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A household randomized-control trial of insecticide-treated screening for malaria control in unimproved houses in Tanzania

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Abstract

Background Installing insecticidal netting on open eaves, windows, and holes in walls of unimproved houses is a potential malaria control tool. It prevents mosquito house-entry, induces lethal and sub-lethal effects on malaria vectors, and may reduce malaria transmission. Therefore, a household epidemiological trial was conducted to assess the efficacy of insecticide-treated screening (ITS) on malaria infection and indoor vectors in Tanzania.

Methods In Chalinze district, Tanzania, 421 households were randomized into two arms. In June-July 2021, one group of households' houses was fitted with ITS (incorporated with deltamethrin and piperonyl butoxide) on eaves, windows, and wall holes, while the second group did not receive screening. After installation, consenting household members (aged ≥ 6 months) were tested for malaria infection using quantitative polymerase chain reaction after the long rainy season (June/July 2022, primary outcome) and the short rainy season (January/February 2022, secondary outcome). Secondary outcomes included indoor total mosquito per trap/night (June–July 2022), adverse effects after one month of ITS installation (August 2021), and chemical bioavailability and retention of ITS samples after one year of field use (June/July 2022). At the end of the trial, the control group received ITS.

Results Malaria prevalence among residents in the ITS arm was 19.9% (50/251) and 28.3% (65/230) in the control arm after the long rains, however, this difference was not significant [adjusted odds ratio (OR) 0.67 (95% CI 0.35–1.28), p = 0.227]. Similarly, no protection was seen for ITS after the short rains, [OR 1.27 (95% CI 0.68–2.38), p = 0.452]. However, school-age children in the ITS arm had lower malaria after the long rains [OR 0.11 (95% CI 0.02–0.73), p = 0.022]. No serious adverse effects were reported. The mean number of female *Anopheles* mosquitoes caught per trap/night was not significantly different between arms [1.7 vs 2.4, crude relative risk: 0.71 (95% CI 0.16–3.09), p = 0.650]. ITS showed reduced chemical bioavailability and retention post-field use. The trial reported high household refusals (17–30%) in both arms in both surveys.

Conclusion The trial was inconclusive because households' refusal resulted in low power. A large cluster randomized trial of the intervention, preferably with screens treated with longer-lasting insecticides installed in houses, is needed.

Trial registry: The trial was registered at ClinicalTrials.gov (NCT05125133) on October 2021

Keywords Insecticide-treated screening, ITS, Insecticide-treated nets, ITNs, Eave nets, Malaria prevalence, Mosquitoes, Trial, House modification, Tanzania

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Background

Since 2020, the global malaria burden, particularly in sub-Saharan Africa (SSA), has increased [1], mainly driven by indoor malaria transmission [2]. Indoor malaria transmission, and mosquito densities in particular, are influenced by housing characteristics [3–7]. The main entry points for malaria vectors into a house are open eaves (gaps between the walls and roof), unscreened windows, and holes in walls [8, 9]. Unimproved houses (houses built with traditional or natural materials, e.g., mud), very commonly have these features in Tanzania and SSA generally [10]. These houses are more commonly found in rural areas [11] and in areas of low socioeconomic status [10], which are frequently the areas with the greatest potential for malaria transmission [10, 12]. Although many people living in unimproved houses are provided with insecticide-treated nets (ITNs) [13], ITN coverage and use are often not optimal [14]. No demographic group is completely covered with nets [15], but some, such as school-age children, are particularly likely to be unprotected [16]. Indoor residual spraying (IRS), i.e., spraying the interior walls of houses where mosquitoes rest, is another recommended vector control tool [17]. Although it does protect all groups equally (unlike ITNs) [18], it is being scaled down in SSA largely due to its operational cost [1]. Therefore, modifying houses to kill mosquitoes and prevent their entry, could fill a gap in vector control by providing a cost-effective method that provides equitable protection.

