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Field evaluation of Veeralin, an alpha-cypermethrin + PBO long-lasting insecticidal net, against natural populations of *Anopheles funestus* in experimental huts in Muheza, Tanzania

Patrick K. Tungu [a,b], John Waweru [c], Sengodan Karthi [d], James Wangai [c], Eliningaya J. Kweka [e,f,*]

[a] National Institute for Medical Research, Amani Medical Research Centre, P.O. Box 81, Muheza Tanga, Tanzania
[b] Department of Disease Control, London School of Hygiene and Tropical Medicine, London, UK
[c] PestNet Kenya Ltd, P.O. BOX 51533-00200, Nairobi, Kenya
[d] Molecular Entomology Laboratory, Department of Biotechnology, Periyar University, Salem 636 011, Tamil Nadu, India
[e] Tropical Pesticides Research Institute, Division of Livestock and Human Health Disease Vector Control, P.O. Box 3024, Arusha, Tanzania
[f] Department of Medical Parasitology and Entomology, School of Medicine, Catholic University of Health and Allied Sciences, P.O. Box 1464, Mwanza, Tanzania

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ABSTRACT

The success of long-lasting insecticidal nets (LLIN) as the primary method for preventing malaria is threatened by pyrethroid resistance in *Anopheles* vectors. New generation long-lasting nets incorporating PBO synergist (piperonyl butoxide) with pyrethroid are designed to control insecticide-resistant mosquitoes. The efficacy of Veeralin® PBO LLINs was evaluated in experimental huts against wild free-flying pyrethroid-resistant *Anopheles funestus* (*s.l.*). Mosquito mortality, blood-feeding inhibition and personal protection were compared between untreated nets, standard LLINs and PBO/pyrethroid combination nets. Blood-feeding rates recorded with 20-times washed Veeralin were not significantly different from those with 20-times washed PermaNet 3.0 LLIN, a WHO Pre-Qualification Team (PQT) approved PBO/pyrethroid LLIN. This provides evidence that Veeralin LLIN provides similar blood-feeding inhibition to the standard approved LLIN and thus meets WHO PQT criteria for blood-feeding. Results show significantly higher mortality for Veeralin PBO LLINs against pyrethroid-resistant *Anopheles funestus* (*s.l.*) compared to DuraNet, a WHO PQT approved standard pyrethroid-only LLIN, both when unwashed and washed 20 times. The improved efficacy over a standard pyrethroid-only LLIN can be attributed to the effect of PBO in the Veeralin LLIN, hence meeting the Vector Control Advisory Group (VCAG) criteria for a resistance breaking LLIN.

1. Introduction

Long-lasting insecticide-treated nets (LLINs) have played a vital role in the decline of malaria incidences and vector populations across malaria endemic countries (WHO, 2019, 2020). In Africa, ownership and usage of bednets have caused an increased percentage of the population sleeping under an ITN between 2000 and 2020, for the whole population (2–46%), for pregnant women (3–52%) and for children aged under 5 years (3–52%) (Bhatt et al., 2011, 2015; WHO, 2019, 2020).

The World Malaria Report in 2020 showed the stagnation in control occurred between 2015 and 2019 with some countries reporting increased malaria cases (WHO, 2020). The rise in malaria vector species' resistance to multiple insecticides is a current major concern (WHO, 2020). There is experimental evidence linking vector resistance to factors suggesting greater transmission efficiency from Benin and Muheza, Tanzania (N'Guessan et al., 2007; Asidi et al., 2012; Kweka et al., 2019). Furthermore, sustained malaria transmission has been recorded in areas with high resistance to multiple insecticides (Surendran et al., 2020; Bartilol et al., 2021; Soma et al., 2021). The frequency of pyrethroid resistance and underlying mechanisms may predict decline in the protection of LLINs against resistant populations of malaria vectors (Asidi et al., 2012; Yewhalaw & Kweka, 2016).

