The non-inferiority efficacy of Piperonyl-butoxide Yorkool® G3 Insecticide treated nets in the experimental huts in Tanzania

Olukayode G. Odufuwa  
ooduifuwa@ihi.or.tz

Ifakara Health Institute

Masudi Suleiman Maasayl  
Ifakara Health Institute

Emmanuel Mbuba  
Ifakara Health Institute

Watson Ntabaliba  
Ifakara Health Institute

Rose Philiipo  
Ifakara Health Institute

Safina Ngonyani  
Ifakara Health Institute

Ahmadi Bakari Mpelepele  
Ifakara Health Institute

Isaya Matanila  
Ifakara Health Institute

Hassan Ngonyani  
Ifakara Health Institute

Godfrey Ligema  
Ifakara Health Institute

Jason Moore  
Ifakara Health Institute

Yeromin P Mlacha  
Ifakara Health Institute

Jennifer C Stevenson  
Ifakara Health Institute

Sarah Jane Moore  
Ifakara Health Institute

Research Article

Keywords: ITN, non-inferiority, insecticide resistance, pyrethroid, PBO, Yorkool® G3, mosquito, malaria, experimental hut, Tanzania

Posted Date: June 15th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4510891/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: Competing interest reported. OGO, MSM, JS, EM, KS, RP, SN, ABM, IM, HN, JM, and SJM test vector control products.
Abstract

Background

Non-inferiority trials are recommended by the World Health Organization (WHO) to demonstrate that health products show comparable efficacy. As part of the WHO Global Malaria Programme (GMP) process of assessment of malaria vector control products, a second-in-class insecticide treated net (ITN) must be shown to be non-inferior to a first-in-class product based on mosquito mortality. The public health impact of the first-in-class pyrethroid-piperonyl butoxide (PBO) ITN, Olyset® Plus, has been demonstrated in epidemiological trials in areas with insecticide-resistant mosquitoes, but there is need to determine the efficacy of other pyrethroid-PBO nets to ensure timely market availability of nets and so to increase access to ITNs. The non-inferiority of a deltamethrin-PBO ITN Yorkool® G3 was evaluated entomologically against Olyset® Plus in experimental huts in Tanzania, following WHO guidelines for non-inferiority trials.

Method

The trial of the two pyrethroid-PBO ITNs was conducted in experimental huts in Lupiro, Tanzania, using a randomised double-blind 7 x 7 Latin Square block design. The study ran for 49 nights in 14 huts assessing the mosquito mortality and blood feeding of wild free-flying resistant Anopheles arabiensis. Using the non-inferiority approach, the efficacy (mosquito mortality at 24 hours and blood feeding) of unwashed and 20 times field-washed pyrethroid-PBO Yorkool® G3 ITNs, were compared with the first-in-class product Olyset® Plus and against a pyrethroid-only ITN, PermaNet® 2.0 ITNs, as a standard comparator.

Results

The experimental hut trial demonstrated the non-inferiority of Yorkool® G3 to Olyset® Plus based on mortality [51% vs 39%, respectively, OR 1.68 (95% CI: 1.50–1.88)], given that lower 95% CI exceeded 0.74 (delta of 39%). Blood feeding inhibition was high for all treated ITNs (> 90%). The pyrethroid-PBO ITNs were superior to the pyrethroid-only net, PermaNet® 2.0, as determined by both the proportion of mortality and blood feeding of mosquitoes (p-value < 0.05).

Conclusion

Yorkool® G3 ITNs demonstrated non-inferiority to the first-in-class Olyset Plus and superiority over the standard pyrethroid-only ITN, PermaNet® 2.0 as measured by mortality and blood feeding inhibition of wild insecticide resistant An. arabiensis mosquitoes. Yorkool® G3 ITNs are therefore potential tools for the control of metabolic insecticide resistant malaria vectors.

Background

Vector control, primarily through the use of insecticide treated nets (ITNs) are the cornerstone in the fight against malaria transmission [1], due to its primary ability for killing mosquitoes and reducing blood feeding success of mosquitoes that come into contact with the insecticide-treated surface. However, due to the extensive use of the same insecticides for mosquito control and agricultural practices [2, 3], mosquitoes have developed resistance to the pyrethroid used on ITNs [4, 5]. Nets treated with both pyrethroid and the synergist - piperonyl butoxide (PBO) have been developed to counteract metabolic resistant in mosquitoes [6]. The PBO inhibits the action of the metabolic enzymes that the mosquitoes use to detoxify pyrethroids [7]. Epidemiological data from a cluster-randomised trial conducted on the pyrethroid-PBO net Olyset® Plus in Tanzania demonstrated a 33% reduction in malaria prevalence over 21 months relative to a standard pyrethroid only net - Olyset® (permethrin incorporated net manufactured by A to Z textiles, Tanzania), in an area of insecticide resistance in mosquitoes [8]. Therefore, in 2017 the World Health Organization (WHO) identified PBO-incorporated nets as a new class of ITNs [9], with Olyset® Plus (the first pyrethroid-PBO incorporated net manufactured by Sumitomo, Japan) being the first-in-class (FIC) product prequalified by WHO [9].

WHO recommends that for new pyrethroid-PBO ITNs to be classified as efficacious second-in-class (SIC) products, non-inferiority trials using experimental huts may be used as a means of evaluation [10]. Mathematical modelling of entomological data from experimental hut trials has demonstrated that such studies can be used to predict the epidemiological effect of ITNs, thus supporting non-inferiority trials for the evaluation of novel ITNs [11]. Given that the FIC pyrethroid-PBO ITN (positive control) has been demonstrated to have an impact on malaria prevalence [12, 13], non-inferiority entomological trials which follow established guidelines and procedures [14, 15], can be used as a proxy to determine whether a new product would produce similar epidemiological results. Such entomological trials can be conducted in a shorter timeframe to epidemiological trials, thereby bringing new products to market sooner and so increasing access to ITNs.

