, therefore referred to as malaria vectors. Five species of the plasmodium parasite can infect human namely *Plasmodium falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi* [3]. Though *P. falciparum* accounts for 80% of all malaria cases [4], transmission by *P. vivax* is overlooked. Mueller, et al., [5] reported that nearly 2·5 billion people are at risk of *P. vivax* infection and an estimated 80 million to 300 million clinical cases occur every year including severe disease and death are attributed. Malaria caused by *P. ovale*, and *P. malariae* causes milder disease in humans that is not generally fatal. A fifth species, *Plasmodium knowlesi*, a monkey malaria that occurs in certain forested areas of South-East Asia, is a zoonosis that causes malaria in monkeys but can also infect humans, [6,7]. All the 5 Plasmodium species differ in various aspects including morphology, location of habitat, relapse pattern and in how do each respond to particular antimalarial. The primary hosts of Plasmodium species are female mosquitoes of the Anopheles genus, while humans and other vertebrates are secondary hosts.

Plasmodium life cycle and Pathogenesis of the disease

Mosquitoes first ingest the malaria parasite by feeding on an infected human carrier [8]. Once ingested, the male and female gametocytes taken up in the blood will then release male and female gametes which fuse in the lumen of the mosquito to produce zygotes which rapidly become an ookinete that penetrates the gut lining within 18-24 hours [9] and produces a single oocysts which is trapped between the endothelium and the basal lamina of the gut wall (Figure 1.1). When the oocyst ruptures into the haemocoel, it releases sporozoites that migrate through the mosquito's body to the salivary glands, where they are then ready to infect a new human host [10]. The sporozoites are injected into the skin, alongside saliva, when the mosquito takes a subsequent blood meal [11]. Each human infection with the parasite begins with an intravenous inoculation of sporozoites by infected female mosquitoes.

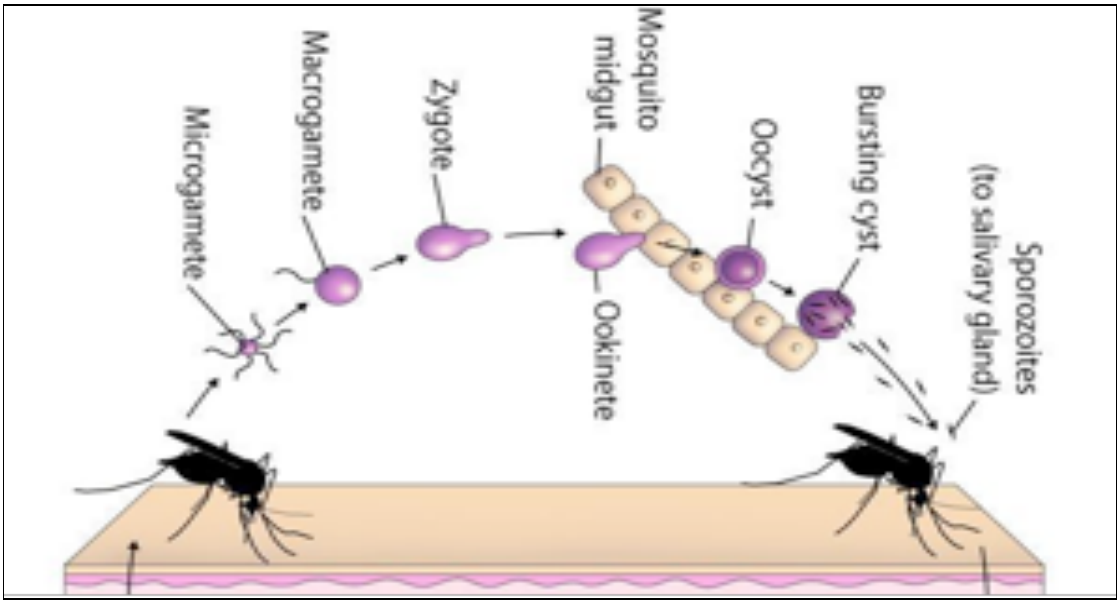

Figure 1.1. The life cycle of malaria parasites in the mosquito body (Image credit: Johns Hopkins Bloomberg School of Public Health).

1.3. Malaria vectors and disease transmission

Malaria in humans is usually transmitted by the bite of an infected female anopheline mosquito of genus Anopheles. Out of the 460 recognized species of Anopheles, 100 species can transmit malaria in humans [12]. The principal malaria vector in east Africa coastal areas are the members of *Anopheles gambiae* complex mosquitoes [13]. The most common and important malaria vectors in Africa are An. gambiae Giles, An arabiensis Patton and An. funestus Giles [14]. *An. gambiae* is more common in humid coastal and highland areas while *An. arabiensis* is concentrated in the arid mainland of Tanzania [15]. Secondary malaria vectors in Tanzania include *Anopheles coustani*, *Anopheles quadriannulatus* species A and B, and *Anopheles merus* [16].

Malaria in human usually develops via two phases: an exo-erythrocytic and an erythrocytic phase (Figure 1.2). The exo-erythrocytic phase involves infection of the hepatic system, or liver, whereas the erythrocytic phase involves infection of the erythrocytes, or red blood cells. The infecting agent is the sporozoite, a microscopic spindle-shaped cell which is in the mosquito's saliva. When an infected mosquito pierces a person's skin to take a blood meal, sporozoites in the mosquito's saliva enter the bloodstream. Within 30 minutes of being introduced into the human host, the sporozoites infect hepatocytes multiplying asexually and asymptomatically for a period of 6–15 days via a process called schizogony. Inside the liver cell, the sporozoites differentiate by asexual fission to form a cyst-like structure called a pre-erythrocytic schizont which contains thousands of merozoites which, following rupture of their host cells, escape into the blood and penetrate red cells, thus beginning the erythrocytic stage of the life cycle [17].

The time between the infecting mosquito bite and the appearance of the parasites in the blood is called the pre-patent period. It is usually 7-30 days in *P. falciparum* (usually around 10 days) and longer in other species; in the case of *P. vivax* and *P. ovale* many months or even more than a year.

Merozoites released into the bloodstream from hepatic schizonts attach themselves to red cells by means of surface receptors. Then they penetrate the red cells and reside in a vacuole with a lining derived from the red cell surface. Within the red cells, the parasites undergo the process of blood schizogony, which for *P. falciparum*, only occurs in capillaries deep within the body whereas for other parasites they are commonly seen in peripheral blood films from infected patients. They then rupture after a fixed period for each parasite, releasing thousands of merozoites, which then invade fresh red blood cells. Several such amplification cycles occur, each causing rapid onset of more severe symptoms. Thus, classical descriptions of attacks of fever and chills arise from repeated waves of merozoites escaping, releasing waste products and degraded cell contents, and the infecting new red blood cells. Some *P. vivax* and *P. ovale* sporozoites do not immediately develop into exoerythrocytic-phase merozoites, but instead produce hypnozoites that remain dormant for periods ranging from several months (6-12 months is typical) to as long as three years.

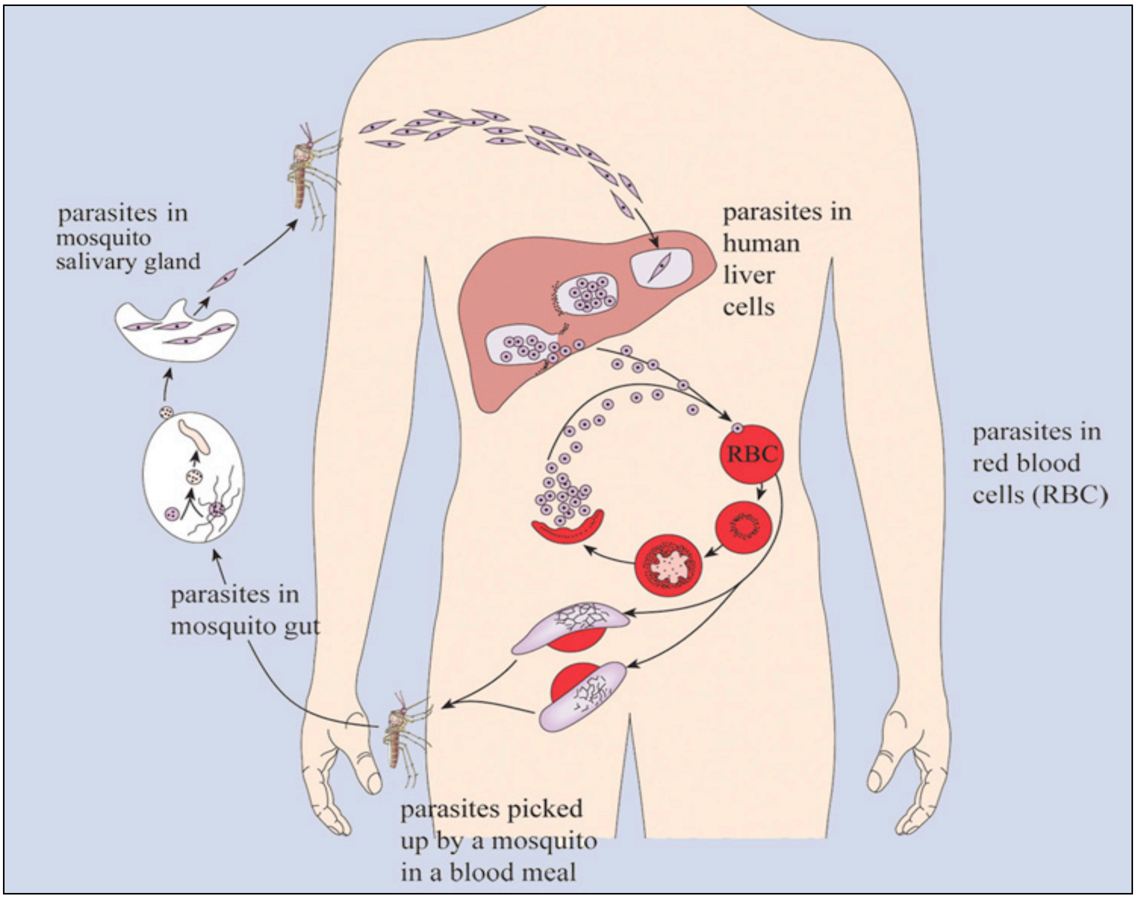

Figure 1.2. The life cycle of malaria parasites in the human body (Image credit: OpenLearn Works).

After a period of dormancy, they reactivate and produce merozoites. Hypnozoites are responsible for long incubation and late relapses in these two species of malaria [18]. Some of the merozoites entering red cells do not develop into schizonts, but develop into male and female gametocytes. These may persists in the blood circulation for many weeks without destroying the red cells containing them, and these are the forms infective to the mosquito. If a female mosquito pierces the skin of an infected person, it swallows the male and female gametocytes in her blood meal. Unlike other diseases and parasites, mosquitoes are the definitive hosts for the malaria parasites due to the fact that it is inside the mosquito's gut where fertilization and sexual recombination of the parasite occurs [18]. New sporozoites develop and travel to the mosquito's salivary gland, completing the cycle. Sometimes, human malaria is transmitted by transfused blood from infected to healthy individuals, sharing infected needles, or from an infected gravid woman to her fetus.

Malarial attacks present over seven days or more (usually 10-15 days after the incubation period) with fever, headache, sweating and chills, often associated with fatigue, headache, dizziness, nausea, vomiting, abdominal cramps, dry cough, muscle or joint pain, and back ache. If not treated within 24 hours, P. falciparum malaria can progress to severe illness and sometimes death [19]. Children in endemic areas with severe disease frequently develop one or more of the following syndromic presentations: severe anaemia, respiratory distress, or cerebral malaria. In adults, multi-organ involvement is also frequent. For both *P. vivax* and *P. ovale*, clinical relapses may occur weeks to months after the first infection, even if the patient has left the malarious area. These new episodes arise from "dormant" liver forms (absent in *P. falciparum* and *P. malariae*), and special treatment targeted at these liver stages is mandatory for a complete cure [20].

1.4. Malaria control

Control of malaria represents one of the world's greatest public health challenges, especially in sub-Saharan Africa where over 80% of the disease occurs [21]. The history of malaria control goes back in late 1800s after the discovery of the connection between Anopheles Mosquitoes and the disease transmission by Ronald Ross [22].

This discovery opened a new chapter in malaria prevention specifically vector control. After the discovery of this connection, major vector control measures included environmental sanitation through drainage to eliminate the larval mosquito habitat, biological control through the use of larvivorous fish in ponds and larviciding with oil. They were all very effective, especially in countries like Brazil [23], Zambia [24], Egypt [25], northern part of Australia and large swath of the south Pacific [26] until 1955 when WHO launched the Global Malaria Eradication Programme with an overwhelming emphasis on widespread use of this indoor DDT spraying against malaria vectors and anti-malarial drugs to treat malaria parasites [27]. This resulted in great success with the elimination of malaria from most parts of Europe.

However, in late 1960's operational problems associated with DDT led to the emergence of fast-evolving resistant Anopheles species. In some places there were instances of overdosing with

DDT, refusal of access to houses to be sprayed and even theft of insecticide for illicit sale on the black market [26]. Insecticide resistance threatened the efficacy of these tools for the control of malaria as DDT began to lose its efficiency in certain places. This led to abandonment of the Global malaria Eradication Programme and WHO changed its policy from world-wide malaria eradication program to malaria control through drug treatment of the parasite in infected humans. The prioritized approaches during this era included the use of new antimalarials like Chloroquine and Quinine against malaria parasites [28], and, later on, use of insecticides in bednet treatment (i.e. synthetic pyrethroids) and in Indoor Residual Spray (IRS) for malaria vector control [29]. They all contributed significantly to the malaria control through reduction of malaria vector density and malaria parasites in 2000s in most malaria endemic African countries including Kenya, Rwanda, Eritrea, Zambia, Gambia, South Africa, Mozambique and Zanzibar [30–34], representing an historical achievement and the most definitive progress since WHO changed its policy from malaria eradication to malaria control [35].

1.5. Insecticide Treated bed nets (ITNs)

Bed nets were primarily developed for the purpose of protection against flies, mosquitoes and other biting insects, as well as to protect against transmission of diseases such as malaria, dengue fever and yellow fever. The idea of using insecticide-treated bed nets came during World War II, when Germans, Russian and US armies started treated their combat uniforms and bed nets to protect them against vector borne diseases, mainly malaria and leishmaniasis [36]. From the late 1970s, common use of synthetic pyrethroids started and studies showed they were safe and could have a dramatic impact on malaria transmission [36–38].

About 68% of the total reduction in *Plasmodium falciparum* parasite rate (PfPR) seen in 2015 were attributed to by insecticide treated nets [39]. Furthermore, in recent years, the ownership and use of insecticide treated nets has increased such that they are now the main important intervention for malaria vector control in most part of sub-Saharan Africa. In Tanzania, according to the recent malaria indicator survey, ownership of at least one ITN per household has increased to 78% in 2017 from 50% in 2010 [40].

Lengeler, (2004) reported that the distribution of mosquito nets impregnated with insecticides was an extremely effective method of malaria prevention, and these remain one of the most cost-effective interventions available in public health [41]. In addition to above, community-wide use of treated nets has been shown not only effective in killing or repelling larger number of malaria vectors to provide personal protection, but also provides communal protection to non-users in the surrounding community by reducing vector population sizes, mean age and infection prevalence [41–49]. Community-wide bed net use can not only reduce child mortality by about 20% but also reduce malaria episodes by about 42% [50].

The need to continual re-treatment nets with insecticides every six to twelve months proved a major challenge to successful implementation in the field and prompted the development of Long-Lasting Insecticide nets (LLINs) which will last for three up to five years without requiring re-treatment [51]. These have been evaluated in comparison with the ITNs and found to last far longer than standard ITNs [46,52].

1.6. LLIN durability in the context of vector control

The effective life of a good bednet is estimated to be three years after which they will be in bad condition and need replacement [53]. However protection provided by treated bed nets is not long lasting from either physical or chemical factors. Physical factors are attributed by wear and tear (holes formation from various sources) of the fabrics over time. Chemical factors are attributed by all factors leading to reduced or loss of insecticides). However, this is based on the assumptions that there are variations in the physical and chemical decay among bed nets, which appears to be significant, and the effective net life could even be less than three years. This assumption was later thought to be over-optimistic [54]. Evidence from different places observed variations in the effective life of bednets, with some observed an effective life of between three and four years [55], four years [56] and seven years [57,58]. Having seen the variations of effectiveness of LLINs in different field settings, WHO established standard guidelines, which will be used to monitor net durability across different places. WHO suggested three elements to consider when assessing net durability namely net survivorship, fabric integrity and insecticidal activity (bioefficacy and insecticidal residual) [54]. A durable insecticidal treated net is one that is still available for use (even after the three years of effective life), in good condition and can prevent blood feeding by mosquitoes (as physical or chemical barrier) and killing mosquitoes (as chemical barrier) and there are standard methods established to assess each of the above components.

Net survivorship is the first component of bed net durability and refers to the proportion of nets that are still available in the households after a certain period following initial distribution. The opposite of survivorship is attrition, which refers to the absence of nets from the household [54]. Following WHO guidelines, absence include either given away, thrown away or used for alternative purposes and can be captured from household questionnaire surveys [59–63]. Information on net survivorship (attrition) is very important for any malaria endemic country even before understanding other components of the durability in order to know whether the net distributed is still available or not and if not, what are the reasons. This will also assist future net replacement campaigns because replacing nets too late puts people at risk of disease, but replacing them too often wastes limited resources.

Physical integrity is another important component when assessing bed net durability. It involves counting the number and size of holes based on hole size categories i.e. smaller than thumb (0.5-2cm), larger than thumb but smaller than a fist (2-10cm), larger than fist but smaller than head (10-25cm) and larger than head (>25cm). The total hole counts are then weighted according to the estimated average area of each hole size category and summarize the counts in a proportionate Hole Index (pHI) weighted by the approximate surface area of the holes to provide a single measure as either good (<79cm^2 if circular or <100 cm^2 if rectangular holes), damaged (80-789 cm^2 if circular or 100-1000 cm^2 if rectangular holes) or too torn (>790cm^2 if circular or >1000cm^2 if rectangular holes) [64]. Fabric integrity assessments are not used by WHO for LLIN durability assessment required for full WHO PQ (Pre Qualification) listing. Currently, a candidate LLIN is considered to meet the criteria for efficacy for testing in Phase III studies, if after 3 years, at least 80% of sampled nets are biologically effective in WHO cone or tunnel tests [65].

Bio-efficacy is also among the components measured when assessing bednet durability. Standard WHO methods used to assess net bioefficacy include cone test, tunnel test and through experimental hut trials. The standard means of ITN bioefficacy evaluation is through cone bioassays, WHO tunnel tests and experimental hut evaluations [65]. Cone test is a contact assay where mosquitoes are held in proximity to the ITN and mosquito knockdown (KD60) and 24-h mortality are recorded after 60 min and 24 h, respectively. The tunnel test uses a live animal as a bait (rabbit or guinea pig), so mosquitoes are able to exercise host-seeking behaviour, and ITN efficacy is assessed by measuring mosquito mortality and blood feeding inhibition [66–68]. Experimental huts are small-scale field (phase II) testing assays used to evaluate ITNs that meet laboratory (phase I) testing criteria [54,69]. Huts are built in areas with high densities of target mosquito species and are designed to resemble small local housing but have features to retain mosquitoes that enter huts such as window traps and baffles [70]. Volunteers sleep underneath the ITNs and wild mosquitoes attempt to feed and interact with the ITNs in the same way as they would in local homes. Both mortality and feeding inhibition are key outcome parameters, which translate to personal and community protection from malaria [71].

1.7. Problems with standard LLIN durability methodologies

Though a lot has been done to ensure wide coverage of bednets in most of these malaria endemic countries, the world cannot claim victory through this intervention, as the distribution alone is not enough unless the distributed nets last longer and are consistently and correctly used. LLINs function as a physical and chemical barrier between mosquito and human being sleeping under the net. As explained above, main components measured when assessing bed net durability includes, attrition/survivorship, physical integrity and bioefficacy (insecticide activity and chemical residual). However, all assays pose some challenges that need to be considered when assessing durability of bednet.

Firstly, although information on net survivorship (attrition) is very important for any malaria endemic country even before understanding other components of the durability in order to know whether the net distributed is still available or not and if not, what are the reasons; but this information usually relies on the reported information from the household heads that may not reflect the real condition of the particular LLIN under field settings.

Secondly, the pHI method was formulated to help determine whether bed nets are still protective or should be replaced after a certain period of field use. The method has been widely used as a standard method to assess physical integrity of different net products in different settings [60,72–78]. However, notable challenges have been observed related to this method including the difficulties in identifying and counting of all holes in a net, the method is laborious and include some assumptions when assessing net integrity [79–81].

Thirdly, the standard WHO bioefficacy methods (cone/tunnel tests and experimental huts) have been observed to face some methodological challenges [66], which may affect the outcome measures of bed net durability. Cone tests may underestimate the induced mortality of irritant insecticides, as mosquitoes do not settle on treated nets [82]. Indeed, comparatively higher mortality is often measured in experimental hut studies of ITNs where mosquitoes make repeated contacts with treated nets as they try to feed on human volunteers sleeping under nets. In tunnel tests, the live host used as bait is not the preferred host for the strongly anthropophilic Afro-tropical vector Anopheles gambiae sensu stricto (s.s.) [83] and may overestimate feeding inhibition. Alternatively, mosquitoes must be reared by feeding them on small mammals to select them for a preference to these non-preferred hosts, which is both expensive and of animal welfare concern. Experimental hut experiments are the gold standard for ITN and IRS evaluations, but wild mosquito populations are often seasonal and have high temporal heterogeneity requiring substantial replication to ensure adequate power to detect true effect differences between products [84].

While collecting data on bednet durability, in the laboratory or in the field, can provide information on net efficacy and damage as well as attrition, however this information is only useful and reliable if collected correctly in a simple, cost effective and reproducible manner in different field settings.

Based on the observed challenges above, we undertook several experiments to understand more the methodologies for LLINs durability and develop improved methods that are realistic, cost effective and reproducible for assessing LLIN longevity under field settings.

1.8. Objectives

General objective

This thesis was motivated by the need to understand and improve the LLIN durability methodologies for the longer and sustained malaria vector control. Therefore the overall aim of this thesis was to understand the current methods used for assessing LLINs durability and associated limitations/challenges and to develop improved methodologies for the same.

Specific objectives:

1. To measure the durability of LLINs in order to; i) better advise the country on procurement of

the most durable LLIN and provide information needed to plan the timing of future net replacement campaigns and ii) understand factors that affect net durability.

2. To use this information to revise the standard WHO bioassays (cone and tunnel tests) as a measure of LLINs bioefficacy and develop a logistically simple, time saving and realistic method for assessing LLIN durability.

3. To use this information to improve understanding of the proportional hole index (phi) as a measure for LLIN effectiveness and to revise this measurement to develop a "location adjusted phi".

2. Investigating mosquito net durability for malaria control in Tanzania - attrition, bioefficacy, chemistry, degradation and insecticide resistance (ABCDR): study protocol

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2.1. Abstract

Background: Long-Lasting Insecticidal Nets (LLINs) are one of the major malaria vector control tools, with most countries adopting free or subsidised universal coverage campaigns of populations at-risk from malaria. It is essential to understand LLIN durability so that public health policy makers can select the most cost effective nets that last for the longest time, and estimate the optimal timing of repeated distribution campaigns. However, there is limited knowledge from few countries of the durability of LLINs under user conditions.

Methods/Design: This study investigates LLIN durability in eight districts of Tanzania, selected for their demographic, geographic and ecological representativeness of the country as a whole. We use a two-stage approach: First, LLINs from recent national net campaigns will be evaluated retrospectively in 3,420 households. Those households will receive one of three leading LLIN products at random (Olyset®, PermaNet®2.0 or Netprotect®) and will be followed up for three years in a prospective study to compare their performance under user conditions. LLIN durability will be evaluated by measuring Attrition (the rate at which nets are discarded by households), Bioefficacy (the insecticidal efficacy of the nets measured by knock-down and mortality of mosquitoes), Chemical content (g/kg of insecticide available in net fibres) and physical Degradation (size and location of holes). In addition, we will extend the current national mosquito insecticide Resistance monitoring program to additional districts and use these data sets to provide GIS maps for use in health surveillance and decision making by the National Malaria Control Program (NMCP).

Discussion: The data will be of importance to policy makers and vector control specialists both in Tanzania and the SSA region to inform best practice for the maintenance of high and cost-effective coverage and to maximise current health gains in malaria control.

Keywords: Long-lasting insecticidal nets, LLINs, Durability, Mosquito net, Hole index, Biological efficacy, Malaria control, Anopheles, Semi-field, Insecticide resistance

2.2. Background

The recent successes in malaria control in sub-Saharan Africa (SSA), specifically in Tanzania where malaria deaths have reduced by 70% since 2003, has been largely attributable to the massive scale up of vector control tools, particularly Long Lasting Insecticidal Nets (LLINs) [1-3]. However, sustained malaria control is costly, and dependent on continuing political and donor support. As political commitment diminishes, the deliveries of life-saving control tools will slow down and risk the reversal of the huge achievements to date. Global commitments for malaria control in 2012 were approxi- mately US$2.5 billion, far below the estimated sum of US $5.1 billion required for the task [4]. Global funding mechanisms are projected to decelerate even further in the coming years, leaving gaps of US$2.25 billion before achieving universal access to malaria interventions [1]. Therefore, maximising the impact of interventions through selection of the most cost effective and long lasting interventions is a health policy priority.

Despite the huge financial and logistical investments in the development, production and distribution of LLINs worldwide, there are still limited data available on the LLIN durability under user conditions. The World Health Organization (WHO) released specific guidance on LLIN durability monitoring [5,6], which was incorporated into guidelines for laboratory and field-testing of LLINs [7] to support national governments with the design of stan- dardised net monitoring and evaluation studies. Effective net life has been estimated to be 3-5 years [8], but some studies indicate that LLIN brands may last less than three years under operational conditions [9-12]. It is only recently that researchers have started to investigate net attrition, i.e. how long nets remain in use in a household, and constructed net survivorship curves [5,13]. Durability of mosquito nets should thus be defined and measured by the whole process of net loss – from attrition and physical damage to the chemical loss of insecticide residue [5].

Net deterioration differs greatly between regions or cultures as care and repair behaviours, maintenance and net use vary from place to place. Thus, nation-wide evaluations of LLINs are required and called for by the WHO [6,14]. Evaluation of PermaNet®2.0 retrieved from six countries [15] and Olyset® nets from seven countries [16] show large between-country variability of LLIN durability. Net products also vary in material, insecticide, or fibre impregnation technology. Such varia- tions are still largely unknown and direct comparisons within sites are scarce [17] (but see [9,11,18]). Reliable data need to be collected by National Malaria Control Programs (NMCPs) to inform national procurement decisions for 1) selection of the most suitable net to plan timely replacement, 2) to understand factors associated with net durability to guide behaviour change communication including care and repair interventions, and 3) to assist industry in product improvement. NMCPs need to under- stand LLIN durability in their local settings because replacing nets too late puts people at risk of disease, but replacing them too often wastes limited resources.

Also, the dramatic increase in pyrethroid resistance in mosquitoes throughout SSA, including Tanzania [19], might be posing a threat to the sustainability of insecticidal control methods [20,21]. A surveillance system to monitor emerging insecticide resistance, for example using Geographical Information Systems (GIS) [22,23], would allow governments and national malaria control pro- grammes to plan resistance control strategies [24]. Spatio-temporal analysis of malaria transmission to identify persistent transmission hotspots may maximise cost- and health-effectiveness of control programmes [23]. The determinants and risk factors for net loss and effectiveness vary spatially, but there is a lack of information of which factors play a role in the attrition and deterioration of LLINs.

Therefore, the current study is conducted in collaboration with the Tanzanian NMCP to inform their procurement decisions. The study will be conducted in eight districts in Tanzania, selected for their demographic, geographic and ecological representativeness of the country as a whole. There will be an initial retrospective evaluation of Olyset® nets distributed by the NMCP two-to-four years previously as part of both a targeted and a universal coverage campaign [25]. The same sampled households will then receive one of three LLIN products (Olyset® with the new knit pattern to improve fabric strength, PermaNet®2.0 or Netprotect®) by random allocation for a prospective follow up study. Effective life of the nets will be assessed at regular intervals for three years using the WHO-recommended set of net durability variables [5] (Table 2.1) and a set of new methodologies (Figure 2.1).

Table 2.1 LLIN durability components

Component	Definition	Response variables for analysis
Attrition	Net loss from household through discarding or use for alternative purpose	- Net presence.
Biological efficacy	Ability of net to incapacitate or kill anopheline mosquitoes after contact with insecticide	- Mosquito knockdown (%) 60 minutes post-exposure. - Mosquito mortality (%) 24 hours post-exposure. - Percentage of bloodfed mosquitoes.
Chemical residue	Amount of active ingredient in fibres	- Proportion of nets with active ingredient equal to WHO standard g/kg
Physical degradation	Physical state of the net defined through number, size and location of holes to estimate protection against mosquito bites	- Proportionate Hole Index (pHI) / hole area by location on net. - Proportion of nets with a pHI exceeding ≥643 [6].

We will also monitor insecticide resistance in mosquito vectors as an additional component for evaluating LLIN effectiveness to contribute to the growing knowledge within Tanzania, which will assist the NMCP on rational selection of insecticides for vector control.

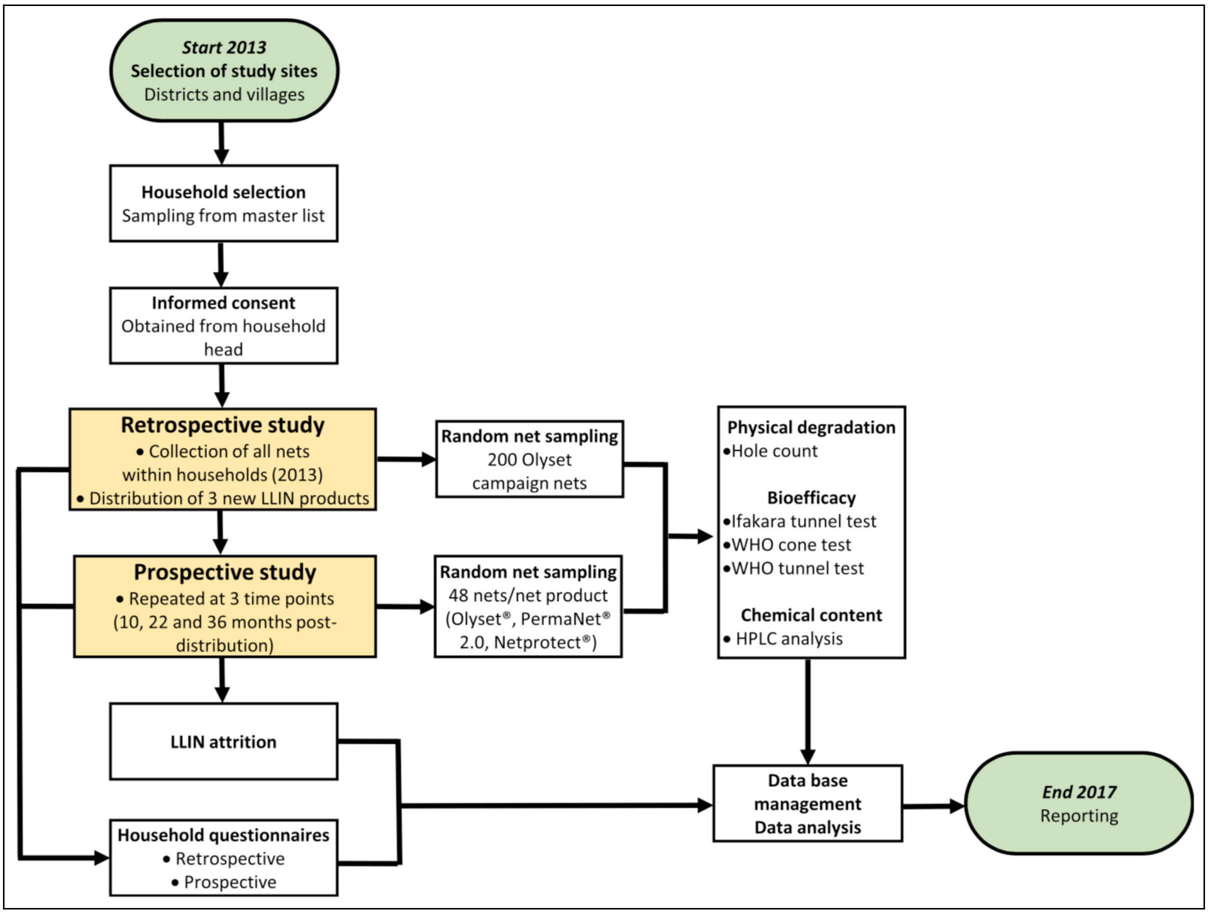

Figure 2.1 Retrospective and Prospective data collection

Spatial risk factors of insecticide resistance and LLIN durability, such as land use patterns, agriculture, altitude or distance to potential breeding sites, will be assessed to determine their usefulness in selecting appropriate malaria control strategies by identifying areas where a particular LLIN intervention may be more effective than another.

2.3. Methods/Design

Study population

The project will be carried out in eight districts represent- ing five of the eight geographical zones of Tanzania and covering variations in malaria epidemiology and ecology. Fifteen districts, i.e. seven districts in addition to the eight previously mentioned, will be included in the mosquito insecticide resistance part of the project (Figure 2.2).

#	District	Malaria prevalence	Zone
		Range	
1	Kinondoni	0.3 - 3.6%	Dar es Salaam
2	Ruangwa	4.0 - 26.0%	Southern
3	Mtwara Urban	2.0 - 17.4%	Southern
4	Songea Urban	0.8 - 12.3%	Southern
5	Mbozi	0 - 4.5%	Southern Highlands
6	Iringa Urban	0 - 0.4%	Southern Highlands
7	Kilosa	6.9 - 13%	Eastern
8	Bagamoyo	7.4 - 10.2%	Eastern
9	Arusha	0%	Northern
10	Kondoa	0.5 - 2.6%	Central
11	Singida Rural	0 - 0.2%	Central
12	Geita	21.0 - 32.9%	Lake
13	Musoma	14.6 - 25.6%	Lake
14	Kahama	4.4 - 6.8%	Western
15	Kasulu	9.9 - 26.1%	Western

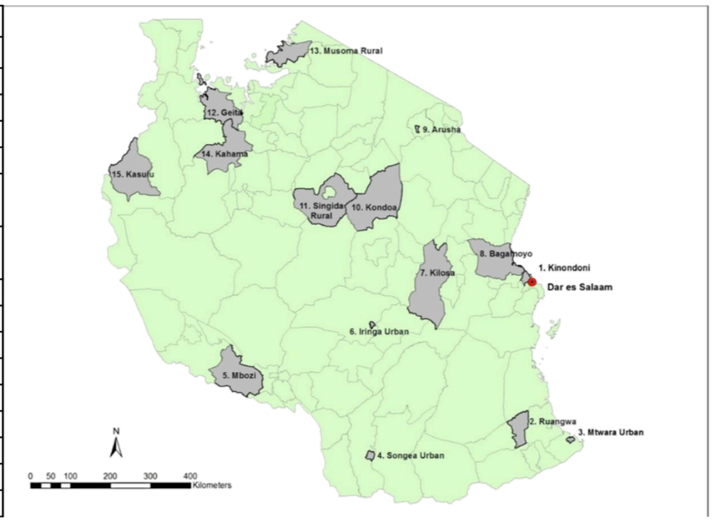

Figure 2.2 ABCDR districts in Tanzania.

Districts selected for LLIN durability (ABCD-components) and the insecticide resistance part of the project (R-component) with malaria prevalence data (% of children aged 6-59 months diagnosed with malaria by Rapid Diagnostic Test and microscopy [29]) and geographical zone. The following eight districts are included in the LLIN study: Kinondoni (1), Mbozi (5), Iringa Urban (6), Kilosa (7), Bagamoyo (8), Geita (12), Musoma Rural (13) and Kahama (14).

The 15 districts were selected from the 23 districts enrolled in the population arm of the Sentinel Panel of Districts (SPD), SAmple Vital registration with Verbal autopsY (SAVVY) [26]. The 23 SAVVY districts were selected using two-stage sampling with probability proportional to size (PPS) of districts and villages/Enumeration Areas (EAs) from the 2002 Population and Housing Census dataset [27]. In each of the eight districts (Figure 2.2), all households within 6-20 villages/EAs were enrolled by the SAVVY programme for national representativeness in 2012/2013. We will select ten SAVVY villages per district based on the proximity to district headquarters, except for Kinondoni (Dar es Salaam) where SAVVY only covered six EAs. Using the SAVVY baseline household information, 45 households per village will be randomly selected using the 'sample' function in the statistical software R 3.1.1 [28], giving a total of 3,420 households nationwide. Fifty percent more households will be randomly selected as substitution households to accommodate for non-consent or household head absence.

The 3,420 study households will be geo-referenced using Global Positioning System (GPS) points to create a GIS database including data on village and house characteristics, socioeconomic variables, net characteristics, and geographical variables, such as environment, land use and potential mosquito breeding sites.

LLIN products

All three products (Table 2.2) that will be tested in the prospective study were recommended by WHO Pesticide Evaluation Scheme (WHOPES) at the point of procure- ment with full approval of Olyset® [16] and PermaNet®2.0 [15], and interim approval of Netprotect® [30]. However, Netprotect® approval was withdrawn in September 2014 [31] and from that point on it was decided to replace all sub-sampled study Netprotect® nets with Olyset®.

Table 2.2 Characteristics of Olyset®, PermaNet®2.0 and Netprotect® net products distributed in the study

Product name	Product type	Insecticide concentration	Denier	Manufacturer	WHO approval	Reference
Olyset®	Permethrin **incorporated** into polyethylene	780 mg/m^2	>150 denier	Sumitomo Chemicals	Full	[16]
PermaNet® 2.0	Deltamethrin **coated** on polyester	55 mg/m^2	100 denier	Vestergaard Frandsen	Full	[15]
Netprotect®	Deltamethrin **incorporated** into polyethylene	68 mg/m^2	110 denier	BestNet	Withdrawn	[30,31]

The WHOPES working group recommends that programmes should monitor efficacy and performance of Netprotect® under local conditions to obtain further information about the product [32]. Ten nets of each product will be assessed at baseline to ensure that they meet WHOPES thresholds for bioefficacy against anopheline mosquitoes using WHO cone and tunnel tests and insecticidal content with high-performance liquid chromatography (HPLC) analysis.
As a consequence of using LLIN products from different materials (Table 2.2), the nets may be able to be distinguished physically. However, each net type will be rectangular, of the same dimensions (190 cm x 180 cm x 150 cm) and colour (white) with six loops per net to prevent household participants, technical staff and field team from knowing the treatment allocation as much as possible. A waterproof unique identifying barcode and a five-digit serial number will be attached to each distributed LLIN with a self-laminating laser tag to a hanging loop of the net. This will allow tracking of the nets once they are distributed.

The field team will record the net serial number on the questionnaire as the net is distributed to allow the matching up of household and unique net identifying numbers on the net master list.

Study design

The general study design is shown in a flow chart in Figure 2.1. One week before the start of the study, a sensitisation meeting will be set up at the district level to inform community leaders (Mwenyekiti and Viongozi), key informants, District Executive Directors (DEDs) and District Medical Officers (DMOs) of the purpose and design of the study. Their permission to work within the community will be sought to inform the com- munity members of the study's objectives and methods.

Retrospective study

Households will be enrolled on written informed consent (Additional file 2.1). Participants' houses and questionnaires will be identified by barcodes associated with numeric codes (six-digit serial numbers) to ensure their anonymity and due care will be taken to ensure that only barcodes and numeric codes are used on LLINs and questionnaires, thus blinding participants and researchers to treatment allocation. All the nets from the participating households will be collected and replaced with one of the three new LLIN products (Table 2.2) chosen at random. The prospective LLIN products will only be known to field teams as net types 1, 2 and 3, thereby blinding and randomising the treatment distribution as much as possible. Each day the field team will receive a household list and a randomly mixed bundle of five sets of type 1, 2 and 3 nets (three nets of same type per set bagged for one household, assuming an average of three sleeping spaces per household). The interviewer will randomly pick one set from the bundle to be distributed when they arrive at each household (modified lottery method). If the household contains more than three sleeping spaces, more nets of the same type will be provided. The interviewer will record the five-digit serial numbers attached to the nets on the questionnaire as described above. Thus, randomisation is conducted by the field workers at the household level, resulting in 15 households per village receiving sufficient nets of one product to cover each sleeping space (Table 2.3).

Table 2.3 Households allocated to each net product per village and district in the prospective study

	Olyset®	PermaNet® 2.0	Netprotect®	Total
Districts	8	8	8	8
Villages per district*	10	10	10	10
Households per village	15	15	15	45
Total households	1,140	1,140	1,140	3,420
Total nets**	3,420	3,420	3,420	10,260

A questionnaire will be conducted in Kiswahili, the local language spoken throughout Tanzania, with household heads, or another adult, by the field team (Additional file 2.2). Respondents will be asked whether they received nets during two NMCP campaigns in 2009-2011. Nets from the campaigns are identifiable by their light-blue colour and size (single), allowing us to differentiate those nets from the campaign and those that might have come from the private sector or Non-Governmental Organisations (NGOs). We will individually assess every net returned to the storage facilities at Bagamoyo Research and Training Centre (BRTC) for its brand label, colour, size, level of cleanliness, and age of manufacture, if available. From those retrospective nets, 200 Olyset® campaign nets will be randomly selected using the 'sample' function in R 3.1.1 for durability testing in the laboratory and semi- field systems (Figure 1; Table 1). All other collected Olyset® nets will be recycled by A to Z Textile Mills Ltd (http://www.azpfl.com/index.php/en/).

Prospective study

Attrition, net use and user behaviour (Additional file 2.2), and physical degradation of study nets will be assessed in every consenting household at three subsequent sampling points (10, 22, and 36 months) after the initial LLIN distribution. All households will be surveyed for attrition and a sub-sample of three nets per household will be assessed for physical degradation. Field interviewers will be trained using an amended version of a recently developed USAID/NetWorks-supported training tool kit to assess the number of different category sized holes under field conditions [33].

At each time point, all nets from 48 randomly selected households for each net product will be taken for sub-sampling to validate the D component (physical degradation) assessment in the laboratory, and for B (biological efficacy against mosquitoes) and C (HPLC analysis) components efficacy testing (Figure 2.1). These households will be randomly selected stratified by district and LLIN product so that six nets from each district per product are evaluated for BCD components. All sampled nets will be replaced with new nets of the same kind except for Netprotect® nets which will be replaced by Olyset®, and the sampled household will be excluded from subsequent sampling rounds.

ABCDR components

Attrition (A component)

Attrition of LLINs is defined as the proportion of LLINs that are no longer in use as mosquito nets to sleep under in the receiving household after a given amount of time. This is commonly due to loss through nets being damaged, discarded, or used for other purposes than sleeping under. Nets that are sold, given away or stolen will be excluded from the attrition analysis following WHO guidelines [6] as they may still be "serviceable".

Trained field interviewers will perform the field visits of all households selected from the master list and voluntarily participating in the study during the sampling points (retrospective sampling, 10, 22 and 36 months after prospective LLIN distribution). Questionnaire data will be collected using Open Data Kit (ODK) Collect software (http://opendatakit.org/use/collect/) on Android tablet computers (Google Nexus One). Observations by the field workers on presence and absence of distributed nets, the location of the net (hanging or stored away), fabric integrity and the net condition are included in the questionnaire (Additional file 2.2).

Physical degradation (D component)

The physical degradation, or integrity, of the nets will be measured by counting the number, location and size of hole(s) in each net. The proportional hole index (pHI) will be calculated using the hole size categories as per WHO guidelines [5,6] (Table 2.4). In addition to the different category sized holes, we will also include five different hole locations on the net by dividing the side panels of the net into a total of four zones from top to bottom, each measuring 37.5 cm, and counting holes in the roof separately as a fifth location (Figure 2.3). Mosquitoes are more likely to aggregate around certain locations on occupied bed nets (e.g. the roof; [34]). In addition, the lower edges of the bed nets are more likely to be severely damaged, but they are also more likely to be tucked in at night, potentially avoiding mosquito entry [35]. By counting the holes by location, we will be able to take into account these factors when analysing the hole index data and give different weights to holes in different locations.