Combination LLINs with pyrethroid and synergist compound piperonyl butoxide (PBO) have been developed and deployed as an alternative tool against pyrethroid-resistant mosquitoes (Kweka et al., 2017a, b). PBO is a synergist compound that aims to block insecticide resistance caused by cytochrome P450 metabolic enzymes which often play a key role in detoxification of insecticides (Bingham et al., 2011; Stevenson

* Corresponding author. Tropical Pesticides Research Institute, Division of Livestock and Human Health Disease Vector Control, P.O. Box 3024, Arusha, Tanzania.
E-mail address: eliningaya.kweka@tpri.go.tz (E.J. Kweka).

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et al., 2011; Yewhalaw & Kweka 2016; Kweka et al., 2017a, b). Pyrethroid-PBO nets have been shown to have improved efficiency to reduce malaria cases in areas with resistant populations of malaria vectors in Muleba, Tanzania, and across Uganda (Protopopoff et al., 2018; Staedke et al., 2020).

The use of pyrethroid-PBO nets was advocated as a new step with an interim recommendation by the World Health Organization (WHO, 2019, 2020) for the efficient control of pyrethroid-resistant mosquito vectors. There are several pyrethroid-PBO net products in the market, some of which have PBO applied on the top of nets or on the whole net. Veeralin® LLINs produced by VKA Polymer Ltd, Tamil Nadu, India, is a new brand of LLINs alpha-cypermethrin-PBO, with PBO-treated on the whole net.

This study reports the phase II experimental hut trial of Veeralin LLINs in Muheza, Tanzania, an area with pyrethroid-resistant populations of malaria vectors *Anopheles gambiae* (*s.l.*) and *An. funestus*.

2. Materials and methods

2.1. Description of the test product

Veeralin LLIN is an alpha-cypermethrin pyrethroid + PBO (incorporated into polyethylene) LLIN made of monofilament yarn (150 denier) containing 6.0 ± 25% g/kg alpha-cypermethrin and 2.2 ± 25% g/kg PBO. Veeralin LLIN is manufactured by Vector Control Innovations Pvt Ltd., India, and has recently passed WHOPES Phase I efficacy criteria with 20 standard washes. Comparison of Veeralin LLIN was done against PermaNet 3.0 LLIN, which is a WHO PQT recommended pyrethroid + PBO LLIN with a top panel made of monofilament polyethylene (100 denier) fabric incorporating deltamethrin at 4 g/kg (*c.*180 mg/m^2) and piperonyl butoxide at 25 g/kg (*c.*1.1 g/m^2). The side panels have 85 mg/m^2 in the netting and 115 mg/m^2 in a 70 cm border (i.e. 2.8 g/kg deltamethrin). DuraNet® is a WHO PQT approved pyrethroid-only LLIN manufactured by Clarke Mosquito Control (USA). Alpha-cypermethrin is incorporated into 150-denier, monofilament, high-density polyethylene fibres, with a target dose of 5.8 ± 25% g/kg AI, corresponding to 261 mg of alpha-cypermethrin per m^2.

2.2. Description of the trial site and hut design

The experimental huts are located at a field site in Zeneti village, 30 km from Muheza District town, in Tanga region, northeastern Tanzania (5°13′24″S, 38°39′96″E), at an altitude of 192.9 m above sea level (Kweka et al., 2019a). The area around Muheza is characterized by high malaria prevalence caused mainly by *Plasmodium falciparum* which is transmitted by *Anopheles gambiae* (*s.s.*) during the rainy season, and by *An. funestus* (*s.s.*) during the dry season (Mboera & Magesa, 2001). The huts are made to a standard traditional East African veranda trap-hut design, with brick walls plastered with mud on the inside, a wooden ceiling lined with hessian sackcloth, corrugated iron roof, open eaves, with window traps and veranda traps on each side. The huts are built on concrete plinths and surrounded by a water-filled moat to deter entry of scavenging ants. There are two screened veranda traps on opposite sides of the huts to capture any mosquitoes that exit *via* the unbaffled open eaves (baffles are funnel-shaped wooded structures that allow mosquito entry but prevent their exit). The eaves of the two open verandas are baffled inwardly to funnel host-seeking mosquitoes into the hut and to deter exiting through these openings. With this modified hut design there is no need to make any correction for escaping mosquitoes (Kweka et al., 2017b; Mahande et al., 2018).