In a WHO-approved experimental hut non-inferiority trial, new vector control products should demonstrate a comparable entomological performance to the WHO prequalified FIC or SIC product with the same entomological mode of action as that of the product that is under investigation [14, 15]. Performance is primarily assessed by mortality of malaria vectors with blood feeding as an additional endpoint [14]. For a new product to be deemed non-inferior to the FIC comparator, the difference in mosquito mortality must not be less than 7% [14]. This threshold is called the non-inferiority margin (NI). To be able to determine this statistically, this margin is translated into an odds ratio (OR) called the 'delta', and for a product to be deemed non-inferior, the lower limit of the 95% confidence interval (CI) of the OR of the candidate should be equal or above the estimated delta from the proportion derived for the FIC comparator [14]. In the assessment, it is also necessary to determine the susceptibility of the wild test mosquitoes to this class of insecticides through the exposure of mosquitoes to a ‘standard comparator’. For the assessment of pyrethroid-PBO products, a WHO prequalified pyrethroid only ITN known to be efficacious against susceptible malaria vectors (e.g. PermaNet® 2.0 treated with deltamethrin only) [16] is often used. In addition, an untreated net (negative control) should be included in the study to assess the quality of the experiment. The candidate product should ideally demonstrate superior efficacy over the standard
A new ITN incorporated with pyrethroid insecticide (deltamethrin) and the synergist PBO named Yorkool® G3, has been developed by Yorkool International Trading Co., Ltd in China, as additional product in the pyrethroid-PBO ITN class. As a potential new product in this class, its non-inferiority to the FIC product, Olyset® Plus, or another prequalified product in this class, is necessary for it to be prequalified by WHO [15]. Robust experimental hut trials of its entomological efficacy are therefore needed, as products differ in terms of manufacturing technique and dosage.

This paper presents experimental hut entomological studies to assess non-inferiority of Yorkool® G3 pyrethroid-PBO nets to the FIC product, Olyset® Plus, and superiority compared to a standard pyrethroid-only net, PermaNet® 2.0 following WHO procedures [14, 15, 17].

Methods

Study area

The experimental hut trial of Yorkool® G3 nets was carried out in Lupiro village (8.385°S and 36.670°E), which is located in Ulanga district, Morogoro region in the south-eastern part of Tanzania (Fig. 1 in grey). The yearly rainfall in the Morogoro region range from 1200 to 1800mm, with temperatures varying between 20°C and 34°C. The most abundant malaria vector in the area is Anopheles arabiensis which is resistant to pyrethroids and carbamates, and accounts for over 99.9% of the Anopheles gambiae complex species [18]. Pre-exposure to PBO resulted in lower knockdown rates and higher mortalities against pyrethroids and bendiocarb, compared to tests without the synergists [19]. The main malaria control intervention in the area is the use of ITNs [20].

Quality assessment of Yorkool® G3 nets were performed at the Vector Control Product Testing Unit (VCPTU) facility that is accredited (SANAS GLP0003) for Good Laboratory Practice, and situated at the Bagamoyo branch of Ifakara Health Institute (IHI) in Tanzania (Fig. 1, in green).

Figure 1: Geographical location of study area

Study design

The experimental hut trial was executed between September and October 2020. The trial used a partially-randomised double-blinded approach; both nets and volunteers were randomly assigned to huts on the first day, with a sequential rotation strategy employed thereafter, with daily rotation of volunteers and rotation of nets at the end of each round (7 nights). The trial implemented two contiguous 7×7 Latin Squares design in fourteen huts, commonly referred to as the "Williams Design" [21]. Each treatment condition was tested 98 times over the course of 49 nights, following WHO guidelines [14, 15], as depicted in Fig. 2.

Figure 2: Experimental hut study design

Description of test items

Yorkool® G3 ITNs are made of 130 denier yarn, polyethylene fibres coated with 120 mg/m² deltamethrin and 440 mg/m² piperonyl butoxide (PBO). It is manufactured by Yorkool International Trading Co., Ltd in China. Olyset® Plus is a knitted monofilament polyethylene net of 150 denier yarn, treated with 20 g Al/kg (800 mg Al/m²) permethrin and PBO contents of 10 g PBO/kg (400 mg PBO/m²). The net is manufactured by Sumitomo Chemical Co., Ltd in Japan [23].

The standard comparator ITN used was PermaNet® 2.0, a 55 mg/m² deltamethrin-coated ITN manufactured by Vestergaard Frandsen SA [24]. To assess the quality of the experiment, Safi Net was used as a negative control, an untreated polyester net manufactured by A to Z Textiles Mills Limited, Tanzania.

Mosquitoes

The experimental hut trial used free-flying wild insecticide-resistant An. arabiensis (Lupiro strain) mosquitoes. The resistance of these mosquitoes is attributed to the overexpression of CYP450 enzymes [25]. Sugar-fed 2–5 day old, insecticide-susceptible An. gambiae sensu stricto (s.s) (Ifakara strain) and pyrethroid-resistant An. arabiensis (Kingani strain) mosquitoes were used for quality of nets’ bioefficacy before and after the experimental hut trial (supplementary material table 1 and 2 for insecticide susceptibility tests conducted at the time of the study). All mosquito colonies are maintained in insectaries at the VCPTU, Bagamoyo, following standard operating procedures (SDPs) adapted from the MR4 guidelines [26].

Net preparation

Nineteen Yorkool® G3 ITNs from three production batches were prepared for the experimental hut trial (Fig. 2) as follows:

- Three nets (one per production batch), samples were removed when nets were unwashed and after 20x-washed for baseline quality checks of bioefficacy.
- Sixteen nets (from across the three production batches), eight of which remained unwashed and eight were washed 20 times. Per wash status, seven nets were used each for the experimental hut trials with a spare in case of need of replacement.

For the pyrethroid-PBO positive control net and the standard positive control pyrethroid only net, an equivalent number of nets from one production batch was used, and all nets were the same colour for blinding of study technicians and investigators.
Nets selected for washing, including those selected for quality checks, were field-washed at their appropriate wash interval day (one day for Yorkool® G3 and PermaNet® 2.0 and two days for Olyset® Plus [23]) in the facilities in Bagamoyo. Nets were washed in aluminium bowls containing 10 litres of filtered well water with a maximum hardness of 5dH and containing 2g/litre of soap ("Jamaa palm oil" soap flakes), using manual agitation of 20 rotations per minute for 10 minutes. Rinsing was done twice using filtered clean well water. Nets were dried horizontally in the shade then wrapped in foil and stored in labelled plastic bags at 21.4–30.9°C in the IHI store between washes. The procedure was repeated twenty times for all ITNs.