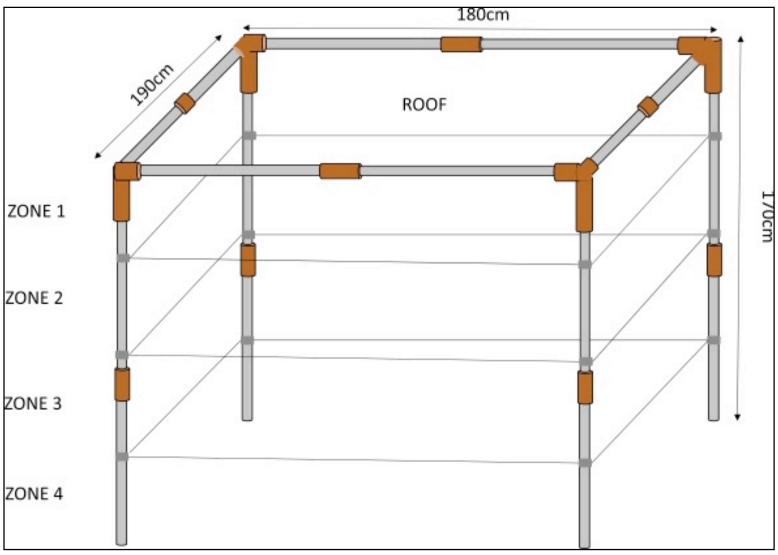

Figure 2.3 Collapsible frame for hanging bednets

To our knowledge, this formula has not yet been developed. One of our aims is therefore to incorporate hole location into the equation, and to compare its relative importance to a simpler model in terms of protection against mosquitoes in semi-field experiments. Holes will be counted both in the laboratory and in the field using a collapsible metal frame made out of locally available economical materials (Figure 2.3). In the field, holes in a maximum of three prospective nets will be counted per household due to logistical and time constraints.

Biological efficacy (B Component)

Testing will be performed at BRTC, Bagamoyo, Tanzania using An. gambiae sensu stricto (s.s.) (Ifakara strain, Njage 1996) mosquitoes that are fully susceptible to insecticides and are reared according to CDC guidelines [36]. Mosquitoes used for testing will be 2-8 days old (depending on the test), nulliparous sugar fed females. Standard WHO cone bioassays will be carried out to evaluate new nets at baseline (ten samples per net product), 200 retrospective Olyset® nets, and a random sub-sample of 48 prospective nets per time point. WHO tunnel tests will be performed if nets fail the cone test [7,37]. To validate these WHO recommended bioassays and help to estimate fully the protection provided by nets under user conditions, those 48 nets will first be tested in a semi-field tunnel (SFT) – the newly developed Ifakara Tunnel Test (ITT) - to measure the protective efficacy of the nets to people rest- ing underneath them [38]. For the WHO tunnel test and ITT, only those mosquitoes that are responsive to human odour on the day of testing will be used. For semi-field tests, mosquitoes will be deprived of sugar solution for six hours prior to experiments and transferred to a screened test cage one hour prior to testing to allow them to acclimatise.

Ifakara tunnel test (ITT) A semi-field enclosure is here defined as an enclosed environment, ideally situated within the natural ecosystem of the target disease vector and exposed to ambient environmental conditions. Semi-field enclosures offer several useful features: 1) participants are safe because they are exposed only to laboratory-reared disease-free mosquitoes, 2) experiments can be run using standard numbers of mosquitoes allowing year round collections regardless of natural vector populations, and 3) using mosquitoes of known age, physiological status and avidity reduces experimental variability allowing for rapid data collection and improved data quality.

The Ifakara tunnel is a 50 m long, 3 m wide and 2.1 m high steel tube frame construction covered by durable UV resistant polyurethane coated netting (Figure 2.4A). The structure is constructed upon a concrete base surrounded by a water channel to prevent entry by ants and spiders. The tunnel sits beneath a simple beamed wooden frame supporting a corrugated steel roof to allow work in all weather conditions.

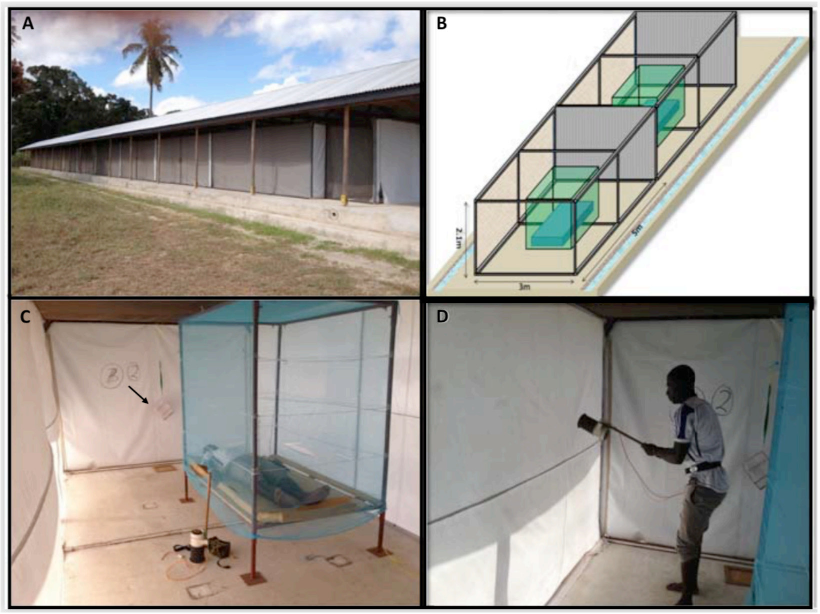

Figure 2.4. Ifakara Tunnel Test (ITT)

The netted tunnel is divided into ten individual test chambers (5 m x 3 m x 2.1 m) with interconnecting doors that are sealed with zips and Velcro to prevent mosquitoes moving from one test chamber to another (Figure 2.4B). Each end of the tunnel contains an additional double door module to prevent loss of laboratory-reared mosquitoes into the wild. Mosquitoes will be released within each compartment by raising the netted holding cages from their removable wooden bases. This is achieved remotely by the volunteer in each compartment pulling a nylon cord to raise the cage whilst remaining beneath the net (Figure 2.4C). After the allotted experimental time period, all mosquitoes within each of the compartments will be removed by mechanical aspiration (Figure 2.4D).

Each of the ten experimental compartments will be provided with a steel bed frame and foam

mattress upon which a volunteer will sleep during each test and over which the LLIN will be draped (Figure 2.4C). A human volunteer will sleep beneath the LLIN from 21.00 hrs to 06.00 hrs to represent user conditions. For each test, 30 nulliparous 2-8 day old, disease-free An. gambiae s.s. mos- quitoes will be introduced. At 06.00 hrs, the mosquitoes within the compartment will be collected using a mechan- ical aspirator (Prokopack; [39]) and scored for knockdown (KD), 24-hour mortality and blood-feeding success.
All participants in ITT experiments will be male staff members of IHI who have received appropriate training and are experienced in conducting semi-field tunnel tests. All participants will be recruited on written informed consent, which explains the risks and benefits of the study and are free to leave the study without explanation. The risk of disease transmission to volunteers is very low.

Chemical residue (C Component)

After biological efficacy and physical degradation testing in semi-field facilities in Bagamoyo has taken place, the same 48 LLINs per product will be used for chemical residue analysis. Chemical residues will be determined by HPLC [40]. The HPLC analysis will be carried out in a WHO Collaborating Centre for Quality Control of Pesticides (Walloon Agricultural Research Centre; CRA-W) following the latest WHO recommendations. Four sub-samples of 30 cm x 30 cm will be taken from each net representing the entire net. Samples will be kept at 4°C in aluminium foil until analysed to determine the total content of permethrin (Olyset®) or deltamethrin (Netprotect® and PermaNet®2.0) in g/kg.

Resistance monitoring (R Component)

The resistance monitoring component builds upon the existing nationwide longitudinal monitoring of insecticide resistance in Tanzania that has already been carried out in 26 selected sentinel districts from different ecological zones of Tanzania [41]. In the current study, insecticide resistance will be assessed in a total of 15 districts (Figure 2.2). Eight of these districts coincide with the ABCD part of the project. Insecticide resistance will be monitored in cross-sectional countrywide surveys conducted annually throughout the project life. These surveys will be carried out in May/June, just after the long rainy season. The susceptibility levels and resistance mechanisms of malaria vectors to insecticides of public health and agricultural relevance in Tanzania will be determined. Results will feed into the online geospatial application

IR Mapper [23]. Anopheles larvae will be collected in easily accessible larval habitats in one or two villages per district. Each breeding site will be geo-referenced using GPS. Larvae will be bred to adult mosquitoes in field laboratories, which will be maintained on 10% glucose solution in mosquito cages. Three- to five-day old F1 generation mosquitoes will be tested using standard WHO insecticide susceptibility testing procedures [42]. Mosquitoes will be exposed to papers impregnated with the WHO-recommended discriminating concentrations (v⁄w) of 0.05% delta-methrin, 0.05% lambda-cyhalothrin, 0.75% permethrin, 0.1% bendiocarb, 1% fenitrothion and 4% DDT prepared at University Sains, Malaysia [42]. During exposure, KD rates will be recorded after a range of exposure times. Mosquitoes will then be provided access to 10% glucose solution and 24 hour mortality will be scored. All mosqui- toes will be identified using keys described by Gillies [43,44] and An. gambiae sibling species identified using established Polymerase Chain Reaction (PCR)-based methods [45]. PCR-based standard methods will also be used to detect kdr mutations [46] and biochemical assays will be used to detect the enzyme-based resistance mechanisms in mosquitoes.

Statistics and data analysis

Sample size calculation

Sample size calculations were based on the primary outcome measure of net attrition using the standard formula for the difference between two proportions [47]. The BCD components were treated as an additional sub-sample to the original calculated sample size. Assuming an average of 3 nets per household and a coefficient of variation of 0.25, then the formula on page 110 of Hayes & Moulton [48] gives a sample size of 973 households per arm to detect a difference in attrition between two brands with attrition rates of 47.5% and 52.5% with 90% power. Therefore, there will be at least 90% power to detect a 5% difference in attrition rates. Loss to follow up and households excluded due to sub-sampling have been added to the final sample size to give (1,140 households * 3 nets/ household)=3,420 LLINs per LLIN product (Table 2.3).

Data analysis

We will collect a set of response variables (Table 2.1) and explanatory variables. The explanatory variables will be collected from household questionnaires and observations and will include time after net distribution, net product, geographical location, patterns of net use (e.g.

type of bed, frequency of net use), net status, washing and handling, perceptions of nets and socioeconomic status of the household. All response variables will be analysed using the statistical programs STATA®13 (http://www.stata.com/) and R (http://www.R-project.org/). Regression modelling including multivariate generalised linear models and generalised linear mixed models will be used to determine covariates affecting net durability components such as LLIN age, geographical location and data collected from household surveys. Principal Component Analysis (PCA) will be used to determine a combination of variables for socioeconomic status to explain the overall observed variation and reduce the complexity of the data. In order to analyse net attrition and physical degradation in more detail, 95% confidence intervals will be calculated for the attrition and 'unserviceable' physical condition of each net product at the three prospective time points. At each point, logistic regression with a category for each brand of net will be performed to assess if there is a difference in attrition between the three net products. If a significant difference is found, then pairwise comparisons will be examined.

Ethical considerations

Full ethical approval has been obtained from ethical review committees at London School of Hygiene & Tropical Medicine (6333/A443), Ifakara Health Institute (IHI/ IRB/AMM/ No: 07-2014) and the National Institute for Medical Research (NIMR/HQ/R.8c/Vol. I/285).

Written informed consent will be obtained from the head of the household of those households selected for participation (Additional file 2.1). If absent, another adult household member (above the age of 18) representing the household head will sign the informed consent form. The informed consent will be obtained before each survey. For participants who cannot read the form, the informed consent form will be read out and explained by the local field staff in Kiswahili or the local language in the presence of a community witness. After consenting, the household head, or his representative, will be asked to mark a thumb impression on the form, and the witness will be asked to sign it. The potential participants will be advised that they can refuse to participate at any point in the future and may still keep their new net.

2.4. Discussion

In addition to following WHO durability guidelines [5], which will allow direct comparison between our study and other ongoing durability investigations in SSA, we are also developing new methodologies to fully assess to what extent physical degradation, chemical decay and biological efficacy actually determine the life of a net, i.e. the duration of its effective protection. LLINs act as a barrier against blood-feeding of anopheline mosquitoes on humans. We will determine the effectiveness of nets as transmission barriers by testing the whole net from the field protecting humans throughout the night against mosquito bites in semi-field Ifakara Tunnel Tests (ITT). This will give us a strong measure of the individual protective efficacy against human biting behaviour. In addition, it will allow us to estimate the mortality of mosquitoes exposed to LLINs under more natural conditions, a methodology that is commonly performed in experimental huts [49]. However, the ITT is designed to increase both data throughput and data power because it evaluates eight nets and two controls per night using mosquitoes of identical physiological status. In addition, the same number of mosquitoes can be released into each of the compartments so that the effect of the efficacy of the nets is measured in the same way in each compartment. In contrast, field tests require far greater numbers of replicates to achieve good statistical power due to both spatial and temporal heterogeneities in mosquito numbers [50]. We will also determine the WHO-recommended hole index (pHI) by location on the net, with the potential of influencing further net product design with strengthened material in the bottom quarter of the net.

National and international public health policy makers may therefore use the information provided by this, and other ongoing studies, to procure the most cost- and health-effective nets. Results will allow the selection of nets that provide protection against disease at optimum costs (trading off LLIN durability, price and insecticide resistance status of local mosquito populations), and to estimate the timing of repeated distribution campaigns to ensure that maximal health gains are maintained.

Current study status

At the time of submission of this manuscript (December 2014), the study had completed the retrospective data collection and random distribution of the three new LLIN products, the establishment of the return net data base, and the prospective household survey after 10 months.

Additional files

Additional file 2.1: English version of the informed consent form that will be used to obtain written informed consent from heads of household in the retrospective study. This informed consent form has been translated into Kiswahili. (https://static-content.springer.com/esm/art%3A10.1186%2F1471-2458-14-1266/MediaObjects/12889_2014_7451_MOESM1_ESM.pdf)

Additional file 2.2: English version of the prospective questionnaire that will be programmed in Kiswahili using ODK Collect on Google Nexus tablet computers to collect basic household and net attrition and use information. (https://static-content.springer.com/esm/art%3A10.1186%2F1471-2458-14-1266/MediaObjects/12889_2014_7451_MOESM2_ESM.pdf)

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

LML, SJM and HJO devised the study. LML wrote the manuscript. JM, HJO, WK, JB, DM, ZM and SJM contributed to the writing of the manuscript and provided valuable comments throughout the protocol development stage. JM, DM and SJM developed the protocol for the semi-field bioefficacy testing. JB and LML performed the sample size calculations. WK and HJO developed the resistance component of the study. JB devised the analysis plan. RM and KK contributed to the setting of the study and advised on the study design. All authors approved the final version of the manuscript.

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3. Durability of Olyset campaign nets distributed between 2009 and 2011 in eight districts of Tanzania.

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3.1. Abstract

Background: Long-lasting insecticidal nets (LLINs) are the first line choice for malaria vector control in sub-Saharan Africa, with most countries adopting universal coverage campaigns. However, there is only limited information on LLIN durability under user conditions. Therefore, this study aimed to assess the durability of Olyset® LLINs distributed during net distributions campaigns between 2009 and 2011 in Tanzania.

Methods: A retrospective field survey was conducted in eight districts in Tanzania mainland to assess the durability of Olyset campaign nets in year 2013. Household questionnaires were used to assess attrition, i.e. net loss. All nets remaining in households were collected. A sub-sample of 198 Olyset campaign nets was examined for bio-efficacy against *Anopheles gambiae s.s.* mosquitoes, permethrin content and physical integrity following standard World Health Organization (WHO) methods.

Results: Of 6,067 campaign nets reported to have been received between 2009 and 2011, 35% (2,145 nets) were no longer present. Most of nets (84%) had been discarded mainly because they were too torn (94%). Of the 198 sub-sampled Olyset LLINs, 61% were still in serviceable physical condition sufficient to provide personal protection while 39% were in unserviceable physical condition according to the WHO proportionate Hole Index (pHI). More than 96% (116/120) of nets in serviceable condition passed WHO bioefficacy criteria while all nets in unserviceable condition passed WHO bioefficacy criteria. Overall mean permethrin content was 16.5g/kg (95% CI: 16.2 – 16.9) with 78% of the sub-sampled nets retaining the recommended permethrin content regardless of their age or physical condition. Nets aged four years and older had a mean permethrin content of 14g/kg (95% CI: 12.0 – 16.0). Physical integrity of the present nets was 4 times significantly lower (OR: 0.4, p-value=0.04, 95% CI: 0.1-1.0) when rats were present in the houses.

Conclusions: Two-to-four years after a mass campaign, only 39% of distributed nets remain both present and in a serviceable physical condition, a functional survival considerably below WHO assumptions of 50% survival of a ‘three-year’ net. However, the majority of nets still retained substantial levels of permethrin and could still be bio-chemically useful against mosquitoes if their holes were repaired, adding evidence to the value of net care and repair campaigns.

Keywords: Durability, LLINs, Olyset, Universal coverage campaign, Attrition, Physical integrity, Chemical content, Bio-efficacy, Tanzania

3.2. Background

Long-lasting insecticidal nets (LLINs) have significantly contributed to the success of malaria control in malaria- endemic countries in Africa [1]. In Tanzania in particular, mosquito nets contributed to a 45 % reduction in all- cause mortality in children less than 5 years of age from 146/1000 live births in 1999 to the recent level of 81/1000 live births in 2010 [2]. Since 2009, two LLIN mass distribution campaigns have been implemented in Tanzania, namely the under-five catch-up campaign (U5CC), which provided Olyset® nets to all children under the age of five between 2009 and 2010 [3], and the universal coverage campaign (UCC), between 2010 and 2011 [4], which provided Olyset nets for all sleeping spaces that had not been previously covered during the U5CC campaign. As recommended by the World Health Organization (WHO) [5], LLINs are expected to provide both personal and community protection resulting in a decline in malaria transmission. In addition to the mass distribution campaigns described above, two continuous distribution strategies have been implemented in Tanzania. The Tanzania National Voucher Scheme (TNVS) from 2004 to 2014 provided pregnant women and infants with LLINs at a greatly reduced price [6]. The currently ongoing annual School Net Programme (SNP) in the Southern zone provides every school child in specific grades one free LLIN for distribution to their households [7]. In addition, a universal replacement campaign (URC) is currently ongoing in 2015 and 2016, and is expected to provide 22 million nets to all households in Tanzania not covered by the SNP. All these distribution campaigns aim to reduce malaria transmission in the country through sustainable distribution mechanisms. Since LLINs have a limited serviceable life through loss of chemical insecticide and physical damage, net replacement campaigns are necessary to maintain high coverage, and the timing of these campaigns is of crucial importance.

The useful life of LLINs depends on properties of the net including physical integrity and persistence of insecticide and is not simply a matter of how long the net remains in the house [8]. Durability of LLINs is affected by variation in physical wear, which in turn depends on environmental and social factors like climate, type of sleeping space, presence of rodents or other animals, frequency of use and washing of nets; all of which vary between locations and populations [9–11]. This means that Tanzania's management decisions regarding LLIN replacement should be based on local LLIN performance data [12]. Information on the durability of different LLIN brands under user conditions will help malaria control programmes by providing information needed to plan the timing of future net replacement campaigns, and the procurement of the most durable LLIN for a country. In addition, information on appropriate net use and care (through improved behaviour change communication) might help to prolong the life of LLINs and reduce the costs of procurement and distribution [13, 14].

In Tanzania, the first choice of LLINs has historically been Olyset nets, developed by Sumitomo in Japan and manufactured by A–Z Textile Mills Limited in Arusha, Tanzania. Olyset nets, made from 150 denier polyethylene material with permethrin incorporated in the yarn, were the first LLINs to receive the full recommendation of WHOPES in October 2001 for use in prevention and control of malaria [15]. This study aimed to assess the durability of Olyset campaign nets (old knitting pattern) distributed between 2009 and 2011 in eight districts in Tanzania by measuring attrition (net loss), biological efficacy against anopheline mosquitoes (blood feeding inhibition and mortality), chemical content (amount of active ingredient) and physical integrity (number of holes and resulting physical condition of nets).

3.3. Methods

Study areas

This study was conducted as part of a long-term project on LLIN durability in Tanzania [16]. The study took place in eight districts (Fig. 3.1) selected from 23 districts enrolled in the population arm of the sentinel panel of Districts (SPD), sample vital registration with verbal autopsy (SAVVY) [17].

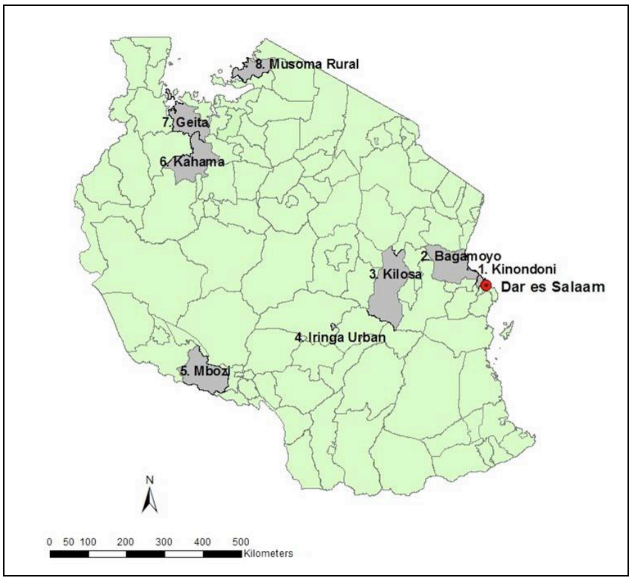

Figure 3.1. Geographical distribution of eight study districts representing five of eight geographical zones of Tanzania and covering variations in malaria epidemiology and ecology

Ten SAVVY villages per district were selected based on their proximity to district council headquarters, except for Kinondoni district where SAVVY only covered six villages. Using SAVVY baseline household information, 45 households per village (3420 households in total) were randomly selected using the 'sample' function in the statistical software R 3.1.1. [16].

Data collection

Cross-sectional household surveys were conducted between October and December 2013. The surveys involved collections of two sets of data. First, household information was collected by a pre-tested semi-structured household questionnaire using Google Nexus tab- let computers programmed with the open-source survey tool kit ODK Collect [18]. Information collected included household characteristics (household assets and housing conditions), number of mosquito nets (including Olyset campaign nets) received, any net lost since initial distribution and reasons for losing them. Second, all nets present in the sampled households were collected and replaced with new nets. All collected nets were returned to Bagamoyo Research and Training Centre (BRTC), part of Ifakara Health Institute (IHI), and their colour, size, product and manufacturing date (if available on label) were recorded to establish the total number of campaign nets that were still present in households at the time of the survey. Government Olyset campaign nets could be distinguished from nets from other sources because they were light-blue in colour and of single size (4 × 6 × 7 feet). However, it was not possible to identify which mass campaign the nets originated from (U5CC or UCC) as very few nets retained labels with legible manufacturing dates. Net age was estimated from data on when nets had been distributed to each district during the UCC campaign. This underestimates the age of nets obtained during the U5CC campaign, which took place approximately 12 months before the UCC campaign. This is believed to be a reasonable assumption since the UCC was a considerably bigger campaign distributing 17.6 million nets compared to U5CC's 7.8 million [4]. From all light-blue single sized Olyset campaign nets collected, 200 nets (25 nets per district) that still had legible manufacturing labels attached were selected for counting holes (for physical integrity) and bio-efficacy testing. All Olyset nets distributed in Tanzania and tested in this study were of the old knitting pattern, which was replaced by a new knitting pattern in 2014.

LLINs testing procedures

Attrition

Net attrition, the inverse of net survivorship, refers here to the proportion of nets distributed to house- holds during the UCC or U5CC campaigns which are no longer in use due to nets either being discarded, used for something else than sleeping under [19] or given away for others to use [8]. It is calculated by dividing the number of nets lost by the number of nets given out to each household. Unfortunately, in this study, it was not possible to establish the number of campaign nets given to each household through official net distribution channels. Therefore, recall information on the number of nets received by each household was collected. Net survivorship was calculated by setting the recalled total number of campaign Olyset nets (U5CC and UCC) received by each sampled household as the denominator, and the total number of light-blue Olyset campaign nets physically collected as the numerator.

Net attrition was calculated using the following formula:

$$1 - \frac{\textbf{Total campaign light-blue Olyset nets present in the household}}{\textbf{Total campaign nets reported to be received by each household}} \times 100\%$$

Physical integrity

Two hundred Olyset campaign nets were sub-sampled for assessment of their physical integrity and repair status. Each sampled net was mounted onto a 180 cm × 160 cm collapsible net frame. The number of differently sized holes of each sampled net was recorded following WHO guideline [20]. The physical integrity of nets was categorized by the proportion- ate hole index (pHI), which is calculated as follows:

$$pHI = (size1holesx1)+(size2holesx23)+(size3holes\ x\ 196) + (size\ 4\ holes\ x\ 578)$$

Based on their pHI value, LLINs were assigned to one of the following WHO categories: "good" (pHI ≤64), "dam- aged" (pHI = 65–642) and "too torn" (pHI ≥643). The first two categories were then combined as "serviceable" while those "too torn" were defined as "unserviceable" nets.

Bioassays

After the physical integrity assessment, two squares of netting (25 × 25 cm) were cut from each of four positions on each of the 200 sampled nets following WHO procedures [8]. One netting sample per position per net was tested with cone bioassays as per WHO guidelines [20], the other netting sample was sent for chemical analysis of permethrin content (see below).

Cone assays were carried out at 27 ± 2 °C and 75 ± 10 % relative humidity. Four standard WHO cones were used per netting sample. These cones were laid on the netting sample pinned to a board, held at a 45° angle to prevent mosquitoes from resting on the cone surface. The mosquitoes used were pyrethroid susceptible *Anopheles gambiae* sensu stricto (*s.s.*) aged 3–8 days old originally colonized from wild-caught gravid females in Njage, South-East Tanzania in 1996. Mosquitoes were reared according to standard procedures [21]. Five mosquitoes were introduced into each of the cones and exposed to the netting samples for 3 min, after which they were transferred to holding cups and held for 24 h with access to 10 % sugar solution. One untreated netting sample was used as a control for each net tested. Mosquito knock- down (any mosquito that cannot stand or fly in a coordinated manner) and mortality (mosquitoes that show no movement) were recorded 60 min and 24 h after expo- sure, respectively.

Net samples that failed cone test cut-off points (i.e. ≤80 % mortality and/or ≤95 % knockdown) were further tested in WHO tunnels assays with *An. gambiae s.s.* Kisumu strain, 3-8 days old at Amani Research Centre (Muheza, Tanzania) using rabbits as bait following WHO guidelines [20]. Of the four squares per net, the square that elicited mosquito mortality closest to the average mortality for the whole net sample was selected and tested in the WHO tunnel. Mosquitoes were scored as alive, dead, blood-fed or unfed. Delayed (24 h) mortality was recorded for the live mosquitoes. Net samples with mortality ≥80 % and/or blood feeding inhibition ≥90 % in tunnel tests were regarded to pass WHO tunnel assay criteria [18]. If mortality in control replicates was between 5–20 %, it was corrected by Abbott's formula [22]. If control mortality was above 20 %, the whole test was discarded and repeated as per WHO guidelines.

Permethrin content

Four netting square samples from each net were individually packed in foil, labelled and stored at 4 °C before being sent for analysis of permethrin content at the WHO Col- laborating Centre for Quality Control of Pesticides, Walloon Agricultural Research Centre (CRA-W) [23]. The analytical method used for determination of permethrin in Olyset samples was the CIPAC method 331/LN/M/3. This method involved extraction of permethrin in a water bath (85–90 °C) for 45 min with heptane in presence of triphenyl phosphate as internal standard and determination by Gas Chromatography with Flame Ionisation Detection (GC-FID). The performance of the analytical method was controlled during the analysis of samples in order to validate the analytical results. The results were recorded as either net sample with permethrin content below the lower WHO tolerance limit of 15 g/kg of the target dose of a new net of 20 g/kg ± 25 % [15–25 g/kg].

Data analysis

Results from WHO cone and tunnel bioassays, insecticide content and physical condition of nets were recorded on standardized forms and double entered in Excel spreadsheet for validation. Cleaning and analysis of data were done using Stata 13.0 statistical software (Stata Corp., College Station, USA).

Socioeconomic status (SES) of each sampled household was assessed by constructing a household wealth index based on household measures that included household assets and housing condition [24, 25]. A weighted sum of the factors and household assets for each sampled house- hold was calculated using principal component analysis (PCA) and the best model was the one with the lowest Akaike Information Criterion (AIC) value [26]. The sample was then divided into wealth quintiles.

Attrition data was analysed by logistic regression with proportion of nets lost as the outcome variable and district as the explanatory variable. Data from the physical integrity assessment were analysed by logistic regression with the binary outcome of proportion of nets in serviceable condition (pHI <643) relative to nets in unserviceable condition (pHI ≥643) and SES wealth quintile, net age in months since UCC distribution grouped into four categories (<25 months group, 25–36 months group, 37–48 months group and >48 months group), number of sleepers per bed, presence of rats, and type of sleeping space set as explanatory variables. A likelihood ratio test was used to compare two models in order to test the significance of particular explanatory variables.

Data from WHO cone and tunnel assays were analysed using logistic regression with the proportion that passed WHO cone or tunnel criteria as outcome variables. Net age categories in months since UCC distribution and net condition by pHI were explanatory variables.

Data from chemical tests were analysed using logistic regression with the proportion of nets exceeding WHO cut-off for permethrin content. Net age categories in months since UCC distribution and net condition by pHI as explanatory variables. Different relationships were also explored in a multivariate analysis model. In all analyses, robust standard errors were used to account for clustering in the data at the district level.

Ethics

Ethical approval was obtained from the London School of Hygiene and Tropical Medicine (LSHTM—UK) Research Ethics Committee (Reference number 6333), Ifakara Health Institute in Tanzania (Reference number IHI/ IRB/No: 19-2013), and the Tanzanian National Institute for Medical Research (Ref: NIMR/HQ/R.8a/Vol. IX/150 and NIMR/HQ/R.8c/Vol. I/285). Before household inter- views, the study was explained in Kiswahili or the local language and written informed consent was obtained from each household head or other adults above the age of 18 years. Questionnaires were coded with a unique ID code and names were not taken to ensure confidentiality. All sampled households received free replacement nets for each sleeping space.

3.4. Results

Demographic characteristics

A total of 3,398 households (out of 3,420 target households) were sampled in 76 villages in eight districts in Tanzania. From these households, 6,529 nets were collected of which 5,047 (77%) were LLINs, which included campaign and non-campaign mosquito nets. The total number of household members was 18,597, with an average household size of 5.7 people (95% CI: 5.6–5.8). Each household had an average of 3.1 (95% CI: 3.0-3.2) sleeping spaces of any type and 2.4 (95% CI: 2.3-2.4) mosquito nets of any kind. Over 66% (95% CI: 64.9 – 68.6%) of the household heads had attained primary school education.

Net attrition

Households reported to have received 6,067 campaign LLINs between 2009 and 2011. In 2013 during the retrospective survey, 3,922 (65%) light-blue Olyset nets were still present, giving a mean net attrition of 35% (95% CI: 34.0 – 37.0 %). Attrition of campaign nets varied significantly between districts ($\chi^2 = 54.56$, $p<0.001$). Bagamoyo and Mbozi lost the fewest campaign nets (28.2%; 95% CI: 25.2 - 31.0 % and 29.3%; 95% CI: 25.8 – 32.6 %, respectively) whereas the districts around Lake Victoria (Figure 3.1) showed the highest attrition (e.g. Geita: 40.6%; 95% CI: 37.4 – 43.7 %; Figure 3.2). Age of nets was confounded by district as the campaigns were staggered geographically and temporally, but there was no observed trend of time on net attrition (Figure 3.2). Of those nets no longer present, 84% were reported to have been discarded, 14% were said to have been given away, sold or stolen and 2% had been used for alternative purposes such as fencing, screening of doors and windows, fishing and protecting chickens. The reasons given for discarding nets were that they were too torn (94%), dirty (3%), or the user did not like them (3%).

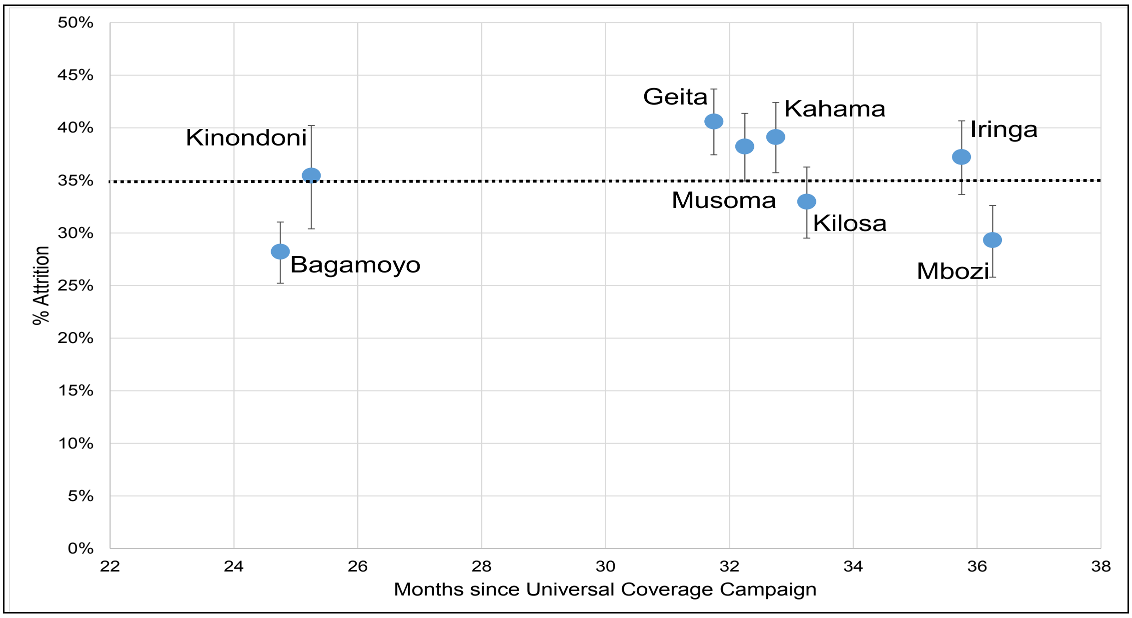

Figure 3.2. Attrition of Olyset campaign nets by age (month) and districts.

The Figure shows the proportion of nets no longer present in the household in each district since initial distribution between 2009 and 2011

Net integrity

Out of the 3,922 light-blue Olyset nets collected, 200 nets were sampled and their physical integrity was assessed. Two of these were excluded from the analysis because their unique identifying labels were lost, leaving a total of 198 sub-sampled nets.

Overall, more than half of the nets (n=106; 54%) were between 25 - 36 months of age, while 17 nets (10%) were more than four years old (Table 3.1). Twelve percent (n=24) had no holes, while 88% (n=174) had at least one hole. The frequency distribution of pHI was right-skewed with a median pHI of 279 (Interquartile range - IQR = 9th – 1220th percentiles). Based on WHO pHI categories, 37% (n=73) were considered in “good” condition, 24% (n=47) were “damaged” while 39% (n=78) were “too torn”.

Table 3.1 Number and proportion of campaign Olyset nets by age and proportionate Hole Index (pHI)

Time of use since distribution (months)	pHI≤64 'Good'	65<pHI≤642 'Damaged'	pHI≥643 'Too torn'	Total
<25 months	15 (31%)	12 (25%)	21 (44%)	**48**
25 – 36 months	41 (39%)	30 (28%)	35 (33%)	**106**
37 – 48 months	13 (48%)	2 (7%)	12 (44%)	**27**
> 48 months	4 (24%)	3 (18%)	10 (59%)	**17**
Total	**73 (37%)**	**47 (24%)**	**78 (39%)**	**198**

Following WHO classification [12], 61% (120/198) of the sampled nets were still in serviceable condition (good and damaged conditions). The only factor that was found to have a significant effect on net integrity was presence of rats in households (Table 3.2). The presence of rats decreased the odds of having a net in good condition by 60% (OR=0.4; 95% CI: 0.1-1.0; p=0.05). Neither SES wealth quintile, type of sleeping space, number of sleepers under a mosquito net nor net age showed any statistically significant relationship with the physical condition of the net (Table 3.2). Two-to-four years after the distribution campaigns, 39% (95% CI: 38 – 40%) of nets were functional i.e. 61% still serviceable of the 65% campaign nets remaining. Only 17% of the sampled nets had been observed to be repaired.

Table 3.2 Multivariable analysis on factors that might affect the good physical condition (proportionate Hole Index≤64) of Olyset campaign nets distributed 2-4 years earlier in Tanzania

Explanatory variables	Likelihood of net being in good physical condition (pHI ≤ 64)			
	Odds ratio	**95% CI**	**P value**	**Overall P value (Likelihood ratio test)**
Socioeconomic status of households				
Least wealthy (1)	1.0			
(2)	0.9	0.4 - 2.7	0.98	
(3)	0.7	0.3 - 2.1	0.59	
(4)	1.1	0.4 - 3.2	0.89	
Wealthiest (5)	1.3	0.5 - 3.5	0.65	0.88
Net age (months)				
≤25	1.0			
26 - 36	1.9	0.8 - 4.2	0.13	
37 - 48	1.0	0.4 - 3.1	0.95	
> 48	0.6	0.1 - 2.1	0.39	0.09

Presence of rat faeces/ rats in household				
No	1.0			
Yes	0.4	0.1 - 1.0	**0.05**	**0.04**
Type of sleeping space				
Reed mat	1.0			
Mattress, no frame	0.6	0.1 – 3.2	0.54	
Bedframe made from sticks	0.7	0.2 - 2.7	0.56	
Wooden and iron bedframe	0.6	0.2 - 1.9	0.32	0.78
Number of persons per net				
1 user	1.0			
2 users	0.7	0.3 - 1.4	0.39	
3 users	0.6	0.2 - 1.7	0.41	
4 users	3.2	0.3 - 33.9	0.33	0.44

Bioassay and permethrin content results of subsample of nets of different age.

Results of the bioassay tests and permethrin content analysis of 198 sub-sampled nets of different ages are presented on Table 3.3. The mean permethrin content was 16.5g/kg (95% CI: 16.2 – 16.9 g/kg). Only nets aged four years and older had permethrin content 14.0 g/kg (95% CI: 12.0 – 16.0 g/kg), which is below the WHO recommended concentration for brand-new nets (i.e. 15g/kg).

Table 3.3. Number and proportion of Olyset nets of different ages with recommended permethrin content and passed WHO cone/tunnel tests criteria

Explanatory variables	**Net age (months)**			
	≤ 25 Months	**26 – 36 Months**	**37-48 Months**	**>48 Months**
Number of sub-sampled nets	48 (24%)	106 (54%)	27 (14%)	17 (8%)
Cone assay				
Proportion of nets passed WHO cone assay criteria[1]	30 (62%)	64 (60%)	20 (74%)	12 (71%)
Pass WHO cone or tunnel tests[2]				
Proportion of nets passed WHO cone or tunnel tests criteria	45 (94%)	105 (99%)	27 (100%)	17 (100%)
Chemical residue				
Proportion of nets with recommended permethrin content	41 (85%)	86 (81%)	20 (74%)	8 (47%)
Mean permethrin content in g/kg (95% CI)	16.8 (16.3-17.2)	16.8 (16.4-17.4)	16.5 (15.7-17.3)	14.0 (12.0-16.0)

[1]WHO Cone assay criteria: ≥ 95% knockdown and/or ≥80% mortality pass rate
[2]WHO tunnel test criteria: ≥ 80% mortality and/or ≥90% blood feeding inhibition pass rate

The cone assay data of sampled nets are presented as proportion of nets that passed WHO cone assay criteria, i.e. nets that caused more than 95% knockdown and/or more than 80% mortality. The average knockdown was 89.5% (range 86.7 – 92.2%) at 60 minutes post exposure while average mortality was 55.7% (range 51.9 – 59.3%) at 24 hours post exposure with susceptible mosquitoes. Overall 63.6% (126/198) of nets passed the WHO cone assay criteria. Age of the net had no significant effect on probability of passing cone criteria (Table 3.4). The 72 nets that failed cone assay criteria were tested in WHO tunnel assays, which is presented as proportion of nets that passed WHO tunnel assay criteria i.e. nets that caused more than 90% blood feeding inhibition and/or more than 80% mortality. Ninety-four point three percent (range 89.9 – 98.8%) of mosquitoes did not blood feed while average mortality was 97.4% (range 96.7 – 98.1%) at 24 hours post exposure. The overall percent of nets passing the tunnel tests criteria was 94.4% (89.0 – 99.9%), which was not statistically explained by net age (Table 3.4).

Table 3.4. Multivariable logistic regression analysis of the bio-efficacy and permethrin content of sampled Olyset campaign nets

Explanatory variables	Pass cone assay criteria			Above 15 g/kg permethrin		
	Odds ratio	95% CI	P value	Odds ratio	95% CI	P value
Net age (in months since initial distribution)						
Old nets (>25months)	1			1.0		
Newer Nets (≤ 25 months)	0.9	0.5 – 1.9	0.9	2.1	0.8 – 5.3	0.11
Physical condition of net						
Net in unserviceable condition	1			1.0		
Net in serviceable condition	2.4	1.3 – 4.4	**0.04**	4.1	2.0 – 8.5	**0.001**

Bioassay and permethrin content results of subsample of nets of different physical condition.

Results of the bioassay tests and permethrin content analysis of 198 sub-sample nets of different physical condition (i.e. good, damaged and unserviceable condition) are presented in Table 3.5. The proportion of nets (of different physical condition) that passed cone assay, tunnel tests and with recommended permethrin content are presented as percentages. Overall, 96% (116/120) of nets in serviceable condition passed WHO cone or tunnel tests cut off criteria while all nets in unserviceable condition passed WHO cone or tunnel tests cut off criteria.

Table 3.5. Number and proportion of Olyset nets of different physical condition with

recommended permethrin content and passed WHO cone/tunnel assay tests criteria

Explanatory variables	Net physical condition (pHI)			
	Good (pHI<64)	**Damaged (pHI= 64 – 642)**	**Serviceable condition** (pHI <643)**	**Unserviceable Condition (pHI >642)**
Number of subsampled nets	73 (37%)	47 (24%)	120 (61%)	78 (39%)
Cone assays				
Proportion of nets passed WHO cone assay criteria[1]	79% (n=58)	60% (n=28)	72% (n=86)	51% (n=40)
Cone assays or tunnel tests[2]				
Proportion of nets passed WHO cone or tunnel tests criteria	97% (n=71)	96% (n=45)	97% (n=116)	100% (n=78)
Chemical residue				
Proportion of nets with recommended permethrin content	92% (n=67)	81% (n=38)	88% (n=105)	64% (n=50)
Mean permethrin content in g/kg (95% CI)	17.8 (17.4 -18.2)	16.5 (15.8-17.2)	17.3 (16.9 – 17.7)	15.4 (14.7-16.0)

[1]WHO Cone assay criteria: ≥ 95% knockdown and/or ≥80% mortality pass criteria

[2]WHO tunnel test criteria: ≥ 80% mortality and/or ≥90% blood-feeding inhibition pass criteria

** Serviceable condition include nets in good condition and those in damaged condition

A multivariable analysis was conducted to explore different relationships (Table 3.4). The odds of a net passing cone assay tests was 2.4 times (OR=2.4, 95% CI: 1.3 – 4.4, p=0.04) greater for nets in serviceable condition as compared to nets in unserviceable condition. Permethrin content was four times higher (OR= 4.1, 95% CI: 2.0 – 8.5, p=0.001) among nets in serviceable condition as compared to those in unserviceable condition.

3.5. Discussion

This retrospective study in 3398 households in Tanzania found that more than a third of campaign nets had been lost since the government campaigns in 2009 and 2011, and that a further 39 % of the nets had large hole surface areas, leading to an urgent need to replace LLINs in Tanzania.

