Effect of long-lasting insecticidal nets with and without piperonyl butoxide on malaria indicators in Uganda (LLINEUP): final results of a cluster-randomised trial embedded in a national distribution campaign

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Summary

Background Long-lasting insecticidal nets (LLINs) are the foundation of malaria control but resistance of mosquito vectors to pyrethroids threatens their effectiveness. We embedded a cluster-randomised trial into Uganda's 2017–18 campaign to distribute LLINs. LLINs with piperonyl butoxide (PBO) reduced parasite prevalence more effectively than conventional LLINs (without PBO) for 18 months. Here, we report the final 25-month survey results.

Methods LLINEUP was a cluster-randomised trial conducted in 48 districts in eastern and western Uganda. 104 health subdistricts (clusters) without ongoing or planned indoor residual spraying with pirimiphos-methyl (Actellic, Basel, Switzerland) were eligible for inclusion in the trial. Clusters were randomly assigned to PBO LLINs (PermaNet 3.0 or Olyset Plus) and conventional LLINs (PermaNet 2.0 or Olyset Net) with proportionate randomisation using STATA version 14.2. LLINs were delivered from March 25, 2017, to March 18, 2018. Between April 23, 2019, and Sept 13, 2019, community surveys were conducted in 50 randomly selected households per cluster; ten households per cluster were randomly selected for entomology surveys. Mosquitoes were collected in the morning from indoor surfaces of households using Prokopack aspirators. Due to COVID-19 restrictions, only 90 of the 104 clusters were surveyed at 25 months. The primary outcome was parasite prevalence by microscopy in children aged 2–10 years, assessed in the as-treated population, determined using the results from the 6-month household survey on the type of LLINs received in each cluster. This trial is registered with ISRCTN, ISRCTN17516395, and is now completed.

Findings In the as-treated analysis, two clusters were excluded (no predominant LLIN received) and four were reassigned; 40 PBO LLIN clusters (30 PermaNet 3.0, ten Olyset Plus) and 48 non-PBO LLIN (36 PermaNet 2.0, 12 Olyset Net) were included. Parasite prevalence was 17·1% (506 of 2958 participants) in the PBO group and 19·8% (701 of 3534) in the non-PBO group (prevalence ratio adjusted for baseline 0·80 [95% CI 0·69–0·93], p=0·0048). Comparing within-treatment group parasite prevalence to baseline, parasite prevalence ratios were lower in the PBO groups at all timepoints, but the difference was greatest at 6 months (PBO LLINs parasite prevalence at baseline 28·8% [1001 of 3472, 95% CI 27·3–30·4] *vs* at 6 months 12·0% [361 of 3009, 10·9–13·2], prevalence ratio [PR] 0·43 [95% CI 0·36–0·52], p<0·0001; non-PBO LLINs parasite prevalence at baseline 25·4% [1015 of 4004, 24·0–26·7] *vs* 6 months 14·8% [526 of 3551, 13·7–16·0], PR 0·60 [0·54–0·68], p<0·0001) and 25 months (PBO LLINs parasite prevalence at 25 months 17·1% [506 of 2958, 15·8–18·5], PR 0·63 [95% CI 0·57–0·71], p<0·0001; non-PBO LLINs parasite prevalence at 25 months 19·8% [701 of 3534, 18·5–21·2], PR 0·79 [0·73–0·86], p<0·0001).

Interpretation In Uganda, PBO LLINs outperformed pyrethroid-only LLINs for 25 months. WHO concluded that PBO LLINs are more effective against malaria than non-PBO LLINs when resistance to pyrethroids is high and issued a conditional recommendation suggesting PBO LLINs should be deployed in areas of pyrethroid resistance.

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Introduction

Over the past 20 years, remarkable progress on malaria control has been achieved following substantial investment in long-lasting insecticidal nets (LLINs), indoor residual spraying, and artemisinin-based combination therapies.[1] Between 2000 and 2019, WHO estimated that 1·5 billion malaria cases and 7·6 million malaria deaths were averted, mostly in Africa.[2] However, progress on malaria control has stalled particularly in high burden countries, including Uganda. WHO reported increased malaria mortality in 24 countries since 2015.[3] Globally, malaria control efforts have been challenged by insufficient funding, the emerging threats of drug and insecticide resistance,[4–6] and, more recently,

Research in context

Evidence before this study

We searched titles and abstracts in PubMed with the terms "piperonyl butoxide, PBO, Olyset* or PermaNet*", and "insecticide-treated bednets, long-lasting insecticidal nets, nets, bednet*, ITN*, LLIN*, insecticide-treated bednet*, or insecticidal net*" for studies published in English on Feb 15, 2022. We found three trials that evaluated the impact of long-lasting insecticidal nets (LLINs) with piperonyl butoxide (PBO) on epidemiological outcomes, including cluster-randomised trials in Tanzania and Kenya, and the results from the first 18 months of follow-up of the LLINEUP trial in Uganda. In the Tanzanian trial, Protopopoff and colleagues compared LLINs with PBO (Olyset Plus) with conventional LLINs without PBO (Olyset Net) using a two-by-two factorial design of 48 clusters from one district. At 9 months, 16 months, and 21 months after LLIN distribution, prevalence of malaria parasites by rapid diagnostic test was lower in children aged 6 months to 14 years who received PBO LLINs than in those who received conventional pyrethroid-only LLINs. In the Kenyan trial, Minakawa and colleagues compared LLINs with PBO (Olyset Plus) with conventional LLINs (Olyset Net) in eight clusters. The primary epidemiological outcome was parasite prevalence measured by PCR in children aged 7 months to 10 years. At 5 months and 12 months after LLIN distribution, the cluster-level adjusted *Plasmodium falciparum* PCR prevalence ratios were lower in children who received PBO LLINs compared with those who received conventional LLINs. In the LLINEUP trial in Uganda, LLINs with PBO (PermaNet 3.0 and Olyset Plus) and conventional LLINs (PermaNet 2.0 and Olyset Net) were randomly assigned to 104 clusters (health subdistricts) covering 48 districts. At 6 months, 12 months, and 18 months after LLIN distribution, parasite prevalence by microscopy in children aged 2–10 years was lower in the PBO LLIN clusters compared with clusters that received conventional LLINs.

A Cochrane systematic review published in 2021 assessed the effectiveness of PBO LLINs on epidemiological and entomological outcomes. 16 studies were included: ten experimental hut trials, four village trials, and two cluster-randomised controlled trials, including the Tanzanian trial and the LLINEUP trial in Uganda. This review concluded that in areas of high-level pyrethroid resistance, PBO LLINs are more efficacious than conventional LLINs, as evidenced by superior reduction in parasite prevalence, mosquito mortality, and reduction in mosquito feeding rates at 21 to 25 months post-LLIN distribution. However, evidence supporting the durability and epidemiological impact of PBO LLINs over the intended 3-year lifespan of the nets, and in areas of lower pyrethroid resistance, was lacking. Further epidemiological evidence of the effectiveness of PBO LLINs is urgently needed to guide WHO recommendations and malaria control policy throughout Africa, where pyrethroid resistance in malaria vectors is widespread.

Added value of this study

In this Article, we provide additional 25-month follow-up data from the LLINEUP trial, making an important contribution to the limited evidence base on use of PBO LLINs and meeting WHO requirements for assessing new vector control products. In this large, cluster-randomised, controlled trial, we found that PBO LLINs provided superior protection against malaria in the setting of high-level insecticide resistance in Uganda up to 25 months post-distribution.

Implications of all the available evidence

This study contributes to the evidence needed to support WHO's final recommendation on use of PBO LLINs. In April, 2021, WHO's Vector Control Advisory Group concluded that in areas with high-level pyrethroid resistance, PBO LLINs are more effective than conventional LLINs, confirming the public health value of PBO LLINs.

the COVID-19 pandemic. To maintain the trajectory of malaria control, interventions must be prioritised alongside COVID-19 and new technologies are needed to address drug and insecticide resistance.