2.3. Bioassays on Veeralin LLIN preparation and washing procedure

The nets were washed according to a protocol adapted from the standard WHO washing procedure used in Phase I. The nets were washed in individual aluminium bowls, one bowl for each net type containing 10 litres of filtered water from the local water provider with a pH of 6.0 and containing 2 g/l of Jamaa palm oil soap, which was grated to create flakes and then dissolved completely in warm water before addition to the basin using manual agitation. Each net was agitated for 3 min, left to soak for 4 min and re-agitated for 3 min. Agitation was done by stirring the net and gently submerging it by hand (wearing heavy duty rubber gloves) with 20 rotations/submersions per minute. Rinsing was performed twice using clean water (10 l per rinsing, i.e. 20 l per net). Nets were dried vertically in the shade then packed in polythene bags, placed inside of metal boxes and stored at 27 ± 2 °C between washes. The interval of time between washes (i.e. regeneration time: the time required to restore the biological efficacy of a net when the surface insecticide has been depleted by washing) was 5 days for Veeralin LLIN and 1 day for PermaNet 3.0 LLIN (WHOPES, 2011).

2.4. Cone bioassays

Bioassays were performed according to standard WHO procedures (WHOPES, 2013). From each treatment arm, one net was sampled. From each of the sampled nets in the baseline and washing evaluation, four pieces of 25 × 25 cm were cut from net sides as per the WHO LLIN guidelines, with the same procedure repeated after the trial. On each netting sample, a standard WHO cone was held in place using masking tape. An untreated control with 5 cones was run for each net. Five laboratory-bred fully pyrethroid susceptible *An. gambiae* (Kisumu strain) female mosquitoes (sugar-fed, 3-day-old) were introduced into each cone and exposed for 3 min. Thereafter they were removed from the cone using a manual aspirator and placed into paper cups supplied with 10% sugar solution provided on cotton wool. Therefore, 25 mosquitoes were exposed to each netting sample. All cone bioassays and subsequent holding periods were conducted at 27 ± 2 °C and 80 ± 10% relative humidity. Outcomes measured were knockdown after 60 min and mortality after 24 h (WHO, 2013).

2.5. WHO insecticide susceptibility tests

The susceptibility tests were carried out using the WHO test kits for adult mosquitoes (WHO, 1998, 2016). Test papers impregnated with the WHO-recommended discriminating dosage of 0.75% permethrin were used because alphacypermethrin and deltamethrin test papers (which would match the net insecticides) were not available. The quality of the test papers was checked against a laboratory susceptible *An. gambiae* Kisumu strain. Wild mosquitoes used in the tests were F1 of adults *An. funestus* and *An. gambiae* (*s.l.*) collected from the untreated experimental huts during and just after this trial. For each test, batches of 15–20 adult females were aspirated from paper cups and transferred into the holding tubes where they were held for 1 h before testing in exposure tubes lined with the test papers. Mosquitoes were exposed for 1 h and the number of mosquitoes knocked down was recorded after 60 min. At the end of exposure period mosquitoes were transferred into holding tubes (lined with untreated papers) and provided with cotton pad soaked in 10% sugar placed on top of the holding tube. Mortality was scored 24 h post-exposure and each test was replicated depending on the number of mosquitoes collected. WHO permethrin and deltamethrin-treated papers at 5× and 10× of the discriminating concentrations were also tested to assess higher intensity of resistance for *An. gambiae* (*s.l.*) and *An. funestus*.

2.6. Experimental huts field study

Washed and unwashed candidate LLINs were evaluated using 6 East African experimental huts for their effects on wild *An. funestus* (*s.s.*) mosquitoes and for their ability to deter entry, drive mosquitoes out of houses, induce mortality and inhibit blood-feeding (WHO, 1998, 2013, 2016). Other mosquito species were also collected but numbers were too few for statistical analysis. Before testing in the experimental huts, preliminary catches (without any treatment) were performed for 4 nights to ensure the field team was fully versed in collection procedures.

The following 6 treatment arms were compared: (i) unwashed Veeralin; (ii) unwashed PermaNet 3.0; (iii) unwashed DuraNet LLIN; (iv) Veeralin washed 20 times; (v) PermaNet 3.0 washed 20 times; and (vi) untreated polyester net.