In this study, attrition of LLINs was higher than that observed in Western Kenya after 5 years [27], but similar to net loss in Rwanda [28] and Nigeria [11]. In Tanzania, net condition (i.e. the number and size of holes) was the

primary reason given by study households for discarding of nets, a finding mirrored in a recent multi-country investigation, which found that 63 % of lost nets had been discarded, primarily because they were perceived as too torn (93 %) [19].

More than half of nets were still in a serviceable physical condition, hence theoretically effective in protecting individuals against mosquito bites. Percent of nets with holes observed in this study was higher than in Western Uganda where the majority (87 %) of polyester nets were in serviceable conditions after three and a half years and 23 % of nets had no holes at all after 36–42 months of use [9]. In Zambia, on the other hand, 30 % of polyester and poly-ethylene nets were classed as 'too torn' after 30 months in the field [29]. Unlike other studies [13, 26], this study did not find a relationship between net age and its physical condition. This may be because very worn nets are more likely to be discarded, resulting in lower hole counts in older nets as was found in Zambia [30]. The only statistically significant determinant of a net being unserviceable was the presence of rats, a parameter often associated with poor physical condition of mosquito nets [14, 31]. With differences in geographical settings between different study villages, assessing degradation using only 198 nets is likely to have been an insufficient sample size to detect differences between nets of different ages, although it is sufficient to demonstrate that nets do need to be replaced.

The low rates of repair observed in this study have also been observed in Ethiopia [10] and in Kenya [14], suggesting that barriers to net care and repair may exist [32]. Such low repair rates, which probably result in more nets becoming unserviceable, are particularly important because more than two-thirds of the unserviceable nets still contained permethrin concentrations above 15 g/ kg—the lowest permethrin threshold set by WHO for brand-new nets. This means that these nets, if repaired, could still provide good individual protection and it is likely that these nets continue to provide community protection by killing mosquitoes [33, 34] or inhibiting blood feeding [35] despite containing holes. However, it should be noted that the nets collected in the households and tested for bio-efficacy are those that have been retained by households, probably precisely because of their better condition and may have been stored for later use.

Despite not being able to plot LLIN survival due to the collection of data during only one time point, this study nevertheless adds a useful data point to the growing table of net durability in various countries and of numerous net products [29, 36]. This study showed a functional LLIN survival of 39 % two-to-four years after the distribution campaigns, which is lower than the median survival of 50 % after 3 years of a 'three-year net' [14]. This survival estimate is based on two facts: (a) Hole counts were performed on a sub-sample of 198 nets; and (b) the WHO cut-off criteria of a 'serviceable' net is in terms of protection against malaria. The relevance of the hole index as a measure of personal protection is currently lacking hard evidence and requires further investigation [37]. The relative contribution of insecticide and pHI to personal and community protection will be further studied by this study team.

The results from this study could have several implications for the LLIN strategy of the Tanzanian National Malaria Control Programme (NMCP). Firstly, a clear challenge observed from this and other studies was that owners discard nets mostly because they are perceived to be in too torn condition (unserviceable). However, two- thirds of the sampled nets in the unserviceable category were found to have permethrin concentrations above

the recommended WHO threshold criteria, which could pose environmental problems when discarded inappropriately [38]. Therefore, the government needs to intro- duce a better mechanism of collecting and disposing of unserviceable nets to prevent environmental pollution and introduction of insecticides to the environment. As a first step, the manufacturer A–Z Textile Mills Limited recycled all Olyset nets collected by the study team, but a more widely applicable system of net recycling by the government or industry should be developed. A second challenge observed was the functional LLIN survival rate, which fell 11 % short of the expected median survival of a 'three-year' net. In addition, target net coverage goals of at least 80 % coverage by 2020 as set by the Tanzanian NMCP will not be maintained through mass campaigns taking place in 3 year cycles. Therefore, continuous net replenishment has been implemented through the TNVS between 2004 and 2014 and through schools in Southern Tanzania since 2013. A new free LLIN distribution mechanism through reproductive and child health clinics will be rolled out in 2016 to replace the TNVS (K. Kramer, *pers. comm.*). Thirdly, given the bio-efficacy and permethrin contents of the collected nets, the government and NMCP need to improve their behavioural change communication strategy so it delivers locally appropriate education messages on net care and repair, which could serve to increase net retention and personal protection and hence prolong the lifespan of LLINs.

A limitation of this study was its retrospective sampling design. Data collection on attrition relied on respondents' recall information (hence recall bias), because it was not known how many Olyset nets had been distributed to each household. In addition, it was impossible to establish the exact age of nets due to difficulties in distinguishing between U5CC and UCC Olyset nets. Therefore, the attrition rate presented in Fig. 2 is a conservative estimate of the smallest possible age gap. A large prospective study is currently on-going in Tanzania to compare three net brands in the same study households over three years [16], which will be able to capture attrition and physical degradation more precisely and accurately.

3.6. Conclusions

The findings from this study highlight that the functional survival of Olyset nets two-to-four years after campaigns is 39%, which is below the median survival of a 'three-year' net of 50% as recommended by WHO. Therefore, LLINs are urgently needed in Tanzania to substantially increase access to serviceable mosquito nets. A universal mass campaign is currently ongoing to increase baseline levels, but high coverage must be maintained through continuous distribution mechanisms. When all the measurements of LLINs durability are taken together, it can be concluded that around 65% of LLINs distributed between 2009 and 2011 were still present in households, and a majority of them had retained target insecticide levels and were biologically effective against anopheline mosquitoes. This means that these nets could still be useful if they were repaired and they may pose environmental problems if incorrectly disposed of. Therefore, it is recommended the implementation of more targeted care and repair campaigns and investigations into means of encouraging net re-use, net recycling and safe disposal.

Competing Interests

The authors declare that they have no competing interests.

Authors' contributions

Conceived and designed the experiment SJM, LML, HJO, JM, KK, RM. Performed the experiments DJM, ZM, EJM, OP. Analysed the data DJM, SJM, LML. Contributed to data analysis JB. Wrote the manuscript DJM, LML. Critically reviewed the final manuscript LML, WK, HJO, KK, OP, JB, SJM. All authors read and approved the final manuscript.

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4. Mosquito net coverage in years between mass distributions: a case study of Tanzania, 2013

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4.1. Abstract

Background: The Government of Tanzania is the main source of long-lasting insecticidal nets (LLINs) for its popula- tion. Mosquito nets (treated and untreated) are also available in the commercial market. To sustain investments and health gains in the fight against malaria, it is important for the National Malaria Control Programme to monitor LLIN coverage especially in the years between mass distributions and to understand what households do if their free nets are deemed unusable. The aim of this paper was to assess standard LLIN indicators by wealth status in Tanzania in 2013, 2 years after the last mass campaign in 2011, and extend the analysis to untreated nets (UTNs) to investigate how households adapt when nets are not continuously distributed.

Methods: Between October–December 2013, a household survey was conducted in 3398 households in eight dis- tricts in Tanzania. Using the Roll Back Malaria indicators, the study analysed: (1) household net ownership; (2) access to nets; (3) population net use and (4) net use:access ratio. Outcomes were calculated for LLINs and UTNs. Results were analysed by socio-economic quintiles and by district.

Results: Only three of the eight districts had household LLIN ownership of more than 80%. In 2013, less than a quar- ter of the households had one LLIN for every two people and only half of the population had access to an LLIN. Only the wealthier quintiles increased their net ownership and access to levels above 80% through the addition of UTNs. Overall net use of the population was low (LLINs: 32.8%; UTNs: 9.5%) and net use:access ratio was below target level (LLINs: 0.66; UTN: 0.50). Both measures varied significantly by district.

Conclusions: Two years after the last mass campaign, the percentage of households or population with access to LLINs was low. These findings indicate the average rate at which households in Tanzania lose their nets is higher than the rate at which they acquire new nets. The wealthiest households topped up their household net ownership with UTNs. Efforts to make LLINs available through commercial markets should be promoted, so those who can afford to buy nets purchase LLINs rather than UTNs. Net use was low around 40% and mostly explained by lack of access to nets. However, the use:access ratio was poor in Mbozi and Kahama districts warranting further investigations to understand other barriers to net use.

Keywords: Long-lasting insecticidal nets (LLINs), Untreated nets, Universal coverage, Net ownership, Net access, Net use, Tanzania

4.2. Background

Since the global resurgence of interest in malaria control about 20 years ago, insecticide-treated nets (ITNs) have been the most widely distributed intervention against malaria and account for a 68% decline in *Plasmodium fal- ciparum* infection prevalence in sub-Saharan Africa [1]. Universal coverage as recommended by the World Health Organization (WHO) is defined as "universal access to, and use of, long-lasting insecticidal nets (LLINs)" of all people at risk of malaria, and is defined operationally as one net for every two people [2]. Tanzania has a long-standing record in the deployment of mosquito nets as an intervention for malaria control [3–7]. The use of ITNs in Tanzania has been associated with the reduction of malaria morbidity and mortality, particularly in children under the age of five [8, 9].

Mass distribution campaigns are the primary source of LLINs in most malaria endemic countries and aim to ensure equitable distribution across all socio-economic groups [1, 10–12]. Given the increasing distribution of large numbers of mosquito nets in communities, the Roll Back Malaria Monitoring and Evaluation Reference Group (MERG) developed indicators to assess and compare LLIN interventions in countries at risk of malaria [13]. Household surveys are widely used to measure the MERG indicators, which determine achievements of uni- versal coverage of LLINs following mass distributions [13].

Between 2004 and 2014, the Government of Tanza- nia distributed nets to pregnant women and infants at a subsidised cost during their routine antenatal and immunization clinic visits through the Tanzania National Voucher Scheme (TNVS) [14–16]. Nationwide, children under the age of 5 received nets free of charge through the Under-Five Catch-up Campaign (U5CC) between 2009 and 2010 [17], and a Universal Coverage Campaign (UCC) was implemented in 2010 and 2011 to reach all remaining uncovered sleeping spaces [18]. Another mass universal replacement campaign (URC) was conducted between 2015 and 2017 to achieve universal coverage in most of the country. Since 2013, the School Net Pro- gramme (SNP) has been ongoing in the Southern Zone to explore sustainable continuous "Keep Up" mechanisms to distribute nets into the community [19, 20, 21].

In addition, both insecticidal and untreated mos- quito nets (UTNs) are available through the private sec- tor at varying costs [22]. A to Z Textile Mills Ltd. holds the biggest market share for mosquito nets in Tanzania, but their commercial market is currently restricted to UTNs (Safinet) and supplies to international funders for mass LLIN campaigns (Olyset and Miranet) within the region and elsewhere (Nick Brown, Business Develop- ment Manager, *pers. comm.*). There are three more local manufacturers of UTNs than LLINs in Tanzania, which increases the accessibility and availability of UTNs in the commercial markets at a cheaper cost [22]. Though not as efficient as LLINs for protection against malaria, UTNs do provide physical protection against mosquitoes if in relatively good condition [8, 23–25].

While many studies focus on evaluating the achieve- ments of the LLIN distributions usually immediately fol- lowing mass campaigns [12, 26–30], this study provides, (1) data on LLIN coverage at a unique time between mass campaigns, and (2) an account of how households adapt when nets are not freely distributed, including the acqui- sition of UTNs. Using the MERG indicators, LLIN and UTN ownership, access and use was assessed to investi- gate the net landscape of Tanzania 2 years since the last mass campaign with particular emphasis on how the population responds to loss of free LLINs and whether this is affected by socio-economic status. The National Malaria Control Programme (NMCP) could use these data to predict current LLIN coverage following the URC in 2015–2017 to better assess target areas and populations for continuous net distribution strategies.

4.3. Methods

Study sites and population sampling

The study was conducted in eight districts in Tanzania (Fig. 4.1) between October and December 2013, during the baseline survey of a long-term LLIN durability study [31]. The eight districts were selected from 23 districts enrolled in the Sentinel Panel of Districts (SPD) for the Sample Vital registration with Verbal Autopsy (SAVVY) project [32], a demographic surveillance platform based at the Ifakara Health Institute (IHI).

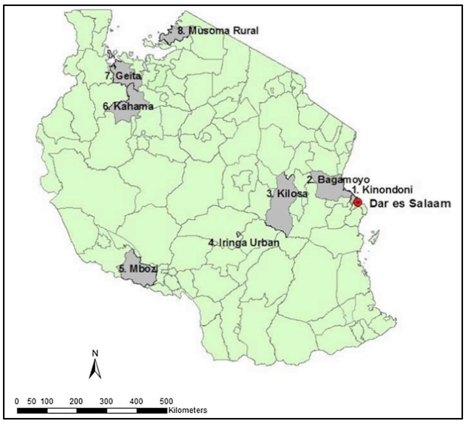

Figure 4.1 Geographical distribution of the eight districts in Tanzania sampled for this study.

The eight districts sampled in this study were: (1) Kinondoni, (2) Bagamoyo, (3) Kilosa, (4) Iringa Urban, (5) Mbozi, (6) Kahama, (7) Geita, and (8) Musoma Rural

The eight districts were selected to represent six of the eight geographical zones of Tanzania with varying malaria prevalence across study sites, excluding the Southern Zone (ongoing SNP) and the Northern Zone (low malaria prevalence at the time) [33]. This study was conducted leading into the short rainy season when transmission is usually lowest. Of the eight districts, two (Kinondoni and Iringa) were urban while the other six were rural. Ten villages in each district were selected for inclusion except for Kinondoni district where only six villages were available. In each selected village, 45 households were randomly selected from the SAVVY database, giving a total of 3420 house- holds. The sample size calculation was for the overall long-term LLIN durability study outcomes [31].

Data collection

A cross-sectional household survey was conducted. The household questionnaire was programmed using Open Data Kit (ODK) [34] and administered using Google Nexus tablet computers. The questionnaire included a household member roster and questions about the mosquito net(s) owned and whether the net(s) had been used the previous night. The number of sleepers under each net the previous night was recorded. Each mosquito net identified in the household was assigned a unique barcode. All participating households were provided with new LLINs to cover all sleeping spaces as part of their enrolment into the net durability study [31]. All mosquito nets present in these households were collected and returned to the IHI laboratories in Bagamoyo where they were sorted by colour, size, product label and manufacturing date (creating a “net database”). The insecticide treatment status of each net was identified using its attached product label and cat- egorized as either LLIN, UTN or unknown (if label was missing). The net database was linked to the question- naire data using the unique barcode assigned to each mosquito net collected.

Data analysis

Mosquito net indicators

This study used the MERG indicators to report the sta- tus of Tanzania’s mosquito net coverage in 2013 (Table 4.1) [13]. Household net ownership, which is defined as the percentage of households owning at least one net, one LLIN or UTN, was determined.

Table 4.1 Descriptions of mosquito net indicators used

Mosquito net indicator	Indicator description
Household ownership	Percentage of households owning at least one net, one LLIN, or one untreated net
Household with enough nets	Percentage of households with at least one net, one LLIN, or one untreated net, for every two people
Population access	Percentage of the population with access to any net, LLIN, or untreated net within their household, assuming each net is used by two people
Population net use	Percentage of the population that used any net, any LLIN, or any untreated net the previous night
Net use:access ratio	Percentage of the population that used a net the previous night divided by the percentage of the population that had access to a net
Net use gap	The proportion of the population who had access to a net within their household, assuming each net is used by two people, but did not sleep under one (1-use:access ratio)

The percentage of households with at least one net for every two people in its household (“households with enough nets”) was also determined for LLINs, any net and UTNs. “Population access”, i.e. the percentage of the population with potential to be protected by a net within their household, assuming a net can be used by two people was deter- mined for LLINs, any net and UTNs (values were corrected to a maximum value = 1 to ensure the value for potential users does not exceed the number of actual household members [A. Kilian pers. comm.]). Population access was calculated using the following equation:

$$Population\ Access = \frac{\text{Number of nets present in household } * \ 2}{\text{Number of people who slept in the household the previous night}}$$

The proportion of the population that reported to have used a net, an LLIN or UTN, the previous night was calculated.

The use:access ratio was calculated by dividing the percentage of the population that reportedly used a net the previous night by the percentage of the population that had access to a net. The mean number of sleepers per net was calculated by multiplying the use:access ratio by two, assuming each net should be used by two people. The net use gap ("1-use:access ratio" [28]), i.e. the proportion of the population who had access to a net within their household, assuming each net is used by two people, but did not sleep under one, was also determined. The net use gap indicates whether people made a choice not to sleep under a net despite having access or whether they were without access to nets in their households [28].

Socio-economic status

The socio-economic status (SES) of each participating household was calculated by creating a wealth index based on measures such as the materials used to construct the house, household amenities and assets owned [35]. Questions to measure assets were adapted from the WHO sample questionnaire for monitoring LLIN durability under operational conditions [36] to fit the current local context. Using principal component analysis (PCA) [37], a weighted score was calculated for each household and the whole population divided into five quintiles, following the methods described by the Demographic Health Survey Comparative Report No. 6 [38].

Statistical analysis

Data analysis was carried out using statistical software package STATA 13.1 (StataCorp LP, College Station, TX). Using the survey suite of commands to account for the clustered sampling design, a single-stage sampling scheme designated the variable 'village' as the primary sampling unit. This was done to account for the highest level of clustering (village) to give the correct standard errors even if the lower levels of clustering (household) were not explicitly modelled [39]. Statistical analysis focused on the effect of socio-economic status on the variation between access to and use of any net (treated and untreated) and LLINs. Logistic regressions were performed to analyse the effect of SES on the following dependent variables: (1) ownership of at least one net (any type), (2) ownership of at least one LLIN, (3) own- ership of at least one UTN, (4) households with enough nets (any type), (5) households with enough LLINs, (6) households with enough UTNs, (7) population access to any net within the household, (8) population access to an LLIN within the household, (9) population access to an UTN within the household, (10) population net use the previous night, (11) population LLIN use the previous night, (12) population use of UTNs the previous night, (13) any net use:access ratio, (14) LLIN use:access ratio, and (15) UTN use:access ratio, adjusting for district variation (Table 4.2).

Variations between net use and access among different districts was assessed for LLINs only. This is because the WHO specifically recommends universal coverage with LLINs [2].

4.4. Results

A total of 6529 nets were collected from 3398 households from 76 villages across eight districts in Tanzania [40]. Seventy-seven percent of nets were LLINs, 16% UTNs, and 7% had no labels attached (Fig. 4.2). The predominant net product was Olyset (74.2%). Other LLIN products included PermaNet (1.5%) and BASF (0.9%).

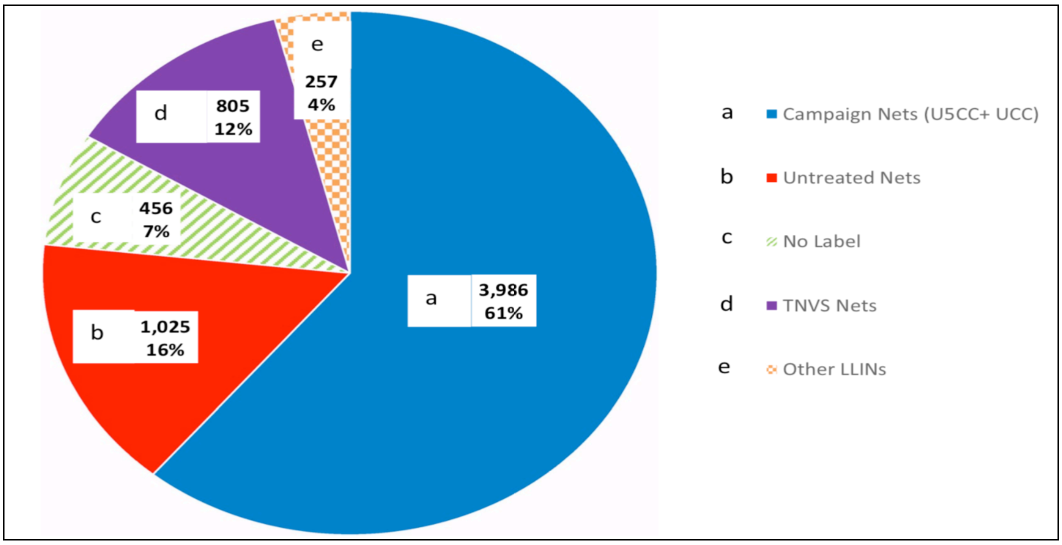

Figure 4.2 Assessment of 6529 nets collected from households.

a Campaign Nets: Under-Five Catch-Up Campaign (U5CC) and Universal Coverage Campaign (UCC); **b** untreated nets; **c** no label; **d** Tanzania National Voucher Scheme (TNVS); and **e** other LLINs

Untreated net products included Safinet (13.5%), SupaNet (1.5%) and Health Net Ltd (0.5%). Seventy-three percent of all nets collected were identified by their colour to have come from a government distribution mechanism (TNVS, U5CC or UCC) (Fig. 2). Of the 3986 campaign nets identified, only 1063 could be distinguished by manufacturing date (U5CC: 135, UCC: 928), the rest had lost their manufacturing label. Of the 6529 nets collected, 85% were single size (3 × 6 feet) while 15% were double size (4 × 6 feet) in dimensions. Eighty-five percent of the single size nets were LLINs. Fifty-one percent of the double-sized nets were UTNs, 35% were LLINs and 14% unknown. Ninety-seven percent of nets were square in shape while 3.3% were conical-shaped. Seventy-one percent of the conical-shaped nets were UTNs. Most of the households in Kinondoni and Iringa (urban districts) ranked among the wealthiest SES quintile while none ranked among the poorest quintile (Table 4.2).

Table 4.2 Number (%) of households by socio-economic quintiles (SES) in the eight districts in Tanzania, 2013

District	Socio-economic quintiles (SES)					Total
	Poorest	Second poorest	Medium	Wealthier	Wealthiest	
Bagamoyo (R)	66 (15.0)	77 (17.5)	114 (26.0)	126 (28.8)	55 (12.6)	438 (100)
Kinondoni (U)	0 (0.0)	0 (0.0)	2 (0.7)	25 (9.3)	242 (90.0)	269 (100)
Kilosa (R)	124 (27.6)	80 (17.8)	85 (18.9)	118 (26.3)	42 (9.4)	449 (100)
Iringa (U)	0 (0.0)	4 (0.9)	24 (5.4)	144 (32.1)	277 (61.7)	449 (100)
Mbozi (R)	49 (10.9)	125 (27.8)	162 (36.1)	95 (21.2)	18 (4.0)	449 (100)
Kahama (R)	164 (36.6)	113 (25.2)	70 (15.6)	64 (14.3)	37 (8.3)	448 (100)
Geita (R)	131 (29.2)	131 (29.2)	120 (26.7)	65 (14.5)	2 (0.5)	449 (100)
Musoma (R)	146 (32.7)	150 (33.6)	102 (22.8)	43 (9.6)	6 (1.3)	447 (100)
Total	680 (20.0)	680 (20.0)	679 (20.0)	680 (20.0)	679 (20.0)	3398 (100)

R rural, *U* urban

Household ownership of at least one government-distributed LLIN (TNVS, U5CC or UCC) was almost twice as high among the poorest quintile at 90.0% [95% CI 86.2–92.8%] compared to the wealthiest quintile at 47.3% [95% CI 42.1–52.6%]. Thirty-five percent of households owned both an LLIN and a UTN.

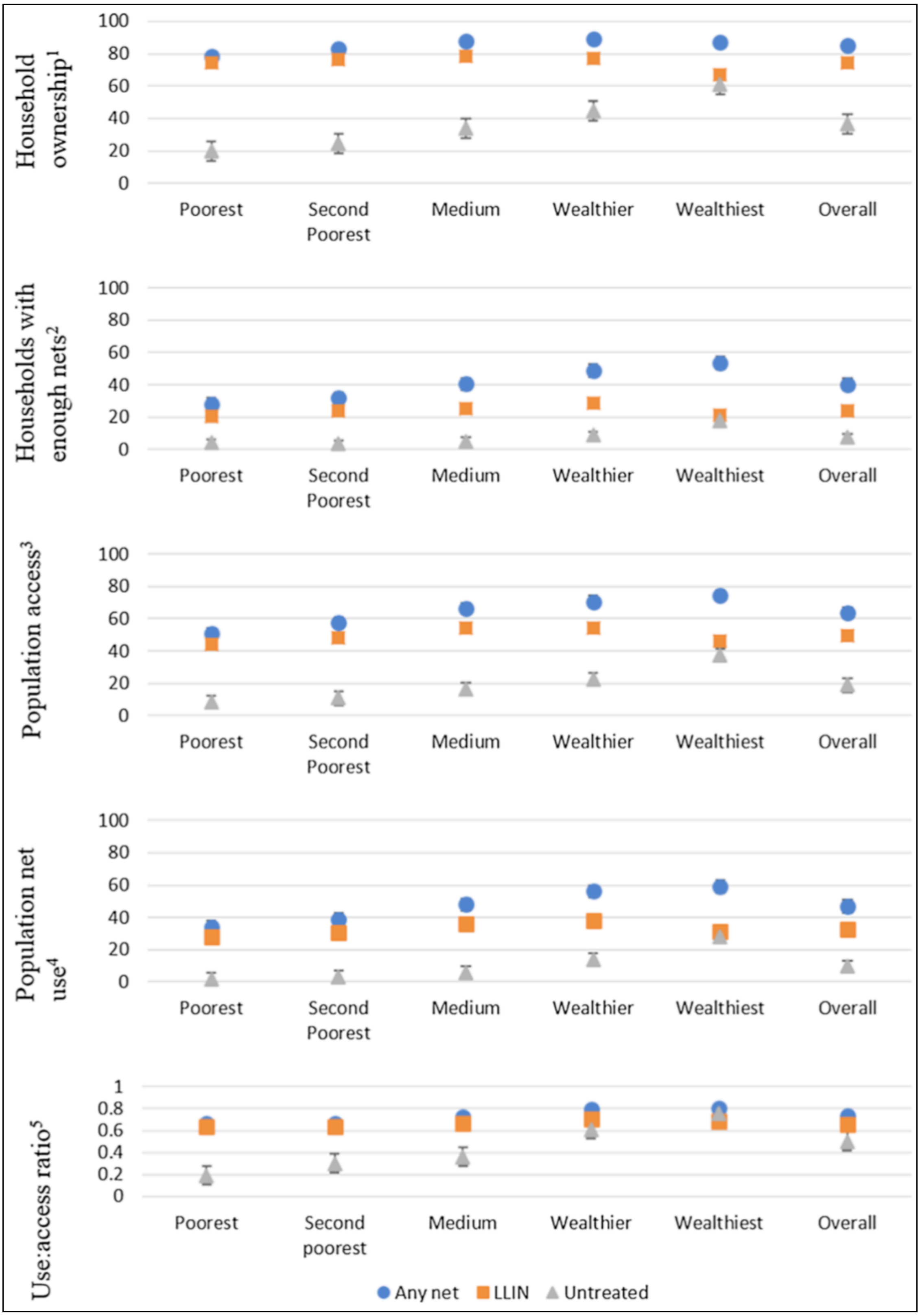

Figure 4.3 Ownership, access, and use of any nets, LLINs and UTNs by socio-economic quintile.

The mean percentage household ownership, access and use of any nets, LLINs and UTNs by socio-economic quintile in Tanzania, October–December 2013 (also see Additional file 4.1 for tabulated data). Error bars represent 95% confidence intervals. Definitions of mosquito net indicators used are listed in Table 4.1

Net ownership

Overall, 85.0% [95% CI 82.3–87.4%] of households owned at least one net (any type) while 74.5% [95% CI 71.0–77.7%] and 36.7% [95% CI 32.6–41.0%] of house- holds owned at least one LLIN and at least one UTN, respectively (Fig. 4.3). The wealthiest quintiles had the highest percentage of household net ownership at 89.3% [95% CI 85.3–92.3%] but the lowest percentage of households owning at least one LLIN at 66.6% [95% CI 59.2–73.2%] (Fig. 4.3). The poorest quintile had the lowest household ownership of any net at 78.1% [95% CI 70.8– 84.0%] while the middle quintile had the highest LLIN ownership at 78.6% [95% CI 72.8–83.5%] (Fig. 4.3). Own- ership of UTNs increased with the increase of wealth quintile.

Socio-economic status was significantly positively associated with ownership of any net (Table 4.3). For those in the wealthiest quintile, the odds of owning a net was 2.62 times the odds of owning any net for those in the lowest quintile. There was no statistically significant association between SES and LLIN owner- ship. However, the odds of the middle quintile to own an LLIN was 1.47 times the odds of owning an LLIN for those in the lowest quintile. Socio-economic status was significantly positively associated with ownership of UTNs. The odds of the wealthiest quintile to own a UTN was 6 times the odds of owning an UTN for those in the lowest quintile (Table 4.3).

Households with one net for every two people

Overall, the percentage of households with enough LLINs to cover every two of its household members was low (Fig. 4.3). Only in the wealthiest quintile did more than half of the households have enough nets (any type) for everyone in the household at 53.3% [95% CI 48.7–57.9%]. The percentage of households with at least one LLIN for every two people was below 30% across all socio-eco- nomic quintiles. The odds of the wealthiest quintile to have households with enough nets of any type was 2.47 times the odds for those in the lowest quintile, but there was no statistically significant effect of SES on house- hold access to LLINs (Table 4.3). There was a significantly positive association between SES and households with enough UTNs (Table 4.3).

Table 4.3 The effect of SES on mosquito net indicators for any net, LLINs and untreated nets

Mosquito net indicator	Variable	SES	Unadjusted odds ratio (95% CI)	P value	Adjusted odds ratio[a] (95% CI)	P value
Household ownership	Any net	Poorest	1	0.013	1	0.005
		Second Poorest	1.38		1.53	
		Medium	2.01		2.33	
		Wealthier	2.33		2.61	
		Wealthiest	1.86		2.62	
	LLIN	Poorest	1	0.070	1	0.053
		Second Poorest	1.13		1.25	
		Medium	1.29		1.47	
		Wealthier	1.15		1.26	
		Wealthiest	0.7		0.87	
	Untreated net	Poorest	1	0.000	1	0.000
		Second poorest	1.31		1.36	
		Medium	2.08		2.18	
		Wealthier	3.24		3.35	
		Wealthiest	6.19		6.95	
Household with enough nets	Any net	Poorest	1	0.000	1	0.001
		Second poorest	1.2		1.22	
		Medium	1.76		1.67	
		Wealthier	2.46		2.04	
		Wealthiest	2.97		2.47	
	LLIN	Poorest	1	0.039	1	0.121
		Second poorest	1.18		1.21	
		Medium	1.27		1.2	
		Wealthier	1.53		1.29	
		Wealthiest	1.04		0.92	
	Untreated net	Poorest	1	0.000	1	0.002
		Second poorest	0.88		0.81	
		Medium	1.31		1.10	
		Wealthier	2.30		1.78	
		Wealthiest	5.09		3.41	
Population access	Any net	Poorest	1	0.005	1	0.005
		Second poorest	1.40		1.53	
		Medium	2.06		2.31	
		Wealthier	2.58		2.68	
		Wealthiest	1.92		2.43	
	LLIN	Poorest	1	0.039	1	0.021
		Second poorest	1.15		1.24	
		Medium	1.31		1.44	
		Wealthier	1.26		1.25	
		Wealthiest	0.7		0.77	
	Untreated net	Poorest	1	0.000	1	0.000
		Second poorest	1.33		1.35	
		Medium	2.15		2.17	
		Wealthier	3.51		3.40	
		Wealthiest	6.52		6.68	

Table 4.3 (continued)

Mosquito net indicator	Variable	SES	Unadjusted odds ratio (95% CI)	P value	Adjusted odds ratio[a] (95% CI)	P value
Population net use	Any net	Poorest	1	0.000	1	0.000
		Second poorest	1.23		1.3	
		Medium	1.8		1.93	
		Wealthier	2.49		2.52	
		Wealthiest	2.82		2.92	
	LLIN	Poorest	1	0.009	1	0.002
		Second poorest	1.13		1.19	
		Medium	1.44		1.51	
		Wealthier	1.54		1.56	
		Wealthiest	1.18		1.23	
	Untreated net	Poorest	1	0.000	1	0.000
		Second poorest	2.05		2.25	
		Medium	3.82		4.08	
		Wealthier	9.62		8.17	
		Wealthiest	23.47		18.89	
Use:access ratio	Any net	Poorest	1	0.014	1	0.050
		Second poorest	1.04		1.23	
		Medium	1.13		1.28	
		Wealthier	1.62		1.77	
		Wealthiest	1.85		1.7	
	LLIN	Poorest	1	0.771	1	0.899
		Second poorest	0.83		0.94	
		Medium	0.88		0.99	
		Wealthier	1.01		1.11	
		Wealthiest	1.07		0.97	
	Untreated net	Poorest	1	0.721	1	0.321
		Second poorest	1.31		3.47	
		Medium	1.97		1.25	
		Wealthier	2.02		0.69	
		Wealthiest	2.37		0.76	

District variation of LLIN coverage

Overall, households with enough LLINs for every two of its household members were 23.8% [95% CI 21.2–26.7%], the percentage of the population with access to an LLIN within their household was 49.2% [95% CI 46.3–52.0%], and the percentage of the population that used an LLIN the previous night was 38.2% [95% CI 29.9–35.8%] (Fig. 4.4). The overall use:access ratio of LLINs was 0.66 and in turn the LLIN use gap was 0.34. Only three districts, namely Bagamoyo, Kilosa and Musoma had more than 80% of households owning at least one LLIN (Fig. 4.4). Kinondoni district had the low- est percent of household ownership of LLINs at 62.5% [95% CI 40.5–80.3%] while neighbouring Bagamoyo had the highest at 83.3% [95% CI 74.3–89.6%]. Geita had the lowest percentage of households with enough LLINs at 16.0% [95% CI 12.2–20.8%] and low population access at 45.6% [95% CI 40.7–50.5%]. Mbozi and Kahama districts, who have the lowest household ownership of LLINs, had the lowest LLIN use:access ratios of 0.39 and 0.52 respectively while Musoma district had the highest at 0.80 (Fig. 4.4).

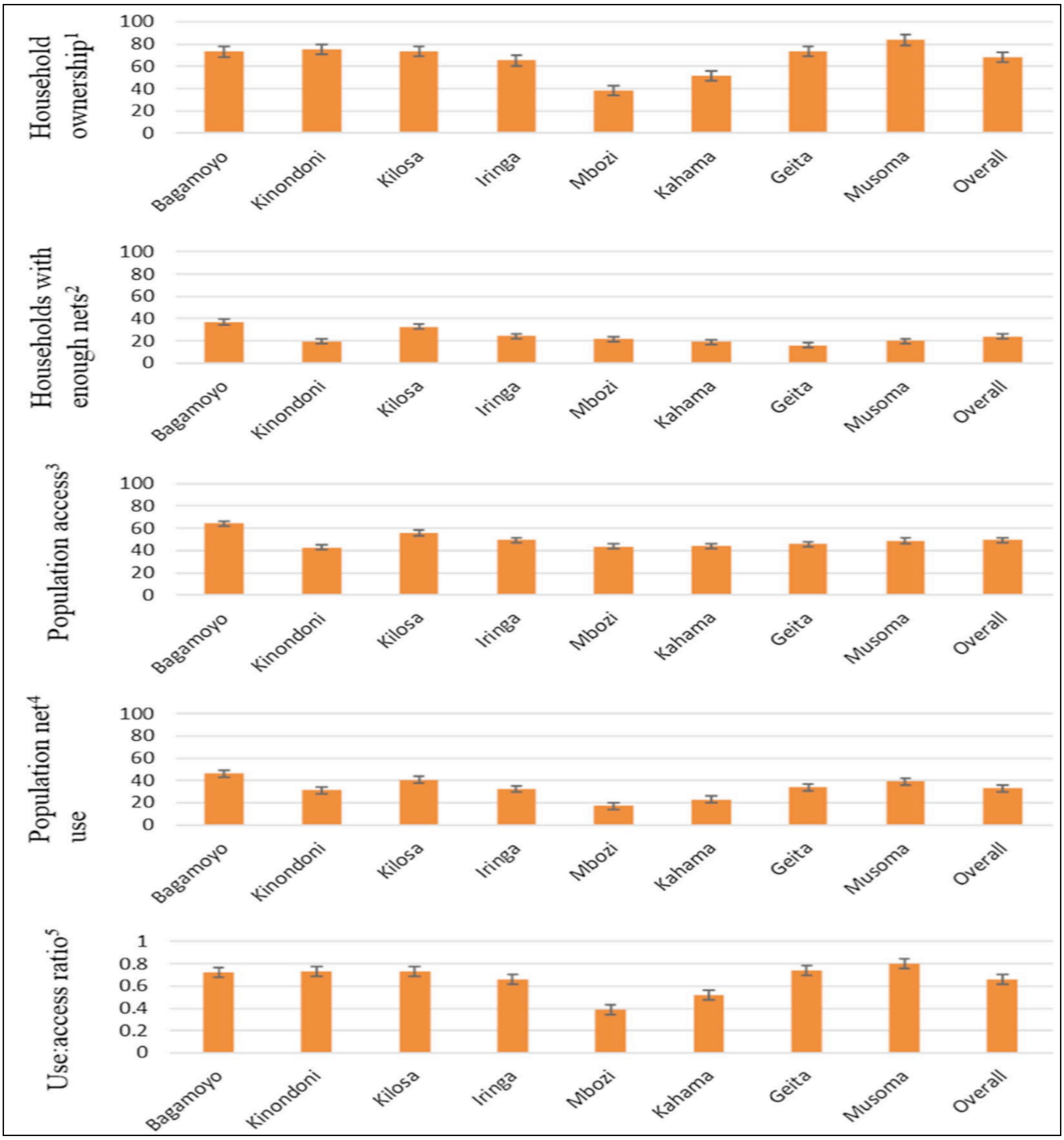

Figure 4.4 Ownership, access, and use of LLINs by district in Tanzania, October–December 2013. The mean percentage household ownership, access and use of LLINs by district in Tanzania, October–December 2013 (also see Additional file 4.2 for tabulated data). Error bars represent 95% confidence intervals. Definitions of mosquito net indicators used are listed in Table 4.1.

4.5. Discussion

Overall, the percentage of households with one LLIN for every two people was below 30%. This finding indicates that 2 years after the mass distribution, many households were without enough nets to cover their population, leading to low population access to LLINs (below 50%). This emphasizes that the URC was long overdue by 2013. Recent national surveys suggest that malaria prevalence in Tanzania may have increased from 9.2% in 2011–2012 to 14.4% in 2015–2016 [33, 41], which could be attributed to poor LLIN indicators although the difference in malaria prevalence could also be attributed to varying transmission intensity between the survey years [42, 43]. The WHO currently recommends mass distribution campaigns to be conducted at 3-year intervals unless there is reliable data to justify longer replacement intervals or as per locally available investments to accommodate population growth and intermittent net loss [2]. This study emphasizes the need for continuous malaria intervention especially during the gap years between mass distributions. Geita district, for example, recorded the highest malaria prevalence (38.4%) in 2015–2016 [41] and lowest percentage of households with enough LLINs (16%) in this study. It is currently profiting from the expansion of SNP to the Western and Lake Zone since late 2016 to maintain high net coverage [44].

Generally, household ownership of any type of net was highest among the wealthiest quintile (89.3%). Sixty percent of the wealthiest households owned at least one UTN, most probably acquired from the commercial mar- ket. This indicates willingness to purchase affordable nets for continued protection against mosquitoes in the absence of free net distributions. A literature review by Koenker and Yukich [45] found that households tend to use the nets available to them irrespective of net characteristics (colour, shape, size or texture), probably because they are restricted to what is distributed or what they have access to. Purchasing their own nets, however, allowed households to exercise choice regarding treatment status, material and size of net. This assessment found that 51% of the double-size nets and 71% of the conical-shaped nets were UTNs.

The inequalities observed across socio-economic quintiles in the acquisition of UTNs was similar to what was observed in Nigeria [46]. The wealthiest households, situated in the urban districts of Kinondoni and Iringa, increased their household access to nets through the commercial markets. Access to a variety of products and affordable prices have been shown to have a significant association with willingness to purchase mosquito nets in Ethiopia [47].

Remotely-located districts are often dis- advantaged by increased costs to cover transport charges [16]. This study found that household ownership of at least one government-distributed LLIN (TNVS, U5CC, UCC), distributed 2–4 years prior to this study, was almost twice as high in the poorest quintile (90%) com- pared to the wealthiest quintile (47%). This indicates that households belonging to the lower socio-economic quin- tiles relied mostly on campaign LLINs and kept them for longer. Hence, there is a need to identify pro-poor methods of targeting net distributions such as the SNP to lower socio-economic quintiles to ensure households have enough nets to cover all members.

It will be important to identify locally and culturally appropriate avenues for behavioural-change campaigns (BCC) to motivate increased purchasing of LLINs while strengthening the local production of LLINs through private–public partnerships [22, 48, 49]. It is also useful to explore factors associated with net retention and how those can be incorporated in the BCC in districts with high net loss. Household net ownership of at least one LLIN in Mbozi district dropped by 28.8% from what was reported by the THMIS 2011–2012, 10 months prior to this study [33].

Population net use of any net type and LLINs was low across all socio-economic quintiles. Any net use was highest among the wealthiest quintile but was still below 60%. Overall, LLIN use:access ratio of 0.66 indicated that not all of the nets collected from households were used [29]. Previous studies have identified reasons for net non- use include lack of access to nets [50, 51] or discomfort, low mosquito density, or sleeping elsewhere [52, 53]. Across districts, the LLIN use:access ratio was lowest in Mbozi at 0.39 (mean number of people per net was 0.7). Mbozi district is in the Southern Highlands, a hypo- endemic zone (with less than 3 months of transmission a year, < 10% malaria prevalence in children 2–9 years old) [54, 55]. Thus, people might not see malaria as a public health threat, explaining the low use rate. Further stud- ies need to be conducted to understand the barriers to net use in specific geographical areas, especially follow- ing the informative “Hang Up” campaign by the Tanzania Red Cross Society after the UCC [56].

This study was unable to match net use with user char- acteristics such as age and gender from the household member roster. Therefore, it was not possible to analyse the person-type most and least likely to sleep underneath a net, to understand those most likely to remain uncov- ered that ought to be targeted in future net distribu- tions [57–59]. The uneven distribution of SES quintiles observed after PCA analysis where most of the house- holds in Kinondoni and Iringa (urban districts) ranked among the wealthiest while no household ranked among the poorest (Table 2), is an important limitation of this study. However, statistical analysis controlled for the variation observed between districts. Decision-makers should adjust by district SES-focused interventions and consult with the Tanzania Social Action Fund on the modalities of pro-poor focused interventions [60].

4.6 Conclusions

In 2013, 2 years after the last mass campaign and 2 years before the URC, the percentage of households or popula- tions with access to LLINs, assuming each LLIN is used by two people, was low (< 30 and < 50%, respectively). These findings indicate that the average rate at which households in Tanzania lose their nets is higher than the rate at which they acquire new nets. There is a need for continuous distribution of LLINs, especially during gap years between mass distributions. The NMCP is currently implementing continuous "Keep Up" strategies delivering LLINs free of charge through the expanding SNP, and through routine health care to pregnant women at their first antenatal clinic (ANC) and at an infant's first vac- cination clinic. Household ownership of any type of net was highest among the wealthier quintile (89.3%), who topped up their ownership with UTNs. Efforts to make LLINs available through commercial markets should be promoted, so that those who can buy nets from the mar- ket purchase LLINs rather than UTNs. Targeted BCC is crucial to motivate net use among those with access to nets within their households. Further investigation is rec- ommended to understand barriers to net use and what can be done to ensure year-round net use.

Additional files

Additional file 4.1. Tabulated data representing household ownership, access and use of any nets, LLINs and UTNs by socio-economic quintile in Tanzania, October–December 2013 also presented in Fig. 4.3. Definitions of mosquito net indicators are listed in Table 4.1. (https://static-content.springer.com/esm/art%3A10.1186%2Fs12936-018-2247-z/MediaObjects/12936_2018_2247_MOESM1_ESM.pdf)

Additional file 4.2. Tabulated data representing household ownership, access and use of LLINs by district in Tanzania, October–December 2013 also presented in Fig. 4.4. Definitions of mosquito net indicators are listed in Table 4.1. (https://static-content.springer.com/esm/art%3A10.1186%2Fs12936-018-2247-z/MediaObjects/12936_2018_2247_MOESM2_ESM.pdf)

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets analysed in this current study are available in part in the addi- tional information files but also from the corresponding author on reasonable request.