WHO recommends distributing one LLIN for every two people at risk of malaria through mass campaigns conducted every 3 years.[7] All conventional LLINs are treated with pyrethroid insecticides; however, resistance to pyrethroids is now a major concern across Africa.[6,8] Pyrethroid resistance in *Anopheles* mosquitoes is mediated through knockdown resistance caused by alterations in the voltage-gated sodium channel where pyrethroids bind, and metabolic resistance due to changes in cytochrome P450s enzymes that detoxify pyrethroids.[9,10] To address metabolic resistance, newer LLINs that combine pyrethroids with the synergist piperonyl butoxide (PBO), which inhibits P450s enzymes and partially restores pyrethroid susceptibility in mosquito vectors, have been developed.[11]

The burden of malaria remains high in Uganda. In 2020, Uganda accounted for 5% of global malaria cases and deaths, with more than 14 million presumed and confirmed malaria cases reported.[3] LLINs are the primary tool for malaria prevention in Uganda and, in line with WHO recommendations, achieving high LLIN coverage nationwide has been prioritised.[7] In 2013–14, Uganda delivered LLINs free-of-charge through a mass distribution campaign, and the Ugandan Government has committed to delivering LLINs through mass campaigns every 3 years. In 2017–18, two brands of LLINs (Permanent [Vestergaard Frandsen, Roskilde, Denmark] and Olyset [Sumitomo Chemical, Tokyo, Japan]), including LLINs with and without PBO, were distributed across Uganda. Together with the Ugandan Ministry

of Health, we embedded a cluster-randomised trial (LLINEUP) within the national LLIN distribution campaign to evaluate the impact of PBO LLINs versus pyrethroid-only LLINs on parasite prevalence in children aged 2–10 years over 18 months of follow-up.[12] This earlier work demonstrated that children from communities that received PBO LLINs, had significantly lower parasite prevalence. Here, we report the results of a final survey conducted 25 months after LLIN distribution; for comparison, data for other timepoints (baseline, 6 months, 12 months, and 18 months) are also shown. These data were intended to provide the WHO with the information they require for assessment of new vector control interventions.[13]

Methods

Study design and setting

LLINEUP was a cluster-randomised trial conducted in eastern and western Uganda. Health subdistricts without ongoing or planned indoor residual spraying with pirimiphos-methyl (Actellic; Syngenta, Basel, Switzerland) were eligible for inclusion in the trial. A cluster was defined as one health subdistrict. Overall, 104 (47%) of the 221 health sub-districts within Uganda, located in 48 districts, were purposely selected for inclusion in the study. Of these, only 90 were evaluated in the 25-month survey (figure 1) and included in this analysis, because of the COVID-19 pandemic restrictions. Clusters were randomly assigned to receive one of four types of LLINs, including two PBO LLINs (PermaNet 3.0 and Olyset Plus), and two non-PBO LLINs treated with pyrethroid insecticides only (PermaNet 2.0 and Olyset Net).[12] Baseline cross-sectional community and entomology surveys were conducted after sensitisation of national and district authorities and local communities.[14–16] At baseline, pyrethroid resistance was high, due in part to knockdown resistance (primarily mediated through the mutation *Vgsc*-1014S) and metabolic mechanisms (characterised by the markers *Cyp4j5–43F* and *Coeae1d*).[15] LLINs were delivered free-of-charge in March 25–26, May 13–14, and July 22–23, 2017, and in March 17–18, 2018, through a mass-distribution campaign led by the Ministry of Health. Parasite prevalence as measured by microscopy in children aged 2–10 years was the primary outcome. Follow-up cross-sectional community and entomology surveys were conducted at 6 months, 12 months, and 18 months; these initial results have been published previously.[12] The 25 month survey was conducted between April 23, 2019 and September 13, 2019.

The trial protocol for this study has been published.[17] The trial was approved by the Ugandan National Council for Science and Technology (UNCST; reference HS 2176), Makerere University School of Medicine Research & Ethics Committee (SOMREC; 2016–133), London School of Hygiene & Tropical Medicine Ethics Committee (LSHTM; reference 12019), and the Liverpool School of Tropical Medicine (LSTM; reference 16–072), which sponsored the study.

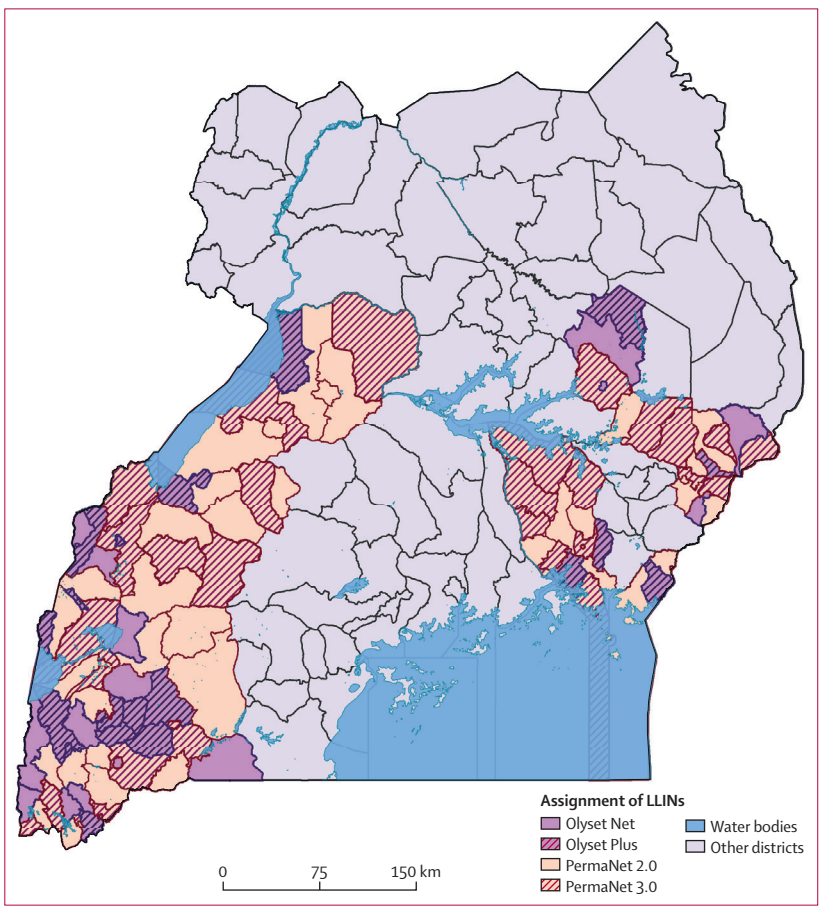


Figure 1: **Map of the study area**
The study included 104 clusters, defined as one health subdistrict.

Randomisation and net distribution

LLINs were procured before randomisation. Due to the size of the trial, the entire production capacity for the PBO LLINs was used; thus, the number of nets available for the four LLIN types varied. Proportionate randomisation was done by a co-investigator (GD) based outside of Uganda using STATA version 14.2 (StataCorp, College Station, TX, USA), as described previously.[17] Briefly, an iterative process was used to assign net types to each cluster using cumulative probability ranges generated for each type of LLIN based on the targeted number of each individual LLIN type, divided by the total number of LLINs; random numbers between 0 and 1 were generated for each cluster. The randomisation was stratified by region, with 66 clusters in the west and 38 clusters in the east, in case regional differences in insecticide resistance were found. However, no significant differences in resistance marker frequency by region or study group were observed at baseline. Allocation of LLINs was not masked.