There is some evidence that some forms of house modification can prevent malaria infection [3–6], and the World Health Organization (WHO) recommends house screening as a supplementary vector control tool [17]. Previous malaria elimination activities in the United States of America, Europe, and elsewhere where house improvements, including screening with netting without insecticide was an essential activity [6]. In addition, it does not need user compliance and has the potential to protect all occupants. Often, house modification involves closing mosquito entry points, especially the eaves, with local building materials [19–21]. While this can sometimes protect household members, randomized trials have not always found an epidemiological effect at a larger scale [22]. Furthermore, even if individual households are protected by closing mosquito entry points, without having a direct insecticidal effect means there is unlikely to be a population-wide reduction of mosquitoes which could otherwise result in a community effect that protects other households. Another disadvantage of closing the eaves of unimproved houses that reduces airflow is increased indoor temperatures, which (1) could reduce the acceptability of the intervention and (2) may influence members to not use ITNs, and/or stay outdoors and be exposed to mosquito bites [19]. Using untreated netting to screen houses may help with the indoor temperature, but, as with closing eaves, it will not induce a mass-killing effect on mosquitoes [19, 23]. Another house modification method is eave tubes, where the existing eaves are blocked with tubes inserted that are covered with an electrostatic gauze wire mesh that binds an insecticide. This allows the inflow of air whilst attracting mosquitoes as human odour plumes are channeled outside through the tubes. Mosquitoes cannot enter due to the mesh and are killed by the insecticide [5]. However, closing eaves and inserting tubes can be time-consuming, and they require timely maintenance to maintain protection, all of which can increase cost [5].

One such form of housing modification is covering eaves, windows, and wall holes with insecticide-treated screening (ITS). The screening generally used is a thicker, more durable version of the netting used in ITNs [6]. ITS has many potential advantages over other housing modifications. It provides a barrier against mosquito entry, it is treated with an insecticide so it kills mosquitoes, which can lead to community protection, it does not restrict airflow as much as blocking eaves, and it does not require regular maintenance [24].

Previous studies of ITS have shown an impact on entomological outcomes. This includes an impact on mosquito density in a randomized controlled trial in Kenya [24] and an impact on mosquito mortality, blood-feeding inhibition, and deterrence in a semi-field system setting in Tanzania [Odufuwa, in press]. Here, the results of the first trial of ITS on malaria epidemiological endpoints are presented.

Methods

Design

This was a two-arm parallel household randomized controlled trial of insecticide-treated screening (ITS) installed in 2021 with the current best practice (absence of indoor residual spraying, mass campaign of ITNs in 2017, and yearly distribution of ITNs through schools) compared to the current best practice alone. The trial followed a published protocol [25], registered at ClinicalTrials.gov (NCT05125133).

Description of participants and study area

The trial was conducted in three selected villages within the Chalinze district of Pwani region, Tanzania (Fig. 1), based on perceived high malaria infection by village leaders. Households from nine hamlets from these villages were enrolled as they provided the required number of households needed for the trial. The primary local occupation of residents is farming. This coastal region

experiences an average temperature of 28°C and 1000 mm of annual rainfall. Two distinct rainy seasons occur: a short season (October–December) and a longer season (March to May). These seasonal patterns correspond with the two peaks of moderate malaria transmission periods, with varied malaria prevalence of 10–30% [14]. The primary local malaria vectors are *Anopheles arabiensis* and *Anopheles funestus* [26], and are highly resistant to pyrethroids [27].

A *household* comprised a house or group of houses with one head of the household, and where residents shared the same cooking facilities [28]. Households were eligible if houses had open eaves and unscreened windows, and all four walls were standing. All household members aged six months and older were eligible for enrolment into the study, except pregnant women, who are routinely screened during antenatal care as per national guidelines [29].

Description of intervention

Households were randomly assigned to either the intervention group or the control group. The households in the intervention group were visited to receive ITS from June 19th to July 8th, 2021 [30]. The intervention was applied to cover eaves, windows, and holes in the walls of consenting houses in the intervention group (Fig. 2). The procedure for installation is detailed elsewhere [30]. The ITS used in the trial was manufactured by Vegro (Denmark). They contain 8 grams per kilogram (g/kg) piperonyl butoxide (PBO) synergist and 2 g/kg deltamethrin insecticide. The control group received no ITS. None of the arms received ITNs from the trial.