Consent for publication

Nick Brown has reviewed and approved the publication of this manuscript.

Ethics approval and consent to participate

Ethical approval was obtained from the Ifakara Health Institute (Ref: IHI/IRB/ No: 19-2013), the National Institute of Medical Research, Tanzania (Ref: NIMR/ HQ/R.8a/Vol I/285) and the London School of Hygiene & Tropical Medicine (Ref: 6333). The household questionnaire was administered upon written informed consent by interviewees above 18 years of age. Initials were used in the household member roster to ensure anonymity. All participating house- holds were given new LLINs in replacement of all nets collected from the household or to cover every sleeping space. This manuscript is published with the permission of the Director-General of the National Institute of Medical Research (NIMR), Tanzania.

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5. Comparing the new Ifakara Ambient Chamber Test (I-ACT) with cone and tunnel assays for bioefficacy and non-inferiority testing of Long Lasting Insecticidal nets (LLINs)

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5.1. Abstract

Background: Insecticide-treated net (ITN) durability, measured through physical integrity and bioefficacy, must be accurately assessed in order to plan the timely replacement of worn out nets and guide procurement of longer- lasting, cost-effective nets. World Health Organization (WHO) guidance advises that new intervention class ITNs be assessed 3 years after distribution, in experimental huts. In order to obtain information on whole-net efficacy cost- effectively and with adequate replication, a new bioassay, the Ifakara Ambient Chamber Test (I-ACT), a semi-field whole net assay baited with human host, was compared to established WHO durability testing methods.

Methods. Two experiments were conducted using pyrethroid-susceptible female adult Anopheles gambiae sensu stricto comparing bioefficacy of Olyset , PermaNet 2.0 and NetProtect evaluated by I-ACT and WHO cone and tunnel tests. In total, 432 nets (144/brand) were evaluated using I-ACT and cone test. Olyset nets (132/144) that did not meet the WHO cone test threshold criteria (≥ 80% mortality or ≥ 95% knockdown) were evaluated using tunnel tests with threshold criteria of ≥ 80% mortality or ≥ 90% feeding inhibition for WHO tunnel and I-ACT. Pass rate of nets tested by WHO combined standard WHO bioassays (cone/tunnel tests) was compared to pass in I-ACT only by net brand and time after distribution.

Results. Overall, more nets passed WHO threshold criteria when tested with I-ACT than with standard WHO bioassays 92% versus 69%, (OR: 4.1, 95% CI 3.5–4.7, $p < 0.0001$). The proportion of Olyset nets that passed differed if WHO 2005 or WHO 2013 LN testing guidelines were followed: 77% versus 71%, respectively. Based on I-ACT results, PermaNet 2.0 and NetProtect demonstrated superior mortality and non-inferior feeding inhibition to Olyset over 3 years of field use in Tanzania.

Conclusion. Ifakara Ambient Chamber Test may have use for durability studies and non-inferiority testing of new ITN products. It measures composite bioefficacy and physical integrity with both mortality and feeding inhibition end- points, using fewer mosquitoes than standard WHO bioassays (cone and tunnel tests). The I-ACT is a high-throughput assay to evaluate ITN products that work through either contact toxicity or feeding inhibition. I-ACT allows

mosquitoes to interact with a host sleeping underneath a net as encountered in the field, without risk to human participants.

Keywords.

Biological efficacy, WHO cone test, WHO tunnel test, I-ACT, Ifakara Ambient Chamber Test, Long Lasting Insecticidal Nets, durability, Non-inferiority.

5.2. Background

National malaria control programmes (NMCPs) must ensure that all people living in malaria transmission areas are protected through the provision, nightly use and timely replacement of high quality long-lasting insecticidal nets (ITNs) and where appropriate, the additional application of indoor residual spraying (IRS) [1]. While it is assumed that all ITNs that have World Health Organization (WHO) prequalification listing last for 3 years, several ITN products are available that may vary in price as well as performance under local conditions [1–7]. Because ITNs are the primary means of malaria control, their durability, measured through physical integrity and bioefficacy against anopheline mosquitoes, needs to be accurately assessed in order to inform NMCPs of the most cost effective products and the correct interval for net replenishment campaigns [8].

Any ITN product is expected to retain its insecticidal activity (bioefficacy) for a minimum number of 20 standard washes or 3 years of use under field conditions as defined by the WHO [9]. However, the durability (years of functional life) of both existing and new net products under development is a crucial consideration. Despite mass distribution of ITNs, currently fewer than 50% of people living in malaria endemic areas are covered by one of the core malaria interventions: either ITNs or IRS [10]. Maximizing ITN access through the provision of the most long-lasting, and cost-effective products remains a critical concern, particularly as a number of countries have shown an increase in malaria in the past year (2016/2017) as investments in malaria control have plateaued [10].

For products within new intervention classes e.g. dual active ITNs, an Evidence Review Group (ERG) report to the WHO Malaria Policy Advisory Commit- tee (MPAC) recommended specific guidance on the assessment of non-inferiority of products within a class [11]. A non-inferiority trial of an intervention aims to demonstrate that the test product is not worse than the comparator/reference by more than a pre-specified margin [12], known as the non-inferiority margin. For ITNs this margin relates to mortality or feeding inhibition. In recognition of the

importance of ITN durability, the WHO recommended that once sufficient test and active comparator ITNs from large-scale field trials have been collected over 3 years of field use, a second set of two non-inferiority trials should be conducted to ensure that the test product continues to be non-inferior to the
comparator/reference product for up to 3 years on both mosquito mortality and blood-feeding inhibition end- points [13]. While this guidance recommended that non- inferiority trials should be conducted in experimental huts it was acknowledged that alternative methodology for non-inferiority testing including the ambient chamber test or the tunnel test should be explored.

The standard means of ITN bio-efficacy evaluation is through cone bioassays, WHO tunnel tests and experimental hut evaluations [14]. The cone test is a contact assay where mosquitoes are held in proximity to the ITN and mosquito knockdown (KD60) and 24-h mortality are recorded after 60 min and 24 h, respectively. The tunnel test uses a live animal as a bait (rabbit or guinea pig), so mosquitoes are able to exercise host-seeking behaviour, and ITN efficacy is assessed by measuring mosquito mortality and blood feeding inhibition [15–17]. Experimental huts are small scale field (phase II) testing assays used to evaluate ITNs that meet laboratory (phase I) testing criteria [8, 18]. Huts are built in areas with high densities of target mosquito species and are designed to resemble small local housing but have features to retain mosquitoes that enter huts such as window traps and baffles [19]. Volunteers sleep underneath the ITNs and wild mosquitoes attempt to feed and interact with the ITNs in the same way as they would in local homes. Both mortality and feeding inhibition are key outcome parameters, which translate to personal and community protection from malaria [20].

However, all assays have some limitations, which need to be considered when assessing bioefficacy of ITNs. WHO cone tests may underestimate the induced mortality of irritant insecticides, as mosquitoes do not settle on treated nets [21]. Indeed, comparatively higher mortality is often measured in experimental hut studies of ITNs where mosquitoes make repeated contacts with treated nets as they try to feed on human volunteers sleeping under nets. In the WHO tunnel test, the live host used as bait is not the preferred host for the strongly anthropophilic Afro-tropical vector *Anopheles gambiae* sensu stricto (s.s.) [22] and may overestimate feeding inhibition. Alternatively, mosquitoes must be reared by feeding them on small mammals to select them for a preference to these non-preferred hosts, which is both expensive and of animal welfare concern. Experimental hut bioassays are the gold standard for

ITN and IRS evaluation, but wild mosquito populations are often seasonal and have high temporal heterogeneity requiring substantial replication to ensure adequate power to detect true effect differences between products [23].

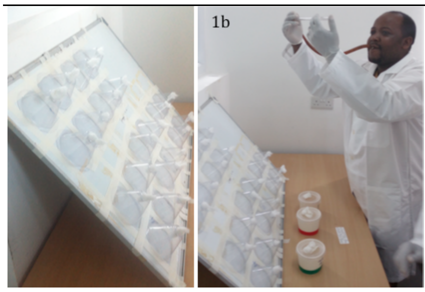
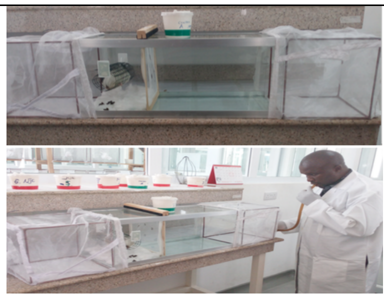
Therefore, presented here is the first evaluation of a new standardized semi-field assay: the Ifakara Ambient Chamber Test (I-ACT) assay. The assay was used to evaluate the bioefficacy of whole ITNs that were returned from the field in a longitudinal durability study. This study measured the bioefficacy of used (field-aged) ITNs using the I-ACT assay and standard WHO durability testing bioassays (cone and tunnel tests). The proportion of nets passing WHO criteria by standard methods and I-ACT was compared. The aim was to demonstrate the utility of this new assay for measuring bioefficacy of different ITN products and to explore its applicability for non-inferiority testing of new ITN products [11]. Further work comparing the I-ACT and experimental hut evaluations of ITNs will be reported separately.

5.3. Methods

Study design

Bioefficacy tests were conducted as part of a 3-year prospective project (the ABCDR-Attrition, Bioefficacy, Chemical residual, Damage and Resistance project) to assess of the useful life of three brands of ITNs in Tanzania (24). The main design characteristics of each bioassay in this study are presented in Table 5.1. The study involved two experiments. In the first experiment, LLINs efficacy measured by cone bioassay and I-ACT were compared. In the second experiment, LLINs bioefficacy measured by WHO tunnel test and the I-ACT was compared. The overall pass/fail rate for each net brand by year were examined following the criteria outlined in both 2005 and the 2013 World Health Organization guidelines for evaluation of long lasting nets [14,25]. The pass rate of each product by standard WHO methods and I-ACT was compared.

Table 5.1. Design characteristics of the WHO cone assay, WHO tunnel assay and I-ACT

Particular	WHO cone assay	WHO tunnel assay	Ifakara Ambient Chamber test (IACT)
Diagram			
Endpoints measured	Knock down mortality (KD 60), 24 hour mortality	12 hour mortality, 24 hour mortality, Feeding inhibition	12 hour mortality, 24 hour mortality, Feeding inhibition
Infrastructure required	Temperature controlled room, boards, aspirators, cones, insect rearing facilities	Temperature controlled room, tunnel, aspirators, insect rearing facilities, animal rearing facilities	Ambient or temperature controlled chambers, temperature controlled holding room, aspirators, insect rearing facilities
Bait Used	No	Rabbit, guinea pig	Human
Cost per net evaluated	$ (i.e. cheaper) (Mosquito rearing, maintenance of facilities)	$$$ (i.e. very expensive than all) (Mosquito rearing, animal rearing permits, veterinary care, maintenance of facilities)	$$ (i.e. expensive than cone assay) (Mosquito rearing, maintenance of facilities, volunteer compensation)
Mosquitoes per net	80	100	15
Exposure time	3 minutes	12-15 hours	12 hours
Holding time	24 hours	None	24 hours
Time to conduct including preparation	25 hours	16 hours	26 hours
Surface area exposed to mosquitoes	$78cm^2$	$625cm^2$	Whole net
Useful for durability monitoring	Measures presence of insecticide	Measures feeding inhibition on a small section of net	Measures the functional efficacy of nets under user conditions
Useful for non-inferiority testing	Not suitable for some products	Works for all nets	Works for all nets

Mosquito rearing

Mosquitoes used during testing were laboratory-reared fully pyrethroid susceptible 3-8 days old unfed female adult *Anopheles gambiae* sensu stricto (Ifakara strain, Njage 1996) reared following standard methods [26].

Mosquito nets

All mosquito nets used in this study came from a three-years prospective longitudinal follow-up study between 2013 and 2016 (ABCDR Project) conducted in eight districts of Tanzania. Net samples were randomly selected from the three surveys conducted between October-December 2014 (Year 1), October-December 2015 (Year 2) and October-December 2016 (Year 3). The detailed description of the ABCDR Project has been reported previously (24). Three net brands were used for this study: 1) Olyset® net (permethrin incorporated into polyethylene fibres @ 1000mg/m2), 2) PermaNet®2.0 net (deltamethrin coated on polyester fibres @ 55mg/m2) and 3) Netprotect® net (deltamethrin incorporated into polyethylene fibres @ 63mg/m2). All nets were rectangular, white, double sized (190cm x 180cm x 150cm) and recommended by WHO (27). All nets were used in the I-ACT as found i.e. with damage due to wear and tear. In the first experiment, to compare between cone test and I-ACT, a total of 432 nets (144 per net brand) were evaluated and results were compared. In the second experiment to compare between tunnel test and I-ACT, nets that failed to meet cone test threshold criteria in the first experiment were assessed using WHO tunnel test and their results were compared with that from I-ACT.

Ifakara Ambient Chamber Test (I-ACT)

This is a 50m long, 3m wide and 2.1m high steel tube frame construction (Figure 5.1a) covered by durable UV resistant polyurethane coated netting with an overlaid polyurethane sheet to minimize wind so that bioassays are conducted in still air (as would occur in a house). The structure is constructed upon a concrete base surrounded by a water channel to prevent entry by ants and spiders that eat mosquitoes during the conduct of experiments. The tunnel sits beneath a simple beamed wooden frame supporting a corrugated steel roof to allow work to continue in all weather conditions. The netted tunnel is divided into ten individual test chambers with interconnecting doors that are sealed by means of zips and Velcro to prevent mosquitoes moving from one test chamber to another. Each compartment contains a white netted chamber 5 m long, 2 m wide, and 2 m high that seals with a zip, in which the ITN is hung from a frame with a human volunteer sleeping underneath (Fig. 5.1b). At each end of the tunnel is an additional double door module to ensure no loss of laboratory-reared mosquitoes into the wild. Mosquitoes are released from the holding cages within each netted chamber by means of raising a netted cage from its removable wooden base.

This is achieved by the technician in situ underneath his net pulling a nylon line attached to the mosquito release cage to elevate it (Fig. 5.1c). After the allotted experimental time period all mosquitoes within each of the compartments are recovered by mouth aspiration (for mosquitoes inside the net) and by a battery powered Prokopack aspirator (for mosquitoes outside the net but inside the compartment). This allows whole ITNs to be tested in a controlled ambient chamber test with a human host sleeping beneath (Fig. 5.1d) to measure the protective efficacy (both personal protection measured by feeding inhibition and community protection measured by mosquito mortality) under user conditions. The design of the chambers allows 100% recovery of released mosquitoes that improves precision of the data, and experiments can be conducted year-round.

Each of the ten testing chambers was randomly assigned one whole net (with wear and tear as found after use for 1, 2 or 3 years) from one of the three net brands using a random number generator. Two chambers were used each night as negative control with untreated SAFI Net (A to Z, Tanzania) holed with six holes 4×4 cm (Additional file SOM 5.1) i.e. two holes on each large side and one hole on each small side (hole surface area of 96 cm2) according to WHO guidance [9]. One adult volunteer per chamber slept underneath the nets from 21:00 to 06:30 h and collected mosquitoes in the mornings. Each volunteer was fixed to the same chamber for the duration of the experiment. On each night of experiment, each volunteer hung the tested net on the bednet frame and tucked it underneath the mattress (between 28 and 35 cm of each net was tucked). At 21:00 h, each volunteer released 30 mosquitoes within the chamber but outside of the ITN by opening the mosquito release cage while remaining beneath their test net. The following morning, at 06:30 h, mosquitoes inside the net were collected first using a mouth aspirator and mosquitoes outside the net but within the chamber (floor and walls) were collected using a 6 V battery driven mechanical aspirator (Prokopack). A study supervisor checked the start and finish of the experiment and intermittently spot checked that the volunteers were in position overnight to ensure good conduct of the experiment. All collected mosquitoes were placed in paper cups and scored as dead-fed, alive-fed, dead-unfed, alive-unfed after which mosquitoes were held for 24 h in the laboratory with access to 10% sugar solution at 27 °C±2 and 80%±10 relative humidity and scored again. After every experimental night, all tested nets were taken out and chambers were aired and bed sheets were washed daily to prevent any carry-over insecticide residue. Each net sample was tested on two consecutive nights (fixed to a chamber and volunteer) to improve the precision of the estimation of performance of each net. Outcome measures were 24 h mortality and blood feeding inhibition. Nets that induced ≥90% blood-feeding inhibition and/or ≥80% mortality were regarded as meeting WHO efficacy criteria. Data were discarded and test repeated if control mortality exceeded 10% or control blood-feeding success was less than 50%.

A Standard Operating Procedure for conducting Ifakara Ambient Chamber Test is provided as an Additional file SOM 5.2.

Figure 5.1. The Ifakara Ambient Chamber Test (I-ACT).

Ifakara Tunnel situated at Bagamoyo Research Training Centre (BRTC) in Kingani, Bagamoyo (A). Net covered tunnel divided into 10 individual compartments each containing netted cage 2 × 2 × 5 meters (B). A human volunteer sleeps beneath the LLIN (C). The volunteer releases mosquitoes by opening the lid of the holding boxes while beneath the tested net (D).

Cone tests

Cone tests were conducted following WHO guidelines [8] to determine the bioefficacy of insecticides on sampled netting fibers. For each of the sampled whole nets, after completion of the I-ACT, four 30cm x 30cm sub-samples were cut as per additional file SOM 5.3. Cone bioassays were held at a 60^{o} vertical angle on the netting sub-samples [28]. *Anopheles gambiae* s.s. (aged 3-5 days old) were exposed for 3 minutes after which they were held for 24 hours with access to 10% sugar solution at 27°C±2. The numbers of mosquitoes knocked down 60 minutes (KD60) and dead 24 hours after the exposure period were recorded. A sub-sampled net that can cause ≥95% KD60 and/or ≥ 80% 24-hour mortality in the cone assay is regarded as meeting WHO efficacy criteria. Tests where control mortality at 24 hours exceeded 10% were discarded and repeated.

WHO Tunnel test

WHO tunnel tests were conducted following WHO guidelines [8] to assess the efficacy of netting sub-samples that failed to meet the WHO threshold criteria for cone assay (95% KD60 and or 80% 24-hour mortality). The surface area of the sample netting accessible to mosquitoes was 625 cm^2 (25 × 25 cm) with nine artificial holes cut, each 1 cm in diameter: one at the centre of the square and the other eight holes were equidistant and located 5 cm from the border. The sampled net piece was inserted on a cardboard frame and positioned across the tunnel, one-third of the length of the tunnel. A total of 100 sugar-starved *An. gambiae* s.s. aged 5-8 days were released in the long section of the glass tunnel at 18:00 hours. A rabbit was used as bait and positioned on the other side of the net so that mosquitoes must pass through the holed net to feed. The following morning, between 0600 and 0900 hrs, mosquitoes were removed (separately from each section of the tunnel) using a mouth aspirator, counted, scored (as alive or dead, blood fed or unfed) after which they were held for 24-hours with access to 10% sugar solution. The main outcome measures were 12-hour immediate mortality (measured in the morning after the experiment) and blood feeding inhibition after this exposure period [14]. After 24-hours holding time, the 24-hour mortality was also recorded as a secondary outcome [25]. Nets that cause ≥90% bloodfeeding inhibition and/or ≥ 80% mortality is regarded as meeting WHO efficacy criteria [25]. Tests were discarded if control mortality exceeded 10% or control blood-feeding success was less than 50%.

Data management and analysis

A sample size calculation for generalized linear mixed effects models (GLMMs) through simulation [12] in R statistical software 3.02 http://www.r-project.org [13] was performed for the semi-field experiments to detect a difference between the nets of 5% mortality (half the smallest anticipated effect size). Simulations were performed using an estimated mosquito mortality of 70% with the Olyset and 80% for the PermaNet 2.0 and NetProtect. With 44 replicates tested on two occasions with an inter-observational variance of 0 for the chamber (controlled environment) and 0.1 for individual and 0.1 for the night of observation based on the variance of the random effects observed in a pilot study. Power was estimated at >90% with a density of 30 mosquitoes per chamber per night using 1000 simulations.

Data were collected on standardized data collection forms and double entered into Microsoft Excel. Data were cleaned and analysed following a predefined analysis plan using STATA 14.1 (Stata Corp., College Station TX, USA) with significance level of ≤0.05 for rejecting the null hypothesis. Descriptive statistics were used to present the comparison of proportion of nets passing WHO threshold criteria as measured by each method. Generalized linear mixed models (GLMM) with a binomial error distribution and logit link function were used to analyse the main outcome measures from cone, tunnel and I-ACT (mortality and blood feeding) as well as the proportion of nets passing WHO criteria in order to detect differences between the two evaluation methods. Net brand, age of net and bioassay method were fitted as fixed effects while date was fitted as a random effect to account for repeated testing of individual nets. Several GLMMs were run for each comparison (with interactions) and the final model selected was that with the lowest Akaike's Information Criterion (AIC). Residuals were plotted using histogram, qnorm plots and comparison with fitted values to ensure appropriateness of the model selection and testing if the residuals are normally distributed. Odds ratio (OR) and 95% confidence interval were calculated for the differences between methods in each comparison.

In addition, non-inferiority between net products (PermaNet® 2.0, Netprotect® with Olyset® as reference/ comparator) measured by I-ACT was analysed using a paired t-test with a 90% confidence interval of the observed effect difference in the mortality and blood- feeding inhibition rates to measure non-inferiority at a margin of 10% and data were presented for comparison using a Forest Plot [29].

Ethics

Participants involved in I-ACT were all IHI staff members who have received appropriate training and being experienced in conducting semi-field tunnel tests. Written informed consent was obtained from all sleeping volunteers. Ethical approvals was obtained from Ifakara Health

Institute in Tanzania (Reference number- IHI/IRB/No: 19-2013) and from the National Institute for Medical Research (Ref: NIMR/HQ/R.8a/Vol. IX/150 and NIMR/HQ/R.8c/Vol. I/285). All persons involved in this project had medical insurance provided by the project in case of any adverse events associated with sleeping under net. The animals used in WHO tunnel assays are cared for with consultation of registered project veterinary doctor.

5.4. Results

Comparison between cone test and I-ACT

The data presented in Table 5.2 show that a smaller percentage of nets passed WHO threshold criteria using cone test 62% (268/432) than passed using by I-ACT 97% (417/432) irrespective of brand and net age (cone test criteria ≥80% 24-h mortality and/or ≥95% KD60; I-ACT criteria ≥ 80% 24-h mortality).

Table 5.2 Percentage and number of nets (by brand and age) meeting the standard 2013 WHO threshold criteria by I-ACT and Cone assays

Age of net	Year 1		Year 2		Year 3		Overall	
	Cone assay	I-ACT	Cone assay	I-ACT	Cone assay	I-ACT	Cone assay	I-ACT
Olyset Net	4% (2/49)	100% (49/49)	8% (4/48)	96% (46/48)	13% (6/48)	86% (42/48)	8% (12/145)	94% (137/145)
PermaNet 2.0	98% (47/48)	100% (48/48)	92% (44/48)	98% (47/48)	73% (35/48)	96% (46/48)	88% (126/144)	98% (141/144)
Netprotect	100% (47/47)	100% (47/47)	100% (48/48)	100% (48/48)	73% (35/48)	92% (44/48)	91% (130/143)	97% (139/143)
Overall	67% (96/144)	100% (144/144)	67% (96/144)	98% (141/144)	53% (76/144)	92% (132/144)	62% (268/432)	97% (417/432)

Using cone bioassays, overall 62% (268/432) of nets met the WHO threshold criteria: Eight percent (12/145) of Olyset® nets, 88% (126/144) of PermaNet® 2.0 nets and 91% (130/143) of NetProtect® nets passed cone test. When tested by the I-ACT, 97% (417/432) of nets passed threshold criteria: 94% (125/143) of Olyset® nets, 98% (141/144) of PermaNet® 2.0 nets and 97% (139/143) of Netprotect® nets (Table 5.2).

Table 5.3 shows that, overall, I-ACT measured higher 24 h mosquito mortality than cone test regardless of net brand (OR: 7.9, 95% CI 7.4–8.4, $p < 0.0001$). Disaggregated by brand the same trend was evident and I-ACT measured higher mortality than cone test: Olyset® nets (OR: 17.8, 95% CI 16.3–19.5%; p < 0.0001), PermaNet® 2.0 (OR: 2.1, 95% CI 1.8–2.3%; p<0.0001) and Netprotect® (OR: 3.6, 95% CI 3.2–4.1, p < 0.0001).

Table 5.3. Measurements of Percentage 24-hour mortality compared between WHO Cone assay and I-ACT by net brand and age

	24h Geometric mean Mortality (95% Confidence interval)				Odds Ratio	95% Confidence Interval	P-value
	Year 1	Year 2	Year 3	Overall			
Olyset®							
Cone assay	19.4 (17.9-20.9)	7.2 (6.2-8.2)	34.1 (32.1-36.2)	20.2 (19.2-21.2)	1		
I-ACT	87.2 (84.1-90.3)	68.9 (64.5-73.4)	69.8 (65.4-74.3)	75.1 (72.5-77.6)	17.8	16.3-19.5	<0.001
PermaNet® 2.0							
Cone assay	93.5 (92.4-94.7)	85.3 (83.3-87.2)	83.2 (81.4-85.1)	87.4 (86.4-88.3)	1		
I-ACT	98.4 (97.7-99.1)	91.5 (88.8-94.2)	87.8 (84.2 - 91.5)	92.5 (90.9-94.1)	2.1	1.8-2.3	<0.0001
Netprotect®							
Cone assay	93.1 (92.1-94.2)	81.1 (78.9-83.3)	82.2 (80.1-83.7)	85.4 (84.4 - 86.4)	1		
I-ACT	99.1 (98.6-99.6)	96 (94.4-97.6)	89.1 (85.9-92.1)	94.6 (93.4 - 95.9)	3.6	3.2-4.1	<0.0001
Overall							
Cone assay	68.2 (66.6-69.8)	58 (56.2-59.8)	66.5 (65.1-67.9)	64.2 (63.3-65.2)	1		
I-ACT	94.9 (93.7-96.2)	85.5 (83.2-87.7)	82.2 (79.8-84.5)	87.4 (86.2-88.6)	7.9	7.4-8.4	<0.0001

Comparison between WHO Tunnel tests and I-ACT

A total of 164 nets (132 Olyset® nets, 19 PermaNet® 2.0 net and 13 Netprotect® nets) did not meet the WHO threshold criteria for cone assay and therefore underwent additional WHO tunnel tests. Only bio-efficacy results of Olyset® sampled nets were used for comparison with I-ACT to ensure an adequately replicated and paired comparison, because the majority of PermaNet® 2.0 and Netprotect® sub-sampled nets passed cone tests.

The overall proportion of Olyset nets meeting WHO thresholds in tunnel test using methods outlined in WHO 2013 guidance (mortality recorded the morning after bioassay) and I-ACT is shown in Table 5.4. In addition, further analysis based on WHO 2005 guidance (mortality recorded after 24 h holding) was also performed and included in the results for comparison. Overall, results from Table 5.4 shows, regardless of the WHO

ITN testing guideline used, Olyset® nets passed when measured in I-ACT than in tunnel test (using 24-h mortality OR: 5.7, 95% CI 2.5–, p < 0.0001).

Table 5.4. Overall (No. of nets passing/No. Tested) sampled Olyset® nets that met the Standard WHO threshold criteria as measured by I-ACT and tunnel bioassays following both 2013 and 2005 WHOPES guidelines

	2013 WHOPES Guideline		2005 WHOPES Guideline	
	Tunnel assay	I-ACT	Tunnel assay	I-ACT
Year 1	62% (29/47)	100% (47/47)	72% (34/47)	100% (47/47)
Year 2	75% (33/44)	95% (42/44)	77% (34/44)	95% (42/44)
Year 3	71% (29/41)	85% (35/41)	76% (31/41)	85% (35/41)
Overall	69% (91/132)	94% (124/132)	75% (99/132)	94% (124/132)

Using 12 h mortality or 24 h mortality, I-ACT recorded higher mortality than the tunnel test (Table 5.5). At 12 h, 64.1% (95% CI: 60.11-68.31%) vs 49.5%, (95% CI: 44.5-54.6%) (OR 1.7 (1.6–1.8), p<0.0001) at 12 h and 71.2%, (95% CI: 67.71-74.89%) vs 64.4%, (95% CI: 59.8-69.4%) (OR 1.3 (1.2–1.4), p<0.0001) at 24h. For Olyset nets mortality was significantly higher measured after a 24-h holding period compared to the morning of collection in WHO tunnel test but not I-ACT (Fig. 5.2).

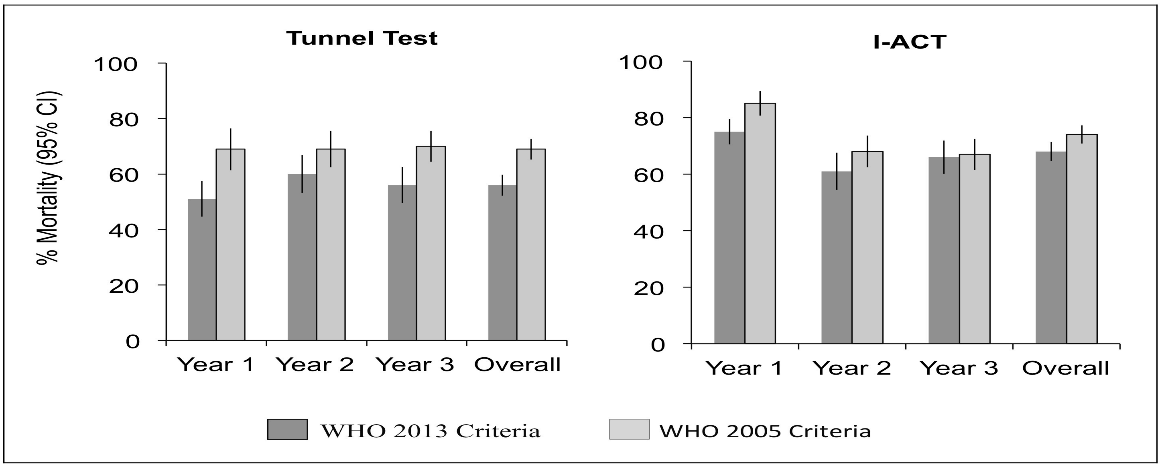


Figure 5.2. Mortality in susceptible Anopheles gambiae s.s. exposed to Olyset® nets by year using the WHO tunnel bioassay (left panel) and the I-ACT (right panel) following WHOPES 2013 [130] and 2005 [53] guidelines for durability monitoring. Error bars indicate 95% Confidence Interval

Table 5.5. Measurement of 12-hour mortality, 24-hour mortality and blood feeding inhibition compared between WHO Tunnel test and I-ACT for sampled Olyset® nets through 3 years of field use.

		Geometric mean (95% Confidence interval)				Odds Ratio	95% Conf. Interval	P-value
		Year 1	Year 2	Year 3	Overall			
Olyset	**% 12-hours mortality**							
	Tunnel test	44.2 (36.5-53.5)	55.1 (47.8-63.5)	50.3 (42.2-60.1)	49.5 (44.9-54.6)	1		
	I-ACT	73.2 (68.3-78.5)	56.1 (48.65-64.74)	63.4 (57.11-70.34)	64.1 (60.11-68.31)	1.7	1.6-1.8	<0.0001
	% 24-hours mortality							
	Tunnel test	61.4 (52.2-72.3)	64.7 (57.2-73.1)	67.7 (61.9-74)	64.4 (59.8-69.4)	1		
	I-ACT	83.8 (78.87-89.12)	65.20 (59.56-71.37)	64.9 (59.15-71.22)	71.2 (67.71-74.89)	1.3	1.2-1.4	<0.0001
	% Blood feeding inhibition							
	Tunnel test	85.3 (80-90.8)	91.9 (89.2-94.7)	90 (84.8-95.6)	88.9 (86.2-91.7)	1		
	I-ACT	99.6 (99.3-100)	98 (95.6-99.9)	91.3 (87.2-95.8)	96.4 (94.73-98.05)	3.6	3.1-4.2	<0.0001

Feeding inhibition (Fig 5.3) of Olyset® nets was also higher as measured by I-ACT (96.4%, 95% CI: 94.7-98.1%) than tunnel test (88.9%, 95% CI: 86.2-91.7%). Similar trends were seen among the deltamethrin nets PermaNet 2.0 and NetProtect but data are not shown due to the imprecision of estimates from the low number of nets evaluated (19 and 13, respectively).

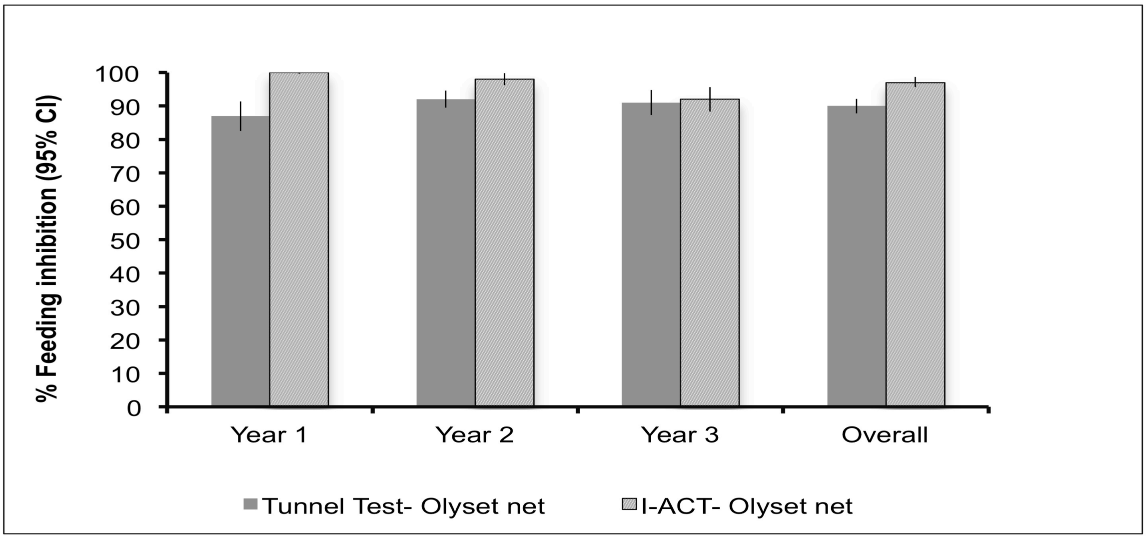


Figure 5.3. Mosquito blood feeding inhibition by year in susceptible An. gambiae s.s. exposed to Olyset® nets using the WHO tunnel bioassay and I-ACT. Error bars indicate 95% Confidence Interval

Proportion of nets meeting the combined WHO methods

Figure 5.4 shows that even after 3 years of field use PermaNet 2.0 and NetProtect killed a greater proportion of mosquitoes than Olyset resulting in a higher pass rate by WHO (combined cone/tunnel) methods, while there was less contract in the performance of the three products overall when tested using I-ACT. The data (Table 5.6) show that overall more nets passed WHO threshold criteria using I-ACT than using standard WHO (combined cone/tunnel) methods irrespective of brand and age (OR: 3.5, 95% CI 1.9–6.5, $p < 0.0001$). The proportion of nets passing using combined WHO methods agreed with I-ACT for NetProtect® and PermaNet 2.0® but different for Olyset® with 94% passing in I-ACT vs 77% by standard bioassays (OR 5.2, 95% CI 2.3–11.8, $p < 0.0001$).

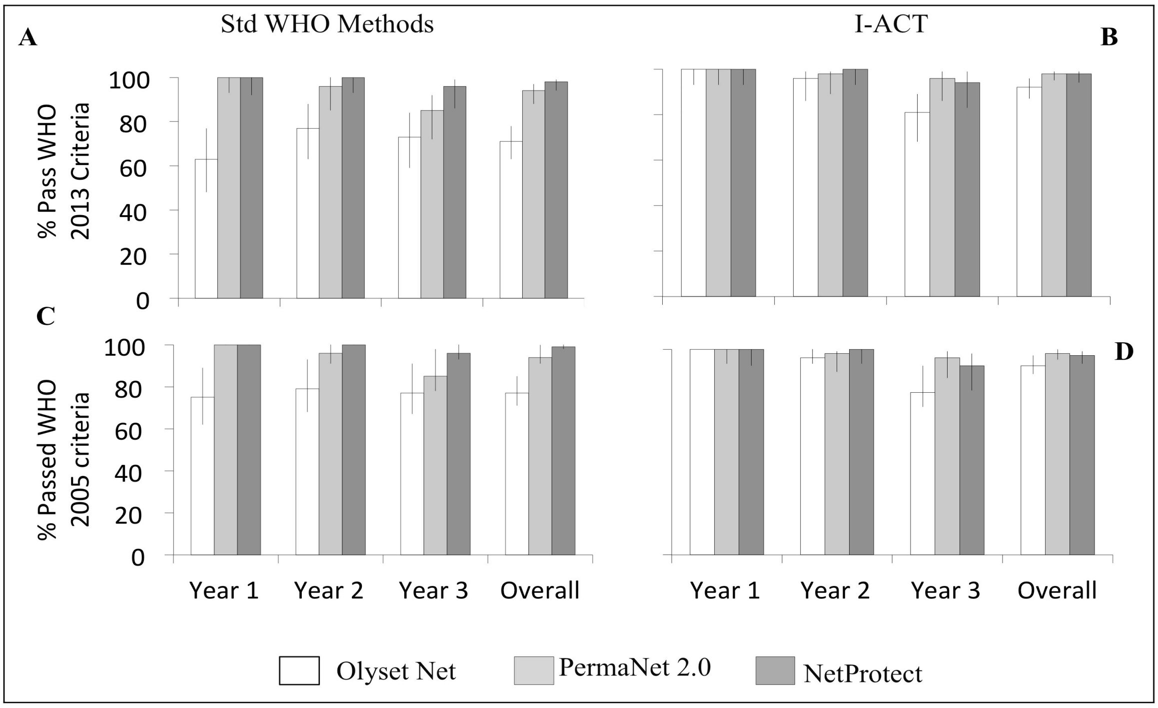


Figure 5.4 Percentage of ITNs by brand and age passing bioassay criteria following WHO 2013 and 2005 guidelines as measured by standard bioassays (**a**, **c**) vs I-ACT (**b**, **d**) against An. gambiae s.s. (Ifakara strain) fully susceptible to all classes of insecticides

Table 5.6. Difference in the proportion of nets passing WHO 2005 threshold criteria by combined WHO cone and Tunnel test methods compared to I-ACT for sampled Olyset®, PermaNet® 2.0 and NetProtect® nets. Odds ratios are calculated using pass / fail with 24hour holding times for all tests.

	No. of Nets tested	% Pass WHO 2013 criteria (n)	% Pass WHO 2005 criteria (n)	Odds Ratio	95% Confidence Interval	p-value
Olyset®						
WHO methods	145	71% (n=103)	77% (n=111)	1		
I-ACT	145	94% (n=134)	94% (n=137)	5.2	2.3-11.8	<0.0001
PermaNet® 2.0						
WHO methods	144	94% (n=135)	94% (n=135)	1		
I-ACT	144	98% (n=141)	98% (n=141)	3.1	0.8-11.8	0.092
NetProtect®						
WHO methods	143	99% (n=141)	99% (n=141)	1		
I-ACT	143	98% (n=140)	97% (n=139)	0.49	0.1-2.7	0.418
Overall						
WHO methods	432	69% (n=379)	90% (n=387)	1		
I-ACT	432	92% (n=415)	97% [(n=417)	3.5	1.9-6.5	<0.0001

Odds ratios are calculated using pass/fail with 24 h holding times for all tests
WHOPES 2005 pass/fail criteria: tunnel test: feeding inhibition and/or ≥ 80% 24 h mortality
I-ACT: ≥ 80% 24 h mortality and/or ≥ 90% blood feeding inhibition

A second notable difference was that holding time was important in determining the pass rate of Olyset with a significant increase in the proportion of Olyset that passed when 24 h mortality vs immediate mortality scoring was used: 77% (95% CI 69–83%) vs 71% (95%
CI 62–78%) pass. A small and not significant difference was observed in the proportion of PermaNet ® 2.0 (94% vs 98%) and NetProtect (98% vs 97%) passing by either WHO methods or I-ACT methods respectively.

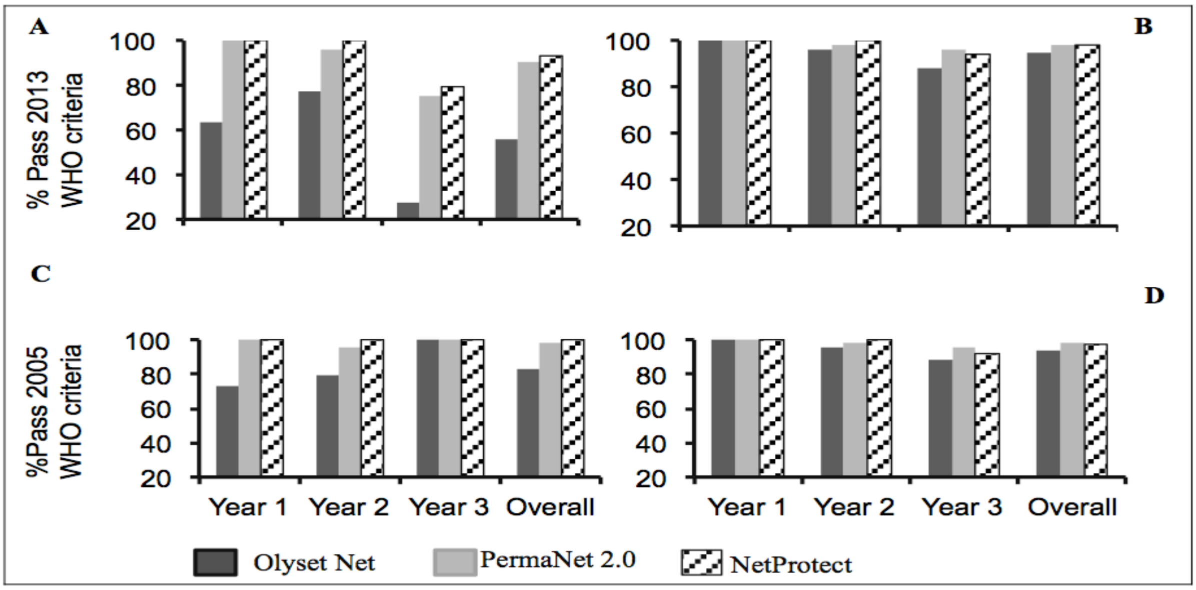


Figure 5.5. Percentage of LLINs by brand and age passing the 2013 (A and B) and 2005 (C and D) WHO criteria in the I-ACT (A and C) versus the combined WHO cone and tunnel assays (B and D) against Anopheles gambiae s.s. (Ifakara strain) fully susceptible to all classes of insecticides

With each year of use, fewer nets met the combined standard WHO criteria, regardless of brand or tests used (Table 5.7 and Figure 5.5). Eighty-eight percent of nets passed after 1 year and 85% passed after 3 years of field use as measured by combined standard bioassays following 2013 WHO guidelines. Importantly, even with natural wear and tear including some large holes. 100% of nets passed WHO criteria (≥ 80% mortality or ≥ 95% feeding inhibition) after 1 year by I-ACT declining to 97% passing (regardless of brand) after 3 years of field use. The most noticeable difference was observed with Olyset® nets that had lower pass rates of combined cone/tunnel test using 2013 WHO guidelines (71% overall) compared to 2005 WHO guidelines (77% overall). This difference was particularly depending if mortality was scored immediately or after 24 hours.