The following LLINs were distributed: PermaNet 3.0 and PermaNet 2.0, and Olyset Plus and Olyset Net. LLINs were distributed nationwide by the Ministry of Health, local government, and partners. LLINs were delivered to the study clusters in 2017 (March 25–26, May 13–14, and July 22–23) and 2018 (March 17–18). An allocation formula was applied, using household registration data collected before the campaign, to determine the number of LLINs for each household (total number of household residents, divided by two, and rounded up if there was an uneven number of residents). After LLINs were delivered to the first 44 clusters, a shortfall of nets was recognised, and additional LLINs were procured. As a result, the final 60 clusters were re-randomised, using the same process of proportionate randomisation,[17] resulting in 52 clusters being assigned to each of the two main study groups (PBO *vs* non-PBO).[17] Only the 90 clusters that received LLINs in 2017 were included in the 25-month survey; the 14 clusters that received LLINs in 2018 could not be surveyed due to COVID-19 restrictions.

Procedures

A two-stage cluster sampling procedure using enumeration areas as the primary sampling unit was used. Ten enumeration areas (defined as a natural village or urban city block) were selected from each cluster; the same areas were used in each survey.[17] All households in the selected enumeration areas were mapped and assigned an identification number and a randomly selected list of households was generated for recruitment for each survey. Households were approached sequentially until five were enrolled from each enumeration area (50 households per cluster). Households were included if at least one child aged 2–10 years resided in the household, at least one adult (aged ≥18 years) was present, the adult was usually resident and slept in the household the previous night, and the adult agreed to provide written, informed consent to take part in the survey. Households were excluded if the house was destroyed or could not be located, the house was vacant, or no adult resident could be located on more than three occasions.

A household survey questionnaire was administered by study personnel to gather information on households, residents, and LLINs.[16,17] Children who met the following selection criteria had a finger-prick blood sample drawn by a clinician: aged 2–10 years, usually resident and slept in the sampled household the previous night, provision of written informed consent by parent or guardian, and provision of assent by the child if they were at least 8 years of age. Children who could not be located were excluded. A thick blood smear was prepared for all children enrolled; haemoglobin was measured in children aged 2–4 years using a portable HemoCue analyzer (HemoCue, Anglom, Sweden).

A subset of households enrolled into the community survey were randomly selected for inclusion into the entomology survey (ten households per cluster). Households were included if at least one adult (aged ≥18 years) was present, the adult was a usual resident who slept in the sampled household the previous night, and the adult resident agreed to provide written informed consent. The household was excluded if no adult resident was home on more than three occasions. Entomology technicians collected mosquitoes resting on indoor surfaces within enrolled households in the mornings (between 0700 h and 1000 h) for 10 min per house, using Prokopack aspirators (John W Hock, Gainesville, FL, USA). Female *Anopheles* mosquitoes were identified morphologically, stored over silica gel, and shipped to the Liverpool School of Tropical Medicine (Liverpool, UK) for molecular analysis (not reported in this Article).

Thick blood smears were delivered to the Infectious Diseases Research Collaboration Molecular Research Laboratory in Kampala within seven days of preparation. Slides were stained with 2% Giemsa for 30 min and read by experienced laboratory technologists. The number of asexual parasites per 200 leukocytes (or per 500 leukocytes if the parasite count was <10 per 200 leukocytes) was counted, assuming a leukocyte count of 8000 per µl. If no asexual parasites were detected after examination of 100-high power fields, a thick blood smear was considered negative. All slides were read by two microscopists, and a third reviewer settled discrepant readings, defined as positive versus a negative thick blood smear, or parasite density differing by at least 25%.

Outcomes

The primary outcome was parasite prevalence (proportion of children aged 2–10 years with asexual parasites detected by microscopy). In this Article, we only report overall results, not stratified by region, because of the reduction in sample size due to the COVID-19 pandemic restrictions.

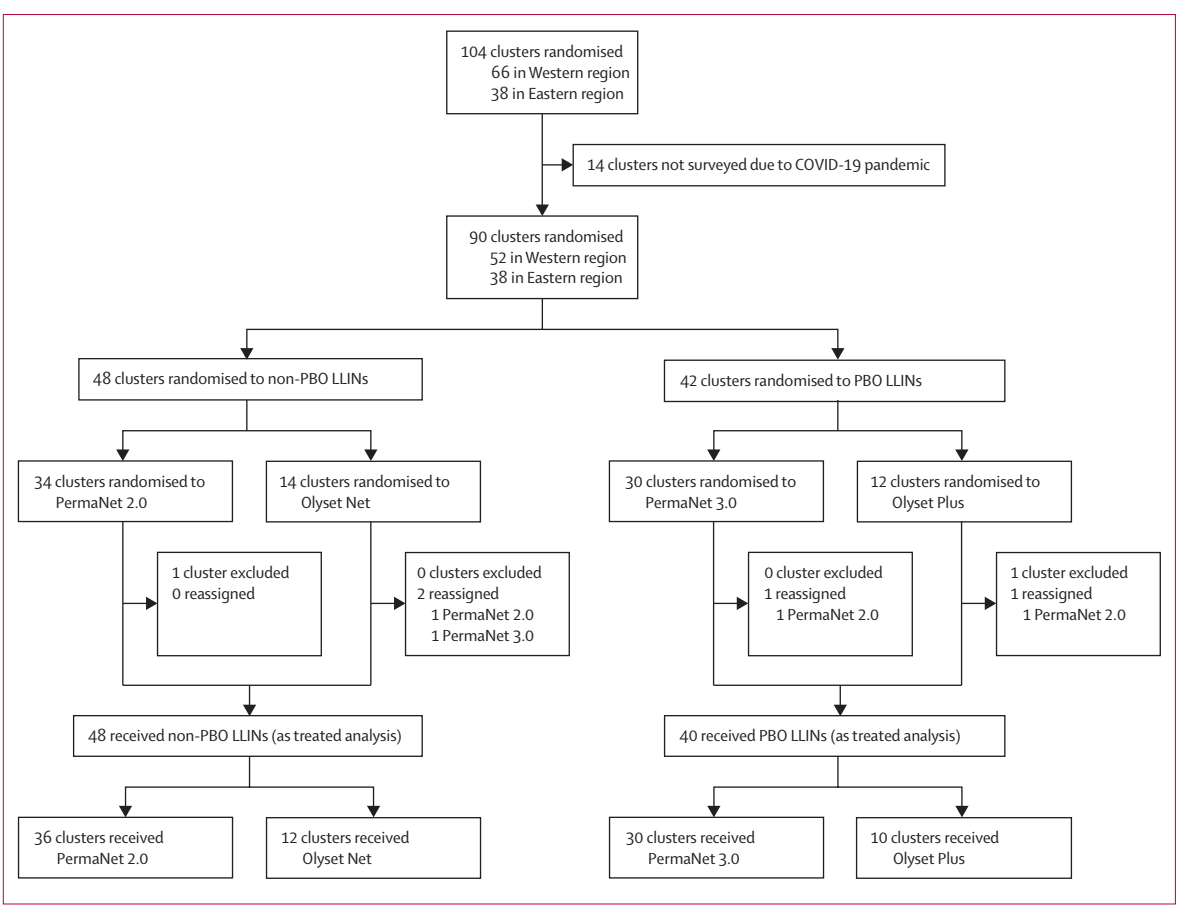
Secondary outcomes included: prevalence of any anaemia (haemoglobin concentration <11 g/dL), vector density (the number of female *Anopheles* collected per household), LLIN ownership (the proportion of households that owned at least one LLIN), adequate LLIN coverage (the proportion of households that owned at least one LLIN for every two residents, as recommended by WHO), and LLIN use (the proportion of household residents who slept under an LLIN the previous night). Results of prevalence of moderate or severe anaemia (haemoglobin concentration <10 g/dL) up to 18 months post-distribution were presented before.[12] Because this outcome was uncommon, which limited the power to detect differences between the study groups, we opted to present only results for prevalence of any anaemia at the 25 month timepoint. More detailed assessments of LLIN integrity and bioefficacy have also been published separately.[20]

Statistical analysis

The number of clusters available for inclusion in the trial was dictated by the population of the clusters and number of LLINs available. In the cross-sectional community surveys, we aimed to sample all eligible children aged 2–10 years from 50 households. Assuming a parasite prevalence of 40% in the non-PBO group (control group),[18] and coefficient of variation between clusters of 0·3 (as determined in the Tanzanian trial),[19] with the original 104 clusters, we had 80% power (with a two-sided significance of 0·05) to detect a relative reduction in parasite prevalence of at least 17%, resulting in a prevalence ratio of 0·83. Given these assumptions, reducing the number of clusters from 104 to 90 had a modest impact on the power of the study to detect a relative reduction in parasite prevalence of at least 17% (reduction in power from 80% to 74%).