Outcomes

This trial assessed the superiority of ITS over malaria control best practice in reducing malaria prevalence in individuals over six months of age, measured by quantitative polymerase chain reaction (qPCR) [31]. The primary outcome was malaria prevalence post-long rainy season (June–July 2022, one year post-ITS installation), with prevalence post-short rainy season (January–February 2022, six months post-ITS installation) as a secondary outcome. An additional secondary outcome was indoor total mosquito measured by Centers for Disease Control

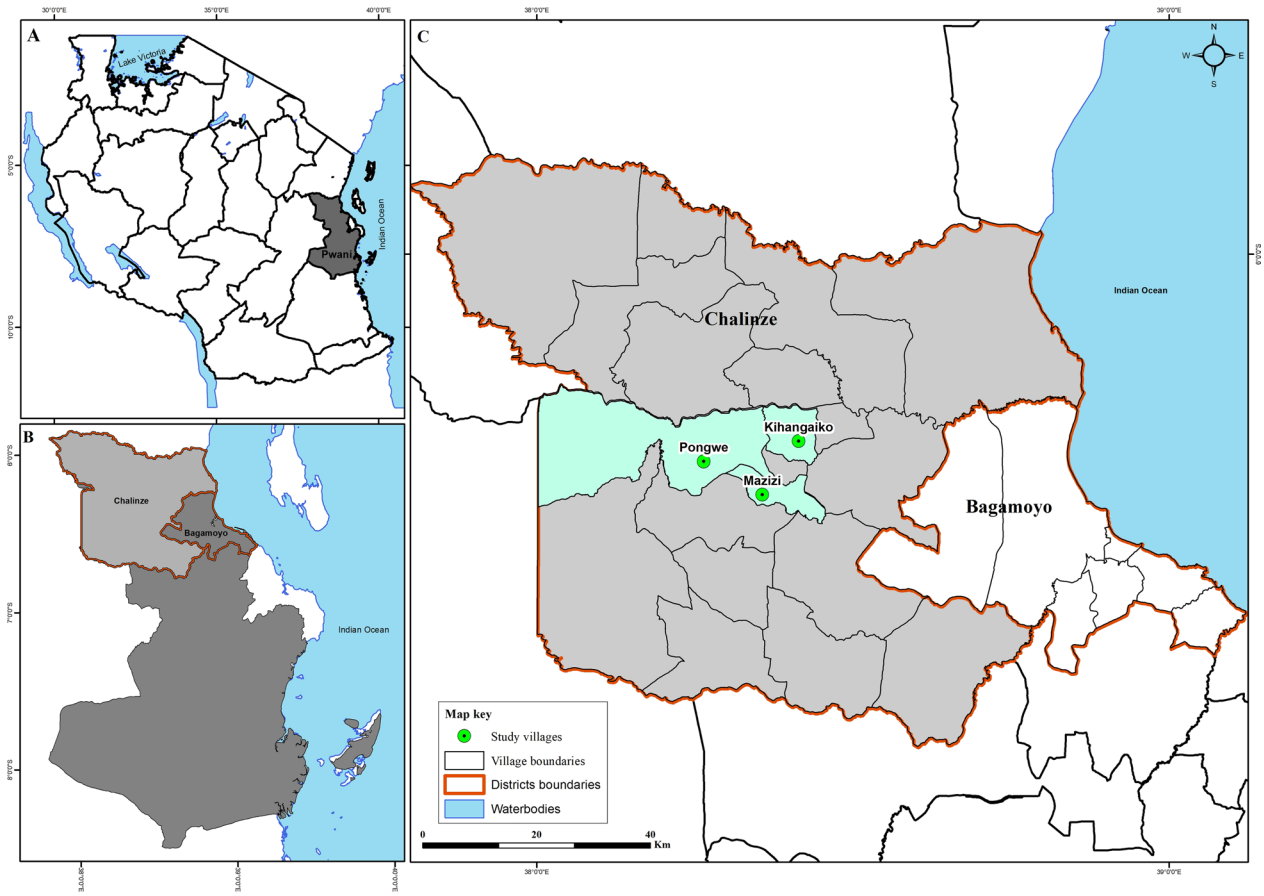

Fig. 1 Location of study villages in Chalinze district, Tanzania

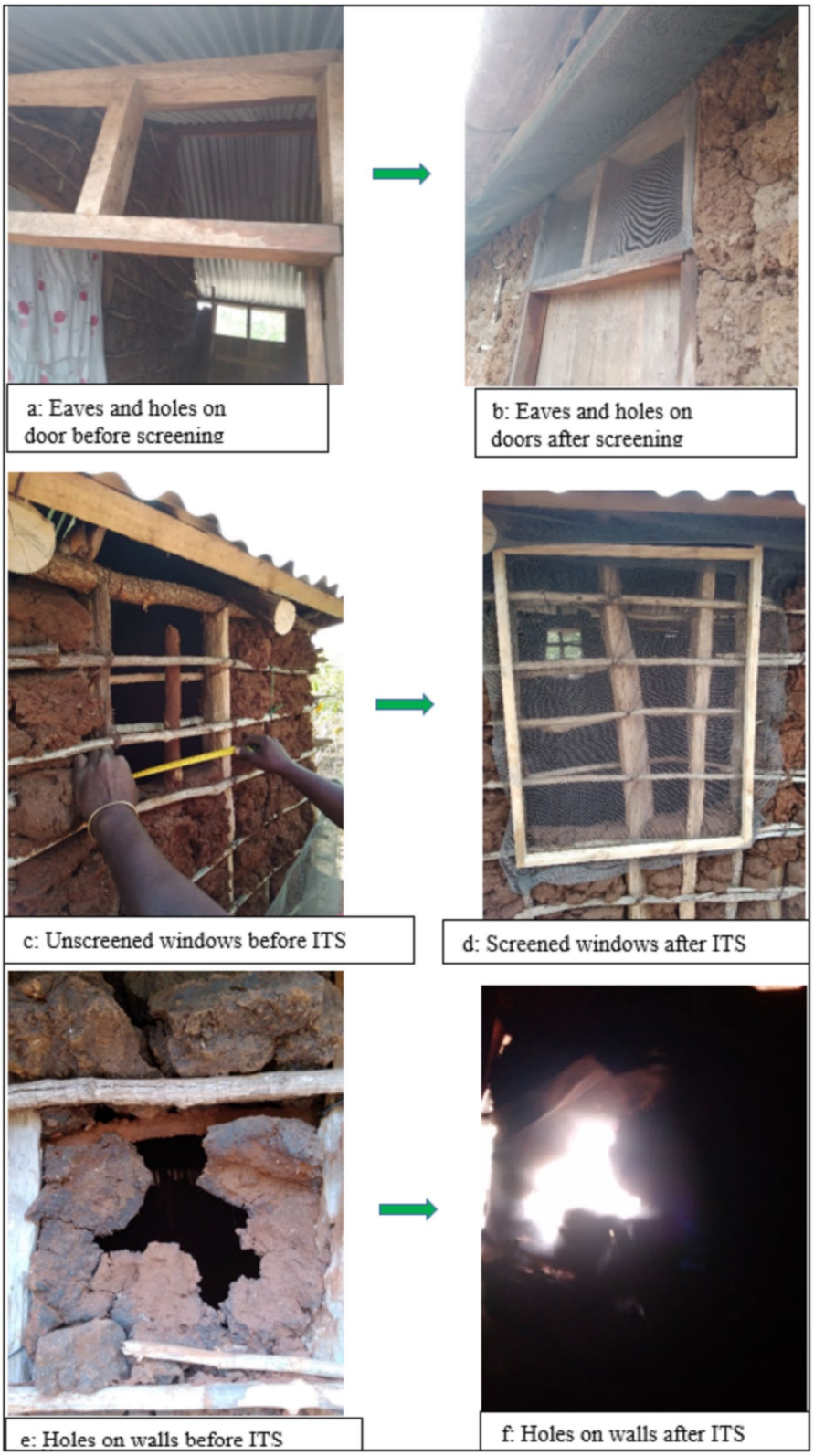

Fig. 2 Insecticide-treated screen installed in a house in the study location

(CDC) light traps [32] set in each house once after the long rainy season (July 2022). Additionally, the number of adverse effects (AEs) was assessed using structured questionnaires one month after ITS installation (August 2022). Furthermore, the quality of ITS was assessed after one year in the field: (1) biologically (by estimating the proportion of mosquitoes that were killed 72 h after three minutes of cone bioassay exposure in November 2022), and (2) chemically (by measuring the chemical retention of ITS in March 2023).

Sample size

To detect a 30% malaria prevalence reduction with 90% power, it was calculated that a sample size of 225 households per arm (4.5 members/household [33]) would be needed. We used an estimated 20% control prevalence [31] and an assumed between-house coefficient of variation of 0.5. No assumptions around non-response or loss to follow-up rates were made. No interim analyses were conducted.

Randomization

Households were randomly assigned to the ITS and control groups, stratified by hamlet, that is, simple randomization within each hamlet to ensure that both ITS and control households were present in each hamlet [25] using STATA (Version 16).