Table 5.7. The Percentage of nets (by brand and age) passing the WHOPES threshold criteria as measured by standard WHO bioassays and in I-ACT against An. gambiae s.s following 2013 and 2005 WHOPES guidelines

	Olyset Net				PermaNet 2.0 net				Net Protect			
	*2013 WHOPES Guidelines		*2005 WHOPES Guidelines		2013 WHOPES Guidelines		2005 WHOPES Guidelines		2013 WHOPES Guidelines		2005 WHOPES Guidelines	
	Standard WHO Bioassays	I-ACT	Standard WHO Bioassays	I-ACT	Standard WHO Bioassays	I-ACT	Standard WHO Bioassays	I-ACT	Standard WHO Bioassays	I-ACT	Standard WHO Bioassays	I-ACT
Year 1	64% (31/49)	100% (49/49)	74% (36/49)	100% (49/49)	100% (48/48)	100% (48/48)	100% (48/48)	100% (48/48)	100% (47/47)	100% (47/47)	100% (47/47)	100% (47/47)
Year 2	77% (37/48)	96% (46/48)	79% (38/48)	96% (46/48)	96% (46/48)	98% (47/48)	96% (46/48)	98% (47/48)	100% (48/48)	100% (48/48)	100% (48/48)	100% (48/48)
Year 3	28% (13/48)	88% (42/48)	99% (48/49)	88% (42/48)	75% (36/48)	96% (46/48)	100% (48/48)	96% (46/48)	79% (38/48)	94% (45/48)	100% (48/48)	92% (44/48)
Overall	56%	95%	83%	95%	90%	98%	98%	98%	93%	98%	100%	97% 43)

2013 WHOPES pass/fail criteria: Cone assay-- ≥95% kd60 and/or ≥ 80% 24h Mortality and Tunnel assay- ≥80% 12-15h Mortality and/or ≥ 90% Feeding Inhibition
2005 WHOPES pass/fail criteria: Cone assay-- ≥95% kd60 and/or ≥ 80% 24h Mortality and Tunnel assay-≥80% 24h Mortality and/or ≥ 90% Feeding Inhibition

Non-inferiority of sampled net product

In order to measure non-inferiority of field aged nets Olyset® was used as the reference net (active comparator for non-inferiority testing) and the other two nets were compared to it. Using I-ACT data it can be seen that, overall, PermaNet® and NetProtect® killed greater proportion of mosquitoes than Olyset®. Using a t-test of the effect difference between the products using the 24 h mortality endpoint with a margin of 10% of non-inferiority it can be seen that both PermaNet® 2.0 and NetProtect® were superior to Olyset® (Figure 5.6). It should be noted that the figure shows a negative value for superior products because the effect difference is calculated by subtracting the induced mortality of the test net from the reference net. However, using the feeding inhibition endpoint, both PermaNet® 2.0 and NetProtect® were non-inferior to Olyset using a 10% margin of non-inferiority. It can, therefore, be concluded that the three products are equivalent based on a combined mortality and feeding inhibition endpoints, as is current WHO practice.

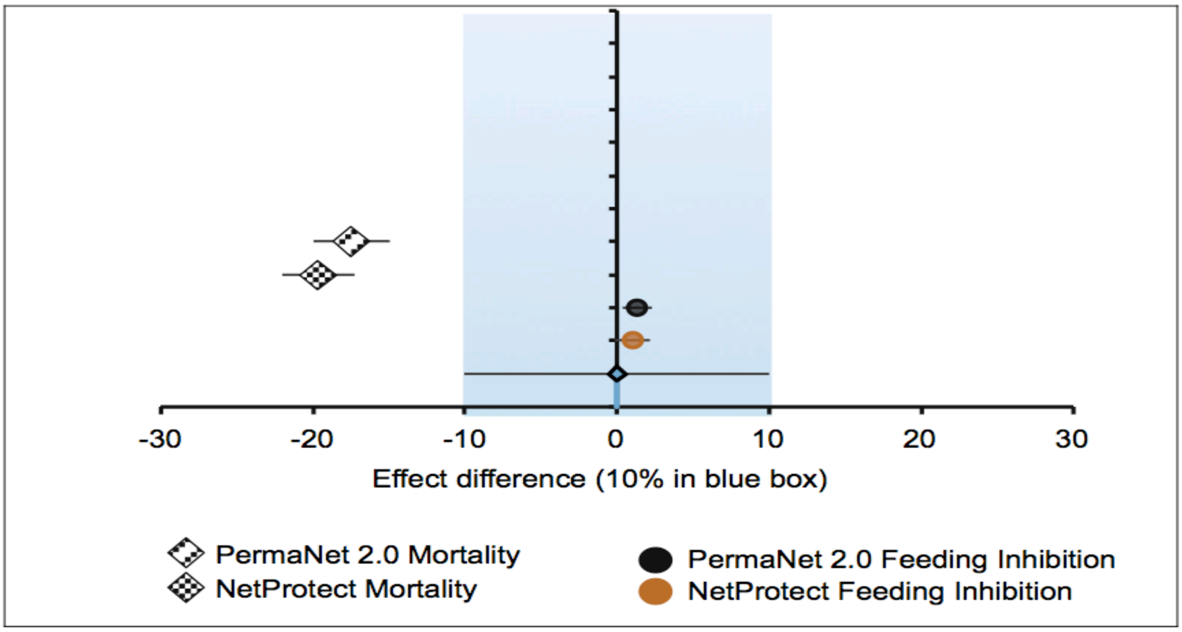


Figure 5.6. Non-inferiority of PermaNet 2.0 net and NetProtect combined 24 hour mortality and feeding inhibition for three years of data with Olyset® as the reference performed in the I-ACT using a 10% margin of non-inferiority

5.5. Discussion

This is the first study to compare bioefficacy of LLINs using standard WHO bioassays (cone and tunnel tests) with data collected from hole nets tested using the I-ACT. We were able to evaluate large numbers of three brands of nets returned from the field with natural wear and tear to measure their protection to users sleeping underneath them. The I-ACT allowed high throughput and gave powerful data due to the low heterogeneity between replicates due to the fact that data for PermaNet and Netprotect largely agrees with the standard WHO methods (cone/tunnel tests) and more Olyset nets passed with I-ACT similar to standard WHO methods (cone/tunnel tests).

It was observed that each of the three net brands showed lower efficacy measured by standard WHO bioassays compared to efficacy measured by I-ACT. This could be due to: 1) duration of exposure (3 min versus 12 h), 2) surface area of treated fabric presented to the mosquitoes (both standard WHO methods use 20 cm^2 samples versus a whole net in the I-ACT) and 3) number of tarsal contacts with the LLIN resulting in exposure to different dose of insecticide due to presence of human host under the net for the I-ACT. In cone test experiments, mosquitoes are exposed to tested LLIN for only 3 minutes which may not allow the tested mosquitoes to exercise natural host seeking behaviour with multiple contacts over the net surface resulting in a higher cumulative dose of insecticide. This has also been measured by other authors in studies to understand behavioural and physiological changes in mosquitoes in relation to responses to insecticides. A series of studies by Angarita-Jaimes and colleagues using a novel video-tracking system to quantify the behaviour of nocturnal mosquitoes attacking human hosts in the laboratory and in field observed that, both *An. gambiae* s.s. and *Culex quinquefasciatus* showed multiple contacts with bednets when a human host was present [30], and this host seeking activity is lower for treated nets than in untreated nets [31,32]. However, the I-ACT study demonstrated that these contacts were sufficient to kill or inhibit feeding among the majority of pyrethroid susceptible mosquitoes used in this study.

In addition, the findings add to the existing data that shows that the cone test underestimate the bioefficacy of Olyset® that contains Permethrin, a contact irritant pyrethroid [15,17,33,34]. During cone tests, permethrin causes mosquitoes to minimise contact with the netting fibres and may sometimes rest on the sides of the cone or cotton plug on the cone and avoid the insecticide and demonstrate frequent take offs from the net [28]

The tunnel test was developed as a consequence of the need to measure feeding inhibition of permethrin treated nets [35] and has also shown some use in evaluating chlorfenapyr products as it allows mosquitoes to exhibit flight and feeding behaviour after night [36]. However, as with cone test, the assay has some limitations. The overall pass rate (using 12 h or 24 h mortality and blood feeding inhibition), as measured following both WHO 2005 and 2013 criteria, was lower compared to when measured by I-ACT. A possible explanation for this observation is that, the baits used in tunnel tests are rabbits that are not the preferred bait for *An. gambiae* s.s that feeds almost exclusively on humans [22,37]. Therefore, during standard tunnel test experiments, mosquitoes may be less responsive to non-preferred bait and remain in the releasing chamber throughout the exposure time without interacting with the LLIN sample resulting in a lower cumulative exposure to insecticide.

Additionally, using a whole net in the I-ACT killed more mosquitoes possibly due to the large surface area of insecticide available for mosquitoes to interact with. It is known from repellent testing that use of a non-preferred bait will overestimate repellent efficacy [38].

However, a similar number of PermaNet 2.0 and NetProtect passed the combined WHO tests and the I-ACT whereas fewer Olyset passed combined WHO tests indicating that the WHO tests are conservative and therefore unlikely to pass a product that is of low efficacy. As the I-ACT is a less conservative test it may have use for early screening of new insecticide treated nets including those with irritant insecticides or those that function through a mode of action other than rapid knockdown before more costly experimental hut tests.

The overall percentage of nets that passed tunnel test following WHO 2013 guidelines was marginally lower than when using the WHO 2005 guidelines [25]. This suggests that reinstating the WHO 2005 pass/fail efficacy criteria may be justified to avoid missing products that are efficacious during early testing. This will also align tunnel test holding times with those of cone bioassays and experimental huts (24 h). It may also be justifiable when testing some products to hold mosquitoes for even longer than 24 h as some authors have done to measure the effects of slow acting insecticides [39,40]. This simple pairwise test between the two guidelines demonstrated the usefulness of exploring the impact of holding time on the outcome of product tests.

The mode of action of insecticides used on LLINs is an important consideration when selecting bioassays. New products with modes of action different from pyrethroids (which are fast acting and neurotoxic) are coming to market and we need suitable means to bioassay them. An example is chlorfenapyr, which acts by disrupting metabolic respiratory pathways (oxidative phosphorylation) in the cells of mitochondria and that require the conversion of the active compound through metabolism [41]. The conversion is optimal at night and is maximised when mosquitoes are metabolically active i.e. flying during host seeking or digesting a blood meal after feeding [42]. Cone test tests are usually conducted during the day and take 3 min exposure time with no bait involved. This means that the cone test may not be suitable to assess the efficacy of chlorfenapyr. Findings from two studies by Oxborough *et al* and Ngufor *et al* observed extremely low levels of mortality caused by chlorfenapyr compared to pyrethroids when assessed by cone test, but excellent effect against resistant mosquitoes when tested in experimental huts [39,43]. These data again suggesting that cone test may be best suited for fast acting non-irritant insecticides [35] and there is a need to be open to exploring new bioassays for new mode-of-action products. The higher pass rate of I-ACT compared to standard WHO tests may be useful when conducting “quick and dirty” tests for new products to avoid early “kill” of promising prod- ucts because they are failing to pass bioefficacy criteria in phase I laboratory tests when they may prove highly efficacious in gold standard experimental hut tests (Phase II).

Ifakara Ambient Chamber Test may be useful in evaluating new products that function through either mortality or feeding inhibition. Tests are conducted at times when mosquitoes are metabolically active, and using the preferred host of Afro-tropical malaria vectors. The advantage of using the I-ACT is that nets are evaluated using mortality and feeding inhibition using just one test rather than having to perform the cone (for mortality) followed by the tunnel test bioassays (for feeding inhibition or mortality at night). Regarding the issue of precision in outcome measure estimates, the durability study performed here in the I-ACT used 30 mosquitoes per chamber per night of experiment and allowed large numbers of nets to be evaluated without exhausting the insectary which is always a concern when product testing. It is important to assess a large number of nets in durability studies to allow a sufficient sample of nets to be returned from the field to capture the large heterogeneity in product performance i.e. fabric integrity and insecticidal content, and using a random sampling framework that is large enough to avoid sampling bias such as the Hawthorne effect [44].

When the efficacy of LLINs was compared using standard WHO assays and I-ACT, it was seen that most of the tested nets were extremely effective against mosquitoes and passed WHO criteria of feeding inhibition and/or mortality using the pyrethroid susceptible *An. gambiae* s.s. (Ifakara) strain even after 3 years of use with natural damage and insecticide depletion from the field. This has also been shown by other research in Tanzania [34,45,46].

Many of the tested nets were damaged. The median hole surface area was 459 cm^2 in Olyset, 295 cm^2 in Permanet and 152 cm^2 in NetProtect in Year 3, which means that most surviving nets were in the “damaged” category but remained highly protective.

In addition, a simple non-inferiority test was conducted using WHO criteria to evaluate the effect difference between products using WHO criteria of mortality and feeding inhibition. Olyset was the reference product (active comparator) against which the two other brands (innovator items) were compared since it is the standard of care in Tanzania. PermaNet® 2.0 and Netprotect® were non-inferior compared to Olyset® on the feeding inhibition endpoint and superior to Olyset® on the 24-h mortality endpoint when measured in the I-ACT. The WHO passes a product based on a combination of either mortality or feeding inhibition, and based on these criteria, PermaNet® 2.0 and Netprotect® were non-inferior to Olyset® based on data for three-year durability. In addition, Olyset® demonstrated lower mortality and similar feeding inhibition to PermaNet® 2.0 and Netprotect® when tested using standard WHO bioassays (cone and tunnel) or in the I-ACT. Estimates of efficacy from the sample of 144 nets per brand were very precise and a 10% effect difference in mortality could be observed. However, it is unlikely that 144 nets per brand could be cost effectively evaluated in experimental huts. A comparison study between Ifakara experimental huts and the I-ACT using 24-h mortality and feeding inhibition outcome measures is in progress (Moore et al., pers. commun.) and will show how I-ACT and gold standard experimental huts compare for non-inferiority evaluation of ITNs. This is important since experimental huts are used to measure entomological correlates of the epidemiological effectiveness i.e. the public health benefit of interventions [47].

Therefore, the I-ACT may become a useful additional method for testing insecticidal materials that can provide a high throughput option for evaluating *functional bioefficacy* of LLINs i.e. the true protection as a function of damage and bioavailability of insecticide in durability studies. This has also been suggested by WHO's Malaria Policy Advisory committee to be incorporated into the net durability assessment [48]. While the methods presented here may not be useful for operational durability monitoring they may be useful for consideration in WHO "Phase 3" community field assessments of ITNs.

In this new assay, recapture of released mosquitoes is 99% so 30 mosquitoes were consistently "captured" every night I every chamber which is unlikely to be the case in standard experimental hut studies [45,49-57]. Experimental hut studies rely on wild mosquitoes entering the hut, and the nightly number of mosquitoes captured is highly variable and consequently substantial replication is required to obtain adequate precision to estimate true effect differences between products [23]. As mosquito densities fluctuate due to seasonality in rainfall it is useful to have a whole net assay that is not dependent on field populations of mosquitoes that may limit the windows of opportunity to conduct tests with adequate mosquito densities to achieve power. Whole net bioassays where the interaction between insecticide and fabric integrity is measured are important for selecting between products or ranking their durability [48]. Bioassays that assess only the insecticidal bioefficacy of a net sample may favour poor quality nets that tear easily reducing user protection and consequently user acceptance, which will eventually lead to the user discarding the net [58].

The experimental hut bioassay that simulates domestic conditions and allows nets to be tested against wild mosquitoes is the definitive test of ITN efficacy [43]. This study had several limitations. Firstly, I-ACT uses laboratory-reared mosquitoes, which means it relies on laboratory strains that may have different resistance mechanisms to those locally or limited genetic diversity. Secondly, the I-ACT test is a more expensive infra- structure to establish compared to small WHO cones and WHO tunnel glass chambers, requires space and it is immovable. The assay must be conducted in climate- controlled chambers or in areas with suitable ambient conditions to conduct the tests. In contrast standard WHO cones and tunnel chambers which can be taken anywhere and tests conducted provided the environment is set to standard conditions for conducting tests. The I-ACT needs to be compared to experimental hut tests, but it did agree well with findings of standard WHO methods using pyrethroid susceptible mosquitoes. Evaluations of ITNs with pyrethroid resistant strains as well as using dual active ITNs will be reported in subsequent publications.

Based on the data here presented, the overnight I-ACT may be a bridge between the lab and the field. Data agreement with standard WHO testing methods was excellent, with high sensitivity and specificity. It allows mosquitoes to host seek during the active phase of the circadian rhythm, and have multiple contacts with treated net- ting in a more realistic way. It uses the preferred human host but allows laboratory-reared mosquitoes to be used. This improves safety for human volunteers because laboratory-reared mosquitoes are disease free and allows sufficient numbers of mosquitoes to be released to reach the power needed to conduct precise comparisons of product performance.

5.6. Conclusions

Findings from this study showed that, I-ACT can be used for high throughput evaluation of whole nets from ITN durability studies. The new assay may provide useful additional information and could act as a link between lab tests and field experiments measuring composite bio- efficacy and net physical integrity with both mortality and feeding inhibition endpoints. For the three products evaluated in this study the bioassay agreed with standard WHO tests for deltamethrin products and measured higher pass for the permethrin-treated nets than standard

WHO tests. I-ACT allows mosquitoes to interact with a preferred host sleeping under a net as it would be encountered in the field using a standard number of mosquitoes released to improve the precision of efficacy estimates and safety of human participants.

Additional files

Additional file SOM 5.1. Location of holes on the deliberately holed SAFI net (https://static-content.springer.com/esm/art%3A10.1186%2Fs12936-019-2741-y/MediaObjects/12936_2019_2741_MOESM2_ESM.pdf).

Additional file SOM 5.2. A standardized operating procedure for conducting experiments to measure feeding inhibition and mortality of different net products using the I-ACT (https://static-content.springer.com/esm/art%3A10.1186%2Fs12936-019-2741-y/MediaObjects/12936_2019_2741_MOESM1_ESM.pdf).

Additional file SOM 5.3. Sampling patterns of the net for cone bioassay and tunnel test (https://static-content.springer.com/esm/art%3A10.1186%2Fs12936-019-2741-y/MediaObjects/12936_2019_2741_MOESM3_ESM.pdf).

Competing Interests

Dr Sarah Moore conducts product evaluation for a number of vector control product manufacturers. The other authors declare that they have no competing interests.

Authors' contributions

Conceived and designed the experiment DJM, SJM, JDM. Performed the experiments DJM, JDM, WSN, ZMM. Analysed the data DJM. Contributed to the analysis SJM, JB. Wrote the manuscript DJM, SJM. Critically revised the final manuscript LML, SM, EM, WNK, ZMM, JB, HJO, SJM. Drew the diagrams JDM, SA. All authors read and approved the final manuscript.

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6.0 General Discussion

The overall aim of this thesis was to provide an overview of the LLIN durability assessment in Tanzania, challenges related to the methodologies and help improving them for sustained malaria control. We used two different projects i.e. ABCDR and Holed Net projects and conduct different field and laboratory experiments in order to achieve this goal.

To the best of our knowledge, this is the first complex study describing the LLIN durability in Tanzania. This study brings together many aspect of net durability in the context of malaria vector control including improved alternative methods for assessing LLIN effectiveness. In the next paragraphs, main findings with policy implications will be discussed.

Main findings from this thesis and their implications

Results from this thesis demonstrated that majority of the Olyset campaign nets (65%) distributed between 2009 and 2010 were still present and 39% of them were in serviceable insecticidal with substantial levels of active ingredients even after two to four years of field use in different geographical settings in Tanzania. This is similar to surveys done in Rwanda and Nigeria: an average attrition rate of 31.6% was observed in Nigeria [1] and 16% to 36% in Masaka, Kinazi and Bungwe settings in Rwanda [2]. Of those nets no longer present, 84% were reported to have been discarded with badly/too torn condition being the main (94%) reason. This mirrored to pooled data findings observed from 14 sub-national post-campaign surveys conducted in Ghana, Senegal, Nigeria (10 states) and Uganda between 2009 and 2012 [3] which reported 93% of the thrown away nets were described as “too torn”. This means discarding nets was primarily associated with condition of the net and not age of the net (median lifespan). Presence of rats in the household was significantly associated with the “too torn” condition of the net i.e. the likelihood of a net being in good physical condition (phi<65) is 0.4 times significantly (p-value=0.04) lower when rats are present in particular household. This was also evident in Kwale County, Coastal Kenya [4] and in India and Nepal [5]. This means, if these households could get rid of rats and repairs their bednets; chances for prolonging lifespan of bednets could be very high and nets could still provide both individual protection (by inhibiting blood feeding) and community protection (by killing mosquitoes).

With this finding, we suggests that, too torn insecticidal nets should never be thrown away unless there are new good nets to replace them. National and International public health policy makers may use this finding and integrate into their social behavioural change communication programs on bednet use, care and repair.

Findings using the "wild" nets from eight districts of Tanzania mainland showed that most of the damage to the bednets were located on lower part (50 cm) of the net. This means this is the part with most damage of the net. Interestingly, using the household questionnaire survey data, most of this bottom part was tucked under the mattresses. Thus, when nets were tucked, holes in this area were sealed by the mattress and hence prevent mosquito from entering the net. Therefore, though most of the damage part of the nets is located on the bottom part and due to tucking behaviour of people in most of sub Saharan Africa before they sleep, these nets still conferred personal protection and hence may contribute to the reduction in malaria transmission. This agrees with the previous finding on presence of substantial levels of insecticides on too torn nets. This finding has also implications for bednet manufacturers on designing bednets with strong netting fibres on the bottom part of the net. In early years of 2000s, some nets in the markets had a piece of white cotton sheet covering the bottom part of the net. Fortunately, the new PermaNet 3.0 has this feature and will make this net last longer. This feature can also be incorporated in other net products too. This finding stresses the importance of not only taking care of the net but also not throwing away the too torn as majority of holes are located on the bottom part which is tucked and hence contribute to some protections against mosquitoes.

Another interesting finding from this thesis was that the roof part of bednet was observed to be less vulnerable to holes, but was the area with the highest risk for mosquito entry and feeding. This is similar to findings of other studies done in an attempt to understand how mosquitoes respond to holes in human occupied bednets [6–9]. This means that holes on the roof should never be ignored. This is another area that has implication for bednet designing to manufacturers of bednets. National Malaria control programs in malaria endemic countries need to improve their social behavioural change communication strategy so it include appropriate education messages on net care and repair (*"care is better than repair"*) which could serve to increase net survival and personal protection and hence prolong the lifespan of LLINs.

Results of comparative efficacy between different net brands demonstrated that, there is variation in LLIN durability and hence functional survival. This has got significant implications on procurement decisions. Through that information on bednet durability among difference net brands, countries' public health policy makers may then use this information to select for the durable and cost effective LLIN. The same information can also be used to plan for the timing of the repeated distribution campaigns to ensure that maximum and sustainable health gains are observed.

In addition, new bioassays have been developed to measure personal protection and mosquito mortality (community protection) with high throughput. One of the new bioassay developed is called the Ifakara Ambient Chamber Test (I-ACT) assay, which is an improved version of the assay known as the Ifakara tunnel test (ITT). I-ACT allows whole LLINs to be tested (in just 20 days compared to 36 days in experimental huts) in an ambient chamber test with a human volunteer sleeping beneath to measure the protective efficacy using laboratory reared mosquitoes (hence no risk of disease for human participants) under user conditions. The design of the chambers allows 99% recovery of released mosquitoes that gives remarkable data power and experiments can be conducted year round using mosquitoes of varying resistance status. Findings from this study showed that, I-ACT is a promising bioassay for high throughput evaluation of bioefficacy of multiple LLINs. The new bioassay provides useful additional information that could otherwise be provided separately with standard WHO bioassays (cone and tunnel tests) and semi-field experiments (experimental huts). In addition, a preliminary exploration done using I-ACT in order to test its application for non-inferiority testing of new LLIN products demonstrated its potential as the novel bioassay for product equivalency testing (i.e. assessment of non-inferiority of products within a class) and durability studies as it measures composite bioefficacy and physical integrity *functional bioefficacy* with both mortality and feeding inhibition endpoints.

The assessment of fabric/physical integrity through counting of holes is, currently, the gold standard method for measuring LLIN durability. Data from hole counts in four hole size categories is then combined in a proportionate Hole Index (pHI) weighted by the approximate surface area of the holes to provide a single measure of damage per net. The PHI is designed to help determine whether bed nets are still protective or should be replaced. However, the HSA / pHI method of fabric integrity assessment assumes equal probability of mosquitoes entering holes per cm^2 of holed area and does not take into account information on: 1) where do holes in nets occur most frequently; 2) how does hole location impact on functional efficacy based on how people use nets; 3) where do mosquitoes most prefer to enter holes in the bednet; 4) how does insecticide interact with holes (of different sizes and locations) to impact on mortality and blood-feeding and 5) how does the pyrethroid resistance status of mosquitoes impact net efficacy as nets age i.e. become more damaged and the insecticide content of nets decreases through time?. A series of experiments using "wild" and "deliberately holed" nets were conducted to answer the above questions and the findings were used to develop a simpler, time saving and realistic method for assessing bed net integrity. The new method is called LaHoSA, which stands for "Location adjusted Hole Surface Area". This is a small modification to the existing pHI. The standard pHI weights hole categories by their average surface area and assume that each squared cm of damage incrementally increases the probability of mosquitoes entering nets. The data from "wild nets" suggests that this holds true for size 1 and 2 holes but that size 3 and 4 holes increase the probability of mosquitoes blood-feeding and surviving-feeding. Therefore, the proportion of mosquitoes surviving feeding for each hole size and location were averaged, and added to the standard weights and suggested as a further calibration to the standard weights used in the WHO durability monitoring guidelines. Thus, this thesis provided a simple method that can be used to assess net integrity *in-situ*. The advantage of this new method is that, you can be able to know when the net is in bad condition right in the field and more houses can be done more rapidly and cheaply to give a wider and more representative population sample.

LLINs are expected to provide both personal (prevent blood feeding) and community (killing mosquitoes) protection resulting in a decline in malaria transmission. Mortality and Blood feeding success have been the main outcomes measured using standard WHO bioassays. However, using findings from this thesis, it was clearly seen that majority of the blood fed mosquitoes did not survive after 24 h post exposure. The current parameter of "*proportion of blood fed mosquitoes*" includes dead mosquitoes that were already counted when scoring for "*mortality*". This means that the current outcome measures used in the standard bioassays needs to be revised in order to reflect the true efficacy of bednet tested. Given this fact, we propose "*survival of feeding*" as useful composite metrics that can be used not only in standard bioassays but also in updating the standard threshold criteria for passing or failing a bednet.

The WHO tunnel test has been used as a gold standard WHO bioassay to evaluate the bioefficacy of LLIN. The test was designed mainly to assess feeding inhibition of sampled nets that failed to meet standard WHO criteria for cone test. It is a very useful test for excito-repelling insecticides. However, one main limitation for this test, which may affect the outcome measure, is the use of small live animals as bait. Use of small live animals poses two complications, which include i) unethical issues, related to the use of animals for experimentations and ii) they are not the preferred hosts for anthropophilic mosquitoes like *An. gambiae* s.s. Interestingly, findings from this thesis showed both bait and exposure time had significant effect on the outcome measures from tunnel tests. Higher mosquito feeding rates were observed when bait was human compared to rabbit. Similarly higher mosquito mortalities were observed when exposure time was 12 hour compared to 1-hour exposure time. This means using the current tunnel test method, the results obtained are misleading as they over estimate the performance of particular LLIN and therefore they should be taken into consideration when assessing efficacy of any net product against malaria vectors using tunnel test.

6.1. Limitations and future studies

From the retrospective study survey, information on bednets collected from surveyed houses relied mainly on respondent's recall explanation (hence recall bias), because it was not known how many Olyset nets had been distributed to each household. In addition, it was impossible to establish the exact age of nets due to difficulties in distinguishing between U5CC and UCC Olyset nets. Therefore, the attrition rate presented in Figure 2.2 is a conservative estimate of the smallest possible age gap. A prospective follow-up study to assess the same may be able to capture the LLIN attrition component.

The I-ACT developed uses laboratory-reared mosquitoes, which means it relies on laboratory strains that may have different resistance mechanisms to those locally or limited genetic diversity. Secondly, the I-ACT test is a more expensive infra- structure to establish compared to small WHO cones and WHO tunnel glass chambers, requires space and it is immovable. The assay must be conducted in climate- controlled chambers or in areas with suitable ambient conditions to conduct the tests. In contrast standard WHO cones and tunnel chambers which can be taken anywhere and tests conducted provided the environment is set to standard conditions for conducting tests. More studies are needed to compare I-ACT with semi field experimental hut studies.

Tunnel test using preferred host (human arm) is an improved assay to the standard WHO tunnel test. However, the assay put human at a risk of mosquito bites, which may lead to itching and paining. The good of this assay, mosquitoes used are non-infected laboratory reared mosquitoes.

8.0. General Conclusion

In conclusion, this thesis has provided a deep insight into many aspects of durability of LLINs and methodologies to assess LLIN durability for sustainable malaria control in Tanzania. Our findings showed that assessing LLIN durability is a very useful for routine monitoring of LLIN durability in different user settings and net brands. Monitoring LLIN durability will help National and International public health policy makers and malaria control programs with information on the extent of physical degradation, biological efficacy and chemical residual which in turn will be used to determine the life of nets i.e. the duration of the functional survival. This will then be used to selection and procuring of the durable cost-effective and longer-lasting sustainable. The new bioassays developed using findings from this thesis can be used to measure personal (prevent blood feeding) and community (killing mosquitoes) protection with high quality, throughput.

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9.0 Annexes

9.1. Annex 1 Curriculum vitae

CURRICULUM VITAE

Surname: Massue
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CONTACT INFORMATION

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PERSONAL PROFILE

Organised, reliable, team player, leader and open minded research scientist with demonstrated expertise in the areas of malaria vector control, monitoring insecticide resistance in Tanzania and durability studies of long lasting insecticidal nets, with over 9 years of experience in entomological field and work in Tanzania. My main strengths are adaptability, dependability and the determination to get a job done.

Career goal:

To work as a Research Scientist or University Instructor in a reputable national/international organisation/institution.

Main interests:

- **Research Interests/expertise:** Malaria vector control research (field, lab and insectary based); Knowledge attitude and practices (KAP) surveys; insecticide resistance; Monitoring and Evaluations in Entomological Surveillance, commercial insecticides and Integrated Vector Management (IVM); Durability of Long lasting Insecticidal Treated Nets (LLINs) in the field and in Laboratory; Testing of vector control products.

- **Teaching Interests:** Medical/Veterinary Entomology; Medical/Veterinary Parasitology; Public health, Durability of LLINs; Biology and Control of Disease Vectors (Malaria); integrated Vector Management, Qualitative and Quantitative Research Methodologies, Basic principles of Bioethics and Basic Epidemiological Concepts/Methods

EDUCATION

2014 - 2018 (**November 2018** Expected): **PhD in Epidemiology and Public Health**- Swiss Tropical and Public health Institute, University of Basel, Switzerland. **Project Title:** Long-lasting insecticide treated bed nets (LLINs) for Malaria control in Tanzania: New methodological approaches to monitor LLINs durability for sustained malaria control. *(Funded by GLOBVAC: ABCDR project no. 220757)*

2009 - 2010: MSc in Biology and Control of Parasite and Disease Vectors- Liverpool School of Tropical Medicine, University of Liverpool, United Kingdom. **Research title:** "New exposure methods for measuring mosquito behavior which influence malaria transmission intensity and the impact of vector control- an experimental case study in Dar es Salaam *(Funded by Welcome Trust Grant Ref No: 089326)*

2002 - 2007: Bachelor of Veterinary Medicine - Sokoine University of Agriculture- Morogoro, Tanzania. Passed with Merit

1999-2001: Advanced Certificate of secondary school. Old Moshi High School- Kilimanjaro, Tanzania. Passed with Merit.

WORK EXPERIENCE

August 2011/2012: Tutor in Medical Entomology course at Vector control training college, Muheza, Tanga-Tanzania.

July 2011: Training Facilitator in International Course on Mosquito ecology and Control under the subject "Mark-Release and Recapture technique". The course was organized by National Institute for Medical Research- Amani Medical research centre, Muheza, Tanga -Tanzania.

November 2008- Amani Medical Research Centre's Acting Centre Director: As a Centre Director, I was in charge of all center's research and administration activities.

January 2008- Todate: Amani Medical Research Centre -Vector Control Project, Muheza. Senior Research Scientist with experience in field and laboratory work covering entomological research especially in malaria vector control and insecticide resistance for malaria vectors.

FELLOWSHIP/ SCHOLARSHIPS

- GLOBVAC through ABCDR Project – PhD study from 2013 to 2017
- Welcome Trust MSc Fellowship award - UK from 2009 to 2012.

RESEARCH EXPERIENCE:

Co-Investigator: The useful life of bednets for malaria control in Tanzania: Attrition, Bioefficacy, Chemistry, Durability and insecticide Resistance **(ABCDR Project). Agency: Research Council of Norway.** It is a multicenter project that involves five institutions: National Institute for Medical Research (Tanzania), Ifakara Health Institute (Tanzania), Swiss Tropical and Public Health Institute (Switzerland), Norwegian University of Life Sciences (Norway) and London School of Hygiene and Tropical Medicine (United Kingdom). (2013 – 2017).

Principal Investigator: Realistic measures of Insecticide resistance and avoidance in African Malaria Vector Mosquitoes. **Agency and grant type:** Welcome Trust-MSc Fellowships **Support level:** £86,248 over two years (2009-2012).

Co-Investigator: Pilot study on sustainable management of long-lasting insecticidal nets (LLINs) used for malaria prevention and control in Tanzania (a case study of Mtwara rural, Kilombero and Muheza districts) **Agency:** World Bank (WB) and the United Nations Environment Programme (UNEP) through World Health Organization (WHO). **Support level:** US$ 63,000.00

Co-Investigator: ENTOMOLOGICAL SURVEILLANCE OF MALARIA VECTORS IN TANZANIA: "National Monitoring of Malaria Vectors Resistance to the Insecticides of Public Health Relevance". **Agency:** Bill and Melinda Gates Foundation through (BMGF) World Health Organisation (WHO) and United States President's Malaria Initiative (PMI) through Research Triangle International (RTI).. **Support level:** US$ 400,000 WHO and US$ 211,750 PMI (2008- 2011).

NIMR Project coordinator: European Union FP7 collaborative project, African Vectors Control: New tools (AvecNet) involving 16 partner institutes in Africa and Europe. Project's objectives were developing and evaluating new tools for malaria vector control, focusing on strategies that will help combat insecticide resistance. **Agency:** European Union's Seventh Framework Programme for Africa (FP7-Africa-2010) **Support level:** €12 million (2011-2016)

Co-Investigator: District Comprehensive assessment (DCA) five year health Impact Evaluation of the scaling up of interventions against HIV/AIDS, TB and Malaria **Agency:** Global Fund **Support level:** 1202062 (2008/2009)

TRAINING WORKSHOPS AND CONFERENCES ATTENDED.

- 6th to 8th October 2015- **Oral presentation** "Factors affecting the durability (attrition, bio-efficacy, chemical residue and physical integrity) of Olyset® nets from national distribution campaigns in eight districts of Tanzania" **at the 2nd Pan-African Mosquito Control Association Annual Conference, Dar es Salaam (Tanzania).**

- 13th to 15th October 2015- **Oral presentation** "Factors affecting the durability (attrition, bio-efficacy, chemical residue and physical integrity) of Olyset® nets from national distribution campaigns in eight districts of Tanzania" **at the 29th NIMR Annual Joint Scientific Conference, Dar es Salaam (Tanzania).**

- 16th to 18th March 2015- Norway **Poster presentation** "Factors affecting the durability (attrition, bio-efficacy, chemical residue and physical integrity) of Olyset® nets from national distribution campaigns in eight districts of Tanzania" **at the 9th Conference on Global Health and Vaccination Research, Oslo (Norway)**

- 25th to 29th October 2015- **Poster presentation** "Factors affecting the durability (attrition, bio-efficacy, chemical residue and physical integrity) of Olyset® nets from national distribution campaigns in eight districts of Tanzania" **at the 64th American Society of Tropical Medicine and Hygiene Annual meeting, Pennsylvania (USA).**

- IVCC 4th Stakeholders forum, 2012 – UK

- 6th European and Developing Countries Clinical trials (EDCTP) forum, 2011- Ethiopia

- June/July 2011: Introductory course on GIS application to Health. The course was organised by NIMR under PEPFAR. Dar es Salaam, Tanzania

- April 2011: The Tanzania Malaria Control Forum: Dar es Salaam, Tanzania.

- October 2010: A training workshop on Capacity building in the Research Methodologies and Writing Skills of Competitive Grant Winning Proposals. Dar Es Salaam, Tanzania

- May 2008: Mosquito Ecology and Vector Control: Tanga, Tanzania

SELECTED PUBLISHED PEER REVIEWED PAPERS

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- Zawadi M. Mboma, Hans J. Overgaard, Sarah Moore, John Bradley, Jason Moore, **Dennis J. Massue**, Karen Kramer, Jo Lines and Lena M. Lorenz Mosquito net coverage in years between mass distributions: a case study of Tanzania, 2013. Malar. J. 2018

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2016

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- **Massue DJ**, Moore SJ, Mageni ZD, Moore JD, Bradley J, Pigeon O, Maziba EJ, Mandike R, Kramer K, Kisinza WN, Overgaard HJ, Lorenz LM. Durability of Olyset campaign nets distributed between 2009 and 2011 in eight districts of Tanzania. Malar. J. 2016. Available from: http://www.malariajournal.com/content/15/1/176

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- Tungu Patrick, Magesa Stephen, Maxwell Caroline, Malima Robert, **Massue Dennis**, Sudi Wema, Myamba Joseph, Pigeon, O., & Rowland Mark. (2010). Evaluation of PermaNet 3.0 a deltamethrin-PBO combination net against Anopheles gambiae and pyrethroid resistant Culex quinquefasciatus mosquitoes: an experimental hut trial in Tanzania. *Malaria Journal*, doi:10.1186/1475-2875-9-21

- Bilali Kabula, Patrick Tungu, Johnson Matowo, Jovin Kitau, Clement Mweya, Basiliana Emidi, **Denis Masue**, Calvin Sindato, Robert Malima, Jubilate Minja, Shandala Msangi, Ritha Njau, Franklin Mosha, Stephen Magesa and William Kisinza (2012). Susceptibility status of malaria vectors to insecticides commonly used for malaria control in Tanzania. Tropical Medicine and International Health 00 (0) April 2012.

- Bilali Kabula, Yahya A. Derua, Patrick Tungu, **Dennis Massue**, Edward Sambu, Grades Stanley, Franklin W. Mosha and William Kisinza (2011). Malaria entomological profile in Tanzania from 1950 to 2010: a review of mosquito distribution, vectorial capacity and insecticide resistance. Tanzania Journal of Health Research. Volume 13 (Suppl 1), December 2011

SELECTED TECHNICAL REPORTS

- Patrick Tungu Robert Malima, Wema Sudi, **Dennis Massue** Joseph Myamba, Stephen Magesa, Caroline Maxwell & Mark Rowland. Laboratory and field evaluation of Syngenta LNs.

- Patrick Tungu, Robert Malima, **Dennis Massue**, Wema Sudi, Elibariki Akyoo, Joseph Myamba, Caroline Maxwell, Stephen Magesa and Mark Rowland. Laboratory and field evaluation of Permanet 3.0 .

- William N. Kisinza, P. Mutalemwa, William J. Kisoka, Michael A. Munga, Kabula Bilali, **Dennis Massue**, Caroline Maxwell, Gaël du Châtellier, David Smith, and Jerry F. Cooper.. Pilot study on sustainable management of long-lasting insecticidal nets used for malaria prevention and control in Tanzania. A case study of Mtwara rural, Kilombero and Muheza Districts.

- Patrick Tungu, Robert Malima, Wema Sudi, **Dennis Massue**, Joseph Myamba, Stephen Magesa, Caroline Maxwell & Mark Rowland. Experimental huts evaluation of Pyrethroids and Deet MC treated blanket developed by Sumitomo chemical company against *Anopheles gambiae* and *Anopheles funestus*.

- Patrick K. Tungu, Stephen M. Magesa, Robert C. Malima, Caroline A. Maxwell, **Dennis Massue**, Wema Sudi, Joseph Myamba and Mark Rowland. Evaluation of the new lambda-cyhalothrin developed by syngenta against *anopheles gambiae* and *anopheles funestus* in experimental huts in muheza, Tanzania.

MEMBERSHIP

Member- Tanzania Public Health Association (TPHA): A non-governmental organization (NGO) established with the goal of promoting health and preventing disease in Tanzania through sound public health practices. **www.tpha.or.tz**

Member- Pan-African Malaria Vector Research Consortium (PAMVERC): A consortium of three African vector research control research sites (located at Moshi and Muheza in Tanzania and in Cotonou, Benin) specializing in the development and evaluation of malaria vector control products and interventions. **www.pamverc.org**

Member- Malaria Research Coordination Network (MalariaRCN): An international network of Scientists established to promote research, communication and training in the biology of malaria (Haemosporida) parasites of vertebrate wildlife populations. **www.malariarcn.org**

Member- Malaria World: The world's scientific and social network for malaria professionals. **www.malariaworld.org**

Member- Tanzania Veterianry Association: A professional association that promotes and protects interests and welfare of veterinarians in Tanzania. **www.tva.or.tz**

Computer skills:
Fully conversant with various software packages, including Windows, Microsoft Office Suite (Word, Excel and Access) and Data Analysis software i.e. Epi Info 2002, SPSS Version 17 and STATA version 13. Basic knowledge and skills in the application of GIS in health practice and research.

REFEREES
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9.2. Annex 2: Journal Paper- The consequences of declining population access to insecticide treated nets (ITNs) on net use patterns and physical degradation of nets after 22 months of ownership

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Abstract

Background: As the number of insecticide-treated nets (ITNs) in households (access) declines when nets wear out some household-members are prioritised to use the remaining ITNs. This study assessed how nets are allocated within households to individuals of different age categories as 1) ITNs are lost or damaged; 2) new ITNs are obtained, and 3) explores how ITN allocation affects ITN durability.

Methods: A cross-sectional household survey and ITN durability study was conducted among 2,875 households across Tanzania to determine the proportion of nets that remain protective (serviceable) twenty-two months after net distribution aiming for universal coverage. Allocation of study nets within houses and re-allocation of ITNs when new Universal Replacement Campaign (URC) nets arrived in study households in Musoma District was also assessed.

Results: Only 57.0% [95% CI: 53.9-60.1%] of households had enough ITNs for every household member (assuming one net covers every 2 members). In households with enough nets, 77.5% of members slept under ITNs. In households without enough nets, pregnant women (45%), children<5 (46%) and adults (42%) were prioritised, with fewer school-age children 5-14 (36%), youths 15-24 (28%) and seniors>65 (33%) sleeping under ITNs. Crowding (≥3 people slept under nets) was twice as common among people residing in houses without enough nets for all age groups apart from children<5. Nets were less likely to be serviceable if ≥3 people slept under them (OR=0.50 [95%CI 0.40-0.63]); if nets were used by school-age children (OR=0.72 [95%CI 0.56-0.93]) and if the net product was Olyset®. One month after the URC, only 23.6% [95%CI 16.7-30.6%] had access to an URC ITN in Musoma district. Householders in Musoma district continued the use of old ITNs even with the arrival of new URC nets.

Conclusion: Users dictated the useful life of ITNs and prioritized pregnant women and children<5 to serviceable ITNs. When household access falls, users adjust by crowding under remaining nets, which further reduces ITN lifespan. School-age children that commonly harbour gametocytes mediating malaria transmission are forced to sleep under unserviceable nets, crowd under nets or remain uncovered. However, they were accommodated by the arrival of new nets. More frequent ITN delivery through the school-net program in combination mass distribution campaigns is essential to maximise ITN effectiveness.

Introduction

Insecticide-treated nets (ITNs) are impactful in the fight against malaria in sub-Saharan Africa [1]. In Tanzania the previous decade, mass distribution campaigns of ITNs have been conducted every four years (2010-2011, 2015-2016 [2, 3]). Through mass distribution, coupled with targeted campaigns, approximately 80 million ITNs have been distributed in Tanzania since 2000 [2-6], resulting in a 12% reduction in malaria deaths and 15% reduction in cases per capita at risk since 2010 [7, 8]. These gains against malaria in Tanzania can also be attributed to early implementation of successful Behavioural Change Communication that has encouraged appropriate and sustained net use among populations at risk of malaria [9].