We applied both an intention-to-treat (based on randomised study group assignments) and as-treated approach to all analyses. We decided to present the as-treated analyses as the main study findings a priori, because this approach most accurately reflected the type of LLINs actually received in each cluster, and was deemed appropriate for this unique, large-scale, effectiveness study. Final as-treated study group assignments were determined using the results from the 6-month household survey on the type of LLINs received in each cluster. If LLIN distribution in a cluster was mixed, the number of dominant nets received (numerator) was divided by the total number of study nets received in that cluster (denominator); non-study nets were excluded. If the predominant net was higher than 75% of all study nets received, the cluster was included in the as-treated analysis; if the predominant net type received was 75% or less, the cluster was excluded. Because of the large number of clusters per group, an individual-level analytical approach was used.

A log-binomial regression model with generalised estimating equations and an exchangeable correlation structure was used for analysis of the primary outcome, allowing for within-cluster correlations and adjustment for baseline cluster-level parasite prevalence. The effect of PBO LLINs was expressed as the prevalence ratio (prevalence in the intervention group [PBO LLINs] divided by prevalence in the control group [non-PBO LLINs]). A within-treatment group analysis of changes in parasite prevalence over time with respect to baseline was also conducted. A log-binomial regression model



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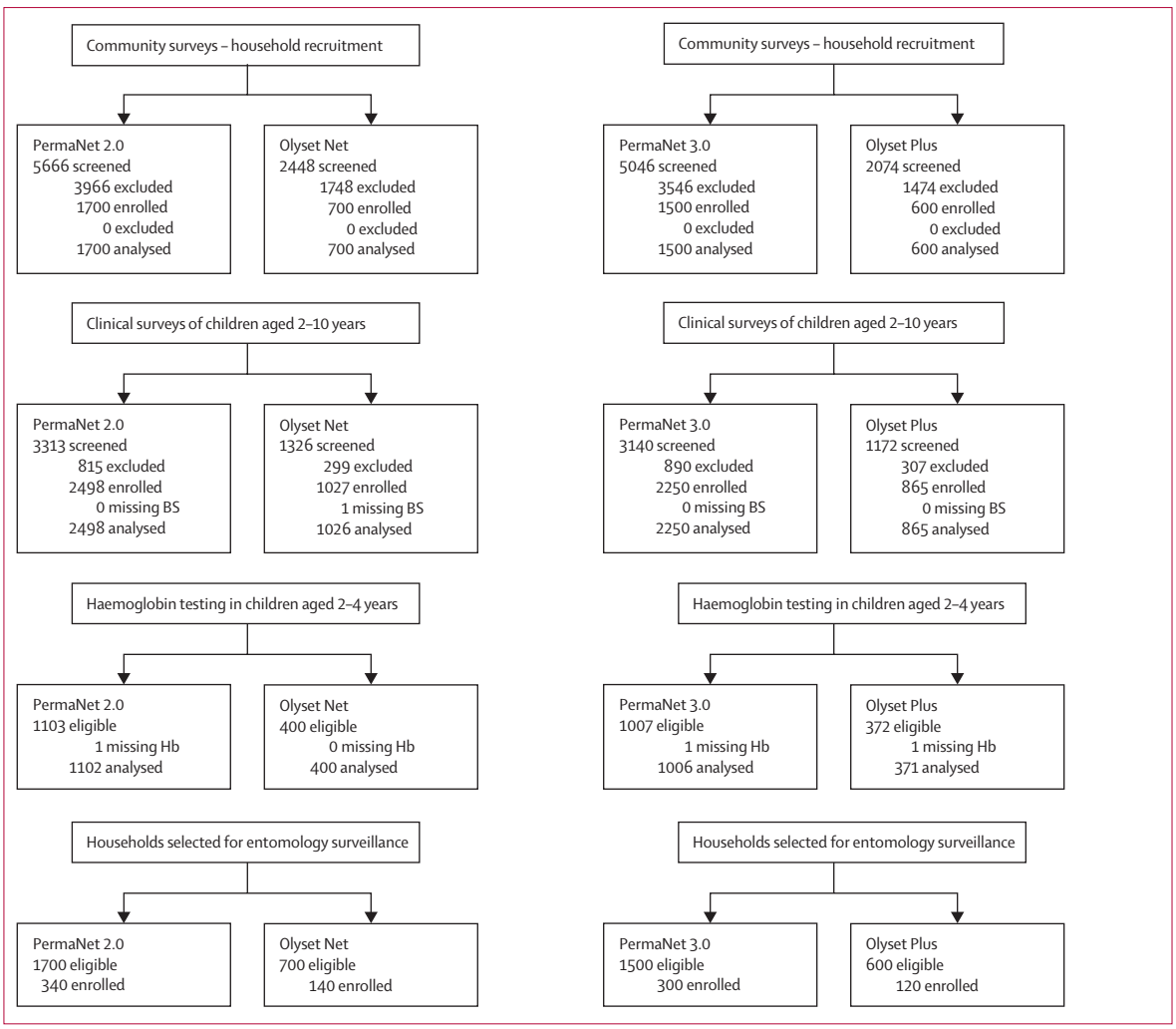

Figure 2: **Trial profile**
LLIN=long-lasting insecticidal net. PBO=piperonyl butoxide. BS=blood slide. Hb=haemoglobin.

with generalised estimating equations and an exchangeable correlation structure was used to allow for within-cluster correlations. The change in parasite prevalence for each of the four cross-sectional surveys was expressed as a prevalence ratio (prevalence measured in each survey post-distribution divided by prevalence at baseline).

For analyses of prevalence of anaemia, the same generalised estimating equation with adjustment for baseline cluster-level values of parasite prevalence and an exchangeable correlation structure was used as for the primary outcome. For comparison of vector density, a negative binomial regression model was used with generalised estimating equations and an exchangeable correlation structure to allow for within-cluster correlations and adjustment for baseline cluster-level vector density. The effect of PBO LLINs was expressed as the density ratio (density in the intervention group divided by density in the control group). A within-treatment group analysis of changes in *Anopheles* density overtime with respect to baseline was also conducted. A negative binomial regression model with generalised estimating equations and an exchangeable correlation structure was used to allow for within-cluster correlations. The change in *Anopheles* density for each of the four cross-sectional surveys was expressed as a density ratio (density in post distribution cross-sectional survey divided by density at baseline).

Proportions for LLIN ownership, coverage, and use were estimated for each cluster at each timepoint. The analysis was done using R.

We also conducted prespecified subgroup analysis stratified by LLIN brand for parasite prevalence and prevalence of anaemia.

Statistical analyses were done with STATA (version 14.2) and R software. A p value of less than 0·05 was considered statistically significant for all analyses.

This trial is registered with ISRCTN, ISRCTN17516395.