Trial procedure

In February 2021, a meeting with village heads was held to discuss the trial objectives. This was followed by community sensitization in each village. Subsequently (April–May 2021), data collectors and semi-skilled carpenters were recruited from each village. Data collectors were trained on trial protocol methodology, ethical enrolment, Global Positioning System (GPS) device/tablet usage, and questionnaire administration. Carpenters were trained on the installation of ITS.

In June-July 2021, intending to enrol all households within each hamlet, data collectors guided by hamlet leaders recruited households door-to-door if their house(s) had open eaves, unscreened windows, and standing walls. An adult household member answered a baseline questionnaire. This questionnaire gathered information on the location and characteristics of the house and the socio-demographic status of household members. A unique identification (UID) was assigned to the informed consent form (ICF) and the questionnaire for consistency, and this UID was printed and laminated and affixed to the front door of each participating household. The baseline data were used to generate a randomization list, which was used as a guide to install ITS in the intervention group.

One month after ITS installation (August 2021), data collectors surveyed 20 technicians who installed ITS in the study houses and 50 randomly selected households that had ITS installed, and had them respond to a questionnaire on adverse effects adapted from WHO guidelines on ITN testing [34] without considering sex or age differences.

To detect malaria infection, cross-sectional door-to-door surveys were conducted on all consented household members aged six months or older who were not pregnant, in June-July 2022 (primary outcome) and January–February 2022 (secondary outcome). During each household visit, blood was taken for both rapid testing for point-of-care and further analysis using qPCR for the malaria prevalence outcome. Firstly, malaria parasite detection was performed using an SD Bioline Malaria Ag Pf/Pan rapid diagnostic test (RDT) [31], and capillary blood was collected into 2.0 ml tubes containing ethylenediaminetetraacetic acid (EDTA). Following blood collection, participants with positive RDT results were treated with artemether 20mg + lumefantrine 120 mg tablets (Ajanta Pharma Limited, India). The blood samples were placed in a cooling box with ice packs during the door-to-door survey. Secondly, 100 microlitre (µl) of whole blood cells from the EDTA tubes were preserved in 300 µl of 1X DNA/RNA Shield (Zymo Research, USA) on the same day of blood collection. These samples were stored in a cold chain for not more than three days before being transported to the Bagamoyo laboratory for deoxyribonucleic acid (DNA) extraction and qPCR analysis for the detection of *Plasmodium falciparum* infection [31]. Deoxyribonucleic Acid (DNA) extraction was conducted by using Quick-DNA™ Miniprep kits (Zymo Research) as per manufacturer guidelines with slight modifications (Supplementary 1), eluted in 50 µl of elution buffer, and used immediately for qPCR or stored at ≤ −20 °C. The *P. falciparum* in the extracted DNA was detected using the PlasQ qPCR assay as described elsewhere [35]. In this assay, two main targets were utilized to detect parasites: *Pan – Plasmodium* 18S rDNA sequence (Pspp18S) and *Plasmodium falciparum* sequence (PfvarATS) with an internal control gene (HsRNaseP). The qPCR amplification was performed on a CFX96 Touch™ Real-Time PCR Detection system (Bio-Rad) in a single reaction mix of 2 µl of sample DNA and 8 µl of reaction mix containing 1X Luna Universal Probe qPCR Master Mix (New England Biolabs, USA) with the cyclic conditions as follows: 95 °C for 1 minute (min); 45 cycles of 15 s (secs) at 95 °C and 45 cycles at 57 °C for 45 secs for polymerase activation, denaturation, annealing and elongation, respectively. All samples were run in duplicate with the negative and positive control (NF54). The parasite density was determined using the WHO International standard for

P. falciparum detection. The standard curves were generated by diluting the standard from 100,000 parasites/µl to 0.0001 parasites/µl and run in triplicate as performed in other studies [31, 35, 36].

Indoor mosquito density was assessed in June–July 2022 using CDC light traps placed overnight once in each house, near the sleeping space, placed at the foot-end where the sleeper's legs would be, and approximately 1.5 m above the ground indoors. A block allocation scheme (allocating an equal number of households per arm for nightly survey) was utilized to ensure equal sampling across groups nightly.