Each net was assigned a unique four-digit code generated by personnel not involved in the study, ensuring that investigators, technicians and participants were blinded to the type of ITN. Before testing in the huts, all nets (unwashed and 20x washed) were deliberately holed. Holes each measuring 4 cm × 4 cm were made on each of four sides of the net: two holes at the centre in each of the long side panels, and one hole at the centre of the short panels. Seven nets per arm, plus a spare, were transferred to the net storage facilities in Lupiro where the experimental hut trial was conducted.

For chemical analysis, five pieces of netting measuring 25 cm x 25 cm were cut from the three unwashed and washed nets used for bioefficacy, to give 15 netting pieces for each wash status to be studied before the experimental hut trial began. On completion of the hut trial, five pieces cut from two unwashed and two washed nets were used. Net pieces for chemical analyses and for bioassays were cut adjacent to one another [27]. All net pieces intended for chemical testing were promptly wrapped in aluminium foil after cutting and stored in a refrigerator at 4 ± 2°C for two weeks before they were shipped to the International Institute of Biotechnology and Toxicology (IIBAT) in India for analysis.

Procedures for experimental hut trial

Before the experimental hut trial, power of the study was estimated using estimates from an earlier trial conducted in the same site following the previous WHO non-inferiority guidelines [28]. A generalised linear mixed model (GLM) simulated 1000 times was used to determine the study power for a 7 x 7 arm trial in fourteen huts for 49 nights, using an estimated median number of 28 mosquitoes per night per hut, 22% mortality for the unwashed pyrethroid-PBO products and 14% mortality for the washed pyrethroid-PBO products, overall study variation of log of 1.034 and non-inferiority margin of 0.7 OR with treatment, volunteer, and hut adjusted for fixed effects, using R software [29].

The experiments were carried out in fourteen Ifakara experimental huts as detailed in a previous study [30]. The trial enrolled fourteen consenting adult male volunteers to occupy the huts at night. The trial had seven arms: (i) Yorkool® G3, unwashed, (ii) Yorkool® G3, washed 20x a 1-day wash interval, (iii) Olyset® Plus, unwashed, (iv) Olyset® Plus, washed 20x at a 2 days wash interval, (v) PermaNet® 2.0, unwashed, (vi) PermaNet® 2.0, washed 20x at a 1-day wash interval, and (vii) untreated Safi Net as negative control. The primary outcome was proportion of 24-hr mortality of mosquitoes and the secondary outcome was proportion of mosquito blood feeding. Data were collected for seven nights, with a one-night airing period between rounds to reduce any chance of any residual effects before introducing the next treatment. During the study, the volunteers adhered to a predetermined roster as they sequentially rotated among the huts. They entered their assigned huts at 19:00 hours (hrs) and slept under the nets until 06:00 hrs the following morning. At 06:00 hrs, mosquitoes were collected from 1) inside the nets, 2) the floor, walls, ceiling, and 3) exit traps using aspirators. Subsequently, the collected mosquitoes were sorted and categorised based on their location, and status (dead and fed, dead and unfed, alive and fed, and alive and unfed). These mosquitoes were then held in a temperature-controlled room for 24-hr with access to a 10% sugar solution at 21.5–33.5°C temperature and 32.5–82.7% relative humidity, to assess mortality at 24 hr after capturing.

Procedures for experimental hut trial quality checks: bioefficacy and chemical analysis

Prior to the experimental hut trial, pre-test quality checks were conducted to assess the bioefficacy (≥ 95% KD60 / ≥ 80% M24) using cone bioassay and nets were stored for chemical analysis of the insecticide on the net at time 0, the day before the first wash. Bioefficacy and storage of samples for chemical analysis tests were again carried out after completing the necessary 20 field washes. Post-experimental checks were conducted at the end of the hut trial using the nets evaluated in the huts (both unwashed and 20x washed) against susceptible An. gambiae sensu stricto (s.s) (Ifakara strain), and strongly pyrethroid-resistant An. arabiensis (Kingani strain) exposed to nets using cone bioassay according to the WHO procedures [15], while samples for chemical content were shipped to IIBAT for insecticide chemical analysis.

Data analysis

Statistical analysis was conducted using STATA 16 software [31]. Descriptive analysis was conducted for cone bioassays for the primary outcomes, knockdown at 60 minutes (KD60) and 24-hr mortality for quality checks. Results were presented as arithmetic mean percentages with 95% Confidence Intervals (CI).

Outcomes (both proportion of 24-hour mortality and blood feeding) in the experimental hut trial were analysed using binomial logistic regression. For the non-inferiority analyses, unwashed Yorkool® G3 nets were compared with unwashed Olyset® Plus nets, 20x washed Yorkool® G3 nets were compared with 20x washed Olyset® Plus nets, and a separate comparison of the average value of the outcomes for unwashed and 20x washed nets was conducted. The delta to assess the non-inferiority for 24-hour mortality was estimated using: 

\[ \frac{x - 7}{100 - (x - 7)} \times \frac{x}{100-x} \]

where x was the 24-hour mortality of mosquitoes captured in the huts with the comparator pyrethroid-PBO nets, Olyset® Plus. The delta for blood feeding was estimated using:

\[ \frac{1.7}{100 - (1.7)} \times \frac{x}{100-x} \]

where x was the proportion of blood feeding of mosquitoes captured from the huts with Olyset® Plus net. Non-inferiority was established when the lower confidence interval model-estimated odds ratio (OR) of Yorkool® G3 nets compared to Olyset® Plus nets was not lower than the delta of the proportion of mosquito mortality, and when the upper confidence interval of the OR of the Yorkool® G3 nets in comparison to Olyset® Plus nets was not higher than the delta of the proportion of mosquito blood feeding.