After every 6 nights, the treatment arms were rotated among the huts according to a Latin square scheme. Six nets were used per treatment arm and each of the 6 nets was tested for one night during the 6 consecutive nights. At the end of the 6 nights, the huts were carefully cleaned and aired to remove potential contamination. Sheets and pillows were also washed to prevent potential contamination. The treatment arm was then rotated to a different hut. The study was performed for 6 rounds over 6 weeks to ensure complete rotation through the huts. The number of mosquitoes that were collected per night was assessed for sufficiency as calculated by power analysis (Johnson et al., 2014) assuming a 70% reduction in feeding inhibition. Nets were stored in labelled polythene bags in the shade at an average temperature of 28 °C between testing.

Six (4 × 4 cm) holes were made in each net, 2 holes in each of the long side panels, and one hole at each end (head- and foot-side panels). Each net was individually coded and labelled with a long-lasting label attached to the one of the hanging loops and also bagged in polythene bags labelled with the same code. Adult male volunteers (> 18-years-old) in good health, who are experienced in these kinds of studies, slept under the nets. Male participants were recruited among the inhabitants of the village close to the site of the experimental huts. Participants were informed on the objectives of this study and recruited upon written informed consent in national language (Kiswahili). Sleepers rotated randomly among huts each night of the study. They entered the hut at 18:00 h and remained inside until 06:00 h. Each morning of the study at 06:00 h, mosquitoes were collected from inside the nets, the floor and walls of the hut as well as from the exit traps (window and eave traps) following the WHO standard operating procedure (WHO, 1975). Mosquitoes were collected from within nets and exit traps using mouth aspirators and from inside the huts (walls, ceilings and floors) using manual aspirators (WHO, 1975).

2.7. Data analysis

The primary outcomes measured in experimental huts were: (i) deterrence (reduction in hut entry relative to the control huts fitted with untreated nets); (ii) induced exiting (the proportion of mosquitoes that are found in exit traps and verandahs relative to control); (iii) blood-feeding inhibition (the reduction in blood- feeding relative to the control); (iv) mortality (the proportion of mosquitoes killed); (v) personal protection, which can be estimated by the calculation of: (a) Personal protection (%) = 100 (Bu – Bt)/Bu, where Bu is the total number blood-fed in the huts with untreated nets, and Bt is the total number blood-fed in the huts with LLIN-treated nets; and (b) the overall killing effect of the treatment was estimated by the calculation: Insecticidal effect (%) = 100 (Kt – Ku)/Tu, where Kt is the number killed in the huts with LLIN-treated nets, Ku is the number dying in the huts with untreated nets, and Tu is the total collected from the huts with untreated nets.

The main analyses were performed using IBM SPSS version 26 (IBM Corp., Armonk, NY, USA) using blocked logistic regression (logit estimation for grouped data) for proportional data and Poisson regression for numerical data. Variance estimates were adjusted for clustering by each hut night of collection. The primary criteria in the evaluation were blood-feeding inhibition and mortality.

3. Results

3.1. Cone bioassay tests

Cone bioassays were conducted using the Kisumu susceptible mosquitoes for all experimental arms: before washing; after washing but before hut trials; and after the hut trials. Before washings, both 1-h knockdown and 24-h mortality for all treated nets was 100%. After washing, but before the hut trial, the 1-h knockdown was 96% and 88% for 20 times washed Veeralin and PermaNet 3.0, respectively (Table 1), and after hut trials the mortality was 100% for all treatments except for the untreated control arm which recorded 0% (Table 1).

3.2. Susceptibility test of *An. funestus* (*s.l.*) and *An. gambiae* (*s.l.*) from untreated huts

WHO susceptibility tests on F1 of the adult *An. funestus* collected from the experimental huts with untreated nets and tested with permethrin papers recorded a mortality rate of 44% (31.8–56.2%), indicating that *An. funestus* (*s.l.*) was resistant to pyrethroids. Susceptibility tests on F1 *An. gambiae* collected from untreated huts recorded percentage mortality of 27% (16.4–37.5%) to permethrin. Insecticide resistance intensity testing showed Zeneti village wild *An. gambiae* displayed over 10-fold resistance to permethrin and deltamethrin (Table 2).