Captured mosquitoes were identified as *Anopheles* or *Culex quinquefasciatus*, or *Aedes* morphologically [37], with *Anopheles* species further analysed into sibling species by conventional multiplex PCR. The PCR for *Anopheles funestus* was based on the methodology described by Koekemoer et al. [38], and that of the *An. gambiae* sensu lato was based on Scott et al. [39]. Briefly, DNA was extracted from individual mosquito legs by placing two mosquito legs in a microcentrifuge tube containing 20 µl of TE (Tris–EDTA) buffer. The tubes were incubated at 95 °C for 10 min. The resulting DNA was stored at −20 °C until PCR analysis. The PCR for the identification of sibling species was carried out in a total reaction volume of 25 µl using 3 µl of DNA template, 12.5 µl of One Taq Quick-load 2X master mix with standard buffer (which contains PCR buffer, dNTP, $MgCl_2$, Taq DNA polymerase, and loading dye), 1 µl of universal primer, and 1 µl of specific primers for the detection of *Anopheles vanedeen*, *An. funestus*, *Anopheles rivulorum*, *Anopheles parensis* and *Anopheles leesoni* (*An. funestus* group) or *An. gambiae s.s*, *An. arabiensis*, *Anopheles merus* and *Anopheles quadrannulatus* (*An. gambiae* group). The cycling conditions were: 94 °C for 2 min followed by 40 cycles of 94 °C for 30 secs, 50 °C for 30 secs, and 72 °C for 40 secs, and a final extension step of 72 °C for 10 min for *An. funestus* group, and 94 °C for 5 min followed by 30 cycles of 94 °C for 30 secs, 50 °C for 30 secs, and 72 °C for 30 secs, and a final extension step of 72 °C for 3 min for *An. gambiae* group. The PCR products for all species were analysed after electrophoresis in a 2.5% agarose gel stained with Safe View Classic. DNA bands were visualized under ultraviolet light using a Kodak Logic 100 imaging system.

At the time of the cross-sectional survey in June–July 2022, 20 houses in the ITS arm were randomly selected for chemical bioavailability and retention assessment of the treated screens. For each of these houses, a minimum of three samples measuring 25 centimetres (cm) × 25 cm were cut from the ITS installed on the eaves and windows, both facing the east or westward direction (to capture the impact of sunrise and sunset). The removed samples were replaced with new ITS. Before testing, the samples were stored in aluminium foil and in a refrigerator at 4 °C to prevent active ingredient (AI) loss while preparing for testing. The entomological bioavailability of these samples was determined in November 2022 using cone bioassays following standard procedures [40] against laboratory strains of malaria (*An. arabiensis* and *An. funestus*), dengue (*Aedes aegypti*) vectors, and those known for nuisance biting (*Culex quinquefasciatus*), all with varying levels of susceptibility to pyrethroids and restored by PBO in [Supplementary Table 1]. Mosquitoes were exposed to ITS samples for three minutes within the cones placed on a board, then aspirated into paper cups and provided with sugar solution. Exposed mosquitoes were then kept in a temperature-controlled room to assess delayed mortality at 72 h. Samples from six houses were randomly selected and shipped to Biolytrics Vietnam Co., Ltd, Hanoi, Vietnam, for chemical analysis in March 2023 using the High-Performance Liquid Chromatography (HPLC) method following WHO guidelines for ITN testing [34]. Despite a four-month difference in the testing of chemical bioavailability and retention, we assume no or minimal loss of the AI during chemical retention analysis. This assumption is based on the storage conditions prior to HPLC (samples wrapped in aluminium foil and kept below 4°C) [34].

Data management and analysis

Malaria infection prevalence data were collected using a questionnaire (Supplementary file 2). These data were entered into electronic tablets with force control (i.e., in-built data entry restrictions) to minimize entry errors.

Data were analysed using STATA (Version 16). Descriptive analysis of the socio-demographic characteristics of the households was presented by arms. An additional characteristic of the household presented by arms was the wealth quintile index, estimated using Principal Component Analysis [41], in which households' asset, livestock, light and cooking energy, means of sanitation, house density (≤ 5 members vs. > 5 members), house building materials and education level of the head of household were combined to make one variable. Descriptive analysis was also utilized for the perceived effect of contact with ITS, mosquito mortality in the cone bioassay, and chemical retention (HPLC) data collected.

Malaria prevalence data were analysed using a per-protocol approach, in that individuals who travelled within the past two weeks or used malaria medication within two weeks before the surveys were excluded from the analysis.

Malaria infection prevalence among individuals aged ≥ 6 months, assessed by qPCR, was compared between households with and without ITS using mixed-effects logistic regression, with a random effect for

household. Additionally, an adjusted model was used which included age groups (< 5, 5–14, and ≥ 15), participant sex, ITN access (household with one ITN for every two members that slept in the household in the previous night of the survey), wall type (mud & stick vs. cement), and roof type (traditional vs. iron sheet) as covariates.