For the superiority analysis, the following comparisons were made for each of the study outcomes: unwashed Yorkool® G3 and unwashed Olyset® Plus nets compared to unwashed PermaNet® 2.0 nets; 20x washed Yorkool® G3 and 20x washed Olyset® Plus nets compared to 20x PermaNet® 2.0 nets; and the

Page 4/16
average value of the study outcomes for unwashed and 20x washed pyrethroid-PBO nets against pyrethroid nets. The pyrethroid-PBO products were deemed superior when the OR for proportion of 24-hour mortality was higher than 1.00 and the p-value < 0.05, and the OR was lower than 1.00 and the p-value < 0.05 for proportion of mosquitoes’ blood feeding.

In these model analyses, fixed effects included the treatment, hut, volunteer, and day of sleeping in the huts. For the analysis performed on the average of the outcomes, the wash status (unwashed and 20x washed) of net was added as a fixed effect.

A post hoc simulation was conducted to check the power of the study following the new guideline [14]. The new guidelines detailed the change of the threshold of non-inferiority from 0.7 OR to 7% difference in proportion of mosquito mortality. This was done by simulating a generalised linear mixed model a thousand times, with treatment, volunteer, hut and day adjusted as fixed effect using R software [29]. Actual estimates of the study were used in the simulation: median number of 26 mosquitoes per hut, overall study variation of 93%, and 40% dispersion of mosquitoes following a negative binomial distribution and a non-inferiority margin of 7% difference in the proportion of mosquito mortality. For unwashed condition, 53% mortality from Yorkool® G3 and 44% for Olyset® Plus, and for 20x washes, 49% mortality from Yorkool® G3 and 35% mortality for Olyset® Plus nets were used in the simulation.

Results

Experimental hut study power

Before the experimental hut trial, power of the study was estimated to be 97% for unwashed nets and 82% for 20x washed nets. Following the latest guideline, the post hoc study power was 98.4% for unwashed nets and 99.9% for 20x washed nets.

Experimental hut trial

Night-time temperature ranged between 21.0–32.3°C and relative humidity was between 45–85% in Lupiro during the study period. Nets were stored at room temperature (< 32°C) throughout the trial. The experimental trial started four days after the last wash (washing ended on the 29th of August 2020 and the trial started on the 2nd of September 2020). A total number of 21,974 An. arabiensis mosquitoes were collected over 49 nights of data collection. Median number of mosquitoes captured per night per hut was 26 (Interquartile range (IQR):14–43), but was heterogeneous and varied by product (Fig. 3).

Figure 3. Violin plot of the distribution of numbers of female Anopheles arabiensis mosquitoes collected per hut-night. 

Mortality

Yorkool® G3 nets induced significantly higher mortality at 24-hr [50.9% (95% confidence interval (CI): 47.5–54.3)] than all other ITNs: Olyset® Plus [39.2% (95% CI: 36.1–42.3]), PermaNet® 2.0 [27.2% (95% CI: 24.7–29.7)] and untreated net [11.1% (95% CI: 8.1–14.1)] (Fig. 4). Based on the WHO non-inferiority margin of 7% difference in mosquito mortality, Yorkool® G3 was non-inferior to Olyset® Plus nets [odds ratio (OR): 1.78 (95% CI: 1.65–1.92)], as the lower confidence interval value was greater than delta (δ = 0.74), with the direction of effect being higher for both the unwashed and 20x washed Yorkool® G3 nets over Olyset® Plus nets (Fig. 5 and Table 1). Furthermore, a superiority analysis demonstrated that pyrethroid-PBO ITNs had a higher killing effect than pyrethroid only ITNs of all wash conditions (Table 2). This was also seen for ITNs over untreated nets (the negative control) (Table 2). Additional results are in supplementary file 3.

Figure 4. Proportion of female Anopheles arabiensis mosquitoes dying at 24 hours (95% CI) after collection from experimental huts. Circles represent unwashed, hollow circles 20x-washed and triangles unwashed and 20x-washed combined.

Figure 5. Mortality (95% CI) of female Anopheles arabiensis mosquitoes at 24 hours after collection from experimental huts demonstrating non-inferiority of Yorkool® G3 compared to Olyset® Plus nets. Circles represent unwashed, hollow circles 20x-washed and triangles unwashed and 20x-washed combined. Pink semi-circle represents the non-inferiority margin, that estimates must not fall within to be deemed non-inferior.

Table 1 Non inferiority analysis of pyrethroid-PBO nets: Yorkool® G3 compared to Olyset® Plus

Blood Feeding

The proportions of mosquito blood feeding were substantially lower for all treated nets than those of the untreated nets, with all ITNs inducing more than 90% feeding inhibition (Fig. 6). The lowest proportions of blood feeding were seen for Olyset® Plus nets (Fig. 6). Yorkool® G3 nets were non-inferior [OR 1.87 (95% CI: 1.46–2.39)] to Olyset® Plus nets; the upper confidence interval value was lower than the delta (δ = 4.85) (Table 1, Fig. 7). As was seen for mortality in the superiority analysis, pyrethroid-PBO ITNs reduced blood feeding significantly more than the pyrethroid only ITNs (Table 2), indicating the additional benefit of the PBO synergist. Likewise, all ITNs significantly reduced An. arabiensis blood feeding compared to untreated net (Table 2). Additional results are in supplementary file 3.

Figure 6. Proportion of female Anopheles arabiensis mosquitoes that blood-fed (95%) after collection.

Figure 7. Blood feeding (95% CI) of female Anopheles arabiensis mosquitoes after collection from experimental huts demonstrating non-inferiority of Yorkool® G3 compared to Olyset® Plus nets. Circles represent unwashed, hollow circles 20x-washed and triangles unwashed and 20x-washed combined. Pink semi-circle represents the non-inferiority margin, that estimates must not fall within to be deemed non-inferior.
Quality assurance bioefficacy

All the pieces of both unwashed and 20x-washed Yorkool® G3 ITNs which were tested before (15 out of 15 samples) and after (10 out of 10 samples) the experimental hut trial met acceptable quality performances of ≥ 95% mosquito knockdown after 60 minutes or ≥ 80% mosquito mortality at 24 hours after exposure (WHO criterion for bioefficacy [17]) of the laboratory-reared resistant *An. arabiensis* and susceptible *An. gambiae* mosquitoes. This was also seen for the pyrethroid only ITNs before and after the trial on susceptible *An. gambiae* mosquitoes. However, for the pyrethroid-PBO positive control, Olyset® Plus ITNs, quality assurance was met based on proportion of knock down (KD60) for unwashed condition only, and not on 20x-washed condition, indicating that Olyset® Plus was not as wash resistant, Table 3.