3.3. Number of mosquitoes collected from huts

Anopheles funestus (*s.l.*) were more abundant than *Anopheles gambiae* during the trial. The average (geometric mean) number of *An. funestus* per hut per night varied between 6 and 8 (Table 3). The number of *An. gambiae* species collected were too few (range: 0.1–0.3 mosquitoes per hut per night) for any meaningful conclusions, and hence have not been included in this paper. All treated arms induced significant deterrence in reference to untreated control arm (Negative binomial, range for Z: 2.7–9.1, $df = 11$, all $P < 0.05$) except Veeralin LLIN washed 20× (Negative binomial, $Z = 0.294$, $df = 11$, $P = 0.769$).

3.4. Exiting rates

All treated arms recorded significantly higher *An. funestus* (*s.l.*) exiting rates as compared to that recorded by the untreated control arm (Logistic regression, range for Z: 7.0–10.9, $df = 15$, all $P < 0.05$). Exiting rates of Veeralin washed 20 times were significantly higher than that of PermaNet 3.0 washed twenty times (Logistic regression, $Z = 3.9$, $df = 15$, $P \leq 0.001$) (Table 3).

Table 1
Cone bioassays of six arms of nets before washing, after washing, before hut trial and after experimental hut trials

Treatment	Before washing			After washing, before hut trial			After hut trial		
	No. of mosquitoes tested	% Knockdown (60 min)	% Mortality (24 h)	No. of mosquitoes tested	% Knockdown (95% CI)	% Mortality (24 h)	No. of mosquitoes tested	% Knockdown (95% CI)	% Mortality (24 h)
UTN	125	0	0	125	0	0	125	0	0
Veeralin, UN	125	100	100				125	100	100
Veeralin, WA	125	100	100	125	96.0 (93.2–99.1)	100	125	96.0 (90.3–97.0)	100
PermaNet 3.0, UN	125	100	100				125	100	100
PermaNet 3.0, WA	125	100	100	125	88.0 (83.2–93.1)	100	125	76.0 (71.0–82.2)	100
DuraNet, UN	125	100	100				125	98.0 (96.1–100)	100

Abbreviations: UN, unwashed net; WA, 20 times washed net; UTN, untreated polystyrene net (a negative control); CI, confidence interval.

Table 2
Permethrin and deltamethrin resistance intensity results

Treatment	N	Mortality (%)	SE
Permetrin 5×	80	83.75	2.4
Permetrin 10×	80	78.75	3.1
Deltamethrin 5×	80	80.75	3.1
Deltamethrin 10×	80	88.50	1.4

Abbreviations: N, number of mosquitoes used; SE, standard error.

3.5. Blood-feeding

Blood-feeding rates recorded in all treatment arms were significantly lower than the untreated control arm, thus providing evidence that all the pyrethroid nets provided protection against mosquito bites in this pyrethroid-resistant population. Moreover, the blood-feeding rate recorded by Veeralin nets after being washed 20× (11.3%) was similar statistically (Logistic regression, $Z = -1.27$, $df = 11$, $P = 0.205$) to that recorded by 20× washed PermaNet 3.0 (13.1%), suggesting that Veeralin LLIN washed 20× is as protective as the 20× washed PermaNet 3.0 net which is the WHO PQT approved PBO/pyrethroid bi-treated LLIN (Table 3). Furthermore, blood-feeding rate recorded for Veeralin LLIN after being washed 20× (11.3%) was statistically similar to unwashed DuraNet LLIN (11.8%) (Logistic regression, $Z = 0.60$, $df = 11$, $P = 0.549$) meaning also that Veeralin LLIN washed 20× provides similar feeding inhibition as the WHO PQT approved standard pyrethroid-only LLIN. Although there were differences in personal protection recorded between treated nets, these differences were not significant (Negative binomial, range for Z: -0.2–0.4, $df = 11$, $P > 0.05$).