Effective malaria protection by ITNs is achieved when at least 80% of household members have access to, and sleep under ITNs [10]. The World Health Organization (WHO) recommends the combination of mass campaigns and targeted mechanisms to ensure continued universal coverage of at least one ITN to cover every two people in a household, for all populations in malaria-endemic countries irrespective of age or gender [11]. To account for differences in household size, one net for every 1.8 persons is recommended during procurement to ensure universal access to ITNs within households [12]. Despite best efforts, population access to ITNs (the percentage of the population with access to an ITN within their household, assuming each ITN is used by 2 people) remains below the target level of 80% in many malaria endemic areas [13]. According to the 2017 Tanzania Malaria Indicator Survey, 63% of the population had access to an ITN while only 52% slept under an ITN the previous night [14]. ITN access in Tanzania has remained around 50% since 2010 with peak access of 75% in 2011 and 63% in 2017 after mass distribution of ITNs [14]. Access to ITNs tends to generally be high after mass distribution but falls rapidly as nets wear out [15]. With time and use, ITNs in households get damaged and when they are no longer perceived to be useful, they are discarded by the householders [16-19], resulting in lower population access to nets [20]. Moreover, an ITN is only effective for as long as it remains serviceable i.e. sufficiently physically intact to provide adequate personal protection against malaria [21]. There is good evidence that when used, ITNs provide personal protection against malaria even in areas of high mosquito resistance to insecticide [22]. Therefore, it is important to understand underlying reasons for the loss of nets from households and reasons why they may not be used in order to maximise the longevity and use of existing ITNs in Tanzania.

There are several factors that affect ITN access and use, including household size [23], user characteristics: age, gender, pregnancy status [24-26], and socio-economic status (SES) [27]. So, as nets wear out and access to nets declines, it is likely that households will prioritize who will use the remaining net(s) based on the number of net(s) currently available in the household and their condition [28-30]. Potential consequences of prioritization could be 1) crowding, i.e. more than the two household members assumed to share a net, sleeping under the same net; and/or 2) some household members being left uncovered. It is important for National Malaria Control Programs (NMCPs) in malaria-endemic countries to understand how households decide on who to prioritize for bed net use within households, so they can inform behavioural change communication strategies, design targeted ITN delivery mechanisms for at risk groups or, if needed, increase the frequency of mass ITN campaigns. This study assessed how nets are allocated within households to individuals of different age categories as 1) ITNs are lost or damaged; 2) new ITNs are obtained, and 3) explores how ITN allocation among houses without enough ITNs further impacts ITN durability.

Methods

In 2015, a cross-sectional household survey was conducted in 2,875 households across eight districts enrolled in a 3-year ITN durability study in Tanzania [31, 32]. The households randomly received one type of ITN from a pool of three products (referred hereafter as study nets): Olyset®, NetProtect®, PermaNet®, to cover every sleeping space identified during enrolment in 2013. Study nets were identifiable by their colour (white) and with a durable waterproof label to allow longitudinal follow up. The average number of sleeping spaces per household among the study population was 3.1 and each household received an average of 3 study nets.

The data presented here are from a survey conducted 22 months after ITN distribution, which coincided with the government's Universal Replacement Campaign (URC) in 2015, creating an opportunity to see how nets are allocated as new nets are received among households. The URC took place in Musoma, one of the eight study districts, one month prior to the study survey. PermaNet® 2.0 was the net product distributed during the URC with a maximum of five ITNs distributed per household among households with ten or more members (Ikupa Akim, pers. comm). PermaNet® 2.0 ITNs distributed by the URC were also identifiable by their blue colour. Additional nets (non-study nets) acquired by household members within those 22 months (regardless of their source) were assessed and all ITNs were included in the analysis. Data was collected using a questionnaire (Supplementary Material 1) on household members and their characteristics (age, gender, pregnancy status and SES), 2) access to and net use including number of people sleeping under a net the previous night, and 3) the physical status (serviceability) of a maximum three study nets per household.

ITN physical degradation (serviceability)

Over time, nets become torn with repeated use. While the inclusion of pyrethroid insecticides helps to prevent mosquitoes entering nets to some extent [33], the more holes in a net, the more mosquitoes will enter the net and reduce the protection given to a net user [34]. It is important to understand how much of the net surface area is available for mosquitoes to pass through. This is often done using a standard metric, the proportionate hole index (pHI), which provides an easy means of comparing this damage by calculating the approximate holed surface area of the net. The study assessed the physical condition of a maximum of three study nets per household. The number and size of holes was assessed at household level using a portable frame [31], following WHO hole categorization [35]. The pHI was calculated for each ITN, and thereafter categorized as either serviceable (pHI: 0-642) or unserviceable (pHI: 643+). A net that is defined as unserviceable has been shown to offer reduced protection from mosquito bites and malaria [36].

Net prioritisation

An in-depth assessment of some of the Roll Back Malaria Monitoring and Evaluation Reference Group (MERG) indicators [37, 38] as well as characteristics of ITN users (Table 1), was performed by the study team in all 8 study districts to understand 1) which users (age category, gender and pregnancy status) were prioritized when ITNs are lost or damaged and 2) how ITN allocation among houses without enough ITNs further impacts ITN durability (age, number of occupants). Data from Musoma where the URC had been conducted was used to understand which users (age, gender and pregnancy status) were prioritized for the allocation of new nets and which users continued to use the older “study nets”. Age categories in years were children under the age of 5, school-age children 5-14, youth 15-24, adults 25-65 and seniors 65+.

Table 1. Roll Back Malaria Monitoring and Evaluation Reference Group (MERG) ITN indicators assessed [37, 38].

ITN indicator	Indicator description
Household with enough ITNs	Percentage of households with at least one ITN for every two people.
Population access	Percentage of the population with access to an ITN within their household (assuming each net is used by two people).
Population ITN use	Percentage of the population that used an ITN the previous night.
ITN use:access ratio	Percentage of the population that used an ITN the previous night divided by the percentage of the population that had access to an ITN

Statistical analysis

Data analysis was carried out using statistical software package STATA 14.1 (StataCorp LP, College Station, TX). Survey weights were used to compensate for unequal sampling units, adjust for non-response, and a multi-level modelling approach. Net use and the proportion of serviceable and unserviceable study nets by user age category, among houses with and without enough nets for every two members, are presented as frequencies and percentages. Statistical analysis of the effect of crowding (more than two people sleeping under a net) on net serviceability were done using logistic regression models with crowding as the main exposure. Other predictor variables specified a priori were user characteristics (age, gender), SES and net product. A forward-selection procedure was applied for modelling and the selection was based on change in main exposure effect estimate (mean square error). The procedure involved three main steps: a) descriptive analysis and preliminary investigations for association between variables while paying attention to the sizes of effects as well as two-sided p-values at 95% significance level; b) variables selection; from prior knowledge, age and sex were considered as forced variables in the model. Then, one variable at a time from a list of candidate variables obtained from univariate analysis was included in the model with and without adjustment of forced variables to understand the effect of forced variables. The choice of the "best" predictor to be included in the model was then decided based on the change in exposure effect estimate. Each time a new variable was added in the model, evidence of confounding and multicollinearity was assessed by comparing the effect estimates and standard errors between the "univariate" and "multivariate" models estimates; and c) multivariable models were fitted by adding explanatory variables that were removed from the models in step "b" one at a time to explore their effect when added to the model in presence of other variables in the model. Variables that resulted in positive changes in the mean square error were then included in the model. The process was repeated until all variables that provided precise estimates of exposure variables were selected.

Results

A total of 2,875 households were visited from eight study districts. Mosquito nets were found in 2,801 (97.4%) households of which, 1,668 (58.0%) had only study nets, 1,126 (39.2%) had both study and non-study nets, and 7 households (0.2%) had only non-study nets. Overall, 9,178 mosquito nets were found, of which 5,899 were in households with enough ITNs and 3,288 in households without enough ITNs. Of these mosquito nets, 6,938 (75.6%) were identified as "study nets" and 2,249 (24.5%) as "non-study nets" since they were obtained from other sources. Of the non-study nets, 712 (31.7%) were identified as ITNs based on their product label. Therefore, a total of 7,650 ITNs (study and non-study) were identified and included in the analyses presented.

ITN access

In 2013, as part of the study design, 100% of sleeping spaces were covered by study nets and this fell to 42.6% of sleeping spaces covered by study nets after 22 months. Including study nets and non-study ITNs, 57% [95% CI: 53.9-60.1%] of the participating households still had enough ITNs i.e. one ITN for every two household members assuming each ITN is used by two people. Eighty-four percent [95% CI: 82.4-86.4%] of the population living in the participating households had access to an ITN, assuming each ITN was used by two people, and 53.2% [95% CI: 52.4-54.0%] of those with access used an ITN the previous night (Table 2). Population access to ITNs among larger households (>10 household members) was 79.0% [95% CI: 72.7%-85.4%] while in smaller households (≤ 10 household members) was 93.2% [95% CI: 91.8%-94.5%]. This data is broadly similar to data collected by the Tanzania Malaria Indicator Survey, two years after the URC (Table 2), indicating that ITNs last around 2 years in Tanzania.

Table 2: Comparison of ITN use and access indicators across study districts in 2015, 2 years after study ITN distribution versus the Tanzania Malaria Indicator Survey in 2017, two years after the Universal Replacement Campaign

District	Households with enough ITNs*		Population Access to ITNs*		Population ITN use		ITN use:access ratio	
	Study**	Malaria Indicator Survey***	Study**	Malaria Indicator Survey***	Study**	Malaria Indicator Survey***	Study**	Malaria Indicator Survey***
Bagamoyo	61.1 (54.1-67.8)	61.8%	82.7 (78.2-86.9)	76.5%	63.5 (61.3-65.6)	83.7%	0.76	1.09
Geita	40.8 (34.2-47.9)	26.8%	70.3 (66.0-74.7)	51.5%	47.6 (45.7-49.6)	78.0%	0.68	1.51
Iringa	71.2 (64.4-77.2)	36.8%	87.7 (83.4-91.9)	55.2%	57.4 (55.0-59.8)	78.1%	0.66	1.41
Kahama	48.1 (38.7-57.7)	28.0%	72.9 (66.4-79.4)	49.1%	42.6 (40.8-44.5)	65.9%	0.58	1.34
Kilosa	61.8 (57.8-65.6)	57.5%	83.8 (80.9-86.7)	73.7%	62.3 (60.0-64.5)	76.7%	0.74	1.04
Kinondoni	53.6 (46.4-60.7)	55.3%	75.1 (67.2-83.0)	70.8%	47.1 (43.6-50.5)	78.2%	0.63	1.10
Mbozi	56.4 (49.8-62.8)	47.7%	79.4 (74.3-84.4)	61.9%	32.1 (30.0-34.3)	44.6%	0.4	0.72
Musoma	61.8 (56.5-60.1)	46.9%	86 (83.2-88.7)	71.8%	71.2 (69.3-72.9)	69.1%	0.83	0.96
Total	57.0 (53.9-60.1)	45.4%	84.4 (82.4-86.4)	62.5	53.2 (52.4-54.0)	66.7%	0.63	1.06

*Assuming each net is used by two people
**Denominator is 7,650 ITNs (study and non-study ITNs) found in all participating households
***Findings from the 2017 Tanzania Malaria Indicator Survey (TMIS)[14]

The effect of household access on ITN prioritisation

Pregnant women and children under 5 years were most likely to sleep under an ITN irrespective of the household's ITN access, while young adults (15-24 years) contributed the lowest percentage of ITN users (Fig 1a). Household access to nets clearly affected how nets were allocated within households. In houses with enough nets 77.5% of members slept under ITNs compared to 37.5% of members who did in households without enough nets. There was prioritisation for children<5 and pregnant women in both access scenarios, but in houses without enough nets this prioritisation was more pronounced (Fig 1a).

In households with enough nets, 91.1%% of pregnant women slept under ITNs, 13.6% higher than the household average of 77.5% use. In houses without enough nets, a 17%-point increase in net use among pregnant women was observed when compared to the average household use (54.6% versus 37.4%). For children <5years, 82.9% slept under an ITN, 5.4% higher than the household average of 77.5% use. In houses without enough nets, 45.8% of children <5years slept under ITNs, which is 8.4% higher than the household average use of 37.4%. A smaller proportion of children 5-14 years slept under ITNs compared to the household average in both houses with enough nets (75.7% versus 75.5%) and in households without enough nets (35.9% versus 37.4%). Youth were also less likely to be prioritised to ITNs in houses with enough nets (5% lower than household average) and this was more pronounced in houses without enough nets (9.3% lower than household average). Seniors were less likely to be prioritised to ITN use in houses without enough ITNs, with only 32.6% of them sleeping under nets which was 4.8% lower than the household average, although this was not seen in houses with enough ITNs.

The variation observed in net use across user categories was related to sleeping space allocations. In descending order; seniors, youths and adults reported the highest percentages of users that slept alone under a net irrespective of whether the household had or did not have enough nets (Fig 1b). Children under the age of 5 and pregnant women were most likely to share a net with another sleeper (Fig 1b).

The effect of household access on the number of people sleeping under an ITN

A total of 2,177 households (1,314 with and 863 without enough ITNs) had ITNs that were used last night. Of the 3,288 mosquito nets found in households without enough ITN's, 25.1% [95% CI: 23.0-27.3] were used by three sleepers while 8.8% [95% CI:8.0-9.7] of the 5,899 nets found in households with enough ITNs were used by three or more people. The proportion of three or more household members sleeping under one net was higher in households without enough ITNs (62.1% [95% CI 60.7-63.6]) compared to those with enough ITNs (30.5% [95% CI 29.2-31.7] (Table 3)). Similarly, use:access ratio of >1 (Table 2) which implies more than 2 people slept under these ITNs [23], was observed in the majority of districts during the TMIS survey, and was more pronounced in Geita, Iringa and Kahama districts which had lower proportions of houses with enough ITNs. When the population net use by three or more sleepers was explored by age category, the trend of crowding in households without enough nets doubled that of households with enough nets for all age categories except for under-fives who are more likely to sleep with their parents (Table 3).

Table 3: Population ITN use by 3 or more people by household access

	Households with enough ITNs			**Households without enough ITNs**		
Number of households with ITNs used last night	1,314			863		
Number of nets found in households	5,899			3,288		
Number of nets used by three or more people	519			824		
% of nets used by three or more people [95%CI]	25.1% [95% CI: 23.0-27.3]			8.8% [95% CI:8.0-9.7]		
Age in years	**n_1**	**n_2**	**Crowded** (95% CI)**	**n_1**	**n_2**	**Crowded ** (95% CI)**
Under 5	612	389	63.6 [59.7-67.3]	814	687	84.4 [81.8-86.8]
5-14	1,446	441	30.5 [28.2-32.9]	1,256	756	60.2 [57.4-62.8]
15-24	945	185	19.6 [17.2-22.2]	630	313	49.7 [45.8-53.6]
25-64	1,880	539	28.7 [26.7-30.8]	1,391	844	60.7 [58.1-63.2]
64+	331	34	10.3 [7.4-14.0]	155	38	24.5 [18.4-31.9]
Total	5,214	1,588	30.5 [29.2-31.7]	4,246	2,638	62.1 [60.7-63.6]

*Assuming each net is used by two people
** Net use by three or more sleepers
n_1=Number of people who slept under net last night
n_2= Number of people who were crowded

ITN serviceability

Holes were counted in 4,783 (68.9%) of the 6,938 study nets 22 months after distribution. Of these, 3,735 (78.1%) nets were still serviceable while 1,048 (21.9%) were unserviceable. Only 3,622 (75.7%) of the 4,783 ITNs assessed for physical damage were used the previous night. Furthermore, 847 (80.8%) of unserviceable nets and 2,775 (74.3) of serviceable nets were used last night. Prioritisation of serviceable nets was also observed. On average, 32.6% people slept under serviceable ITNs last night. Of which around 7% more among pregnant women (40.5%), adults (39.2%), seniors (39.3%) while 5% fewer children 5-14 (27.8%), and 6 % fewer youth 15-24 (26.6%) slept under a serviceable ITN (Fig 1c). Pregnant women reported the highest use of nets irrespective of serviceability (54.2%) followed by adults (49.2%) and children under-five (47.5%) (Fig 1c). Children (5-14 years) and young adults (15-24 years) were less likely to sleep under an ITN and if they did sleep under an ITN it was more likely to be unserviceable (Fig 1c).

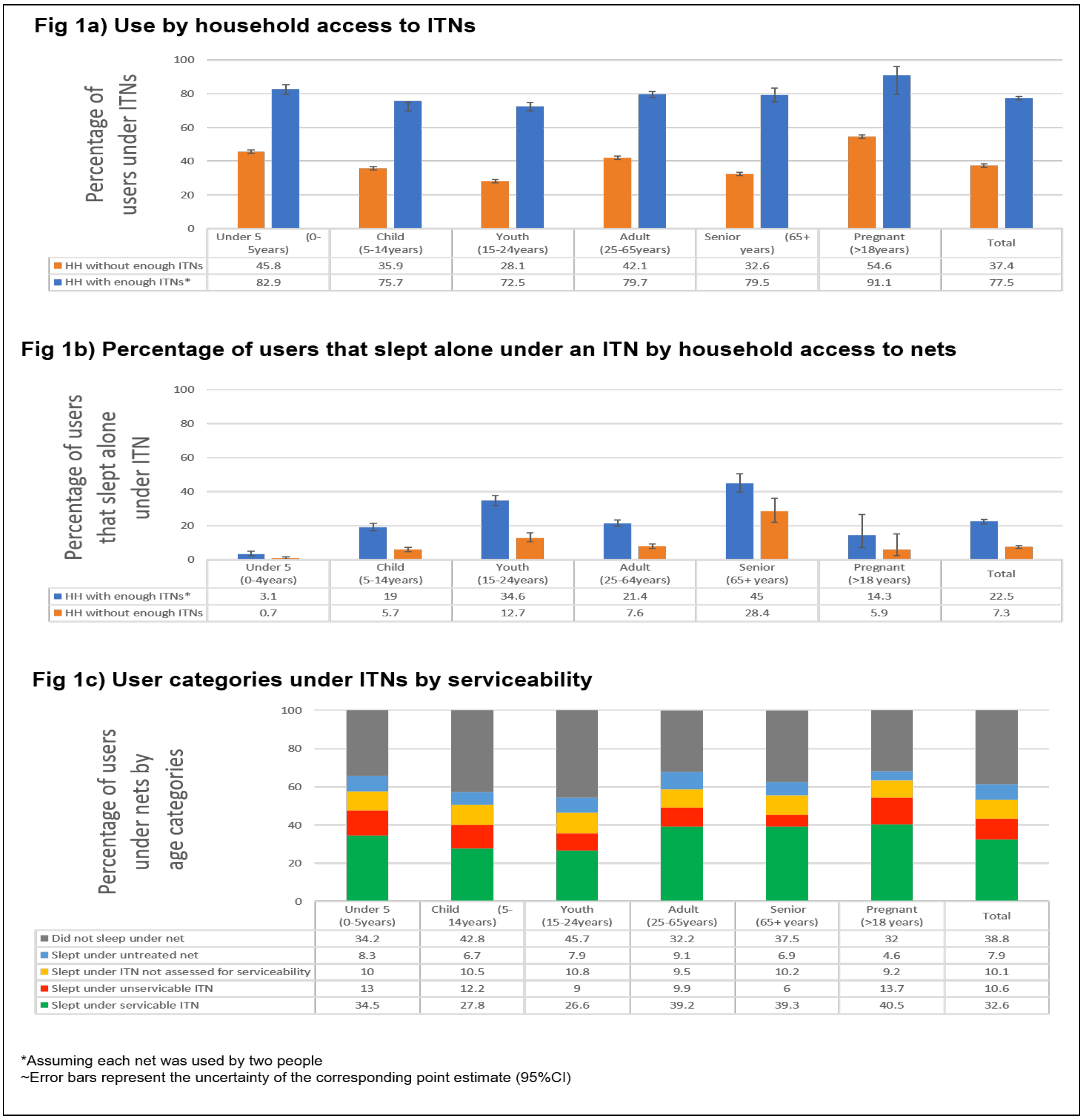

Fig 1: ITN use assessment by user categories and serviceability: *1a) the denominator used is 7,650 ITNs found in the participating households; 1b) while some sleepers slept under an ITN their appropriate age could not be accounted for; and 1c) denomminator includes all 9,178 nets found in households during the survey.*

Results of univariable and multivariable analyses exploring the consequences of net allocation on ITN serviceability are presented in Table 4. The number of people that slept under an ITN, the age category of net users, and socio-economic status were all significantly associated with ITN serviceability (p<0.001) in the univariate analysis. The odds of NetProtect® nets being serviceable was two times the odds of Olyset® nets. ITNs used by children (5-14years) had lower odds of being serviceable compared to those used by under-fives. Controlling for net product and user characteristics (age, gender and socio-economic status), crowding was significantly associated with unserviceable ITNs (P<0.001). Compared to one person under a net, having two people under the net reduced the odds of serviceability to OR=0.75 [95% CI: 0.60-0.83] and having three people under the net reduced the odds of serviceability to OR=0.50 (95% CI: 0.40-0.63).

Table 4: Univariable and multivariable analysis of factors associated with serviceability of study ITNs

			Crude Estimates		**Adjusted Estimates***	
	N	**Number serviceable, n (%)**	**OR (95% CI)**	**P-Value**	**OR (95% CI)**	**P-Value**
# of people under net						
1	1254	1006(80.2)	1	<0.001	1	<0.001
2	866	611 (70.6)	0.60 (0.46-0.77)		0.75 (0.60-0.83)	
3+	788	497 (63.1)	0.45 (0.33-0.59)		0.50 (0.40-0.63)	
User characteristics						
Age (years)						
Under 5	450	312 (69.3)	1		1	
5-14	786	493 (62.7)	0.74 (0.58-0.95)	<0.001	0.72 (0.56-0.93)	<0.001
15-24	392	286 (73.0)	1.19 (0.88-1.61)		1.06 (0.78-1.45)	
25-65	1118	879 (78.6)	1.63 (1.27-2.08)		1.29 (0.99-1.68)	
65+	162	144 (88.9)	3.54 (2.08-6.01)		2.62 (1.51-4.54)	
Socio-economic Status						
Poorest	640	479 (74.8)	1	0.009	1	0.012
Poor	550	393 (71.5)	0.84 (0.65-1.09)		0.85 (0.66-1.11)	
Middle	510	365 (71.6)	0.85 (0.65-1.10)		0.81 (0.62-1.06)	
Wealthy	635	435 (68.5)	0.73 (0.57-0.93)		0.71 (0.55-0.91)	
Wealthiest	537	442 (77.1)	1.13 (0.87-1.48)		1.09 (0.83-1.43)	
Gender						
Male	1,338	951 (71.1)	1	0.070	1	0.081
Female	1,570	1,163 (74.1)	1.16 (0.99-1.37)		1.16 (0.98-1.38)	
Net product						
Olyset®	1520	1066 (70.1)	1	<0.001	1	<0.001
PermaNet®	1667	1317 (79.0)	1.26 (1.04-1.53)		1.32 (1.08-1.61)	
NetProtect®	1596	1349 (84.5)	1.95 (1.58-2.40)		2.08 (1.68-2.58)	

*adjusted for other factors in the table

Universal Replacement Campaign in Musoma

A total of 398 households were visited in Musoma district by the study team in 2015 of where seven households were found with no nets. The average number of sleeping spaces per household was found to be 3.3 and the average number of people per household was 6.1. Forty-four percent [95% CI: 38.8-48.8%]) of households had at least one URC net with an average of 1.4 URC nets per household. Ten percent [95% CI: 9.2-12.6%] of the households had "enough" URC nets, 23.6% [95% CI 16.7-30.6%] of the population in those households had access to a URC and 27.7% [95% CI 25.9-29.5%] of the population used a URC net the night before the survey (Supplementary Material 2). Of the 1,971 total nets identified in Musoma district, 48.4 % were distributed by the study, 17.0% from URC, 1.9 % from Shop/Market, 0.9% from non-governmental/charity organizations and 31.9% from other sources (unknown to the respondent at the time of the survey). Overall, 84.1% of 1,971 nets were used in the night preceding the survey indicating a use:access ratio of 0.78. The distribution of nets used by source was 47.6% study nets, 18.5% URC and 33.9% of other non-study nets.

Houses with enough nets

In households with enough nets in Musoma district, 85.0% of the nets used were study nets (Table 5). Adults (25-64 years) and children under five reported the highest use of study nets. Youth (15-24) were the main users of nets from other sources when households had enough nets while children (5-14 years) had the highest URC net use (Table 5).

Houses without enough nets

Sixty-four out of 398 households in Musoma district did not have enough nets. All of these households were among lowest two SES groups. Majority of these household members were reported to have slept under a study net (75.0%) the previous night in comparison to the 13.0% under URC nets and 12.0% under nets acquired from other sources (Table 5). Among the study nets used by households that do not have enough nets, Olyset® product was the most used at 36.0% (Table 5). Houses without enough nets had a lower percentage of use of URC nets at 13.0% compared to 18.9% of houses with enough nets.

Table 5: Net use by source of net in Musoma District

				Study Nets N (%)					
	Number of households	**Total Nets**	**Nets used last night (%)**	**Olyset®**	**PermaNet®**	**NetProtect®**	**Total Study nets**	**URC N (%)**	**Other N (%)**
Households with enough ITNs*	334	1833	1558 (85.0)	231 (32.3)	240 (33.6)	243 (34.0)	714 (45.8)	294 (18.9)	550 (35.3)
Households without enough ITNs	64	145	100 (72.5)	27 (36.0)	25 (33.0)	23 (30.7)	75 (75.0)	13 (13.0)	12 (12.0)

*Assuming each net is used by two people

The effect of household access on ITN prioritisation

Pregnant women and children under 5 years were most likely to sleep under an ITN irrespective of the household's ITN access, while young adults (15-24 years) contributed the lowest percentage of ITN users (Fig 1a). Household access to nets clearly affected how nets were allocated within households. In houses with enough nets 77.5% of members slept under ITNs compared to 37.5% of members who did in households without enough nets. There was prioritisation for children<5 and pregnant women in both access scenarios, but in houses without enough nets this prioritisation was more pronounced (Fig 1a).

In households with enough nets, 91.1%% of pregnant women slept under ITNs, 13.6% higher than the household average of 77.5% use. In houses without enough nets, a 17%-point increase in net use among pregnant women was observed when compared to the average household use (54.6% versus 37.4%). For children <5years, 82.9% slept under an ITN, 5.4% higher than the household average of 77.5% use. In houses without enough nets, 45.8% of children <5years slept under ITNs, which is 8.4% higher than the household average use of 37.4%. A smaller proportion of children 5-14 years slept under ITNs compared to the household average in both houses with enough nets (75.7% versus 75.5%) and in households without enough nets (35.9% versus 37.4%). Youth were also less likely to be prioritised to ITNs in houses with enough nets (5% lower than household average) and this was more pronounced in houses without enough nets (9.3% lower than household average). Seniors were less likely to be prioritised to ITN use in houses without enough ITNs, with only 32.6% of them sleeping under nets which was 4.8% lower than the household average, although this was not seen in houses with enough ITNs.

The variation observed in net use across user categories was related to sleeping space allocations.

In descending order; seniors, youths and adults reported the highest percentages of users that slept alone under a net irrespective of whether the household had or did not have enough nets (Fig 1b). Children under the age of 5 and pregnant women were most likely to share a net with another sleeper (Fig 1b).

The effect of household access on the number of people sleeping under an ITN

A total of 2,177 households (1,314 with and 863 without enough ITNs) had ITNs that were used last night. Of the 3,288 mosquito nets found in households without enough ITN's, 25.1% [95% CI: 23.0-27.3] were used by three sleepers while 8.8% [95% CI:8.0-9.7] of the 5,899 nets found in households with enough ITNs were used by three or more people. The proportion of three or more household members sleeping under one net was higher in households without enough ITNs (62.1% [95% CI 60.7-63.6]) compared to those with enough ITNs (30.5% [95% CI 29.2-31.7] (Table 3)). Similarly, use:access ratio of >1 (Table 2) which implies more than 2 people slept under these ITNs [23], was observed in the majority of districts during the TMIS survey, and was more pronounced in Geita, Iringa and Kahama districts which had lower proportions of houses with enough ITNs. When the population net use by three or more sleepers was explored by age category, the trend of crowding in households without enough nets doubled that of households with enough nets for all age categories except for under-fives who are more likely to sleep with their parents (Table 3).

Table 3: Population ITN use by 3 or more people by household access

	Households with enough ITNs			**Households without enough ITNs**		
Number of households with ITNs used last night	1,314			863		
Number of nets found in households	5,899			3,288		
Number of nets used by three or more people	519			824		
% of nets used by three or more people [95%CI]	25.1% [95% CI: 23.0-27.3]			8.8% [95% CI:8.0-9.7]		
Age in years	**n_1**	**n_2**	**Crowded** (95% CI)**	**n_1**	**n_2**	**Crowded ** (95% CI)**
Under 5	612	389	63.6 [59.7-67.3]	814	687	84.4 [81.8-86.8]
5-14	1,446	441	30.5 [28.2-32.9]	1,256	756	60.2 [57.4-62.8]
15-24	945	185	19.6 [17.2-22.2]	630	313	49.7 [45.8-53.6]
25-64	1,880	539	28.7 [26.7-30.8]	1,391	844	60.7 [58.1-63.2]
64+	331	34	10.3 [7.4-14.0]	155	38	24.5 [18.4-31.9]
Total	5,214	1,588	30.5 [29.2-31.7]	4,246	2,638	62.1 [60.7-63.6]

*Assuming each net is used by two people
** Net use by three or more sleepers
n_1=Number of people who slept under net last night
n_2= Number of people who were crowded

ITN serviceability

Holes were counted in 4,783 (68.9%) of the 6,938 study nets 22 months after distribution. Of these, 3,735 (78.1%) nets were still serviceable while 1,048 (21.9%) were unserviceable. Only 3,622 (75.7%) of the 4,783 ITNs assessed for physical damage were used the previous night. Furthermore, 847 (80.8%) of unserviceable nets and 2,775 (74.3) of serviceable nets were used last night. Prioritisation of serviceable nets was also observed. On average, 32.6% people slept under serviceable ITNs last night. Of which around 7% more among pregnant women (40.5%), adults (39.2%), seniors (39.3%) while 5% fewer children 5-14 (27.8%), and 6 % fewer youth 15-24 (26.6%) slept under a serviceable ITN (Fig 1c). Pregnant women reported the highest use of nets irrespective of serviceability (54.2%) followed by adults (49.2%) and children under-five (47.5%) (Fig 1c). Children (5-14 years) and young adults (15-24 years) were less likely to sleep under an ITN and if they did sleep under an ITN it was more likely to be unserviceable (Fig 1c).

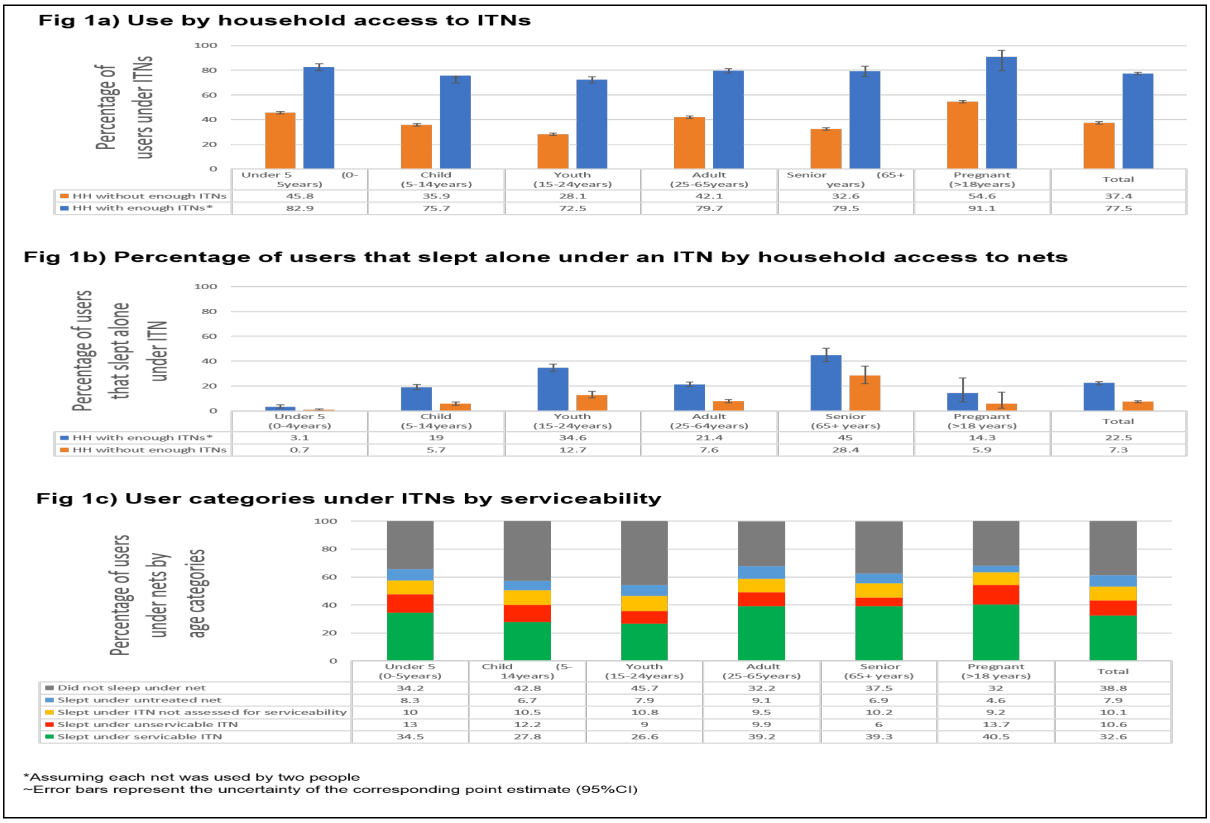

Fig 1: ITN use assessment by user categories and serviceability: *1a) the denominator used is 7,650 ITNs found in the participating households; 1b) while some sleepers slept under an ITN their appropriate age could not be accounted for; and 1c) denomminator includes all 9,178 nets found in households during the survey.*

Results of univariable and multivariable analyses exploring the consequences of net allocation on ITN serviceability are presented in Table 4. The number of people that slept under an ITN, the age category of net users, and socio-economic status were all significantly associated with ITN serviceability (p<0.001) in the univariate analysis. The odds of NetProtect® nets being serviceable was two times the odds of Olyset® nets. ITNs used by children (5-14years) had lower odds of being serviceable compared to those used by under-fives. Controlling for net product and user characteristics (age, gender and socio-economic status), crowding was significantly associated with unserviceable ITNs (P<0.001). Compared to one person under a net, having two people under the net reduced the odds of serviceability to OR=0.75 [95% CI: 0.60-0.83] and having three people under the net reduced the odds of serviceability to OR=0.50 (95% CI: 0.40-0.63).

Table 4: Univariable and multivariable analysis of factors associated with serviceability of study ITNs

			Crude Estimates		**Adjusted Estimates***	
	N	**Number serviceable, n (%)**	**OR (95% CI)**	**P-Value**	**OR (95% CI)**	**P-Value**
# of people under net						
1	1254	1006(80.2)	1	<0.001	1	<0.001
2	866	611 (70.6)	0.60 (0.46-0.77)		0.75 (0.60-0.83)	
3+	788	497 (63.1)	0.45 (0.33-0.59)		0.50 (0.40-0.63)	
User characteristics						
Age (years)						
Under 5	450	312 (69.3)	1		1	
5-14	786	493 (62.7)	0.74 (0.58-0.95)	<0.001	0.72 (0.56-0.93)	<0.001
15-24	392	286 (73.0)	1.19 (0.88-1.61)		1.06 (0.78-1.45)	
25-65	1118	879 (78.6)	1.63 (1.27-2.08)		1.29 (0.99-1.68)	
65+	162	144 (88.9)	3.54 (2.08-6.01)		2.62 (1.51-4.54)	
Socio-economic Status						
Poorest	640	479 (74.8)	1	0.009	1	0.012
Poor	550	393 (71.5)	0.84 (0.65-1.09)		0.85 (0.66-1.11)	
Middle	510	365 (71.6)	0.85 (0.65-1.10)		0.81 (0.62-1.06)	
Wealthy	635	435 (68.5)	0.73 (0.57-0.93)		0.71 (0.55-0.91)	
Wealthiest	537	442 (77.1)	1.13 (0.87-1.48)		1.09 (0.83-1.43)	
Gender						
Male	1,338	951 (71.1)	1	0.070	1	0.081
Female	1,570	1,163 (74.1)	1.16 (0.99-1.37)		1.16 (0.98-1.38)	
Net product						
Olyset®	1520	1066 (70.1)	1	<0.001	1	<0.001
PermaNet®	1667	1317 (79.0)	1.26 (1.04-1.53)		1.32 (1.08-1.61)	
NetProtect®	1596	1349 (84.5)	1.95 (1.58-2.40)		2.08 (1.68-2.58)	

*adjusted for other factors in the table

Universal Replacement Campaign in Musoma

A total of 398 households were visited in Musoma district by the study team in 2015 of where seven households were found with no nets. The average number of sleeping spaces per household was found to be 3.3 and the average number of people per household was 6.1. Forty-four percent [95% CI: 38.8-48.8%]) of households had at least one URC net with an average of 1.4 URC nets per household. Ten percent [95% CI: 9.2-12.6%] of the households had "enough" URC nets, 23.6% [95% CI 16.7-30.6%] of the population in those households had access to a URC and 27.7% [95% CI 25.9-29.5%] of the population used a URC net the night before the survey (Supplementary Material 2). Of the 1,971 total nets identified in Musoma district, 48.4 % were distributed by the study, 17.0% from URC, 1.9 % from Shop/Market, 0.9% from non-governmental/charity organizations and 31.9% from other sources (unknown to the respondent at the time of the survey). Overall, 84.1% of 1,971 nets were used in the night preceding the survey indicating a use:access ratio of 0.78. The distribution of nets used by source was 47.6% study nets, 18.5% URC and 33.9% of other non-study nets.

Houses with enough nets

In households with enough nets in Musoma district, 85.0% of the nets used were study nets (Table 5). Adults (25-64 years) and children under five reported the highest use of study nets. Youth (15-24) were the main users of nets from other sources when households had enough nets while children (5-14 years) had the highest URC net use (Table 5).

Houses without enough nets

Sixty-four out of 398 households in Musoma district did not have enough nets. All of these households were among lowest two SES groups. Majority of these household members were reported to have slept under a study net (75.0%) the previous night in comparison to the 13.0% under URC nets and 12.0% under nets acquired from other sources (Table 5). Among the study nets used by households that do not have enough nets, Olyset® product was the most used at 36.0% (Table 5). Houses without enough nets had a lower percentage of use of URC nets at 13.0% compared to 18.9% of houses with enough nets.

Table 5: Net use by source of net in Musoma District

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Households with enough ITNs*	334	1833	1558 (85.0)	231 (32.3)	240 (33.6)	243 (34.0)	714 (45.8)	294 (18.9)	550 (35.3)
Households without enough ITNs	64	145	100 (72.5)	27 (36.0)	25 (33.0)	23 (30.7)	75 (75.0)	13 (13.0)	12 (12.0)

*Assuming each net is used by two people

Discussion

Twenty-two months post ITN distribution, 57% of households still owned enough ITNs and 84% of the population had access to an ITN within their household assuming each net was used by two household members. These results agree well with a multi-country survey assessment [39] and shows that, distributing nets to cover sleeping spaces identified in the households or limiting the number of nets a household can receive at a time results in a low percentage of households with enough nets. This delivery strategy however, has potential to ensure most of the population residing in the household have access to a net. In Mozambique [40], assumptions on user characteristics, such as age and gender, to assess the likelihood of sharing a sleeping space were used by the NMCP to guide allocation of nets per sleeping spaces available in a household. Net use among children under the age of five in Uganda was reported to be influenced by whom they shared the sleeping space with [41]. Availability of a suitable place to hang net and/or sleeping space also affect ITN use [42].

This study also showed evidence that as the number of people sleeping under an ITN increases ("crowding"), the number of serviceable nets in a household decrease. Eighty percent of household members were observed to sleep under a net when the person:net ratio was 3:1 and this decreased to 50% of the population using a net when four or more people slept under a single net 3:1 [30]. While the use:access ratio observed in Table 2 may vary due to season of data collection, the high (>1) ratio indicates that as access to nets decreases within households, crowding increased which in turn will hasten net damage and increase risk of malaria incidence. In Yemen, non-use of ITNs was associated with ownership of multiple damaged nets [43]. In Liberia [24], a 32% reduction in ITN use was associated with increase in household size while having three or more nets was associated with increased odds of ITN use. Importantly, mosquitoes are more attracted to households with a large family [44], so family size does need to be considered in the design of ITN distribution campaigns. Higher parasitaemia was observed among those with low ITN use in Tanzania [45] while malaria incidence in Senegal [46] rose after the third year when ITNs were assumed to have decreased protection. Therefore, it may be beneficial to distribute slightly too many nets rather than too few nets to ensure households have enough serviceable ITNs to cover the population available to slow the process of net damage as the protective effect of ITNs declines through time as nets age [47].

Physical degradation of the net products was also observed to vary by product after 22 months of ownership. NetProtect® was two times more likely to be serviceable when compared to Olyset® in this setting. When compared to PermaNet®, Olyset® nets have been observed to have more holes in both Mozambique [48], Zambia [49] and Zanzibar [50] and mainland Tanzania [32]. In Madagascar [51], 55.6% of NetProtect® ITNs were in good condition after a year when compared to Royal Sentry® (56.8%) and Yorkool® (69.2%), which is lower than in the current study. The longitudinal assessment of Olyset®, PermaNet® and NetProtect® efficacy [32] showed NetProtect® has a mean (95% CI) pHI of 152 (13-838) after three years agreeing with the findings of the current study. However, an analysis of PMI-country surveys found that the variation of overall durability of ITNs was larger between countries than among net types, although the durability of net types does vary within countries [52, 53]. A literature and data review by Koenker and Yukich [54] found that product attributes do not affect use, agreeing with this study which shows NetProtect® was used equally to the other products but was only found to be more durable in Tanzania. The Tanzania NMCP should consider procuring the most appropriate longer-lasting ITN product to be distributed to ensure those nets distributed last for the intended interval between campaigns.

Population access was 84.4% just prior to the URC campaign in the study population with the exception of Musoma district who had already received their campaign nets in addition to study nets that increased access to 94.3%. Unfortunately, despite the URC that was conducted August 2015 - Jan 2017, none of the participating districts recorded an increase in population access according to the Tanzania Malaria Indicator Survey [14] that was conducted October-December, 2017, two years after the first district received their URC nets(Ikupa Akim, pers Comm). A 10% annual decrease in population access was also observed by Odufuwa et al [55] in both Ulanga and Bagamoyo districts. These findings suggest that the current 4-year universal coverage distribution intervals are too widely spaced, not in line with the WHO recommendations for mass distribution campaigns [11], and will provide suboptimal impact of ITNs for malaria control in Tanzania. Mass distribution campaigns distribute one ITN for every two household members, and generally result in lower access so it may be worth following the WHO recommendation of 3-year intervals to maintain health gains. Fortunately, Tanzania has adopted continuous distribution channels through the antenatal and immunization clinics, and the school-program [56], which will be essential to maintain universal coverage as also recommended by WHO [11]. The school-net distribution program is particularly important as the current study found that children of school age are most likely to be unprotected with either no net at all, or an unserviceable net and this age group has been reported as an infectious reservoir [57-59]. This is not a new finding as it was shown as early as 2009 that school age children are not prioritised for ITNs [60]. However, it was seen that in houses with enough nets all age groups are likely to have access to ITNs. It is therefore prudent to maximise household ITN access during mass campaigns to ensure that all household members use nets and are not forced crowding under nets that is associated with decreased net serviceability.

Increasing access to nets within a household increases net use, which in turn will eliminate inequalities between age and gender [29]. Contrary to the study by Tsuang et al [30], where infants were prioritized to use new nets, in Musoma, children and youth had the highest use of newly acquired URC or nets from other sources. Therefore, while the school-aged children were less prioritized to use existing study nets irrespective of the household's access to enough nets, they were accommodated by the arrival of new nets. Attributed to both studies is the observation that each targeted group was reached by its respective distribution mechanism (Tanzania National Voucher Scheme [targeted pregnant women and infants] [61, 62] or SNP [5, 56, 63]) while the lack of sufficient access to nets in the households left older children to use unserviceable nets or remain uncovered.

Study limitations

The study distributed one ITN for every sleeping space identified during enrolment instead of using the recommended practice of one ITN for every two household members. While this distribution mechanism may have prevented distribution of excess ITNs to household members without unique sleeping spaces, it biased household and population access to ITNs to higher levels than would be achieved by national campaigns from enrolment.