### Chemical content

The chemical content of all the nets tested before washing were within the 25% +/- of target dose as per WHO guidelines [17]. However, after washing, only Yorkool® G3 nets, both the deltamethrin and PBO content, were within the target dose, while the pyrethroid and PBO Al target doses of none of the other positive controls were within the range. Although all the nets had high (> 95%) wash resistance indices, washing resulted in lower concentration of the active ingredients with Yorkool® nets retaining 94% and 76% of deltamethrin and PBO, respectively. Olyset® Plus 69% of permethrin and 57% of PBO, and PermaNet® 2.0 61% of deltamethrin. The greater retainment of the pyrethroid compared to PBO indicates that the PBO is lost more quickly after 20 washes (Table 4).

### Discussion

Yorkool® G3 nets, treated with deltamethrin and PBO are considered a new product under the second class of nets described by WHO, ITNs designed to kill host-seeking insecticide-resistant mosquitoes’, and that provide greater protection than pyrethroid-only nets [15]. For prequalification of this product by WHO, we investigated the wash interval of Yorkool® G3 nets in the laboratory following 2013 WHO guidelines [17] and entomological efficacy in experimental huts in Tanzania according to WHO prequalification guidance [15], and the non-inferiority guidelines of Global Malaria Programme (GMP) at WHO [14] with a conservative budget to fulfil both of their requirements.

Yorkool® G3 nets were found to be non-inferior to the first-in-class pyrethroid-PBO ITNs, Olyset® Plus, in terms of inducing mosquito mortality, and superiority over the first-in-class ITNs. Reasons to this finding could be the technique of dosing, as Yorkool® G3 nets utilise coating technique which allows the insecticide to be readily available on the surface of the net while for Olyset® Plus nets, the insecticide is incorporated into the fibres of the net which is gradually released overtime. The higher mortality seen with the Yorkool® G3 nets may also be attributed to the different pyrethroids used in each net. Olyset® Plus nets are treated with permethrin which is known to have high repellency effect [32]. This may result in less contact with the net and therefore less time to pick up a lethal dose, compared to deltamethrin with less of a repellency effect. The lower blood-feeding inhibition of Yorkool® G3 nets compared to Olyset® Plus nets is consistent with a recent study comparing Olyset® Plus with another deltamethrin-PBO net, PermaNet® 3.0 [32], which was also attributed to the effect of the different pyrethroids used. For an ideal controlled experiment, products with identical chemicals should be used, however the availability of such products may be limited particularly if manufacturers are reluctant to provide their net for testing. For quality purposes, nets used in non-inferiority analyses should be sourced from the manufacturers directly.

Entomological efficacy of enhanced mortality and reduced blood feeding induced by the addition of PBO to pyrethroid ITNs to metabolic pyrethroid resistant malaria vectors was demonstrated in this study for both PBO products, confirming the utility of such nets for malaria control. Similar effects have been reported in previous experimental hut trials in northern Tanzania [34], Benin [13, 33, 35], Burkina Faso [36] and Ivory coast [37]. However, the magnitude of the observed effects can vary depending on factors such as hut design, location, and the resistance intensity and mechanism of vector in different areas [38, 39]. The current study was conducted in an area where *An. arabiensis* is the most dominant vector and which is metabolic resistance to all classes of pyrethroid [40].

The epidemiological efficacy of pyrethroid-PBO nets have been confirmed in large-scale cluster randomised-controlled trials in Tanzania and Uganda [8, 41]. Given the comparable performance of Yorkool® G3 nets to the first-in-class pyrethroid-PBO ITN, Olyset® Plus, the net evaluated in these trials, it could be assumed that Yorkool® G3 nets would also be of public health value, however community durability studies are needed to ensure that the ITNs are durable such that they provide sustained protection and remain cost-effective over three years, usually the interval of ITN campaigns [42]. Durability in the field (the strength of the fabric and insecticide resistance to abrasion, wear and tears) of an ITN is not equal to the simulated ‘aging’ of the nets through washing (20x-washes). Factors that affect net durability when used in the community, and not accounted for through laboratory or field washing, including how the nets are used and maintained (human behaviour) and environmental conditions that affect insecticide evaporation loss, washing, and abrasion, among many others [43].

One of the factors that makes a net “long lasting” is the ability of the net to consistently have sufficient chemical content available at its surface to induce high mortality of mosquitoes following contact. This was seen for Yorkool® G3 in the quality checks, 100% of the original chemical content was observed...
after 20x-washes. In bioecacy evaluations before the hut trials, 67% of exposed insecticide resistant mosquitoes died, whilst mortality following the studies was 95%. This was not the case for the pyrethroid-PBO comparator net; none of the net pieces evaluated were within the target dose range for a chemical retention for the pyrethroid-PBO comparator, and poor mortality recorded in the cone bioassay experiment. This may be due to the difference in the treatment technique, as one utilised coating of the insecticide on the net surface and the other incorporated the insecticide within the fibres. Furthermore, different pyrethroids were compared, deltamethrin in the Yorkool® G3 nets and permethrin in the Olyset® Plus nets. As indicated before, the first-in-class product in this case differed in a number of aspects and may have been considered an inappropriate control in non-inferiority and superiority studies generally. One potential limitation of non-inferiority analyses where a first-in-class product has to be the standard comparator, is when the entomological efficacy in terms of mosquito mortality is lower than any other product to be compared. In this case second or third in class products may always appear non-inferior. Conversely, if the first-in-class product results in very high mosquito mortality, then future products that may still have public health value, may be shown to be inferior in many instances. This was taken into account by WHO and resulted in the non-inferiority margin being widened from 5–7% [14].