3.6. Mortality

Mortality of *An. funestus* (*s.l.*) recorded in all treated arms was significantly higher (Logistic regression, range for Z: 4–7, $df = 15$, $P < 0.05$) as compared to the untreated control arm (Table 4). Mortality recorded for unwashed PermaNet 3.0 LLIN (22.9%) was significantly higher than other treatments; however, mortality recorded for PermaNet 3.0 and Veeralin after being washed 20 times (12.6% and 8.5%, respectively), were not significantly different. Moreover, mortality rates induced by the unwashed Veeralin (12.1%) and 20× washed Veeralin (8.5%) were significantly higher (Logistic regression, $Z = 3.6$ and 5.0, respectively, $df = 15$, $P < 0.05$) than the rates recorded by the WHO approved pyrethroid-only standard unwashed DuraNet LLIN (1.5%). Other net treatments had similar results except for DuraNet that recorded significantly lower killing effect (Table 4).

4. Discussion

This study was designed to investigate the efficacy of the Veeralin LLIN after being exposed to 20 washings according to the WHO standardized washing procedure (WHO, 1998). Contrary to most studies of PBO nets, that have been evaluated in areas dominated by *An. gambiae* (*s.s.*) or *An. arabiensis* (N'Guessan et al., 2010; Koudou et al., 2011; Pennetier et al., 2013), this evaluation trial was conducted in an area dominated by *An. funestus* (*s.s.*) malaria vectors (Derua et al., 2015; WHO, 2017; Kweka et al., 2018, 2020). The assessment of the laboratory-based knockdown effect (using susceptible *An. gambiae* (*s.s.*)) before washing, after 20 washes and after hut trials have shown the Veeralin PBO LLINs elicit high knockdown and mortality. Results presented here of bioassays done after 20 washes following the WHO standard washing protocol (WHO) revealed efficacy of Veeralin LLIN nets in producing > 80% mortality in all test mosquitoes up to 20 washes, thus meeting WHO wash-fastness criteria for a LLIN.

The present study shows that the dominant population of malaria vector mosquitoes during this trial was *An. funestus* (95.01%) with *An. gambiae* (*s.l.*) population (0.52%) being not enough to be included in data analysis, and *Culex quinquefasciatus* (4.47%). This study revealed similar findings to previous studies conducted in the same area, where *An. funestus* (*s.l.*) dominated in the experimental huts (Tungu et al., 2015; Kweka et al., 2019, 2020). There are studies from different ecological areas of Tanzania indicating that recently *An. funestus* (*s.s.*) has emerged as the dominant malaria vector, replacing *An. arabiensis* and *An. gambiae* (*s.s.*), perhaps related to differential effects of intervention tools among species (Lwetoijera et al., 2014; Kweka et al., 2020).

Table 3
Results for experimental huts against Zeneti wild free-flying *Anopheles funestus* (number entering, proportions deterred, exiting, blood-feeding, BFI and personal protection)

Treatment	Untreated net	PermaNet 3.0	PermaNet 3.0	Veeralin	Veeralin	DuraNet
No. of washes	0	Unwashed	20	Unwashed	20	Unwashed
Total no. of females caught	374	256	328	319	364	288
Geometric mean females caught/night (95% CI)	8 (5.2–10.8)	5.9 (3.7–7.1)	7.7 (5.7–9.7)	7.4 (5.1–9.7)	7.8 (5.1–10.5)	6.3 (3.8–9.8)
% Deterrence	–[a]	31.5[b]	12.3[b]	14.7[b]	2.7[a]	0[a]
Total no. of females in verandah and exit traps	134	179	211	223	275	220
% Exophily (95% CI)	35.8[a] (31.0–41.0)	70[bc] (64.3–75.5)	64.3[c] (59.2–69.5)	69.9[cd] (64.9–74.9)	75.5[bd] (71.1–80.0)	76.4[bd] (71.5–81.3)
Total no. of blood-fed females	111	52	43	49	41	34
% Blood-fed (95% CI)	29.7[a] (25.1–34.3)	20.3[b] (15.4–25.2)	13.1[bc] (9.5–16.8)	15.4[bc] (11.4–19.3)	11.3[c] (8.0–14.5)	11.8[c] (8.1–15.5)
% Blood-feeding inhibition	–	31.6	55.9	48.1	62.6	40.3
% Personal protection	–[a]	53.2[a]	61.3[a]	55.6[a]	63.1[a]	70.3[a]

Note: Within a row, treatments not sharing a superscript letter differ significantly by blocked logistic regression ($P < 0.05$).
Abbreviation: CI, confidence interval.