	Combined study groups		Individual study groups			
	Non-PBO LLIN	PBO LLIN	PermaNet 2.0	PermaNet 3.0	Olyset Net	Olyset Plus
Number of clusters	48	42	34	30	14	12
Number of households surveyed	2399	2098	1699	1498	700	600
Household in the lowest tertile of wealth*	32·9% (17·6)	36·3% (20·9)	30·7% (16·2)	35·9% (20·0)	38·1% (20·2)	37·3% (24·1)
Households with modern house construction*	25·1% (21·4)	27·5% (22·8)	27·6% (21·0)	27·0% (21·1)	19·0% (22·0)	28·8% (27·5)
Households with at least one LLIN*	63·9% (14·7)	64·6% (14·1)	63·7% (13·2)	63·2% (12·6)	64·6% (18·4)	67·8% (17·6)
Households with adequate LLINs*	17·5% (11·4)	17·2% (11·2)	16·9% (11·0)	16·3% (11·2)	18·9% (12·8)	19·5% (11·1)
Number of children aged 2–10 years tested for parasitaemia	3973	3676	2881	2674	1092	1002
Parasite prevalence in children aged 2–10 years*	17·8% (9·2–40·7)	24·3% (3·1–45·1)	24·5% (12·5–42·0)	25·8% (9·5–42·0)	12·5% (1·5–24·7)	14·6% (1·4–47·6)
Number of children aged 2–4 years tested for anaemia	1714	1569	1221	1149	493	420
Anaemia prevalence in children aged 2–4 years*†	33·3% (22·2–46·0)	28·7% (20·0–45·2)	36·9% (24·0–47·1)	27·2% (20·0–42·1)	27·8% (17·1–35·9)	36·1% (16·1–50·0)
Number of households selected for entomological collections	473	414	334	296	139	118
Household vector density‡	0·3 (0–2·5)	0·5 (0–9·3)	0·3 (0–4·4)	0·8 (0·2–10·2)	0·2 (0–1·4)	0·3 (0–5·2)

Data are n, median (SD), or median (IQR). PBO LLIN=piperonyl butoxide long-lasting insecticidal net. *Proportions calculated at the level of each cluster. †Anaemia is defined as haemoglobin concentration less than 11 g/dL. ‡Median values calculated at the level of each cluster.

Table 1: **Baseline characteristics**

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Of the original 104 clusters, 14 clusters were not surveyed at the 25-month timepoint due to COVID-19 restrictions (figure 2). Of the 90 clusters surveyed, 48 were randomised to receive non-PBO LLINs (36 PermaNet 2.0, 12 Olyset Net), and 42 to PBO LLINs (30 PermaNet 3.0, 12 Olyset Plus). In the as-treated analysis, two clusters were excluded because no dominant LLIN type was received, and four were reassigned to a different study group; thus, 48 non-PBO LLIN clusters (36 PermaNet 2.0, 12 Olyset Net) and 40 PBO LLIN clusters (30 PermaNet 3.0, ten Olyset Plus) were included in the analysis. The LLINs were distributed by the Ministry of Health, so we do not have an accurate account of number of people covered by each LLIN. Cross-sectional community surveys were conducted 25 months post-LLIN distribution from April 23, to Sept 13, 2019, in the 90 clusters.

Characteristics of households included in the baseline survey, before LLIN distribution, in the 90 clusters (n=4497, median 50 per cluster [IQR 50–50]) were similar in both study groups (table 1). Mean cluster-level household ownership of at least one LLIN was 64·2% (SD 17·4), but adequate coverage was much lower (17·3% [11·3]). At baseline, in children aged 2–10 years tested for parasitaemia (n=7649, median 83 per cluster [IQR 77–92]), median cluster-level parasite prevalence was similar between the study groups (24·3% [IQR 3·1–45·1] PBO group *vs* 17·8% [IQR 9·2–40·7] non-PBO group), but ranged widely (0–76·7%); there was no difference in median cluster-level parasite prevalence between clusters randomly assigned to one of the PermaNet groups and those assigned to one of the Olyset groups (25·3% [IQR 10·9–42·0] *vs* 13·7% [1·4–37·5]). In children aged 2–4 years tested for haemoglobin (n=3283), median cluster-level prevalence of anaemia (haemoglobin concentration <11 g/dL) was similar in the PBO (28·7% [IQR 20·0–45·2]) and non-PBO (33·3% [22·2–46·0]) study groups. Of households included in the baseline entomology survey (n=887, median ten per cluster [IQR 10–10]), median cluster-level household vector density was low and similar between the study arms (0·5 [IQR 0–9·3] female *Anopheles* per house in the PBO group vs 0·3 [0–2·5] female *Anopheles* per house in the non-PBO group, p=0·13).

In both the PBO and non-PBO groups, parasite prevalence decreased from baseline at 6 months, remained low at 12 and 18 months, but began to rise again at 25 months (figure 3A). In the as-treated analysis, parasite prevalence 25 months after LLIN distribution was 17·1% (95% CI 15·8–18·5; 506 of 2958 participants) in the PBO group *vs* 19·8% (18·5–21·2; 701 of 3534 participants) in the non-PBO group (prevalence ratio adjusted for baseline values 0·80 [95% CI 0·69–0·93], p=0·0048; table 2). Comparing within-treatment group parasite prevalences to baseline, parasite prevalence ratios were lower in the PBO groups at all timepoints, but the difference was greatest at 6 and