Given the current situation of increasing resistance in malaria-vectors, that contributes to the stalling or increase in malaria prevalence in certain malaria-endemic regions [46], rotation of pyrethroids has been suggested for management of insecticide resistance [47], however studies have shown no significant effect in the restoring of susceptibility of malaria vectors to pyrethroid insecticides [48, 49]. A promising strategy is to explore the rotation of classes of AI, such as using Yorkool® G3 ITNs in rotation with other limited classes of AI, instead of ignoring pyrethroid-PBO products due to the evidence of a superiority efficacy of a chlorfenapyr product over a pyrethroid-PBO product [50]. In a nutshell, it is pertinent to encourage more classes of AI for development to ensure the swift feasibility of rotation of AI for insecticide resistance management.

Our studies fulfilled WHO recommended requirements for conducting non-inferiority studies in experimental huts [14] as follows: 1) selection of a study site where the primary vectors against which the nets are designed to target dominate, i.e. for pyrethroid-PBO nets the primary malaria vectors resistant to pyrethroids. In Lupiro, the dominant malaria vector is Anopheles arabiensis and has been shown to be resistant to several pyrethroids. Studies of insecticide susceptibility at the time of the experiment demonstrated mortalities of 16%, 48% and 22% against permethrin, deltamethrin and alpha-cypermethrin, respectively (Supplementary Table 2), 2) using recommended hut designs. Our studies were conducted in Ifakara experimental huts, one of the listed designs by WHO [15], 3) using a study design that reduces variability. Using a 7×7 Latin Square design in fourteen huts, our study aimed to minimise variability as far as possible. With the use of many huts, multiple replicates could be undertaken in a short time period with minimal budget implications, 4) ensuring the study is of sufficient power, i.e. >80%, Using a thousand simulation of the model of the data analysis and accounting for estimates (using data from previous studies, the number of mosquitoes per night, % mortality of mosquitoes, and variation between huts, volunteers and day) it was estimated that the study had greater than 98% power. 5) using mortality as the primary outcome, assess non-inferiority and superiority of products using appropriate statistical analyses where the treatment, hut, volunteer and day are included in the model as fixed effects [14].

Conclusion
Yorkool® G3 ITNs were shown to be non-inferior to the first-in-class product within the pyrethroid-PBO ITN intervention class, Olyset® Plus, based on induced mosquito mortality and blood-feeding inhibition. Yorkool® G3 nets were also found to be superior to the standard pyrethroid-only ITN – PermaNet® 2.0. Yorkool® G3 ITNs therefore meet the WHO’s criteria for being prequalified as an efficacious vector control tool to be used in areas with metabolic resistant malaria vectors. Our non-inferiority and superiority evaluations were easy to implement and could be feasibly conducted within the recommended time frame and to a reasonable cost, following the guidelines set by the prequalification team and GMP at WHO.

Abbreviations
AI  Active Ingredient
An  Anopheles
FIC  First-in-Class
IHI  Ifakara health institute
ITN  Insecticide-treated net
M24  Mortality at 24 hours
PBO  Piperonyl butoxide
RT  Regeneration time
SIC  Second-in-class
VCPTU  Vector Control Product Testing Unit
WHO  World health organization

Declarations
Acknowledgements
Heartfelt appreciation goes to the entire team of VCPTU both based in Bagamoyo and Lupiro for their dedication and implementation of the study. A huge thanks to the people of Lupiro for their cooperation during the study. We extend our gratitude to Fu Haili, Yin Qing and the entire team of the Yokool® International Trading Co., Ltd, China for the funding provided to implement the study.

Authors’ contributions

OGO conceptualised and conducted the experimental hut trial, analysed all data and drafted the manuscript; MSM co-drafted the manuscript; EM substantially advised on the implementation of the study and reviewed the manuscript; WN supervised the experimental hut trial and reviewed the manuscript; RP provided logistics support and reviewed the manuscript; SN, ABM, IM conducted all bioassays testing and reviewed the manuscript; HN and GL supervised volunteers, record observations and reviewed the manuscripts, JM advised on the implementation of the study designs and reviewed the manuscript; YM and JS substantially reviewed the manuscript; and SJM was the principal investigator who conceptualised the study and substantially reviewed the manuscript. All authors approved the final draft of the manuscript.

Funding

Tianjin Yokool International Trading Co., Ltd, China provided fund for the testing of the products.

Availability of data and materials

Data are available in supplementary files. File 4 contains cone bioassay data on unwashed net pieces before experimental hut trial, file 5 contains cone bioassay data for 20x wash net pieces before experimental hut trial, file 6 contains cone bioassay data for unwashed and 20x washed net pieces after experimental hut trial, file 7 contains experimental hut trial data and file 8 contains chemical analysis data.

Ethical approval and consent to participate

The study was approved by the IHI Review Board with certificate number IHI/IRB/No: 29-2020 and the National Institute for Medical Research-Tanzania (NIMR) with a certificate number NIMR/HQ/R.8a/Vol.IX/3521.

Consent for publication

Permission to publish was granted by the National Institute for Medical Research-Tanzania (NIMR) referenced BD.242/437/01B/14.

Competing interests

OGO, MSM, JS, EM, KS, RP, SN, ABM, IM, HN, JM, and SJM test vector control products.