Table 4
Experimental huts results: percentage mortality and killing effect of *Anopheles funestus*

Number of washes	Untreated net	PermaNet 3.0	PermaNet 3.0	Veeralin	Veeralin	DuraNet
	0	Unwashed	20	Unwashed	20	Unwashed
Total no. of females caught	374	256	328	319	364	288
Total no. of females dead	10	64	49	46	40	12
% Mortality corrected for control (95% CI)	–[a]	22.9[b] (17.8–28.1)	12.6[c] (9.0–16.2)	12.1[c] (8.5–15.6)	8.5[c] (5.7–11.4)	1.5 (−0.8–3.9)
% Overall killing effect	–[a]	14.4[bc]	10.4[bc]	9.6[bc]	8[c]	0.5[e]

Notes: Percentage mortality and 95% CIs are back-transformed from values calculated by the blocked logistic regression model. Within a row, treatments not sharing a superscript letter differ significantly by blocked logistic regression ($P < 0.05$).

The deterrence effect observed for the Veeralin unwashed (14.7%) and Veeralin washed 20 times (2.7%) was found to be low, as in previous studies where PermaNet 3.0 and DuraNet LLINs were evaluated in experimental huts against wild populations in Magugu, Moshi and Muheza (Tungu et al., 2010; Mahande et al., 2018; Kweka et al., 2019). The findings of the present study contrast with the findings in M'bé, Côte d'Ivoire, West Africa, where unwashed Veeralin, and Veeralin washed 20 times showed deterrence rates of 65.3% and 64.2%, respectively (Oumbouke et al., 2019b). The difference in the experimental hut outcomes from Côte d'Ivoire and Tanzania might be attributed to differences in the degree of pyrethroid resistance and vector species composition, whereby typically East African mosquitoes are less resistant to pyrethroids compared to those in West Africa and are thus more likely to be killed than deterred (Oumbouke et al., 2017, 2019a, b), or to the differences in hut designs.

The Veeralin washed 20 times had a higher exophily rate (75.5%) in the experimental hut compared with the PermaNet 3.0 washed 20 times (64.3%). Unwashed Veeralin and unwashed Permanent 3.0 had similar exophily rates of 70%. The highest (but similar to Veeralin washed 20 times, see Table 3) exophily value was recorded by a pyrethroid-only net (DuraNet). The pyrethroid nets with PBO (PermaNet 3.0, Veeralin) and pyrethroid-only LLIN (DuraNet) induced higher exophily in this study than in a similar study conducted in West Africa where the Veeralin washed 20 times had an exophily effect of 64.7% and unwashed had 55.5% effect (Kweka et al., 2019). The exophily assessed for DuraNet in the study by Kweka et al. (2019) is similar (78.4% *vs* 76.4%) in the present study.

The highest blood-feeding inhibition was found for Veeralin (62.6%) followed by 55.9% for PermaNet 3.0, both washed 20 times. In addition, another study conducted with Magnet LLIN (alphacypermethrin without PBO) in the same area as the present study recorded a similar blood-feeding inhibition of 58.1% against the *An. funestus* (*s.l.*) population (Kweka et al., 2019).

The percentage mortality corrected for control was found to be lowest in pyrethroid-treated net (DuraNet) unwashed (1.5%). In 2007 when the alphacypermethrin-only DuraNet LLIN was evaluated in the Muheza huts for WHOPES and *An. funestus* (*s.l.*) and *An. gambiae* were still pyrethroid-susceptible, the percentage mortality corrected for control of *An. funestus* and *An. gambiae* were respectively 93% and 96% with the unwashed DuraNet and 83% and 81% with the 20 times washed DuraNet (Kweka et al., 2019). This major difference in mortality with the alphacypermethrin-only LLIN can be attributed to elevation of pyrethroid resistance in Muheza in both *An. funestus* (*s.l.*) and *An. gambiae* over the interim period (Kweka et al., 2019). Mortality induced by 20 times washed Veeralin was significantly higher than that for the unwashed DuraNet LLIN, a WHO PQT approved standard pyrethroid-only LLIN. Mortality induced by 20 times washed Veeralin LLIN was similar to that recorded for the unwashed and 20 times washed PermaNet 3.0, a PBO LLIN and a positive control in this trial. Taken together, these results provide evidence that Veeralin LLINs have met PQT mortality criteria for a LLIN.