There is also a challenge in the definition and measurement population access in assuming each ITN is used by two people. For example, if a woman of 25 years old is living with her uncle and they have only one net, in principle as per the MERG indicators for measuring household mosquito net distribution, population access is complete. However, in practice, these two people are unlikely to sleep under the same net, leaving one household member uncovered and population access incomplete. Therefore, this was a challenge while assessing population access that couldn't be changed or controlled for.

While even torn nets still offer chemical protection against mosquitoes [64, 65], including unserviceable nets (which require replacement soon) in the calculation of population access, overestimates the proportion of household members with access to a net that is fully protective within their household.

A maximum of only three nets per household were assessed for their physical condition. The three nets were randomly chosen potentially missing out 1) the most damaged nets in households, and 2) how sleeping arrangements of the population are affected by the physical status of the other nets. Quantifying all the ITNs would further inform the prioritization of net use in larger households with more than 3 nets.

Conclusion

Twenty-two months post ITN distribution of nets to all sleeping spaces, the percentage of the population with access to ITNs among the study population was above the target of 80%, and 57% of households had enough ITNs. The URC mass campaign helped to further maintain universal access to ITNs in Musoma district. These findings indicate that households hold on to their ITNs despite the arrival of new ones. Crowding under ITNs was associated with lower ITN serviceability most likely due to physical stress on the ITN fabric that causes physical damage to occur faster, thereby reducing the serviceable life of the net. When households have enough nets, around 80% of members from all age categories have access to a net. However, when there are insufficient nets, children (5-15years) and youth (15-24years) were least likely to use any ITN or have access to a serviceable ITN. This is of significant biological importance since school age children carry gametocytes that cause transmission of malaria from humans to mosquitoes and maintain malaria transmission. Therefore, there is a need to refine delivery strategies to ensure households, including larger households to receive enough nets to cover all sleeping spaces. Larger households will always be at a disadvantage and have lower ITN access due to current limitations on the number of ITNs they can receive or use at a time. As fewer nets are distributed or if households have limited number of sleeping spaces, more people are forced to either crowd under nets or remain uncovered. Hence, more frequent and more informed ITN distribution through keep up strategies such as the school-net program is essential to address these coverage inequalities and ensure continued protection against malaria transmission for all household members.

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9.3. Annex 3: Journal Paper- Comparative functional survival and equivalent annual cost of 3 long-lasting insecticidal net (LLIN) products in Tanzania: A randomised trial with 3-year follow up

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Abstract

Background

Two billion long-lasting insecticidal nets (LLINs) have been procured for malaria control. A functional LLIN is one that is present, is in good physical condition, and remains insecticidal, thereby providing protection against vector-borne diseases through preventing bites and kill- ing disease vectors. The World Health Organization (WHO) prequalifies LLINs that remain adequately insecticidal 3 years after deployment. Therefore, institutional buyers often assume that prequalified LLINs are functionally identical with a 3-year lifespan. We mea- sured the lifespans of 3 LLIN products, and calculated their cost per year of functional life, to demonstrate the economic and public health importance of procuring the most cost-effective LLIN product based on its lifespan.

Methods and findings

A randomised double-blinded trial of 3 pyrethroid LLIN products (10,571 nets in total) was conducted at 3 follow-up points: 10 months (August–October 2014), 22 months (August–October 2015), and 36 months (October–December 2016) among 3,393 households in Tan- zania using WHO-recommended methods. Primary outcome was LLIN functional survival (LLIN present and in serviceable condition). Secondary outcomes were (1) bioefficacy and chemical content (residual insecticidal activity) and (2) protective efficacy for volunteers sleeping under the LLINs (bite reduction and mosquitoes killed). Median LLIN functional sur- vival was significantly different between the 3 net products ($p = 0.001$): 2.0 years (95% CI 1.7–2.3) for Olyset, 2.5 years (95% CI 2.2–2.8) for PermaNet 2.0 (hazard ratio [HR] 0.73 [95% CI 0.64–0.85], $p = 0.001$), and 2.6 years (95% CI 2.3–2.8) for NetProtect (HR = 0.70 [95% CI 0.62–0.77], $p < 0.001$). Functional survival was affected by accumulation of holes, leading to users discarding nets. Protective efficacy also significantly differed between prod- ucts as they aged. Equivalent annual cost varied between US$1.2 (95% CI $1.1–$1.4) and US$1.5 (95% CI $1.3–$1.7), assuming that each net was priced identically at US$3. The 2 longer-lived nets (PermaNet and NetProtect) were 20% cheaper than the shorter-lived prod- uct (Olyset). The trial was limited to only the most widely sold LLINs in Tanzania. Functional survival varies by country, so the

single country setting is a limitation.

Conclusions

These results suggest that LLIN functional survival is less than 3 years and differs substantially between products, and these differences strongly influence LLIN value for money. LLIN tendering processes should consider local expectations of cost per year of functional life and not unit price. As new LLIN products come on the market, especially those with new insecticides, it will be imperative to monitor their comparative durability to ensure that the most cost-effective products are procured for malaria control.

Author summary

Why was the study done?

- Over 2 billion long-lasting insecticidal nets (LLINs) have been procured for malaria control. Modelling has shown that longer-lasting LLINs would save stakeholders between US$500 million and US$700 million over a period of 5 years, yet LLIN tendering processes currently assume that all LLINs have the same lifespan.
- A functional LLIN must remain in the household, in good physical condition, and with adequate insecticidal activity to give good protection against malaria by preventing bites and killing mosquitoes.
- Before this study, only a few small studies in distinct geographical areas had compared the functional life of alternative LLIN products, mostly retrospectively.
- This 3-year randomised trial was designed to accurately compare the functional life of 3 leading LLIN brands, in order to help the Tanzanian government and other LLIN buyers to choose the most cost-effective LLINs.

What did the researchers do and find?

- We randomised 3,393 households in Tanzania to 1 of 3 LLIN products (10,571 nets in total) and followed them for 3 years using methods recommended by the World Health Organization.

- This study showed that the functional life of LLINs in domestic use is less than 3 years and differs substantially between products. The main reason for different lifespans between brands was differential accumulation of physical damage that results in users discarding nets that they think are no longer protective. However, tests showed that all LLIN products were still partially protective against pyrethroid-susceptible mosquitoes after 3 years.

- In this trial, the most durable LLIN product was 20% more cost-effective (economic cost per year of effective life) than the least durable.

What do these findings mean?

- Based on direct observation of a large number of nets in a range of study areas, our findings support previous studies suggesting that the functional life of LLINs may be less than 3 years.

- Our findings reveal that the lifespans of competing products can differ to a substantial and economically important degree.

- More durable LLINs would reduce the rate of loss of nets and the operational costs of malaria control, ultimately improving population access to this life-saving intervention.

- This study provides justification that measurement of the functional survival of new LLINs coming to market is an essential component of product evaluation for decision making. Functional survival affects LLIN cost; therefore, tendering processes should include a net durability component not just unit price.

Introduction

The use of long-lasting insecticidal nets (LLINs) remains the most cost-effective way to control malaria and reduce mortality [1], notwithstanding insecticide resistance [2]. However, despite the procurement of 254 million LLINs in 2017 alone, global LLIN coverage remains inade- quate, with only 56% of the population in endemic areas estimated to have access to a LLIN [3]. LLINs are mostly distributed through periodic mass distribution campaigns, and as a result, population access to LLINs fluctuates over time. Access is typically high directly after a mass campaign and then declines as nets wear out, often to 50% or less, until the next cam- paign. This fluctuating pattern of coverage, caused by nets wearing out, is seen across the Afri- can region [4], where gains in malaria control have stalled, and fewer than 50% of endemic countries remain on track to reach critical malaria reduction targets [3]. Investment in malaria control has stagnated and was US$2.3 billion (50%) below the resources required to meet the World Health Organization (WHO) targets of 40% reductions in malaria case incidence and mortality rates by 2020 [5]. These gaps in funding and coverage emphasise the need to deploy products that present the best value for money.

A report to the Malaria Policy Advisory Committee (MPAC) advised that increasing the functional life of LLINs by 1 or 2 years would reduce the cost of malaria control by between US$500 million and US$700 million over a period of 5 years [6]. A functional LLIN is one that is present, is in good physical condition, and remains insecticidal, thereby providing protec- tion against vector-borne diseases through preventing bites and killing disease vectors [6]. Durability, or functional survival, of LLINs varies between geographical regions [7] and envi- ronments [8,9] and remains an undervalued but critical determinant of the success and effi- ciency of malaria control programmes [10,11]. How long LLINs remain protective under user conditions will dictate how frequently they should be replaced, which has both public health and economic implications [12]. In 2011, it was calculated that in Tanzania, for mean LLIN lifespans of 2, 3, and 4 years, 89, 63, and 51 million LLINs, respectively, would be needed over 10 years to achieve national access targets [10].

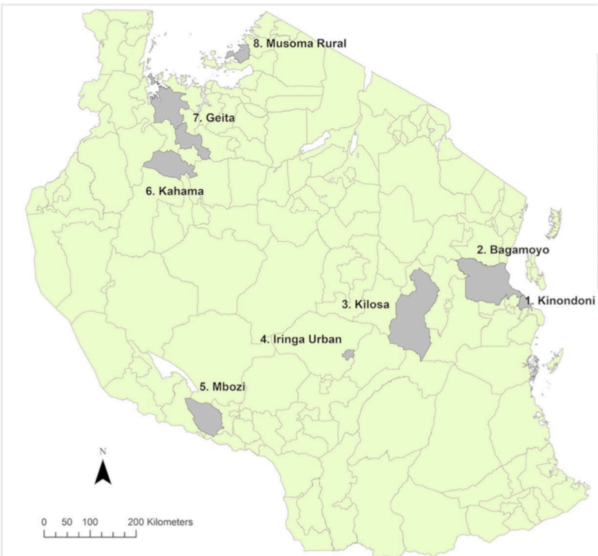
Currently, WHO prequalifies products that demonstrate adequate insecticidal activity 3 years after deployment, but does not appraise the physical deterioration of nets over time as part of the LLIN prequalification assessment [13]. Historically, pyrethroid-treated LLINs were assessed in multi-country studies for physical and chemical durability over an anticipated life- span of 3 years and 20 washes. In the mid-2000s, when these procedures were designed, we did not yet know the relative importance of attrition—the disappearance of nets from study house- holds—as one of the main factors limiting the duration of protection from LLINs. Unfortu- nately, even after the importance of attrition had become very clear, the evaluation criteria were never changed to take account of it. Thus, of the nets tested in the current study, Perma- Net 2.0 received WHO recommendation (now prequalification) based on pooled prospective data from 6 countries, where 80% of remaining nets met bioefficacy and net fabric integrity criteria [14]; Olyset received recommendation based on pooled retrospective data from 7 countries, where 77% of nets passed bioefficacy criteria, although net loss and damage could not be accurately assessed [15]; and NetProtect did not receive full recommendation due to inconsistencies in data between WHO-sponsored studies [16,17], and was withdrawn from the market after the trial reported here had started.

The WHO prequalification website lists a number of newly prequalified products as long- lasting (LLINs) [18], including some with active ingredients other than pyrethroids. The listing of these products was based on experimental hut data from 2 or 3 sites. Fabric integrity, resid- ual chemical content, and bioefficacy data for products after operational household use through longitudinal studies or post-marketing surveillance are requested, but are not a requirement for prequalification. This has resulted in a tendering process where donors assume LLINs are identical, and procurement is weighted by the unit price of the commodity without regard to actual product lifespan [19]. However, all the available data suggest that the assumption of a uniform 3-year lifespan for all LLIN products is unrealistic [4]. There is a clear need for a more integrative economic approach, with purchasing decisions based on value for money and cost per effective unit of LLIN coverage [6,19]. New product classes of LLINs with novel active ingredients for insecticide resistance management are becoming available [20], but they remain susceptible to the same forces of physical disintegration, being discarded, and losing insecticidal activity. Moreover, in most cases, they are more expensive. This emphasises the need to consider the price of LLINs in terms of cost per year of functional life [12].

Here we report results from a large randomised trial of 3 LLIN products (PermaNet 2.0, Olyset, and NetProtect), conducted in 8 epidemiologically and ecologically distinct districts in Tanzania. The proportion of LLINs remaining in use and still protective against malaria mos- quitoes was measured over 3 years of follow-up after deployment. We calculated relative LLIN cost-effectiveness in terms of the equivalent annual cost (EAC), which is a conventional financial indicator used to compare products with different effective lifetimes. The median functional survival of each product and its EAC were calculated to inform optimal procurement of cost-effective LLINs.

Methods

The trial has been described in detail previously [21]. It took place in 8 districts in Tanzania, selected to be representative of national environmental, ecological, and epidemiological settings (Fig 1). Within each district, 10 villages were randomly selected (except for Kinondoni [Dar es Salaam], where only 6 areas were available), and within each village, 45 households were recruited to participate in the trial.



	District	Malaria Prevalence Min	Malaria Prevalence Max	Zone
1	Kinondoni	0.3	3.6	Dar es Salaam
2	Bagamoyo	7.4	10.2	Eastern
3	Kilosa	6.9	13.0	Eastern
4	Iringa Urban	0	0.4	Southern Highlands
5	Mbozi	0	4.5	Southern Highlands
6	Kahama	4.4	6.8	Western
7	Geita	21.0	32.9	Lake
8	Musoma Rural	14.6	25.6	Lake

Fig 1. Map of trial districts with 2015 malaria prevalence data (percent of children aged 6–59 months diagnosed with malaria by rapid diagnostic test and microscopy) [22]. Open-access shapefiles from https://www.nbs.go.tz/index.php/en/census-surveys/gis.

All households were randomised to receive 1 of 3 LLIN brands on a 1:1:1 ratio, stratified by village. The 3 brands were Olyset (manufactured with an enhanced knitting pattern that was introduced in 2013; permethrin incorporated in 150 denier polyethylene; Sumitomo Chemicals, Japan), PermaNet 2.0 (deltamethrin coated on 100 denier polyester; Vestergaard Frandsen, Switzerland), and NetProtect (deltamethrin incorporated in 110 denier polyethylene; BestNet, Denmark). Distribution of trial nets took place between October and December 2013. All nets owned by the participating households were collected and replaced with enough nets to cover all sleeping spaces. Before distribution, a sample of 10 nets per product was quality tested. Nets were the same size and colour and labelled by a 5-digit serial number so that participants and investigators remained blinded to the LLIN product until data collection was complete. In total, 3,393 households were randomised (1,132 to Olyset, 1,127 to PermaNet 2.0, and 1,134 to NetProtect), to which 10,571 nets were distributed.

Surveys were conducted among all consenting trial households when the LLINs were distributed and at 3 follow-up points: 10 months (August–October 2014), 22 months (August– October 2015), and 36 months (October–December 2016) (S1 Table). The serial numbers of the nets, linked to household-identifying codes in a master list, enabled follow-up of each net at each time point. At each follow-up visit, information on each LLIN was collected, including whether the net was present in the house, whether the net was in use, and, if the net was not present, reasons why it was not present. Physical integrity of LLINs was measured on a random sample of 3 nets per household by counting the number, location and size of holes [13,23]. Socioeconomic variables and a household member roster were also recorded. Electronic data capture was used for all surveys.

In addition to the data collected as part of the household surveys, at each time point 48 LLINs from each brand were randomly sampled from the master list and returned to a laboratory in Bagamoyo, Tanzania, for bioefficacy and chemical analysis using standard WHO methods [13,23] and, additionally, the Ifakara Ambient Chamber Test (IACT) [24]. Households received new nets to replace those removed for destructive sampling. Once a house had been sampled, it was eliminated from the master list to prevent confounding of results. Table 1 describes the different components of LLIN durability, the tests conducted to obtain the data, the outcome indicators for statistical analysis, and the corresponding WHO threshold criteria [6,13,23]. The numbers of LLINs tested for each of the components of LLIN durability are listed in S1 Table.

Table 1. LLIN durability components.

Component	Definition	Test conducted	Outcome indicators	WHO criteria or industry standard
Attrition	Net loss from household through discarding or alternative use	Household survey	Net presence	
Physical integrity	Physical state of the net to estimate bite protection	Count number, location, and size of hole(s) of a maximum 3 nets per household	Holed surface area measured by pHI [6] or MHSA (cm^2)	pHI 0–64, MHSA ≤ 79 cm^2: good
				pHI 65–642, MHSA 80–789 cm^2: damaged
				pHI ≤ 642, MHSA ≤ 789 cm^2: serviceable
				pHI ≥ 643, MHSA ≥ 790 cm^2: too torn/unserviceable
Functional survival [6]	Estimation of nets still in households in serviceable condition	Median survival analysis	(Number of nets present and serviceable)/(number of nets originally received and not given away or lost to follow-up)	Median net survival in years = time point at which the estimate of functional survival crosses 50%
Biological efficacy	Ability of net to incapacitate or kill susceptible anopheline mosquitoes after contact with insecticide	IACT: whole nets [24]	Proportion of mosquitoes dead at 24 hours	
			Proportion of mosquitoes not blood fed	
		WHO cone/tunnel test: 25 × 25 cm pieces [13]	Net samples meeting optimal bioefficacy criteria	1-hour knock-down ≥ 95% or 24-hour mortality ≥ 80% or blood feeding inhibition ≥ 90%
Insecticide content	Amount of active ingredient in the net	Permethrin: GC-FID Deltamethrin: HPLC-DAD	Compliance of nets with WHO specifications at baseline; loss of active ingredient over time	Olyset: 20 g/kg ± 25% [15–25 g/kg] PermaNet: 1.4 g/kg ± 25% [1.05–1.75 g/kg] NetProtect: 1.8 g/kg ± 25% [1.35–2.25 g/kg]

GC-FID, gas chromatography with flame ionisation detection; HPLC-DAD, high-performance liquid chromatography with diode array detection; IACT, Ifakara Ambient Chamber Test; LLIN, long-lasting insecticidal net; MHSA, median hole surface area; pHI, proportionate hole index; WHO, World Health Organization.

First, the protective efficacy of whole nets returned from the field was evaluated using IACT [24]. Each night, 10 male volunteers slept underneath 1 of the nets (or an untreated control net to monitor the quality of the bioassay) between 9 PM and 6 AM in a small chamber similar in size to a bedroom, within a screened compartment. At 9 PM, 30 laboratory-reared mosquitoes were released into the chamber. The next morning, all mosquitoes within the compartment were recaptured, and scored for 24-hour mortality and blood feeding inhibition. Each LLIN was tested twice on 2 consecutive nights. Subsequently, net pieces (25 × 25 cm2) were cut fol- lowing the WHO sampling pattern and standard WHO cone bioassays were carried out [13]. If nets did not meet WHO optimal bioefficacy criteria for cone tests (Table 1), WHO tunnel tests were conducted [13]. All mosquito assays were conducted with fully pyrethroid-suscepti- ble 2- to 8-day-old nulliparous female *Anopheles gambiae* sensu stricto (Ifakara strain). Insecti- cide content analyses were performed at Walloon Agricultural Research Centre (CRA-W) using standard Collaborative International Pesticides Analytical Council Limited (CIPAC) methods for determining LLIN insecticide content (Olyset, 331/LN/M/3; PermaNet 2.0, 333/ LN/(M)/3; NetProtect, 333/LN/(M2)/3).

Statistical analysis

All statistical analyses were conducted using Stata release 13 (StataCorp, College Station, TX). Data from the surveys at 10, 22, and 36 months were used to calculate attrition and functional survival (Table 1) using Kaplan–Meier estimators. For both attrition and functional survival, nets reported as given away, sold, or stolen were treated as lost to follow-up. Hazard ratios (HRs) for the difference in attrition and functional survival were calculated using discrete time survival analysis using a complementary log-log model [25]. Robust standard errors were used to account for the highest level of clustering (district) [26]. Of nets that were present, net condition was defined, following WHO recommendations, as ‘good’ or ‘damaged’ (combined as ‘serviceable’) or ‘too torn/unserviceable’ (Table 1). Negative binomial regression was used to compare hole surface area between net products. Data on WHO bioassays and the IACT test came from the 48 nets sampled at each time point. For WHO bioassays and the IACT test, if control mortality for an assay of a section of net was over 10%, the data from that section were not included in the analysis. A chi-squared test assessed the proportion of nets of each product passing the WHO bioefficacy criteria based on combined cone and tunnel tests. Logistic regression was used to analyse mortality and blood feeding inhibition from the IACT test; results were adjusted for chamber and experimental night, and robust standard errors were used to take account of nets being tested multiple times. A further analysis was conducted to test for differences in mortality between net brands in the IACT test based on net condition, in which net condition (defined above) was adjusted for as a fixed effect.

Economic analysis

The EAC of an LLIN was calculated according to the standard formula [27]. To assess the value of longer functional survival, we used Eq 1, where b is the ratio of the lifespan of the more durable product to the lifespan of reference net n. The variable r is the discount rate. This relationship shows, for any change in net lifespan from n to bn, the relative increase in price, a, that would yield an identical EAC for the 2 products. Other factors being equal, a relative price increase less than a would favour the new, longer-lasting LLIN, while relative price increases greater than a would favour the reference net.

$$a = \frac{1-(1+r)^{-bn}}{1-(1+r)^{-n}} \tag{1}$$

Simulation of EACs for products tested in the trial was conducted using Monte Carlo methods, assuming a 3% discount rate, as is standard in health economic analysis. The baseline survival function for LLINs was estimated by regressing the survival proportions of Olyset nets derived from Kaplan–Meier analysis against time. The survival function was converted into a baseline hazard, and net failure lifetimes were simulated for a cohort of 500 LLINs assuming a Weibull distribution of time to failure (in terms of functional survival). The results of the cohort were summarised by estimating the median lifetime, and this process was repeated 10,000 times for each net type, yielding an estimate of the expected median lifetime and quantiles of its expected distribution. Results were converted into EACs with 95% quantiles. Distributional assumptions for the baseline hazard and the parameters of the Weibull distribution were fitted to the results. The baseline hazard and proportional hazard were simulated with log normal distributions (S2 Table).

Ethics

Ethical approval was granted by ethical review committees at the London School of Hygiene & Tropical Medicine (6333/A443), Ifakara Health Institute (IHI/IRB/AMM/No: 07–2014), and the Tanzanian National Institute for Medical Research (NIMR/HQ/R.8c/Vol. I/285). Community sensitisation meetings were held prior to trial inception, and written informed consent was obtained from the head of the household or another adult household member of participating households before each survey. Volunteers for the IACT experiment were all Ifakara Health Institute staff members with appropriate training who gave written informed consent.

Results

A total of 3,393 households were randomised, to which 10,571 nets were distributed (3,520 Olyset [33%], 3,513 PermaNet 2.0 [33%], and 3,538 NetProtect [33%]). The 3 trial arms were similar in number of participants, number of nets allocated, household characteristics, house design, and socioeconomic characteristics (Table 2). The proportion of households lost to follow-up was 20% over the 3 years of the trial.

Table 2. Household and socioeconomic characteristics of participating households in each trial arm.

Characteristic	Olyset	PermaNet 2.0	NetProtect
Number of nets distributed	3,520	3,513	3,538
Number of participants	6,061	6,024	6,200
Number of households	1,132	1,127	1,134
Average household size	5.8	5.8	6.5
Mean sleeping spaces per household	3.65	3.55	3.55
Mean nets per household	2.92	2.96	3.04
Male household members (%)	49	48	49
Female household members (%)	51	52	51
Age distribution of household members (%)			
≤5 years	16.64	17.21	17.56
6–17 years	33.16	33.27	34.19
18–50 years	37.61	39.16	37.73
≥51 years	12.60	10.36	10.52
Highest level of education of household head (%)			
No education	21.62	19.99	20.69
Some primary education	30.23	29.26	20.69
Completed primary school	32.60	33.54	39.66
Secondary education	6.45	6.75	5.17
Housing materials (%)			
Roof: thatch	19.88	17.11	17.08
Roof: tin	79.89	82.60	82.56
Walls: mud and sticks	17.30	14.96	14.65
Walls: mud brick	24.15	21.81	22.18
Walls: burned brick	40.32	43.54	43.98
Walls: cement brick	18.23	19.69	19.19
Floor: mud	52.97	48.42	49.89
Floor: cement	43.17	46.13	44.48
Socioeconomic quintile (%)			
1 (least wealthy)	21.90	18.99	19.23
2	20.59	19.06	20.60
3	19.85	20.12	20.29
4	19.70	20.65	19.52
5 (most wealthy)	17.96	21.18	20.37

Functional survival

There were significant differences in functional survival (defined as presence of serviceable net) of the 3 products (Table 3). Estimated median functional survival was 2.0 years (95% CI 1.7–2.3) for Olyset, 2.5 years (95% CI 2.2–2.8) for PermaNet, and 2.6 years (95% CI 2.3–2.8) for NetProtect ($p < 0.001$). There was no significant difference in net use by net product (S3 Table).

Economic analysis

Simulation results show that the expected EAC in US dollars of the 3 LLINs in the trial varied between $1.2 (95% CI $1.1–$1.4) for PermaNet and NetProtect and $1.5 (95% CI $1.3–$1.7) for Olyset, assuming that each net was priced identically at $3.0 (Table 3). The longer-lived net products (PermaNet and NetProtect) were approximately 20% lower in EAC than the shorter-lived Olyset product.

Table 3. Percentage net functional survival (defined as presence of the net in the house and in serviceable condition) and simulated equivalent annual cost (assuming S$3.0 purchase price) by net product and time point.

Net product	Percent functional survival (95% CI)			Median survival in years (95% CI)[†]	Hazard ratio (95% CI), *p*-value	Simulated equivalent annual cost in US dollars (95% CI)
	10 months	22 months	36 months			
Olyset	82 (79, 85)	54 (47, 62)	27 (20, 34)	2.0 (1.7, 2.3)	1	1.5 (1.3, 1.7)
PermaNet	88 (85, 90)	65 (57, 72)	38 (31, 46)	2.5 (2.2, 2.8)	0.73 (0.64, 0.85), $p = 0.001$	1.2 (1.1, 1.4)
NetProtect	88 (84, 91)	67 (61, 72)	40 (34, 45)	2.6 (2.3, 2.8)	0.70 (0.62, 0.77), $p < 0.001$	1.2 (1.1, 1.4)
					$p = 0.001$*	

[†]Details of the survival analysis are provided in S4 Table.

*p-Value for the comparison between the 3 nets. For the difference between PermaNet and Netprotect, $p = 0.199$.

Components of functional survival and secondary outcomes

Attrition. There were significant differences in attrition between net products. Olyset nets were lost at a faster rate than PermaNet 2.0 and NetProtect nets (Table 4).

Table 4. Percentage attrition (defined as net loss due to discarding or alternative use of nets) and hazard ratios after 36 months by net product and time point.

Net product	Percent attrition (95% CI)			Hazard ratio (95% CI), *p*-value
	10 months	22 months	36 months	
Olyset	7 (5, 8)	25 (21, 29)	55 (49, 61)	1
PermaNet	5 (3, 6)	20 (17, 24)	42 (38, 46)	0.71 (0.64, 0.79), $p < 0.001$
NetProtect	6 (4, 8)	22 (18, 26)	46 (43, 50)	0.81 (0.71, 0.93), $p = 0.008$
				$p < 0.001$*

Details of the analysis are provided in S5 Table. Number of nets remaining in households by time point: 10 months, 8,269 nets; 22 months, 6,324 nets; 36 months, 3,942 nets.

*p-Value for the comparison between the 3 nets. For the difference between PermaNet and NetProtect, $p = 0.006$.

After 3 years, 55% of Olyset nets were no longer present in households, compared to 42% of PermaNet 2.0 and PermaNet 2.0 nets met WHO optimal bioefficacy criteria, compared to 73% of Olyset nets ($p < 0.001$). Nets decreased in bioefficacy through time, but even after 3 years, 96% of NetProtect, 85% of PermaNet 2.0, and 75% of Olyset nets met WHO criteria for bioefficacy ($p = 0.017$; Table 5).

Table 5. Percentages of net products meeting optimal WHO bioefficacy criteria by time point.

Net product	WHO cone test			WHO tunnel test			Overall (cone + tunnel)		
	10 months	22 months	36 months	10 months	22 months	36 months	10 months	22 months	36 months
Olyset	4	8	14	72	78	71	73	79	75
	(1, 14)	(2, 20)	(5, 27)	(57, 84)	(62, 89)	(54, 85)	(58, 85)	(65, 90)	(60, 87)
	[2/48]	[4/48]	[6/44]	[33/46]	[34/44]	[27/38]	[35/48]	[38/48]	[33/44]
PermaNet	98	92	73	100	50	46	100	96	85
	(89, 100)	(80, 98)	(58, 85)	(3, 100)	(7, 93)	(19, 75)	(92, 100)	(85, 99)	(72, 94)
	[46/47]	[44/48]	[35/48]	[1/1]	[2/4]	[6/13]	[47/47]	[46/48]	[41/48]
NetProtect	100	100	73	n/a	n/a	85	100	100	96
	(92, 100)	(93, 100)	(58, 85)			(55, 98)	(92, 100)	(93, 100)	(86, 99)
	[47/47]	[48/48]	[35/48]			[11/13]	[47/47]	[48/48]	[46/48]
							<0.001*	<0.001*	0.017*

95% confidence intervals in parentheses. Numbers passing/numbers tested in square brackets [*n/N*]. Nets are tested by cone test, and those that fail WHO optimal insecticide effectiveness criteria of ≥95% knock-down after 60 minutes or ≥80% 24-hour mortality are then further tested by tunnel test. Optimal criteria for the tunnel test are ≥80% 24-hour mortality or ≥90% blood feeding inhibition. Overall pass (cone and tunnel) is based on a net achieving 1 or more of these 4 criteria.

**p*-Value for the comparison between the 3 nets. For the differences between Olyset and PermaNet, the *p*-values were <0.001, 0.014, and 0.208 at 10, 22, and 36 months, respectively. For the differences between Olyset and NetProtect, the *p*-values were <0.001, <0.001, and 0.004 at 10, 22, and 36 months, respectively. For the differences between PermaNet and NetProtect, the *p*-values were 1.0, 0.153, and 0.080 at 10, 22, and 36 months, respectively.

When whole nets were tested after 3 years using IACT, 88% of Olyset, 96% of PermaNet 2.0, and 92% of NetProtect nets passed WHO optimal criteria of 80% mortality and 90% blood feeding inhibition. There were differences between products in 24-hour mortality. Olyset showed lower mortality ($p < 0.001$), but all 3 products showed similar levels of feeding inhibition (Fig 3; S7 Table). Mosquito mortality was higher for nets defined as 'too torn' (odds ratio = 0.65 [95% CI 0.49–0.88], $p = 0.005$), and the differences in mosquito mortality between the net products remained significant after adjusting for physical condition. Similarly, protection from mosquito bites (feeding inhibition) was considerably lower among nets that were 'too torn' (OR = 0.12 [95% CI 0.08–0.18], $p < 0.001$), but the differences between the net products remained non-significant after adjusting for physical condition.

Active ingredient content. At baseline, 100% (10) of Olyset and PermaNet 2.0 and 50% (5) of NetProtect samples complied with their target doses of active ingredient (S8 Table).

At 10 months, 22 months, and 36 months, mean permethrin content in Olyset nets decreased to 16.2 g/kg, 14.8 g/kg, and 13.0 g/kg, corresponding to a loss of 20%, 27%, and 36% of the original dose, respectively. Mean deltamethrin content of PermaNet 2.0 nets decreased to 0.75 g/kg, 0.47 g/kg, and 0.40 g/kg, corresponding to a loss of 48%, 68%, and 72% of the original dose, respectively. Mean deltamethrin content of NetProtect nets decreased to 0.91 g/kg, 0.52 g/kg, and 0.40 g/kg, corresponding to a loss of 33%, 61%, and 70% of the original dose, respectively (S8 Table). While this loss of insecticide did not negatively impact the bioefficacy of the nets against a pyrethroid-susceptible strain of mosquito, it is plausible that it would impact the efficacy of the nets against more resistant mosquitoes.

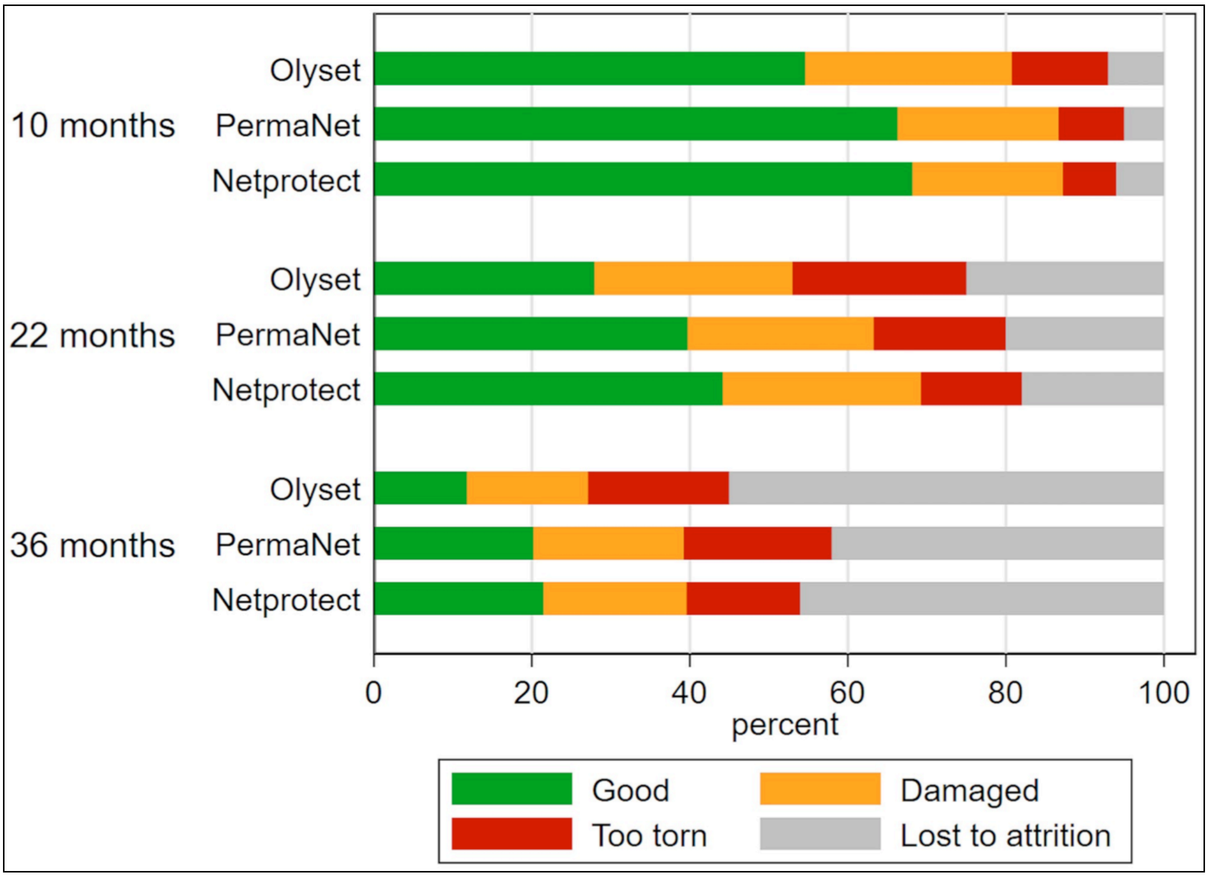


Fig 2. Physical condition of long-lasting insecticidal nets remaining in households at time of survey according to WHO categorisation using proportionate hole index (pHI) [5] for the 3 net products and time points. Green shows percent of nets in good condition (pHI 0–64), orange shows percent nets in damaged condition (pHI 65–642), and red shows percent of nets defined as ‘too torn’ (pHI 643). The sample sizes at 10 months were as follows: Olyset, 3,520; PermaNet, 3,513; NetProtect, 3,538. The sample sizes at 22 months were as follows: Olyset, 2,592; PermaNet, 2,622; NetProtect, 2,617. The sample sizes at 36 months were as follows: Olyset, 1,687; PermaNet, 1,827; NetProtect, 1,746.

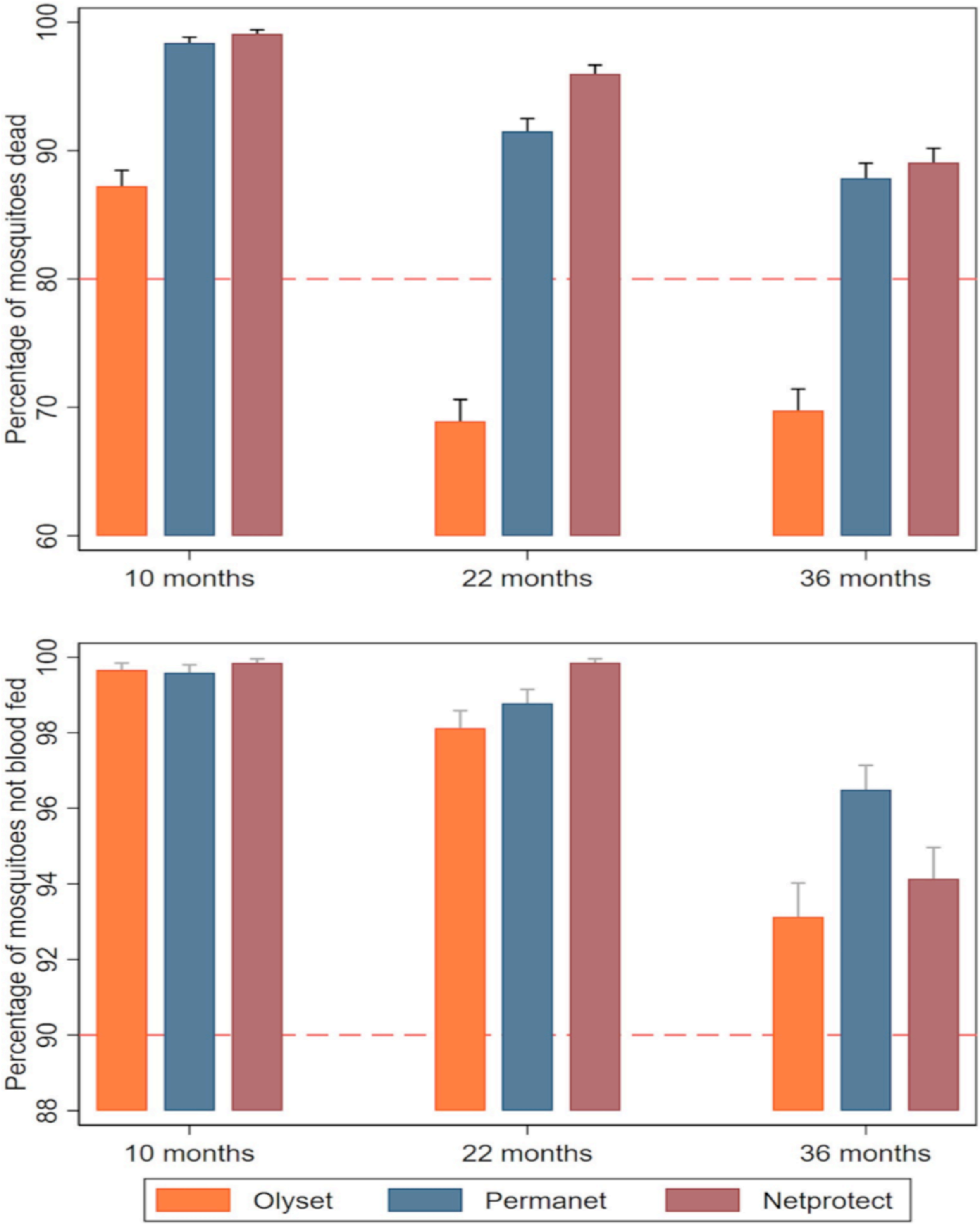


Fig 3. Ifakara Ambient Chamber Test (IACT) results for mosquito mortality and blood feeding inhibition by net product and time point. Mosquito mortality (top panel) and blood feeding inhibition (bottom panel). Orange, Olyset; blue, PermaNet; maroon, NetProtect. Optimal WHO criteria (80% mortality; 90% blood feeding inhibition) are indicated by dashed lines. The number of mosquitoes used at 10 months was as follows: Olyset, 2,700; PermaNet, 2,730;

NetProtect, 2,730. The number of mosquitoes used at 22 months was as follows: Olyset, 2,880; PermaNet, 2,880; NetProtect, 2,880. The number of mosquitoes used at 36 months was as follows: Olyset, 2,880; PermaNet, 2,880; NetProtect, 2,880.

Discussion

We conducted a randomised trial with 10,571 new LLINs of 3 brands (3,520 Olyset, 3,513PermaNet, and 3,538 NetProtect) distributed among 3,393 households in 76 villages in 8 districts in Tanzania and followed up annually for 3 years. This was done to measure the rate at which the 3 net brands became damaged, lost bioefficacy, and were discarded by households. The findings of this trial demonstrate that there is considerable variability in the lifespan of pyrethroid-treated LLIN products. Our data also confirm that the median functional life of the LLINs in our study was less than 3 years in Tanzania, as also suggested by a systematic review of LLIN retention data in 39 sub-Saharan African countries [4]. A WHO-sponsored evaluation of NetProtect and PermaNet 2.0 conducted in Kenya showed very similar results to those found here, with a median time to failure of 2.5 years for PermaNet 2.0 and 2.5 years for Net- Protect [16]. A full literature review of durability data available for the products evaluated in this trial is included in S1 Text. Summary net durability data available from peer-reviewed publications and WHO reports agree with the data in our trial for estimates of bioefficacy and fabric integrity after 3 years of operational use. The proportions of nets passing WHO bioefficacy criteria were above 80% for NetProtect and PermaNet 2.0 and slightly below 80% for Olyset. NetProtect and PermaNet had similar fabric integrity after 3 years of domestic use, with a higher proportion of serviceable nets relative to Olyset.

While there have been substantial economic investments to find new active ingredients, insecticide combinations, and synergists to combat the negative effects of insecticide resistance [28], the importance of durability for LLIN effectiveness has been side-lined. Consideration of its importance in vector control by key stakeholders such as the WHO may re-awaken the LLIN market to reward more durable products. This should, in turn, create incentives for investments in technological advances, research, and development by LLIN manufacturers [19]. There are indications that LLINs can be made substantially more durable for a small increase in unit price [29], and rapid technological evolution may be possible if there are appropriate market incentives.

The WHO's *Guidelines for Procuring Public Health Pesticides* [30] recommends that procurement decisions consider 'operational cost' rather than unit price, and an appropriate measure to compare value for money of LLINs would be 'cost per median year of net life under local conditions'. We measured the relative durability of nets using functional survival estimates, in terms of the EAC, and demonstrated that this approach outlined by WHO would indeed be useful. The cost analysis showed approximately 20% lower EAC when a longer-lasting LLIN (PermaNet 2.0 or NetProtect) was chosen over a shorter-lasting LLIN (Olyset), assuming prices for the products were identical. The economic modelling showed that the relative increase in price that is acceptable for a new product coming to market is also much smaller when the lifetime of the standard product increases (S1 Fig). Thus, the extension of the life of an innovator product is much more valuable if the standard product is relatively short- lived, as was seen in this study.

WHO requests LLIN manufacturers to provide data from 3 longitudinal field evaluations in different ecologies (e.g., West Africa, East Africa, and Asia) to retain prequalification listing. While it is recognised that durability is context-specific, we argue that it is possible to routinely generate median functional survival estimates and EACs for at least 3 locations using the WHO methodology outlined [13,23], albeit with a more limited sample size than the present study. The EAC may be a useful metric to compare cost-effectiveness of products, rather than the current practice of assessing products based simply on a minimum threshold of insecticidal activity after 3 years.

The limitation of the EAC metric is that it only captures the relative weighting of price and effective lifetime, while full cost-effectiveness and cost (including non-commodity costs) will result from a complex interaction of net durability, distribution modality, cost, and effectiveness. A limitation of the simplified approach here is that it does not fully consider these interactions, but it presents a straightforward and easily applicable approach to judging the relative cost and lifetime of a product.

Attrition and fabric integrity, the 2 factors that define physical survival of LLINs [6,31], differed significantly between the 3 net products. Olyset demonstrated more rapid accumulation of damage and faster attrition. In the current study and in previous work, we demonstrated that most LLINs were discarded because they were perceived by users as too damaged to offer protection against mosquito bites or malaria [32]. Attrition and fabric integrity are highly variable between contexts, and information on these factors is simpler to collect than bioefficacy or chemical content data. Further consideration should be given to developing simple tools to allow countries to assess attrition and fabric integrity during routine surveys (e.g., Malaria Indicator Surveys or Demographic and Health Surveys) to inform planning of intervals between mass distribution campaigns.