References


Tables

Table 1 Non inferiority analysis of pyrethroid-PBO nets: Yorkool® G3 compared to Olyset® Plus

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Product</th>
<th>Condition</th>
<th>Delta for 7% difference</th>
<th>OR (95%CI)</th>
<th>Test outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality at 24 hrs</td>
<td>Olyset® Plus</td>
<td>Pooled*</td>
<td>0.74</td>
<td>1.78 (1.65 – 1.92)</td>
<td>Non-inferior</td>
</tr>
<tr>
<td>(Primary Outcome)</td>
<td>Yorkool® G3</td>
<td></td>
<td></td>
<td>1.69 (1.51 – 1.90)</td>
<td>Non-inferior</td>
</tr>
<tr>
<td></td>
<td>Olyset® Plus</td>
<td>Unwashed</td>
<td>0.75</td>
<td>1.69 (1.51 – 1.90)</td>
<td>Non-inferior</td>
</tr>
<tr>
<td></td>
<td>Yorkool® G3</td>
<td></td>
<td></td>
<td>1.85 (1.68 – 2.05)</td>
<td>Non-inferior</td>
</tr>
<tr>
<td></td>
<td>Olyset® Plus</td>
<td>20x washed</td>
<td>0.72</td>
<td>1.49 (1.08 – 2.05)</td>
<td>Non-inferior</td>
</tr>
<tr>
<td>Blood feeding (Secondary outcome)</td>
<td>Yorkool® G3</td>
<td></td>
<td></td>
<td>1.87 (1.46 – 2.39)</td>
<td>Non-inferior</td>
</tr>
<tr>
<td></td>
<td>Olyset® Plus</td>
<td>Pooled*</td>
<td>4.85</td>
<td>2.55 (1.73 – 3.75)</td>
<td>Non-inferior</td>
</tr>
<tr>
<td></td>
<td>Yorkool® G3</td>
<td></td>
<td></td>
<td>1.49 (1.08 – 2.05)</td>
<td>Non-inferior</td>
</tr>
</tbody>
</table>

*unwashed and washed nets combined.

Odds ratio for the effect of Yorkool® G3 ITNs compared to the pyrethroid-PBO positive control, Olyset® Plus, estimated using logistic regression adjusting for the effect of volunteer, day, and hut as fixed effects. For the pooled analysis, wash condition was adjusted for as a fixed effect. For 24-hr mortality, lower margin of the confidence interval must be higher than delta. For blood feeding, upper margin of the confidence interval must be lower than delta.

Table 2 Superiority analysis of pyrethroid-PBO products
<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Product</th>
<th>Condition</th>
<th>OR (95%CI)</th>
<th>P-value</th>
<th>Test outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality at 24 hrs (Primary Outcome)</td>
<td>PermaNet® 2.0</td>
<td>Pooled*</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Olyset® Plus</td>
<td></td>
<td>1.63 (1.52 – 1.76)</td>
<td>&lt;0.0001</td>
<td>Superior</td>
</tr>
<tr>
<td></td>
<td>Yorkool® G3</td>
<td></td>
<td>2.91 (2.69 – 3.14)</td>
<td>&lt;0.0001</td>
<td>Superior</td>
</tr>
<tr>
<td></td>
<td>PermaNet® 2.0</td>
<td>Unwashed</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Olyset® Plus</td>
<td></td>
<td>2.16 (1.93 – 2.43)</td>
<td>&lt;0.0001</td>
<td>Superior</td>
</tr>
<tr>
<td></td>
<td>Yorkool® G3</td>
<td></td>
<td>3.66 (3.24 – 4.13)</td>
<td>&lt;0.0001</td>
<td>Superior</td>
</tr>
<tr>
<td></td>
<td>PermaNet® 2.0</td>
<td>20x washed</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Olyset® Plus</td>
<td></td>
<td>1.33 (1.20 – 1.47)</td>
<td>&lt;0.0001</td>
<td>Superior</td>
</tr>
<tr>
<td></td>
<td>Yorkool® G3</td>
<td></td>
<td>2.46 (2.23 – 2.72)</td>
<td>&lt;0.0001</td>
<td>Superior</td>
</tr>
<tr>
<td>Blood feeding (Secondary outcome)</td>
<td>PermaNet® 2.0</td>
<td>Pooled*</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Olyset® Plus</td>
<td></td>
<td>0.36 (0.29 – 0.45)</td>
<td>&lt;0.0001</td>
<td>Superior</td>
</tr>
<tr>
<td></td>
<td>Yorkool® G3</td>
<td></td>
<td>0.68 (0.55 – 0.83)</td>
<td>&lt;0.0001</td>
<td>Superior</td>
</tr>
<tr>
<td></td>
<td>PermaNet® 2.0</td>
<td>Unwashed</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Olyset® Plus</td>
<td></td>
<td>0.28 (0.19 – 0.40)</td>
<td>&lt;0.0001</td>
<td>Superior</td>
</tr>
<tr>
<td></td>
<td>Yorkool® G3</td>
<td></td>
<td>0.71 (0.52 – 0.96)</td>
<td>0.027</td>
<td>Superior</td>
</tr>
<tr>
<td></td>
<td>PermaNet® 2.0</td>
<td>20x washed</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Olyset® Plus</td>
<td></td>
<td>0.44 (0.33 – 0.58)</td>
<td>&lt;0.0001</td>
<td>Superior</td>
</tr>
<tr>
<td></td>
<td>Yorkool® G3</td>
<td></td>
<td>0.65 (0.50 – 0.86)</td>
<td>0.002</td>
<td>Superior</td>
</tr>
<tr>
<td>Outcomes</td>
<td>Product</td>
<td>Condition</td>
<td>OR (95%CI)</td>
<td>P-value</td>
<td>Test outcome</td>
</tr>
<tr>
<td>----------</td>
<td>--------------</td>
<td>-----------</td>
<td>-----------------</td>
<td>---------</td>
<td>--------------</td>
</tr>
<tr>
<td>Mortality at 24 hrs (Primary Outcome)</td>
<td>Untreated net</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PermaNet® 2.0</td>
<td>Pooled*</td>
<td>3.80 (3.14 – 4.60)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Olyset® Plus</td>
<td></td>
<td>6.21 (5.15 – 7.50)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yorkool® G3</td>
<td></td>
<td>11.05 (9.13 – 13.97)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PermaNet® 2.0</td>
<td>Unwashed</td>
<td>3.17 (2.60 – 3.87)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Olyset® Plus</td>
<td></td>
<td>6.85 (5.65 – 8.31)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yorkool® G3</td>
<td></td>
<td>11.59 (9.51 – 14.13)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PermaNet® 2.0</td>
<td>20x washed</td>
<td>3.12 (2.57 – 3.78)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Olyset® Plus</td>
<td></td>
<td>4.14 (3.42 – 5.01)</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Blood feeding (Secondary outcome)</td>
<td>Untreated</td>
<td>PermaNet® 2.0 Pooled*</td>
<td>Olyset® Plus</td>
<td>Yorkool® G3</td>
<td></td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-----------</td>
<td>-----------------------</td>
<td>--------------</td>
<td>------------</td>
<td></td>
</tr>
<tr>
<td>Yorkool® G3</td>
<td>7.68 (6.34 – 9.30)</td>
<td>0.38 (0.30 – 0.49)</td>
<td>0.14 (0.10 – 0.18)</td>
<td>0.26 (0.20 – 0.34)</td>
<td></td>
</tr>
<tr>
<td>Superior</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Note: Estimations were done using logistic regression adjusting for the effect of volunteer, day, and hut as fixed effects. For the pooled analysis, wash condition was adjusted for fixed effect. For superiority on mortality, OR must be higher than 1.00 and p-value <0.05, and for blood feeding, OR must be lower than 1.00 and p-value <0.05.