A candidate LLIN meets the WHO PQT phase II efficacy criteria if it performs as well as or better than the reference LLIN when washed 20 times in terms of blood-feeding inhibition and mortality. The WHO Vector Control Advisory Group (VCAG) advises the WHO on new vector control tools and stipulates that for use against pyrethroid-resistant vector populations, the mixture LLIN should demonstrate efficacy (mosquito mortality or prevention of blood-feeding) significantly greater than standard pyrethroid-only LLIN. Significantly higher mortalities of pyrethroid-resistant *An. funestus* (*s.l.*) were recorded by the unwashed and 20 times washed Veeralin compared to DuraNet LLIN in experimental hut; the incorporation of PBO in Veeralin has shown increased mortality impact than in non-PBO DuraNet LLIN. High killing effect similar to WHO-approved standard PBO LLINs against pyrethroid-resistant mosquitoes have been recorded by Veeralin in a previous study (Oumbouke et al., 2019b). The significantly higher mortalities over pyrethroid-resistant *An. funestus* (*s.l.*) recorded by both unwashed and 20 times washed Veeralin compared to the unwashed ordinary DuraNet LLIN can be attributed to the effect of Piperonyl Butoxide (PBO), a chemical synergy contained in the whole Veeralin net thus meeting the VCAG criteria for a resistance-breaking LLIN.

Although this study reports superior efficacy of the Veeralin LLIN than a pyrethroid-only treated net over pyrethroid-resistant mosquitoes, the improvement, though significant, was 12–22% (corrected) mortality compared to 1.5% (for the non-PBO net) which may not be meaningful for public health impact. This apparently marginal improvement of PBO nets in hut trials was also observed last year in a non-WHOPES trial of Olyset Plus against Olyset in Muheza (Dr Patrick K. Tungu, personal communication). In Bagamoyo, Tanzania, another experimental hut study detected a similar result with Veeralin nets (WHO, 2016). However, the results from a cluster randomised trial have shown that PBO-LLINs provide better control of malaria than standard nets in Tanzania, whereby Olyset plus (PBO) nets provided a 33% malaria case reduction compared to Olyset nets at 21 months (Protopopoff et al., 2018). This indicates that the marginal improvement documented in the hut trials may indeed translate into a major improved in malaria control. Indeed, the study conducted in Muleba, Tanzania, provided sufficiently compelling evidence for efficacy of PBO-LLINs in reducing malaria cases for the WHO to give an evidence-based interim decision to approve PBO-LLIN to be used for malaria control (WHO, 2017).

5. Conclusion

The findings of this study have shown that blood-feeding rates recorded for 20 times washed Veeralin LLIN were statistically similar to those of the 20 times washed PQT-approved PBO/pyrethroid and a positive control in this trial. Also, the mortality induced by 20 times washed Veeralin LLIN was statistically similar to that recorded for the 20 times washed PermaNet 3.0 LLIN. This provides evidence that Veeralin LLIN have met WHOPES mortality criteria for LLIN.

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Ethical approval and consent to participate

This study had experimental permit issued by the Registrar of Pesticides in Tanzania. The hut sleepers who slept in each individual hut under net per night were recruited among the inhabitants of the villages close to the site in Zeneth. They were informed on the objective of this study and signed an informed consent. All participants took Mefloquine prophylaxis as per Tanzanian guidelines for the duration of the study and were screened weekly for malaria parasites with SD Bioline Malaria Ag Pf/Pan rapid diagnostic test (RDT) as per Tanzanian guidelines (MoHSW, 2006). No participants were found to be malaria-positive during the study period.

CRediT author statement

PKT and EJK conceived and designed the study, coordinated the experiments and analysed the data. PKT, JW, SK, JW and EJK drafted the manuscript, revised and reviewed throughout to final version. All authors read and approved the final manuscript.

Declaration of competing interests

JW and JW work for PestNet who are registration agents for the manufacturer, but had no influence on the trial design, data analysis and interpretation, also the conclusion made herewith. PKT, SK and EJK declare that they have no competing interests.

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