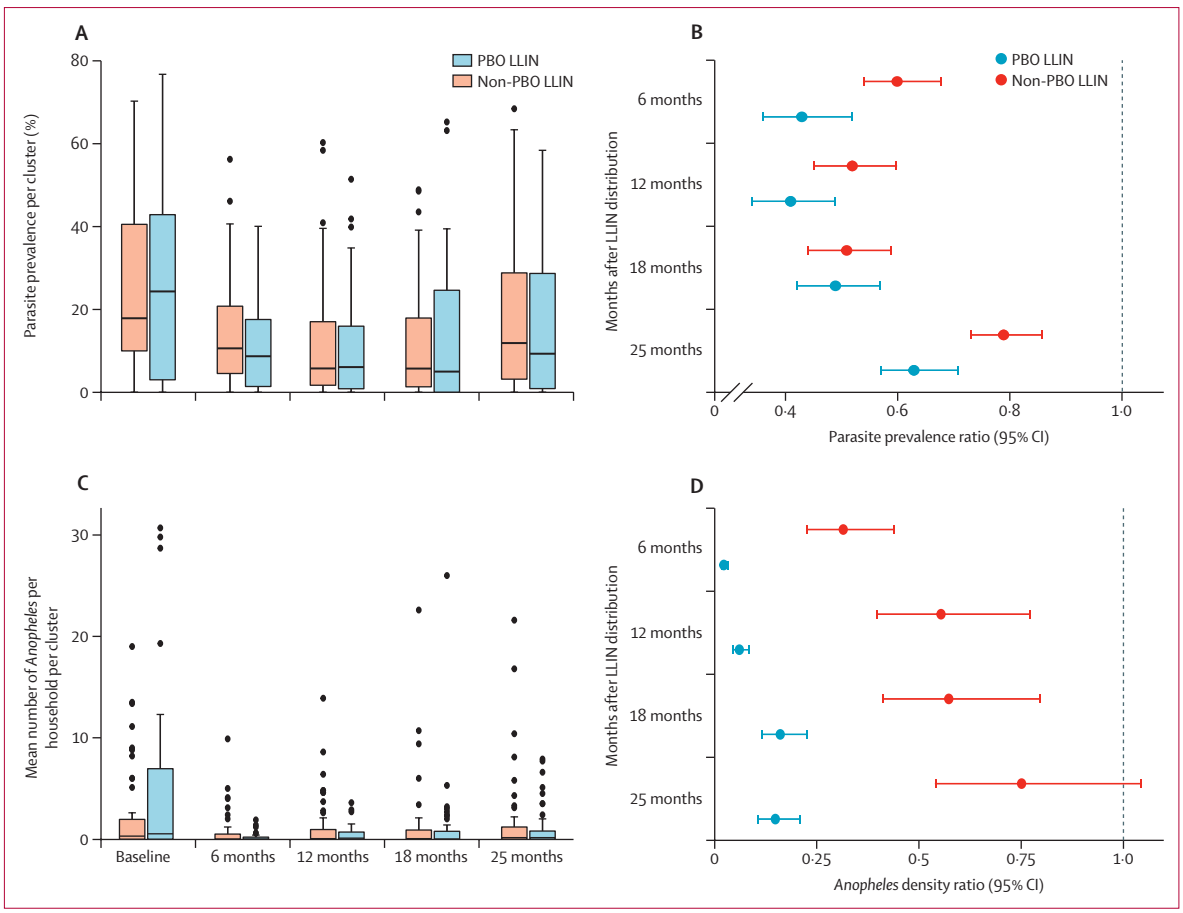

Figure 3: **Trends in parasite prevalence and vector density for PBO versus non-PBO groups (at baseline, 6, 12, 18, and 25 months)**
(A) Parasite prevalence per cluster in children aged 2–10 years as measured by microscopy; the upper whisker represents upper Q3 plus 1·5 × IQR and the lower whisker represents Q1 minus 1·5 × IQR. (B) Parasite prevalence ratios (95% CI) comparing results at each of the 4 follow-up timepoints to baseline. (C) Mean number of *Anopheles* mosquitoes captured per household per cluster; the upper whisker represents upper Q3 plus 1·5 × IQR and the lower whisker represents Q1 minus 1·5 × IQR. (D) *Anopheles* density ratios (95% CI) comparing results at each of the 4 follow-up timepoints to baseline. LLIN=long-lasting insecticidal net. PBO=piperonyl butoxide.

25 months (figure 3B). In the subgroup analysis stratified by brand, parasite prevalence was lower in the PermaNet 3·0 (with PBO) group than the PermaNet 2·0 (non-PBO) group at 25 months (table 2). No difference was observed between the Olyset Plus (with PBO) and Olyset Net (non-PBO) groups. Results of the intention-to-treat analysis are shown in table 2.

During the 25-month follow-up period, trends in LLIN ownership (households with at least one net), coverage (households with one net per two people), and usage (self-reported used of net previous night) were similar in both study groups (appendix p 1). At 6 months, most households (4251 [96·7%] of 4396) owned at least one LLIN. LLIN ownership remained high at 12 months (4192 [95·3%] of 4400) and 18 months (4065 [92·4%] of 4400), but decreased at 25 months (3686 [83·8%] 4400). By 6 months, adequate LLIN coverage had increased sharply from baseline (from 764 [17·4%] of 4400 to 3114 [70·8%] of 4396), but steadily decreased at 12 (2841 [64·6%] of 4400) and at 18 months (2396 [54·5%] of 4400), decreasing even further at 25 months (1530 [34·8%] of 4400). Despite this decline in adequate LLIN coverage, most study participants reported sleeping under a LLIN the previous night at 6 months (20 636 [85·2%] of 24 218 participants), 12 months (19 230 [79·5%] of 24 195) and 18 months (18 568 [75·7%] of 24 518), but with a notable decline at 25 months (14 534 [60·2%] of 24 161).

In the as-treated analysis, there was no difference in prevalence of anaemia at 25 months between the PBO and non-PBO groups (347 [26·7%] of 1299 participants *vs* 384 [25·6%] of 1502], prevalence ratio adjusted for baseline values 1·07 [95% CI 0·77–1·49], p=0·67; table 2). In the subgroup analysis stratified by brand, no statistically significant differences in prevalence of anaemia were observed between the PermaNet groups, or the Olyset groups.

In the households included in the as-treated analysis of the entomology surveys, 483 female *Anopheles* mosquitoes were identified at 25 months in 400 household collections in the PBO group compared with 866 in 480 collections in the non-PBO group (table 3). In the

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	Intention-to-treat analysis			As-treated analysis		
	n/N (%; 95% CI)	PR (95% CI)	p value	n/N (%)	PR (95% CI)	p value
Parasite prevalence						
Non-PBO LLIN	692/3524 (19·6%; 18·3–21·0)	Ref	..	701/3534 (19·8%; 18·5–21·2)	Ref	..
PBO LLIN	552/3115 (17·7%; 16·4–19·1)	0·86 (0·74–1·00)	0·054	506/2958 (17·1%; 15·8–18·5)	0·80 (0·69–0·93)	0·0048
PermaNet 2.0	580/2498 (23·2%; 21·6–24·9)	Ref	..	596/2629 (22·7%; 21·1–24·3)	Ref	..
PermaNet 3.0	415/2250 (18·4%; 16·9–20·1)	0·82 (0·69–0·98)	0·028	414/2246 (18·4%; 16·8–20·1)	0·79 (0·66–0·93)	0·0055
Olyset Net	112/1026 (10·9%; 9·1–13·0)	Ref	..	105/905 (11·6%; 9·6–13·9)	Ref	..
Olyset Plus	137/865 (15·8%; 13·5–18·4)	1·02 (0·74–1·41)	0·89	92/712 (12·9%; 10·5–15·6)	0·90 (0·63–1·29)	0·57
Anaemia prevalence						
Non-PBO LLIN	385/1502 (25·6%; 23·4–27·9)	Ref	..	384/1502 (25·6%; 23·4–27·9)	Ref	..
PBO LLIN	379/1377 (27·5%; 25·2–30·0)	1·15 (0·84–1·57)	0·39	347/1299 (26·7%; 24·3–29·2)	1·07 (0·77–1·49)	0·67
PermaNet 2.0	290/1102 (26·3%; 23·7–29·0)	Ref	..	303/1151 (26·3%; 23·8–29·0)	Ref	..
PermaNet 3.0	280/1006 (27·8%; 25·1–30·7)	1·17 (0·82–1·69)	0·39	285/991 (28·8%; 26·0–31·7)	1·14 (0·79–1·65)	0·47
Olyset Net	95/400 (23·8%; 19·7–28·2)	Ref	..	81/351 (23·1%; 18·8–27·8)	Ref	..
Olyset Plus	99/371 (26·7%; 22·3–31·5)	1·04 (0·55–1·97)	0·90	62/308 (20·1%; 15·8–25·0)	0·79 (0·41–1·54)	0·49

PR=prevalence ratio. PBO=piperonyl butoxide. LLIN=long-lasting insecticidal net.

Table 2: **Parasite and anaemia prevalence at 25 months**

	Intention-to-treat analysis				As-treated analysis			
	Number of mosquitoes	Number of collections	DR (95% CI)	p value	Number of mosquitoes	Number of collections	DR (95% CI)	p value
Non PBO LLIN	905	480	Ref	..	866	480	Ref	..
PBO LLIN	542	420	0·38 (0·29–0·49)	<0·0001	483	400	0·27 (0·21–0·36)	<0·0001
PermaNet 2·0	511	340	Ref	..	472	360	Ref	..
PermaNet 3·0	375	300	0·36 (0·25–0·53)	<0·0001	374	300	0·38 (0·26–0·55)	<0·0001
Olyset Net	394	140	Ref	..	394	120	Ref	..
Olyset Plus	167	120	0·49 (0·31–0·78)	0·0024	109	100	0·14 (0·09–0·23)	<0·0001

DR=density ratio. PBO=piperonyl butoxide. LLIN= long-lasting insecticidal net.

Table 3: **Vector density per study group at 25 months**

as-treated analysis the *Anopheles* density ratio was 0·27 (95% CI 0·21–0·36, p<0·0001). In both the PBO and non-PBO groups, the mean *Anopheles* per household per cluster decreased from baseline at 6 months and remained lower over time in each group up to 25-months after LLIN distribution (figure 3C). Comparing vector density at all four survey rounds to baseline, *Anopheles* density ratios were lower in the PBO groups at all timepoints (figure 3D). In the subgroup analysis, vector density was lower in the PBO than the non-PBO group for both PermaNet and Olyset nets (table 2).