Table 3 Cone test data before washing, after washing 20 times, and after experimental hut trial with pyrethroid susceptible Anopheles gambiae (Ifakara strain) and pyrethroid resistant Anopheles arabiensis (Kingani strain). Number of mosquitoes exposed (No.), number (No.), percent (%) knockdown at 60 minutes (KD60), % 24-hour (24hr) mortality (adjusted for control).
Table 4 Insecticidal content of nets by wash: Number of samples tested (N), active ingredient (AI) content (g/kg) with standard deviation (SD), % of nets within 25% +/- of target dose, AI within net spatial variation (% RSD), and wash retention index of Yorkool® G3, Olyset® Plus and PermaNet® 2.0 net samples before and after 20 washes.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Treatment Arm</th>
<th>N</th>
<th>AI content (g/kg) before trial</th>
<th>% Within target dose range</th>
<th>AI Within Net Variation (% RSD)</th>
<th>AI retained after 20 washes (%)</th>
<th>Wash Resistance Index (%) (AI retained per wash)</th>
<th>N</th>
<th>AI content (g/kg) after trial</th>
<th>Arithmetic Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Susceptible An. gambiae s.s.</strong></td>
<td>Baseline quality check</td>
<td>300</td>
<td>3.08 (0.04)</td>
<td>100</td>
<td>1.30</td>
<td>94.3</td>
<td>99.7</td>
<td>5</td>
<td>3.07 (0.04)</td>
<td>2.91 (0.02)</td>
</tr>
<tr>
<td></td>
<td>20x washed before hut</td>
<td>300</td>
<td>6.0 (3.1–8.9)</td>
<td>100</td>
<td>0.62</td>
<td>75.7</td>
<td>98.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>unwashed after hut</td>
<td>200</td>
<td>6.0 (3.1–8.9)</td>
<td>100</td>
<td>0.62</td>
<td>75.7</td>
<td>98.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>20x washed after hut</td>
<td>200</td>
<td>143 (64.6–78.4)</td>
<td>100</td>
<td>0.67</td>
<td>69.0</td>
<td>98.2</td>
<td>5</td>
<td>19.35 (0.08)</td>
<td>13.22 (0.08)</td>
</tr>
<tr>
<td><strong>Resistant An. arabiensis</strong></td>
<td>Baseline quality check</td>
<td>300</td>
<td>9.84 (0.13)</td>
<td>100</td>
<td>0.62</td>
<td>75.7</td>
<td>98.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>20x washed before hut</td>
<td>300</td>
<td>6.0 (3.1–8.9)</td>
<td>100</td>
<td>0.62</td>
<td>75.7</td>
<td>98.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>unwashed after hut</td>
<td>200</td>
<td>6.0 (3.1–8.9)</td>
<td>100</td>
<td>0.62</td>
<td>75.7</td>
<td>98.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>20x washed after hut</td>
<td>200</td>
<td>143 (64.6–78.4)</td>
<td>100</td>
<td>0.67</td>
<td>69.0</td>
<td>98.2</td>
<td>5</td>
<td>19.35 (0.08)</td>
<td>13.22 (0.08)</td>
</tr>
</tbody>
</table>

**Table 4 Insecticidal content of nets by wash: Number of samples tested (N), active ingredient (AI) content (g/kg) with standard deviation (SD), % of nets within 25% +/- of target dose, AI within net spatial variation (% RSD), and wash retention index of Yorkool® G3, Olyset® Plus and PermaNet® 2.0 net samples before and after 20 washes.**

**Supplementary File**
Figures

Figure 1

Geographical location of study area
Figure 2
Experimental hut study design

Image not available with this version

Figure 3
Violin plot of the distribution of numbers of female *Anopheles arabiensis* mosquitoes collected per hut-night.

Image not available with this version

Figure 4
Proportion of female *Anopheles arabiensis* mosquitoes dying at 24 hours (95% CI) after collection from experimental huts. Circles represent unwashed, hollow circles 20x-washed and triangles unwashed and 20x-washed combined.
Figure 5

Mortality (95% CI) of female *Anopheles arabiensis* mosquitoes at 24 hours after collection from experimental huts demonstrating non-inferiority of Yorkool® G3 compared to Olyset® Plus nets. Circles represent unwashed, hollow circles 20x-washed and triangles unwashed and 20x-washed combined. Pink semi-circle represents the non-inferiority margin, that estimates must not fall within to be deemed non-inferior.

Figure 6

Proportion of female *Anopheles arabiensis* mosquitoes that blood-fed (95%) after collection.

Figure 7

Blood feeding (95% CI) of female *Anopheles arabiensis* mosquitoes after collection from experimental huts demonstrating non-inferiority of Yorkool® G3 compared to Olyset® Plus nets. Circles represent unwashed, hollow circles 20x-washed and triangles unwashed and 20x-washed combined. Pink semi-circle represents the non-inferiority margin, that estimates must not fall within to be deemed non-inferior.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfile1.docx
- Supplementaryfile2.docx
- Supplementaryfile3.docx
- Supplementaryfile4.xlsx
- Supplementaryfile5.xlsx
- Supplementaryfile6.xlsx
- Supplementaryfile8.xlsx