Of those nets still present after 3 years, 25%–40% were categorised as no longer physically serviceable, depending on the brand. However, even after 3 years, nets remained highly insecticidal when tested by bioassays against insecticide-susceptible malaria vectors. Damage actually increased the mortality of mosquitoes that entered nets through holes and became trapped, as also observed in other studies [33]. Indeed, torn LLINs continue to provide a degree of individual and community protection from malaria [34,35]. Our IACT experiments demonstrated that the 3 brands were all highly protective, although Olyset killed significantly fewer mosquitoes than PermaNet 2.0 and NetProtect. It is of note that the most common location for damage to the nets is on the bottom section of the nets at the point where they are tucked under a mat or mattress (S2 Fig). The act of tucking makes these holes inaccessible to mosquitoes even though the net appears to be badly damaged to the user, which may motivate them to discard the net.

A limitation of the trial is that only susceptible mosquitoes were used for bioefficacy testing. Pyrethroid resistance is widespread and increases feeding success and reduces mortality of mosquitoes [33]. Another limitation is the fact that the trial was only conducted in Tanzania (albeit in a wide range of epidemiological settings). Functional survival varies by country (S1 Text), so the single country setting is a limitation. However, the setting is more likely to affect absolute net survival rates than the comparison between LLIN products. Furthermore, the trial only included 3 brands of LLINs, all of which are treated with pyrethroids. As new LLIN products come on the market treated with different insecticides, insecticide combinations, or synergists, such as piperonyl butoxide (PBO), it will be imperative to monitor their comparative durability to ensure that the most cost-effective products are procured for malaria control. Functional life will have important implications for the selection of new products for resistance management that have higher unit costs. New pyrethroid plus PBO nets may not be as durable as standard pyrethroid nets because PBO is lost rapidly from nets during washing, which reduces their efficacy [36]. However, in Tanzania, PBO nets continued to have superior public health benefits 2 years after distribution [20]. If the median functional survival of pyrethroid LLINs is 2 years, then PBO nets may remain cost-competitive.

Our findings confirm that even after 3 years, nets that are still in households, despite holes, still give partial protection against mosquito bites and continue to kill mosquitoes, providing some personal and community protection. However, if nets are discarded, or no longer used because they are perceived as too damaged, then they have no public health benefit at all. While it is possible to encourage users to retain their damaged, but still insecticidal, nets through behavioural change communication, a more effective and safer strategy would be to distribute more physically durable LLINs [29]. LLINs are the largest single cost item in the global malaria control budget. If LLIN effective lifespans became longer, net replacement needs would be substantially reduced, aiding in improving population access to this life-saving intervention despite the current stagnation in financial support for malaria control. It is technically feasible to manufacture more durable LLINs. However, this will happen only if institutional buyers consider cost-effectiveness for coverage [30] and give greater market share to longer-lasting and better value-for-money products.

Supporting information

S1 Fig. Relationship between increased net lifetimes in years and the acceptable increase in price. https://doi.org/10.1371/journal.pmed.1003248.s001 (TIF)

S2 Fig. The location of damage on nets by year after distribution and net brand measured by proportionate hole index. https://doi.org/10.1371/journal.pmed.1003248.s002 (PDF)

S1 STROBE Checklist. https://doi.org/10.1371/journal.pmed.1003248.s003 (PDF)

S1 Table. Study flow. The number of interviews completed each year, loss to follow-up, and the number of study nets evaluated for each durability component is shown. https://doi.org/10.1371/journal.pmed.1003248.s004 (PDF)

S2 Table. Parameters used in simulation of lifetimes for equivalent annual cost simulation analysis. https://doi.org/10.1371/journal.pmed.1003248.s005 (PDF)

S3 Table. Reported net use the previous night by net product and time point. Data represent numbers of respondents (percent) reporting use of nets. https://doi.org/10.1371/journal.pmed.1003248.s006 (PDF)

S4 Table. Number at risk (functional survival). https://doi.org/10.1371/journal.pmed.1003248.s007 (PDF)

S5 Table. Number at risk (attrition). https://doi.org/10.1371/journal.pmed.1003248.s008 (PDF)

S6 Table. Median hole surface area (in cm2) and interquartile range (IQR) by net product and time point. https://doi.org/10.1371/journal.pmed.1003248.s009 (PDF)

S7 Table. Ifakara Ambient Chamber Test (IACT) results for mosquito mortality and blood feeding inhibition by net product and time point (in months). https://doi.org/10.1371/journal.pmed.1003248.s010 (PDF)

S8 Table. Number of nets, mean active ingredient (AI) content (g/kg), range (g/kg), and between net variation (%RSD); percentage of active ingredient lost over time; mean *R*-alpha isomer content (g/kg); and percentage of deltamethrin (only for PermaNet 2.0 and NetProtect) in net samples at baseline and 3 follow-up time points. https://doi.org/10.1371/journal.pmed.1003248.s011 (PDF)

S1 Text. Literature review on durability of PermaNet 2.0, Olyset, and NetProtect nets. (PDF)

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9.4. Annex 4: Journal Paper- Exploring factors affecting the functional efficacy of LLINs (long lasting insecticidal nets) during operational durability monitoring

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Abstract

Background

It is assumed that long-lasting insecticidal nets (LLINs) with World Health Organisation Prequalification listing have a 3-year life. However, effectiveness of different LLIN products varies and assessment of duration of functional efficacy under user conditions is required. Therefore this study was conducted to understand the effects of size and location of holes and insecticidal content on mortality, bloodfeeding success and survival feeding against pyrethroid susceptible and resistant mosquitoes.

Methods

Three experiments were conducted in two phases using the Ifakara Ambient Chamber Test (I-ACT). Outcome measures were 24h-mortality, blood-feeding and surviving-feeding. Experiments evaluated include i) bioefficacy of three sampled field LLIN brands (Olyset® with new knitting pattern, PermaNet® 2.0 and NetProtect®) after 1, 2 and 3 years use; ii) assessing the effect of hole surface area, hole-location and insecticide-concentration using treated and deliberately holed nets and iii) understanding the impact of tucking damaged nets with pyrethroid resistant *An. arabiensis* (Mbita-low resistant and Ifakara-high resistant strains) and susceptible mosquitoes *Anopheles gambiae* sensu stricto (Ifakara strain, Njage 1996).

Results

Irrespective of net brand, the bottom quadrant of the net (zone 4) where the net is tucked under the sleeping mattress showed the highest risk of damage (pHI: 208.55, 95% CI: 177.62-239.48). Assessing the impact of physical condition of sampled nets showed that, the odds of mosquito feeding and surviving feeding were significantly higher in damaged (OR:2.6; 95% CI: 1.84-3.67; $p<0.0001$) and too torn (OR: 16.5; 95% CI: 12.27-22.32; $p<0.0001$) nets relative to the nets in good conditions. Similarly, the odds of mosquito surviving feeding were significantly higher in damaged (OR:4.37; 95% CI: 2.30-8.29; $p<0.0001$) and too torn (OR:30.14; 95% CI: 16.83-53.96; $p<0.0001$) nets relative to the nets in good conditions irrespective of insecticide concentration. The probability of mosquito dying is significantly low in damaged (OR:0.48; 95% CI: 0.444-0.53; $p<0.0001$) and too torn (OR:0.29; 95% CI: 0.26-0.32; $p<0.0001$) nets compared to nets in good conditions. Similar findings were also observed using deliberately treated holed

nets. Again, significantly fewer mosquitoes managed to feed when a "too torn" Permanet® 2.0 with damage in the lowest 40 cm was tucked (Geo. Mean: 1.70%, 95% CI: 1.1-2.4) than when it was untucked (Geo. Mean: 8.9%, 95% CI: 6.8-11.1).

Conclusions

The findings from this study clearly showed that i) the size of holes on the LLIN matters. This means that increase in hole surface area increased chances for mosquito entry, mosquito feeding and survived feeding irrespective of location and insecticide concentration. In addition, this study found that, most of damage occurred in the bottom part of the net, but interestingly, this part is usually tucked in under the mattress, and from laboratory findings, it had little effect on mosquito entry and feeding.

Keywords: Long-lasting Insecticidal nets, LLIN, hole index, physical integrity, net holes, personal protection, mortality, feeding inhibition, bioassay, I-ACT, functional efficacy.

Background

The *World Malaria Report* 2019 estimates increase of malaria cases by nine million in 2018 compared with the previous year [1]. Between 2015 and 2018, substantial increases in case incidence occurred in the World Health Organisation (WHO) Region of the Americas, and marginally in the WHO South-East Asia, Western Pacific and African regions. This coincides with a reduction in malaria control investment. Main malaria control interventions include long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). Furthermore, while population access to LLINs has increased from 33% (2010) to 57% (2018), this is still below the target of at least 80% access in endemic areas. Malaria control funding is around $2.32 per person, and 578 million nets were delivered globally between 2016 and 2018. It therefore remains incumbent upon the malaria control community to use this investment as wisely as possible to ensure maximal population access to effective LLINs.

Currently, a candidate LLIN is considered to meet the criteria for efficacy for testing in Phase III studies, if after 3 years, at least 80% of sampled nets are still effective in killing mosquitoes and prevent blood feeding by mosquitoes when measured in WHO cone or tunnel tests [2]. LLIN efficacy is assessed by measuring its durability through counting number and size of holes over period of field use and then that data is combined with survivorship data as per WHO recommendations [2]. Physical integrity assessment is based on counting number of holes in four hole size categories (size 1: <2cm, size 2: 2-10cm, size 3: 10-25cm, size 4: >25cm) and summarizes the counts to a hole surface area (HSA), to provide a single metric measure of nets as either good (<79cm^2 if circular or <100 cm^2 if rectangular holes), damaged (80-789 cm^2 if circular or 100-1000 cm^2 if rectangular holes) or too torn (>790cm^2 if circular or >1000cm^2 if rectangular holes) [3]. Data may also be weighted to the proportionate hole index (pHI) using a weighting based on median area of hole sizes weights are 1, 23, 196 and 576 for the four size categories of holes 0.5-2 cm, 2-10 cm, 10-25 cm, >25cm, respectively [3]. Based on a number of historical studies carried out with permethrin dipped nets and at a time before pyrethroid resistance, nets are then classified as good (<64 pHI), damaged (65-642 pHI) or too torn (>643 pHI) regardless of the assumed functional shape of the hole [4].

Information on LLIN durability is not only important for providing information needed when planning for replacement of worn out LLINs and procurement decisions to ensure that the most cost effective nets, as measured by dollar cost per year of effective life, are procured, but to improve understanding of factors associated with improved net durability (e.g. net care practices), and to provide LLIN manufacturers information needed for product improvement [3].

Although the assessment of fabric/physical integrity through counting of holes is widely used as the gold standard method for measuring LLIN durability, such method has shortcoming by assuming equal probability of mosquitoes entering holes per cm^2 of holed area and does not take into account: 1) where do holes in nets occur most; 2) where do mosquitoes prefer most when entering holes in the bednet; 3) how hole location impacts on functional efficacy based on how people use nets; 4) how does insecticide interact with holes (of different sizes and locations) to impact on mortality and blood-feeding and 5) how does the pyrethroid resistance status of mosquitoes impact net efficacy as nets age. It was recognized in 2013 [4] that several factors may influence mosquito mortality and blood-feeding, and consequently the degree of programmatic malaria control elicited by nets [5].

We therefore propose three hypotheses, which need to be tested as a matter of urgency;

1) As access and use of LLINs among people in different settings in Tanzania, this may have influence on functional efficacy of particular LLIN based on how people use them.
2) Sizes and location of holes on the net and the interaction with insecticide concentration will have influence on the functional efficacy of LLIN which can not be seen if they are all taken together when assessing physical integrity of LLIN.

The aim of the study was therefore to; i) demonstrate that the functional efficacy of a particular LLIN may be affected by people's behavior of access and use of LLINs in different settings and ii) to understand other factors that may have influence on functional efficacy of LLIN even insitu and hence can be used for operational monitoring and WHO Phase III testing.

Methods

Study design

The study was conducted in two phases. First phase was through understanding the influence of potential factors on the overall functional efficacy of PermaNet 2.0 nets that were randomly collected from the community (190cm × 180cm × 150cm made of 100 denier polyester netting from Vestergaard Frandsen S.A.) after one, two, three years of use using standard WHO assays. Second phase was through bio-efficacy evaluation of SAFI nets (190 cm × 180 cm × 150 cm) that were deliberately impregnated (with a concentration series of Deltamethrin using ICON Maxx, Bayer) and holed to simulate aging nets. Then followed by i) assessing influence of different factors on the functional efficacy of LLIN and ii) assessing the impact of tucking-in nets that were used to test the above hypotheses. All the experiments were conducted in the Ifakara Ambient Chamber Test (I-ACT) (figure 1) with three main entomological outcome measures: 24-hour mortality (proportion of dead mosquitoes at 24-hours post exposure), blood feeding success (proportion of blood fed mosquitoes) and survival of feeding (proportion of mosquitoes surviving feeding at 24-hours post exposure).

Mosquitoes

Mosquitoes used in I-ACT were laboratory-reared, 5-8 days old fully pyrethroid susceptible nulliparous female *Anopheles gambiae* sensu stricto (Ifakara strain, Njage 1996) and *An. arabiensis* (Mbita and Ifakara strains respectively) with different susceptibility status i.e. Insecticide low (80% mortality at WHO discriminating doses) and high (20% mortality at WHO discriminating doses) pyrethroid resistant mosquitoes of the species *An. arabiensis* (Mbita and Ifakara strains respectively). Both resistant strains have metabolic resistance with overexpression of CYP6P1 (Lorenz Hofer *pers. Comm*). Only *An. gambiae* s.s. (pyrethroid susceptible species) was used in the first experiment with nets from the community. The other two experiments used all three mosquito strains. The three colonies are reared according to standard rearing techniques [6]. The larvae are fed with finely ground Tetramin fish food and the emerging adults are maintained on 10 % sugar solution. The insectary is maintained between 25 – 29^{o}C and 70 - 80% relative humidity with 12:12 light: dark ambient light. Mosquitoes were selected from a minimum of three cages by holding a hand close to the cage and aspirating those mosquitoes that were actively probing. The mosquitoes were then sugar starved for 6 hours prior to the start of experiments to ensure their avidity.

Ifakara Ambient Chamber Test (I-ACT)

The I-ACT consists of a series of ten experimental compartments (Figure 1). Each compartment contains a netted chamber 5 m long, 2 m wide, and 2 m high, in which the LLIN is hung from a frame with a human volunteer sleeping underneath. The chambers are made of white cloth and netting and sealed with a zipper. Standard Operating Procedure for the bioassay is available in SOM 1. Volunteers slept on mattresses on the floor between 21.00h and 06.30h. On every test night, two chambers acted as control chambers with standard untreated nets (Safi Nets, A to Z, Tanzania) deliberately holed following WHO recommendations (six 4x4cm holes, two on the long sides and one on the short sides, hole surface area of 96cm^2) while the remaining eight compartments contained treated nets.

Each night, mosquitoes were released into each compartment once sleepers were in position at 21.00h. For phase I experiment, a total of 30 mosquitoes (susceptible strain only) were used per chamber per night. For phase II experiment, a total of 45 mosquitoes from three strains (15 mosquitoes from each strain) were used. As mosquitoes are morphologically identical, a different coloured fluorescent powder (Swada, Cheshire, UK), shown in preliminary experiments to not affect mosquito survival or host seeking was gently applied to each strain before release. At 06.30h mosquitoes were collected from each compartment. Collections started from inside the net using a mouth aspirator and then outside the net but within compartments using 6V mechanical aspirators [20] for a maximum of three minutes per cup to minimize mechanical damage to the resting mosquitoes. Three cups were used to collect mosquitoes per chamber per night. Mosquitoes were then scored as alive, dead, blood-fed or unfed. Delayed (24-hour) mortality and 24-hour survival after feeding was recorded for the mosquitoes held with access to 10% sugar solution under insectary conditions. After every night of experiment, all chambers were left open and aired to prevent any insecticide carry over. Sheets were washed between every replicate to avoid carry over effects.

Phase 1 experiment

This experiment was conducted (using "naturally holed nets after 1, 2, 3 years of field use to understand i) where do holes on nets occur most, ii) impact of physical condition of sampled nets on probability of mosquito outcome measures (An. gambiae s.s.). A total of 432 LLINs (46 per brand per year) collected using random sampling from all nets collected annually between 2013 and 2016 from a three-year prospective longitudinal follow-up study (ABCDR Project) in eight districts of Tanzania were evaluated [7]. Three LLIN brands used were 1) Olyset® (new knitting pattern), 2) PermaNet® 2.0 and 3) NetProtect®. The assessment of the physical integrity was performed on all sub-sampled LLINs. Each LLIN was hung over a 190 x 180 x 170 cm collapsible bed net frame with elastic guides, which divide the frame into four zones of 42cm width (i.e. zone 1-top side, zone 2-lower top side, zone 3-lower middle side and zone 4 bottom side) and roof (Figure 2). Information on number, size and location of holes by zone and roof was recorded (Figure 3) following the template and tally sheet from Vector works [8] designed to collect the pHI and modified for counting holes using zones (SOM 2). The impact of physical condition of sampled nets on probability of mosquito outcome measures was assessed using I-ACT bioassay using 30 mosquitoes per chamber per night using a randomized balanced semi factorial design to account for bias introduced by inter- human variation in attraction to mosquitoes. Each net was tested on two different nights and data on mosquito outcome measures from the two replicates was averaged to improve the precision of estimates.

Phase II experiment

This experiment was designed to understand the effect of hole area, hole location and insecticide concentration on personal and community protection using mosquitoes of varying resistance status. Untreated nets (Safi Net, A to Z, Tanzania) polyester nets were artificially holed and treated with a concentration series of deltamethrin (using ICON Maxx, Bayer) to simulate aging nets. This study was also conducted in I-ACT assay using a randomized balanced semi factorial design to account for bias introduced by inter- human variation in attraction to mosquitoes. The hole sizes, holed surface area and deltamethrin concentrations were derived from field observations and included the thresholds used by WHO to define nets as good, damaged and too torn (Table of the categories tested is in SOM 3) [3]. Bednets were artificially holed with six circular holes; hole sizes and locations (but not number of holes) was varied to limit the number of experimental permutations and allow greater replication of each condition. The experiment was carried out for 75 nights using 15 mosquitoes per strain per chamber, per night. Each night, one untreated net (Safi Net A to Z, Tanzania with six 4x4cm holes (two on each of the long side and one on each of the short side of the net) was used as a control. Nets were rotated between chambers on a nightly basis while volunteers were fixed for the duration of the study and have their own sheets washed every morning to prevent any small risk of contamination. After every night of experiment, chambers were left open and aired to prevent any insecticide carry over. **Hole sizes:** The four standard (i.e. currently used for net durability studies) hole sizes were used: size 1 (1cm diameter i.e. smaller than a finger), size 2, (4 cm diameter i.e. thumb size), size 3, (10 cm diameter i.e. fist size), and size 4 (25 cm diameter i.e. head size) holes. Further, three more hole sizes of 2cm, 15cm and 35cm diameter were tested. Hole sizes were designed to examine the good, damaged and too torn cut offs of 79cm, 80-789 and >790cm, respectively. Holes were either cut on each side of the net or on the roof to explore the effect of hole location on mosquito feeding behaviour. **Insecticide concentrations:** Four concentrations of deltamethrin using (ICON Maxx, Bayer) were used, resulting in the following concentrations on the net: 0 mg/m^2, 2 mg/m^2, 5 mg/m^2, 15 mg/m^2, 25 mg/m^2, 55 mg/m^2 and PermaNet 2.0 as a comparison long lasting insecticidal net. These concentrations were selected based on values of chemical content measured in a previous durability project conducted at IHI with deltamethrin nets samples 12, 24 and 36 months after distribution (*unpublished data*). **Hole location:** To study the possible effect of hole location, nets were holed either in the roof panel or the side panels to investigate species-specific net entry preference. Nets with holes had one hole in each of the short side panel and two holes in each of the long side panel or six holes in the roof panel. This was done to manage the number of permutations in the experiment so a larger number of replicates per net condition could be conducted, to increase power.

This was followed by an experiment to investigate the impact of tucking on net efficacy i.e. measurement of the functional area of the net. It is commonly observed that nets that are regularly tucked have much of their damage in the zone of tucking and this experiment measured whether this tucked area impacts LLIN efficacy. The risk of mosquitoes feeding and surviving feeding in tucked and untucked PermaNet 2.0 nets of different hole sizes compared to untreated bednet was measured. The following treatment arms were tested; 1) two untreated SAFI net-control; 2) four PermaNet 2.0 nets with 6 circular holes 25cm diameter on the bottom quadrant (zone 4 location); 3) four PermaNet 2.0 nets with 6 circular holes 25cm diameter on the sides. Two PermaNets with zone4 holes were tucked while other two were left untucked. Similarly, two PermaNets with side holes were tucked while other two left untucked. The experiment was conducted for 10 nights using 45 mosquitoes (i.e. 15 from each of the three strains) per night per chamber. After every night of experiment, chambers were left open and aired to prevent any insecticide carry over.

Data Analysis

All data were recorded on paper data entry forms in the laboratory and double entered by two different individuals into Excel. Data was validated, cleaned and analysed using Stata 13.0 software (Stata Corp., College Station, USA). Descriptive statistical analysis was undertaken to describe and summarise the main parameters from the data. All the outcome measures were recorded as mean (with 95% confidence interval) and medians (with Interquartile range). Data were analysed using logistic regression model. The influence of different factors on functional efficacy of LLIN were assessed against the main outcome variables which are: 1) proportion of dead mosquito at 24h post exposure, 2) proportion of blood fed mosquitoes, 3) proportion of fed mosquitoes alive at 24h post exposure (see SOM 4 for models). As mosquitoes were scored as fed, unfed alive or dead at 24 hours, blood-feeding inhibition and 24hour mortality can be combined into a single metric called “survival of feeding” so as to make this simpler to conceptualize as it has taken into account both standard mosquito outcome parameters used by WHO to estimate LLIN efficacy.

Phase 1 experiment: Independent variables fitted in the model were number of holes of each size and location permutation (i.e. size 1,2,3 or 4 in zone 1; 1,2,3 or 4 in zone 2; 1,2,3 or 4 in zone 3; 1,2,3 or 4 in zone 4; 1,2,3 or 4 in roof) treated as a continuous variable, and net brand and year were included as categorical variables. Night of experimentation was a random effect to account for the repeated testing of the same net on two contiguous nights.

Phase II experiment: In understanding the effect of hole area, hole location and insecticide concentration on personal and community protection, independent variables fitted in the model were hole area, location of holes on the net, mosquito strain and dose of deltamethrin (all as categorical variables). In assessing the effect of tucking in of bednet, independent variables fitted in the model were tucking, sleeper and chamber (all as categorical variables). For each experiment the initial model was run with all fixed effects and interactions (as per SOM 4) and the final model selected was that with the lowest Akaike's information criterion (AIC). Plotting the Pearson's residuals was also performed to check model fit. A likelihood ratio test was done to compare two models in order to test the relative contribution of particular explanatory variables to the model.

Ethics

Ethical approval was given from Ifakara Health Institute in Tanzania (Reference number-IHI/IRB/No: 19-2013) and the National Institute for Medical Research (Ref: NIMR/HQ/R.8a/Vol. IX/150 and NIMR/HQ/R.8c/Vol. I/285).

Results

Where do holes on nets occur most?

The summary of the net integrity assessment on where do holes occur most by brand and by year is shown on Table 1 and in Figure 4. Irrespective of net brand, the bottom quadrant of the net (zone 4) where the net is tucked under the sleeping mattress showed the highest risk of damage (pHI: 208.55, 95% CI: 177.62-239.48) which equals to 50% of the overall damage on the nets compared to other zones while roof had the lowest risk of damage (pHI: 42.47, 95% CI: 34.10-50.85). Irrespective of net brand, there was consistent deterioration of net integrity over three years of field use. The red line shows the pHI cutoff for "too torn" nets.

Impact of physical condition of sampled field nets on probability of mosquito outcome measures

The probability of mosquitoes to either feed, die or survive feeding on sampled field nets of different physical conditions, i.e. good, damaged and too torn conditions, is shown on Figure 5. The figure shows that, in overall, the odds of mosquito feeding and surviving feeding were significantly higher in damaged (OR:2.6; 95% CI: 1.84-3.67; p<0.0001) and too torn (OR: 16.5;

95% CI: 12.27-22.32; p<0.0001) nets relative to the nets in good conditions. Similarly, the odds of mosquito surviving feeding were significantly higher in damaged (OR:4.37; 95% CI: 2.30-8.29; p<0.0001) and too torn (OR:30.14; 95% CI: 16.83-53.96; p<0.0001) nets relative to the nets in good conditions irrespective of insecticide concentration. The probability of mosquito dying is significantly low in damaged (OR:0.48; 95% CI: 0.444-0.53; p<0.0001) and too torn (OR:0.29; 95% CI: 0.26-0.32; p<0.0001) nets compared to nets in good conditions. The increased survival of feeding associated with damaged and too torn nets is possibly because they allow more mosquitoes to enter the net, feed and leave without being killed.

Impact of hole sizes on probability of mosquito feeding, dying and survived feeding using sampled field nets.

The influence of different sizes of holes for sampled field nets on probability of mosquito feeding, dying and surviving feeding irrespective of location and insecticide concentration was assessed in I-ACT experiment and results are shown on Figures 6. It should be noted that, since the nets are tucked, only the "functional area" i.e. the holed surface area of nets available to mosquitoes was thus evaluated in the experiments. Assessment of sampled field nets showed that, the odds of mosquito feeding and surviving feeding were strongly related to the size of holes irrespective of their location or insecticide concentration. For small hole sizes (size 1 and size 2 holes), the odds of mosquito feeding and survived feeding consistently remained around 1. On average, the probability of mosquito feeding for size 1 holes was OR:1.01 (95% CI:1.0-1.02; p<0.0001) and size 2 holes was OR:0.99 (95% CI: 0.98-1.01; p=0.34) and for mosquito survived feeding- size 1 holes was OR:1.0 (95% CI: 0.99-1.01; p=0.017) and size 2 holes OR:0.99 (95% CI: 0.98-1.02; p=0.979). However large sized holes had greater probability of mosquito feeding and survived feeding i.e. probability of mosquito feeding for size 3 holes OR: 1.21 (95% CI: 1.15-1.27; p<0.0001) and size 4 holes OR: 1.28 (95% CI: 1.21-1.35; p<0.0001) and for mosquito survived feeding- size 3 holes OR: 1.20 (95% CI: 1.12-1.29;p<0.0001) and size 4 holes OR: 1.24 (95% CI: 1.15-1.33; p<0.0001). The increased survival of feeding associated with larger holes is possibly because they allow more mosquitoes to enter the net, feed and leave without being killed. This was true as the probability of mosquito dying was reduced as the size of the hole increased irrespective of location and insecticide concentration

Impact of hole surface area on probability of mosquito feeding, dying and survived feeding using treated and deliberately holed nets.

The summary findings of the impact of hole surface area on probability of mosquito feeding and survived feeding using treated and deliberately holed nets in I-ACT experiments is shown on figure 7. Like in sampled field nets, there was a clear trend such that increase in hole surface area increased mosquito feeding and survived feeding irrespective of location and insecticide concentration ($p<0.0001$). However, hole surface area had no significant impact to the probability of mosquito dying ($p>0.05$). This could possibly mean that hole surface area (hole size) is the greatest predictor of mosquito entry into nets irrespective of location, mosquito phenotypic resistance and insecticide concentration.

Overall, as a net gets more damage, there is significant increase in proportion of mosquito feeding (OR: 23.8; 95% CI: 5.75-98.45; $p<0.0001$) but has a more limited effect on the odds of mosquito mortality (OR: 0.79; 95% CI: 0.44-1.42; p=0.44) (Table 2). The hole categories in which there was a rapid increase in feeding success and the log odds of feeding was high occur when holed surface area increases from 75cm^2 (Geometric mean 17.79% fed, 95% CI: 16.05-19.72% and OR: 1.76; 95% CI: 0.39-7.80) to 471cm^2 (Geometric mean 30.39%; 95% CI: 26.76-34.52% and OR: 4.77; 95% CI: 1.06-21.45; $p<0.05$). This cut off roughly corresponds to the values currently used to define “good” nets (hole area <79cm^2). The second increase occurred from 1060 cm^2 to 5773cm^2 (mean 35.27 to 38.59% fed and Odds ratio increases from 5.86 to 23.80, $p<0.0001$). This means that “damaged” nets 76-789cm^2 and “too torn” nets >790cm^2 are still fairly protective until the nets is extremely badly damaged. At HSA >1060 cm^2 this very high level of damage the nets allowed mosquitoes to enter and exit nets without contacting the treated surface with odds of survival of feeding of 5.57 (95% CI: 1.29- 24.08, $p<0.05$) irrespective of insecticide concentration.

Impact of location of holes was not done due to very few large holes on the top of the nets that would bring statistically meaningful analysis.

Impact of insecticide concentration on probability of mosquito outcome measures using nets from deliberately treated and holed nets.

Results on figure 8 show the impact of insecticide concentration on mosquito outcome measure using deliberately treated and holed nets after 75 nights of experiment in I-ACT. Irrespective of size and location of holes and the phenotypic resistance of mosquitoes treating a net with insecticide drastically reduce the percentage of mosquito feeding from 48.61% (95% CI: 45.23-52.24) (untreated) to 15% (95% CI: 13.67-16.65) and the OR: 0.02 (95% CI: 0.002-0.22; $p<0.05$) for 55mg/m^2. Surprisingly even the addition of small amounts of insecticide (2mg/m^2) reduced feeding success from 48.61% (95% CI: 45.23-52.24%) for untreated to 18.38% (95% CI: 15.51-21.76%) and the OR: 0.06 (95% CI: 0.01-0.71; $p<0.05$) (Table 3). However, mosquito mortality was strongly affected by insecticide loading dose with mortality rapidly increasing in a linear fashion with increased dose of insecticide i.e. from 69.57% (95% CI: 64.75-74.75) for 2mg/m^2 to 91.72% (95% CI: 90.30-93.17%) for 55mg/m^2.

How do people use nets and its impact to personal protection?

Results from Figure 8 showed that, overall, significantly fewer mosquitoes managed to feed when a "too torn" Permanet® 2.0 with damage in the lowest 40 cm was tucked (Geo. Mean: 1.70%, 95% CI: 1.1-2.4) than when it was untucked (Geo. Mean: 8.9%, 95% CI: 6.8-11.1). This could possibly mean, a treated too torn tucked net could provide more protection than an untreated new nets.

Discussion

This study was conducted to understand factors that could influence the overall functional efficacy of bednet using whole net bioassays. The information collected has improved understanding of the pHI and has clearly shown that some factors could highly affect the functional efficacy of the LLIN.

Although pHI is a very useful and standard method for assessing LLIN's physical integrity, however, as pointed out previously, however, results from this study using nets collected from the community (field sampled nets) showed that hole sizes were not uniformly distributed, as it is now assumed, but rather concentrated more on the bottom side of the net. This finding is consistent with other studies elsewhere. A study by Smith et al. in Ghana when evaluating bednets after 38 months of household use, found that the number of holes increased towards the bottom of the net [9]. Studies by Vanden Eng, et al [10] and Sutcliffe & Yin [11] observed similar findings.

Findings from field observations using nets from community and other studies have shown that, tucking the lower part of bed net has been a common behaviour in Sub-Saharan Africa [12–14]. Unpublished data from a retrospective durability study in Tanzania showed that, over 94% people tuck the bottom part of their bednets under the mattress (ABCDR project, Lorenz, pers comm). With this finding, it is logical to assume that the act of tucking is what causes the damage to the nets so nets damaged in the bottom quadrant are probably regularly tucked. The range of the median height of the net that is tucked is between 26 cm to 35 cm (*Unpublished data)*. Tucking a net, hide the most damaged part of the net and may change the categorisation of a net deemed "too torn" based on hole surface area into a serviceable protective net category. This means that, if most holes are located on the lower part of the LLIN and due to people's behaviour of tucking LLINs correctly, then the approximate total hole surface area will be lower than estimated, as majority of the bottom part will be hidden under the mattress. Therefore, holes in the bottom of the net pose very small risk for mosquito entry leading to significant variations in the overall pHI of LLIN. This can be concluded by saying that tucking can make even a badly torn net as protective as a good net, when the majority of damage is in the bottom of the net which is tucked irrespective of mosquito phenotypic resistance and insecticide concentration.

The current pHI method assumes that, the probability/risk of mosquito entry through holes on the net is the same irrespective of size and location. However, results from this study using sampled field nets and treated and deliberately holed nets showed that, the probability/risk of mosquito entry through holes on the net depend mainly on size of the hole on the net (hole surface area). It was clearly observed that increase in hole size increased mosquito entry, feeding and survive feeding. This is because more mosquitoes will enter the net freely, feed blood and exit without being killed. In other words, a net with holes (say a too torn net) provide less protection compared to nets with no holes. Similar findings were also observed by Port & Boreham [15] and Irish et al. [16]. Port and Boreham observed that mosquitoes had greatest success in feeding when the nets were in poor condition (with many holes) compared to nets in very good condition. Irish and colleagues observed that, irrespective of insecticide, there was loss of protection when a net become holed compared to intact net. Findings from a study by Asidi and colleagues also observed that LLINs provide little protection if they have holes [17].

Although very few large holes were observed on top side of the field sampled nets to make statistically meaningful analysis, findings using treated and deliberately holed nets showed that holes (in particular large sized holes) located on the roof panel were most risky location for mosquito entry, and that any holes in the roof were more likely to result in successful feeding than the same holes on the side of nets. Similar findings have been reported previously by Sutcliffe and Yin in their study to explore how host-seeking mosquitoes behave around occupied bed nets [11]. They observed that, the pressure of *An. gambiae* and *An. albimanus* was greatest on the roof compared to other sides of the net. Similar results were also observed in other studies that used infrared video tracking system in an attempt to understand more how mosquitoes respond to holes in human occupied bed nets [18–21].

In addition, as the concentration of insecticide on the net increases, mosquito mortality increased but feeding and survive feeding decreased proportionally. The presence of insecticide makes a too torn new PermaNet 2.0, with holed surface area (HSA) >790, being more protective than a good untreated net (HSA<79) irrespective of location or size of holes. Therefore, too torn treated nets should never be thrown away unless the home has enough good net for all the sleeping spaces. A treated net, if correctly used and well cared, remains effective in providing personal protection irrespective of mosquito resistance status. Similar findings was also observed in India, where a high LLIN use, in the presence of pyrethroid resistant mosquitoes, was observed to significantly reduce proportion of subclinical malaria among the cohort children [22].

The amount of protection is inversely related to mosquito resistance intensity, with increased resistance undermining the protective efficacy of nets. In other words, the duration of the useful life of a good net is highly affected by resistance status of mosquitoes. A study by Asidi et al. also observed similar findings that sleeping under an Insecticide treated net in settings with resistant mosquitoes (compared to setting with susceptible mosquitoes) was no more protective than sleeping under an untreated net, regardless of its physical condition [17]. With the loss of net integrity over time and increase in mosquito resistance to insecticides, malaria transmission will still remain. Nets will more rapidly lose effectiveness in areas with insecticide resistant compared to susceptible malaria vectors as they become torn and insecticide is depleted. Further analysis is ongoing to model these data to estimate the impact of resistance and net damage on the proportion of malaria that nets may avert. The information will then be used to calibrate and validate the new method.

This study highlights the association between potential factors (hole sizes, hole location, insecticide content and mosquito resistance status) on probability of mosquito entry, feeding and survive feeding. Findings from this study on factors that can affect functional efficacy of bednets can be used by countries through social behavioural change campaign (SBCC) not only to educate people on how to better care their bednets but also helps countries' programs on procurement decision by spending money on only true too torn nets.

Despite of useful findings observed, there are several limitations posed by the current study which include; ii) the study was conducted using rectangular-shaped bednets which will likely to give different findings when using round-shaped nets and iii) data collected comparing three net brands (i.e. sampled field nets) did not use resistant mosquitoes and therefore more research on the above may help to improving the understanding of the factors that could affect the functional efficacy of LLINs. Due to fewer holes on the top of field sampled nets, more researches could be conducted so as to get a statistically meaningful analysis on the impact of location of holes on the functional efficacy of LLINs.

Conclusions

The findings from this study clearly showed that i) the size of holes on the LLIN matters. This means that increase in hole surface area increased chances for mosquito entry, mosquito feeding and survived feeding irrespective of location and insecticide concentration. In addition, this study found that, most of damage occurred in the bottom part of the net, but interestingly, this part is usually tucked in under the mattress, and from laboratory findings, it had little effect on mosquito entry and feeding. This is very useful finding and can be incorporated into the existing country's malaria control advocacy platforms i.e. using Social Behavioural Change (SBC) campaigns to community in order to increase net care and hence lasting longer, providing more protection leading to reduction in malaria transmission.

Additional files

SOM 1: SOP for I-ACT

SOM 2: Hole Assessment tally sheet

SOM 3: Treatment arms

SOM 4: Model outcome and independent variables

Competing interests

Sarah Moore conducts product evaluation for a number of vector control product manufacturers. The other authors declare that they have no competing interests.

Authors' contributions

Conceived and designed the experiment DJM, SJM, LML, HJO, JDM, KK, OJTB. Performed the experiments DJM, JDM, WSN. Analysed the data DJM, SJM, OJTB. Contributed to data analysis JB. Wrote the manuscript DJM. Critically revised the final manuscript HJO, LML, JB, SJM. All authors read and approved the final manuscript.

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Figures

Figure 1. The I-ACT. Ifakara Tunnel situated at Bagamoyo Research Training Centre (BRTC) in Kingani, Bagamoyo (A). Net covered tunnel divided into 10 individual compartments each containing netted cage 2 x 2 x 5 meters (B). A human volunteer will sleep beneath the LLIN (C) and mosquitoes will be released by opening the lid of the holding boxes while beneath the tested net (D).

Figure 2. A rectangular collapsible bed net frame with zones, which divided the frame into four equal parts of 42.5 cm in length each.

Figure 3. One of the sampled nets from field draped over the collapsible frame ready for hole assessment exercise

Figure 4. Summary results of the assessment of sampled field nets on where do holes occur most by brand and by year. The red line indicate the cutoff of the most damaged zone (upper part) after three years of field use which is usually tucked

Figure 5. Impact of physical condition of sampled field nets on probability of mosquito outcome measures. Error bar represent 95% confidence interval.

Figure 6. Influence of hole sizes on probability of mosquito feeding, dying and surviving feeding using sampled field nets tested in I-ACT assay against *An. gambiae* s.s. Error bar represent 95% confidence interval.

Figure 7. Influence of hole sizes on probability of mosquito feeding, dying and surviving feeding using PermaNet 2.0 and nets dipped in KO Tabs (N=75nights). Error bar represent 95% confidence interval.

Figure 8. The effect of net tucking on bloodfeeding success and 24-hour mortality of *Anopheles gambiae* s.s. fully susceptible to pyrethroids using Permanet 2.0 over 15 nights. Error bar represent 95% confidence interval.

Table 1 Results of the Mean (Arithmetic) of phi by zone and year of field use (irrespective of the net brand) and their relative contribution to overall phi

	Year 1	**Year 2**	**Year 3**	**Overall phi**
phi zone1	7.07 (3.37-10.71)	45.13 (27.34-62.93)	93.04 (66.27-119.82)	49.19 (38.00-60.38)
phi zone 2	21.3 (10.3-32.35)	29.42 (18.28-40.56)	81.02 (55.89-106.14)	44.35 (34.25-54.45)
ph izone 3	40.95 (26.44-55.45)	136.03 (98.59-173.47)	200.45 (152.04-248.85)	127.41 (105.74-149.07)
phi zone 4	97.21 (69.71-124.71)	233.77 (172.25-295.29)	288.5 (228.10-348.89)	**208.55 (177.62-239.48**)
phi roof	17.99 (8.63-27.35)	38.64 (24.81-52.47)	69.42 (51.35-87.49)	42.47 (34.10-50.85)
Overall phi	184.53 (143.03-226.05)	483.01 (392.12-573.89)	732.44 (604.81-860.08)	471.98 (415.32-528.65)

Table 2 Multivariate analyses on factors affecting blood-feeding success using "deliberately holed nets" tested with I-ACT method for 75 nights

		Geometric Mean	95% Confidence Interval			95% Confidence Interval		
			Lower limit	**Upper limit**	**Odds Ratio**	**Lower limit**	**Upper limit**	**p-value**
Hole surface area in square cm (Circular holes)	0	13.35	11.24	15.86	1			
	19	21.60	18.08	25.79	1.42	0.32	6.40	0.647
	75	17.79	16.05	19.72	1.76	0.39	7.81	0.458
	471	30.39	26.76	34.52	4.77	1.06	21.45	***0.042***
	1060	35.27	31.76	39.17	5.86	1.29	26.39	***0.021***
	2945	31.68	28.68	34.99	5.13	1.16	22.64	***0.031***
	5773	38.59	35.11	42.41	23.80	5.75	98.45	***<0.0001***
Hole location	Side	26.72	25.08	28.47	1.00			
	Roof	30.09	27.99	32.34	2.77	1.37	5.60	***0.004***
Deltamethrin concentration in mg/m^2	0	48.61	45.23	52.24	1.00			
	2	18.38	15.52	21.76	0.06	0.01	0.71	***0.026***
	5	30.18	26.78	34.02	0.06	0.01	0.68	***0.023***
	15	24.36	22.09	26.85	0.05	0.01	0.58	***0.016***
	25	20.06	17.92	22.45	0.04	0.01	0.29	***0.002***
	55	15.15	12.69	18.09	0.08	0.01	0.46	***0.005***
	PermaNet 2.0	15.08	13.67	16.65	0.02	0.002	0.22	***0.002***
Mosquito strain	*An. gambiae* s.s fully susceptible	27.32	25.02	29.84	1.00			
	An. arabiensis (Mbita) low resistant	26.18	24.18	28.34	1.16	0.10	13.42	0.904
	An. arabiensis (Ifakara) high resistant	27.52	25.46	29.74	2.01	0.18	22.45	0.569

Table 3 Multivariate analyses on factors affecting mortality using "deliberately holed nets" tested with I-ACT method for 75 nights

Categorical variables		**Geometric Mean**	**95% Confidence Interval**			**95% Confidence Interval**		
			Lower limit	Upper limit	Odds Ratio	Lower limit	Upper limit	p-value
Hole surface area in square cm (Circular holes)	0	54.35	48.75	60.60	1			
	19	37.83	30.94	46.26	0.69	0.27	1.77	0.442
	75	60.84	56.41	65.62	0.76	0.32	1.79	0.531
	471	46.86	41.07	53.45	0.43	0.15	1.21	0.110
	1060	41.93	36.57	48.07	0.43	0.15	1.21	0.110
	2945	47.57	42.29	53.50	0.56	0.24	1.32	0.182
	5773	50.23	44.79	56.31	0.79	0.44	1.43	0.440

Hole location	Side	52.43	49.58	55.44	1			
	Roof	50.44	46.79	54.36	1.45	0.53	3.99	0.467
Deltamethrin concentration in mg/m2	0	9.82	9.12	10.57				
	2	69.57	64.75	74.75	427.64	111.00	1647.48	***<0.0001***
	5	44.02	38.73	50.02	266.01	33.92	2086.23	***<0.0001***
	15	51.56	47.65	55.79	521.80	66.78	4077.24	***<0.0001***
	25	66.41	62.56	70.49	1238.80	157.91	9718.54	***<0.0001***
	55	78.87	74.19	83.84	1300.36	160.39	10542.52	***<0.0001***
	PermaNet 2.0	91.72	90.30	93.17	3288.89	442.69	24434.50	***<0.0001***
Mosquito strain	*An. gambiae* s.s fully susceptible	66.64	62.19	71.39	1			
	An. arabiensis (Mbita) low resistant	51.58	48.10	55.31	0.06	0.01	0.42	***0.005***
	An. arabiensis (Ifakara) high resistant	40.56	37.77	43.55	0.02	0.003	0.14	***<0.0001***