Discussion

LLINs are the foundation for malaria control in Africa, but widespread resistance of mosquito vectors to pyrethroid insecticides threatens their effectiveness. PBO LLINs are a promising new tool, but evidence supporting their implementation is incomplete. Here, we report the final epidemiological results of the LLINEUP trial, collected 25 months after LLIN distribution. Despite the COVID-19 pandemic, which affected our ability to obtain a full set of data from all study clusters, we found that PBO LLINs provided greater protection than conventional, non-PBO LLINs for more than 2 years after delivery. During the first 18 months of follow-up, reduced parasite prevalence and vector density relative to baseline were observed in both study groups; this effect was sustained at 25 months, but appears to be waning. Importantly, by 25 months, adequate LLIN coverage (at least one net for every two people) dropped to about 35%, and the proportion of residents who used an LLIN the previous night declined to about 60%.

Ensuring access to LLINs is key to achieving adequate coverage and maximising use of LLINs. In this study, we found that household ownership of at least one LLIN remained high during the first 18 months, declining at 25 months. The decline in LLIN use was temporally associated with the increase in parasite prevalence observed in both study groups at 25 months, suggesting that the waning effect of LLINs might be associated with the combined effects of net attrition and inadequate LLIN use. Studies in Uganda and elsewhere have demonstrated that the lifespan of LLINs is often less

than 3 years, highlighting the important challenge of net attrition.[16,21,22] In an assessment of LLIN access and use from 2000 to 2020 in 40 African countries, median LLIN retention times were calculated to be less than 3 years in 35 countries, with an overall median of 1·64 years.[23] This analysis also found that LLIN use was high among individuals with access to a net, suggesting that LLIN use can be optimised by ensuring adequate coverage and access to LLINs.[23] The results of the LLINEUP trial add to the evidence supporting more intensive LLIN distribution, through more frequent mass distribution campaigns, ideally every 2 years, and by strengthening routine LLIN distribution channels, such as delivering LLINs through antenatal clinics and schools. Inconsistent LLIN use is another challenge, which might be due to lack of awareness of the potential benefits of LLINs, low perceived risk of malaria, and discomfort due to heat.[23–25] To target improved adherence, the Ministry of Health should prioritise continuous educational messaging on LLIN use, care, repair, and re-purposing to improve LLIN use and reduce attrition over time. Further qualitative and mixed-methods research would help to improve our understanding of net attrition and barriers to LLIN use.

Chemical integrity and bioefficacy are key determinants of LLIN effectiveness. Our study was designed to compare the two WHO defined product types, namely PBO (pyrethoid-PBO) nets and non-PBO (pyrethroid-only) nets. Although both PermaNet 3.0 and Olyset Plus incorporate PBO, the characteristics of these LLINs, and of the non-PBO nets produced by both companies (PermaNet 2.0 and Olyset Net), vary in terms of insecticide used, concentrations, net weight and construction.[26–27] Both PermaNet nets incorporate deltamethrin, but at different concentrations; PermaNet 2.0 incorporates 1·8 g/kg deltamethrin throughout the net, whereas PermaNet 3.0 has 2·8 g/kg on (75 denier) side panels and 4·0 g/kg on the roof panel, along with PBO. Thus, it is not possible to disaggregate the effect of increased deltamethrin concentrations on PermaNet 3.0 and the presence of PBO. In contrast, both Olyset nets incorporate a similar concentration of permethrin throughout the nets, with Olyset Plus including PBO. These differences in net composition, in addition to the differences in sample size and geographical distribution of the different LLINs in the trial, limit our ability to make direct comparisons between the different net brands in this study. Further research designed to specifically make such comparisons would be required. As part of the LLINEUP trial, we withdrew LLINs from the community at 12 months and 25 months after LLIN distribution, assayed the chemical content of the nets (pyrethroid insecticide and PBO), and conducted bioassays on the same nets with pyrethroid-resistant mosquitoes.[17,20] We observed that that the concentration of PBO on the net was strongly predictive of mosquito mortality. These results have been published separately.[20]

Limitations to the design of this pragmatic trial embedded with a LLIN distribution campaign led by the Ugandan Ministry of Health have been discussed.[12] Additional limitations were present in the current study. First, we were only able to survey 90 of the 104 clusters included in the main trial due to COVID-19 restrictions. This reduction in cluster number had modest impact on the power of the study. Second, the trial was not powered to directly compare the different LLIN brands, preventing us from drawing any conclusions about the superiority of either brand (PermaNet or Olyset). The sample size assessed in the Olyset groups (22 clusters, 1891 participants) was lower than in the PermaNet arms (66 clusters, 4748 participants). Although subgroup analyses stratified by manufacturer do suggest some differences in LLIN performance, these analyses are limited by small sample size, uneven distribution of the LLIN brands, geographical imbalances in LLIN distribution, and differences in baseline parasite prevalence. Third, LLINs were distributed over 12 months, and consequently, the follow-up surveys were also spread out over the year. Although malaria transmission in Uganda is seasonal, we think that prolonged distribution is unlikely to have resulted in bias, because the trial was randomised. Fourth, in this pragmatic trial, we detected some contamination in our 6-month follow-up surveys possibly due to errors in net distribution, movement of nets between clusters, or reporting errors. However, the low level of contamination remaining would have likely biased towards the null. Finally, although we relied on self-report to measure LLIN use, which might have led to reporting bias with residents reporting that they slept under a LLIN because they believe this to be the correct answer, we doubt that self-reporting LLIN use would have affected the primary outcome of the trial. Parasite prevalence was assessed by experienced technologists in a reference laboratory; although the trial was not blinded, we do not suspect this would have led to measurement bias.

WHO's initial interim endorsement of PBO LLINs was supported by the results of a cluster-randomised trial conducted in Tanzania.[19] A systematic review of PBO LLINs also found that PBO LLINs were more effective in areas of high-level insecticide resistance.[28] Based on these results, WHO recommended PBO LLINs for areas of intermediate-level pyrethroid resistance mediated at least partly by metabolic mechanisms.[29] The results of the LLINEUP trial provide evidence that PBO LLINs offer superior protection against malaria in the setting of high-level pyrethroid resistance up to 25 months after distribution. These data were shared with the Cochrane Infectious Diseases Group in advance of publication and were integrated into the revised Cochrane Review on PBO LLINs.[11] This review concluded that "In areas of high insecticide resistance, pyrethroid-PBO nets have greater entomological and epidemiological efficacy compared to conventional LLINs, with sustained reduction in parasite

prevalence, higher mosquito mortality and reduction in mosquito blood feeding rates 21 to 25 months post intervention". The findings of a subsequently published cluster-randomised trial conducted in western Kenya in 2009–11 were consistent with these conclusions; parasite prevalence measured by PCR in children aged 7 months to 10 years was significantly lower at 5 and 12 months post-LLIN distribution in clusters that received PBO LLINs (Olyset Plus) compared with conventional LLINs (Olyset Net).[30] Considering the available data, in April 2021, WHO's Vector Control Advisory Group concluded that PBO LLINs are more effective than conventional non-PBO LLINs in areas with high-level pyrethroid resistance in malaria mosquito vectors, confirming the public health value of PBO LLINs.[31]

In conclusion, we found that PBO LLINs provide superior protection against malaria prevalence and vector abundance 25 months post-distribution. Given widespread metabolic resistance of *Anopheles* vectors to pyrethroid insecticides across sub-Saharan Africa, and the falling price differential of PBO LLINs, we recommend widespread of distribution of PBO LLINs in Africa. These results provide some positive news in the time of COVID-19; new improved tools are available for malaria control. We provide this recommendation with the important caveat that the benefits of both PBO and non-PBO LLINs appear to be waning at 25 months. To maximise the benefits of LLINs, strategies to ensure high LLIN coverage, including more frequent mass campaigns, distribution of adequate numbers of LLINs during campaigns, and strengthened routine distribution channels, should be deployed, supported by intensified social and behaviour change communication messaging.

Contributors

SGS, GD, MRK, and MJD conceived the study with input from JO and JH. SGS, GD, and MJD developed the procedures and drafted the protocol with MRK and JH. CM-S, SG, and AL developed the standard operating procedures. SG, AK, IB, and AL led the data collection in the field, with oversight from SGS, CM-S, JO, MRK, and MJD. PM and SPK managed the data, with support from SGS, SG, AL, and GD. PM and GD had full access to all the data in the study and verified all data. GD led the data analysis, with support from SGS, MJD, MRK, and JH. All authors had access to all of the data in this study, reviewed the manuscript, and gave permission for publication. SGS, the corresponding author, had final responsibility for the decision to submit for publication.

Declaration of interests

We declare no competing interests.

Data sharing

De-identified participant data and a data dictionary defining each field in the set will be made publicly available at the time of publication on the ClinEpiDB website. The study protocol has been published.[17]

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References

1 Bhatt S, Weiss DJ, Cameron E, et al. The effect of malaria control on *Plasmodium falciparum* in Africa between 2000 and 2015. *Nature* 2015; **526:** 207–11.
2 WHO. World malaria report 2020: 20 years of global progress and challenges. Geneva: World Health Organization, 2020.
3 WHO. World malaria report 2021. Geneva: World Health Organization, 2021.
4 Dondorp AM, Nosten F, Yi P, et al. Artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med* 2009; **361:** 455–67.
5 Balikagala B, Fukuda N, Ikeda M, et al. Evidence of artemisinin-resistant malaria in Africa. *N Engl J Med* 2021; **385:** 1163–71.
6 Hemingway J, Ranson H, Magill A, et al. Averting a malaria disaster: will insecticide resistance derail malaria control? *Lancet* 2016; **387:** 1785–88.
7 WHO. Achieving and maintaining universal coverage with long-lasting insecticidal nets for malaria control. Geneva: World Health Organization, 2017.
8 Ranson H, Lissenden N. Insecticide resistance in African Anopheles mosquitoes: a worsening situation that needs urgent action to maintain malaria control. *Trends Parasitol* 2016; **32:** 187–96.
9 Muller P, Warr E, Stevenson BJ, et al. Field-caught permethrin-resistant Anopheles gambiae overexpress CYP6P3, a P450 that metabolises pyrethroids. *PLoS Genet* 2008; **4:** e1000286.
10 Weetman D, Wilding CS, Neafsey DE, et al. Candidate-gene based GWAS identifies reproducible DNA markers for metabolic pyrethroid resistance from standing genetic variation in East African Anopheles gambiae. *Sci Rep* 2018; **8:** 2920.
11 Gleave K, Lissenden N, Chaplin M, Choi L, Ranson H. Piperonyl butoxide (PBO) combined with pyrethroids in insecticide-treated nets to prevent malaria in Africa. *Cochrane Database Syst Rev* 2021; **5:** Cd012776.
12 Staedke SG, Gonahasa S, Dorsey G, et al. Effect of long-lasting insecticidal nets with and without piperonyl butoxide on malaria indicators in Uganda (LLINEUP): a pragmatic, cluster-randomised trial embedded in a national LLIN distribution campaign. *Lancet* 2020; **395:** 1292–303.
13 WHO. Design of epidemiological trials for vector control products, Report of a WHO Expert Advisory Group. Geneva: World Health Organization, 2017.
14 Rugnao S, Gonahasa S, Maiteki-Sebuguzi C, et al. LLIN Evaluation in Uganda Project (LLINEUP): factors associated with childhood parasitaemia and anaemia 3 years after a national long-lasting insecticidal net distribution campaign: a cross-sectional survey. *Malar J* 2019; **18:** 207.
15 Lynd A, Gonahasa S, Staedke SG, et al. LLIN Evaluation in Uganda Project (LLINEUP): a cross-sectional survey of species diversity and insecticide resistance in 48 districts of Uganda. *Parasit Vectors* 2019; **12:** 94.
16 Gonahasa S, Maiteki-Sebuguzi C, Rugnao S, et al. LLIN Evaluation in Uganda Project (LLINEUP): factors associated with ownership and use of long-lasting insecticidal nets in Uganda: a cross-sectional survey of 48 districts. *Malar J* 2018; **17:** 421.
17 Staedke SG, Kamya MR, Dorsey G, et al. LLIN Evaluation in Uganda Project (LLINEUP) - impact of long-lasting insecticidal nets with, and without, piperonyl butoxide on malaria indicators in Uganda: study protocol for a cluster-randomised trial. *Trials* 2019; **20:** 321.
18 Uganda Bureau of Statistics. Uganda malaria indicator survey 2009. Calverton, MD: ICF Macro, 2010.
19 Protopopoff N, Mosha JF, Lukole E, et al. Effectiveness of a long-lasting piperonyl butoxide-treated insecticidal net and indoor residual spray interventions, separately and together, against malaria transmitted by pyrethroid-resistant mosquitoes: a cluster, randomised controlled, two-by-two factorial design trial. *Lancet* 2018; **391:** 1577–88.

For the **ClinEpiDB website** see https://clinepidb.org/ce/app/workspace/analyses/DS_7c4cd6bba9/new/details

20 Mechan F, Katureebe A, Tuhaise V, et al. LLIN evaluation in Uganda project (LLINEUP): the fabric integrity, chemical content and bioefficacy of long-lasting insecticidal nets treated with and without piperonyl butoxide across two years of operational use in Uganda. *Curr Res Parasitol Vector Borne Dis* 2022; **2:** 100092.

21 Gnanguenon V, Azondekon R, Oke-Agbo F, Beach R, Akogbeto M. Durability assessment results suggest a serviceable life of two, rather than three, years for the current long-lasting insecticidal (mosquito) net (LLIN) intervention in Benin. *BMC Infect Dis* 2014; **14:** 69.

22 Lorenz LM, Bradley J, Yukich J, et al. 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. *PLoS Med* 2020; **17:** e1003248.

23 Bertozzi-Villa A, Bever CA, Koenker H, et al. Maps and metrics of insecticide-treated net access, use, and nets-per-capita in Africa from 2000–2020. *Nat Commun* 2021; **12:** 3589.

24 Strachan CE, Nuwa A, Muhangi D, Okui AP, Helinski ME, Tibenderana JK. What drives the consistent use of long-lasting insecticidal nets over time? A multi-method qualitative study in mid-western Uganda. *Malar J* 2016; **15:** 44.

25 Rek J, Musiime A, Zedi M, et al. Non-adherence to long-lasting insecticide treated bednet use following successful malaria control in Tororo, Uganda. *PLoS One* 2020; **15:** e0243303.

26 WHO. Report of the fifteenth WHOPES working group meeting: WHO/HQ, Geneva, 18-22 June 2012: review of Olyset plus, Interceptor LN, Malathion 440 EW, Vectobac GR. Geneva: World Health Organization, 2012.

27 WHO. Report of the 12th WHOPES Working Group meeting – review of Bioflash® GR, ermanent® 2.0, ermanent® 3.0, ermanent® 2.5, Lambda-cyhalothrin LN, 8—11 December 2008. Geneva: World Health Organization, 2008.

28 Gleave K, Lissenden N, Richardson M, Choi L, Ranson H. Piperonyl butoxide (PBO) combined with pyrethroids in insecticide-treated nets to prevent malaria in Africa. *Cochrane Database Syst Rev* 2018; **11:** CD012776.

29 WHO. Conditions for deployment of mosquito nets treated with a pyrethroid and piperonyl butoxide. Geneva: World Health Organization, 2017.

30 Minakawa N, Kongere JO, Sonye GO, et al. Long-lasting insecticidal nets incorporating piperonyl butoxide reduce the risk of malaria in children in Western Kenya: a cluster randomized controlled trial. *Am J Trop Med Hyg* 2021; **105:** 461–71.

31 WHO. Fourteenth meeting of the WHO Vector Control Advisory Group. Geneva: World Health Organization, 2021.