To determine the impact of ITS in different house types, ITN use, and age groups, stratified analyses were performed. Malaria infection prevalence in households with ITS was compared to those without ITS within specific strata: mud walls, brick walls, traditional roofs, iron sheet roofs, individuals with ITNs the previous night of the survey, individuals without ITN the previous night of the survey, young children, school-age children and adults. In each stratified analysis, age group, sex, and the respective household stratification variable (wall type, roof type, ITN use, or age group) were included as fixed effects. Household was included as a random effect to account for clustering. Importantly, the stratification variable itself was not included as a covariate within its own stratified analysis.

For the number of indoor mosquitoes, only an unadjusted negative binomial regression model on the daily number of mosquitoes caught per trap/night was performed due to the low number of mosquitoes caught throughout the evaluation.

Results

Household recruitment

Of the 450 eligible households recruited in all nine hamlets combined, nine were excluded due to not having both open eaves and window(s) before randomization. Simple randomization stratified by hamlet was carried out on 441 households in May 2021, with 221 households assigned to receive ITS and the remaining 220 households assigned to the control arm. Installation of ITS was completed in 208 recruited households, while 195 households remained as controls (Fig. 3).

During the first survey conducted after the short rainy season to assess malaria prevalence (secondary outcome), of the 195 and 208 households in the control and ITS arms, 49 (25.1%) and 36 (17.3%) withdrew consent, while 20 (10.3%) and 28 (13.5%) were not available during the visit in the control and ITS arm, respectively. Therefore, only 126 (72%) and 144 (80%) households consented to malaria testing in the control and ITS arms, respectively (Fig. 3), with a similar proportion of refusals between arms.

For the assessment of malaria prevalence during the survey conducted after the long rainy season (primary outcome), 59 (30.3%) and 53 (25.5%) withdrew consent, and 28 (14.4%) and 32 (15.4%) households were unavailable in the control and ITS arms, respectively. Consequently, only 108 (64.7%) households in the control arm and 123 (69.9%) households in the ITS arm consented to malaria testing (Fig. 3), and substantially lower than the estimated sample size. As observed in the short rains, the proportions of refusals were similar between arms.

Demographic-socioeconomic characteristics of study households

The demographic-socioeconomic characteristics of households were similar in both the ITS and control arms during ITS installation, after six months, and after 12 months (Table 1). The primary wall materials of the majority of houses in the study were built with traditional materials, including sticks, iron sheets, and mud (Table 1).

Malaria infection prevalence

A total of 230 eligible participants in the control and 251 in the ITS arm were included in the analysis. Malaria infection prevalence after ITS installation and after the long rainy season (primary outcome) was 19.9% (50/251) among members in the ITS arm, and 28.3% (65/230) in the control arm. However, the difference was not significant, both in the unadjusted [Odd ratio (OR) 0.64 (95%

(See figure on next page.)

Fig. 3 Trial flow diagram. Enrolment: Informed consent was obtained from household heads or adult family members (≥ 18 years) in all hamlets with houses having open eaves, unscreened windows, and walls in good condition by a door-to-door approach. Exclusion: was done by physical inspection of all recruited houses, and ineligible household codes were removed from the randomization list. Randomization: households within each hamlet were equally randomized to ITS and control arms. During installation, in the control arm, two households notified technicians of their withdrawal, one household had a damaged house and 34 households were used to replace installation refusals in the ITS arm. In the ITS arm, 27 households refused, 7 had damaged houses and members of 15 households were unavailable for consent throughout the installation period. Study population (post-installation): The control group comprised the remaining households after excluding refusals, damaged houses, and replacements, and 15 ITS arm (n = 180). The ITS arm included the 172 originally assigned households, 34 control households installed with ITS, and 2 replacement households (originally excluded for lacking open eaves and unscreened windows during installation). Malaria surveys of households for malaria infection testing at the short and long rains, showing households visited, refusals, consented, loss to follow-up due to participants' relocation and long travel, participants' refusals, use of malaria medication and travel history. Indoor mosquito surveys of houses using CDC light traps once, showing houses visited, refusals, consented, loss to follow-up due to relocation of members, or not present throughout the survey period. Installation of ITS in the control households

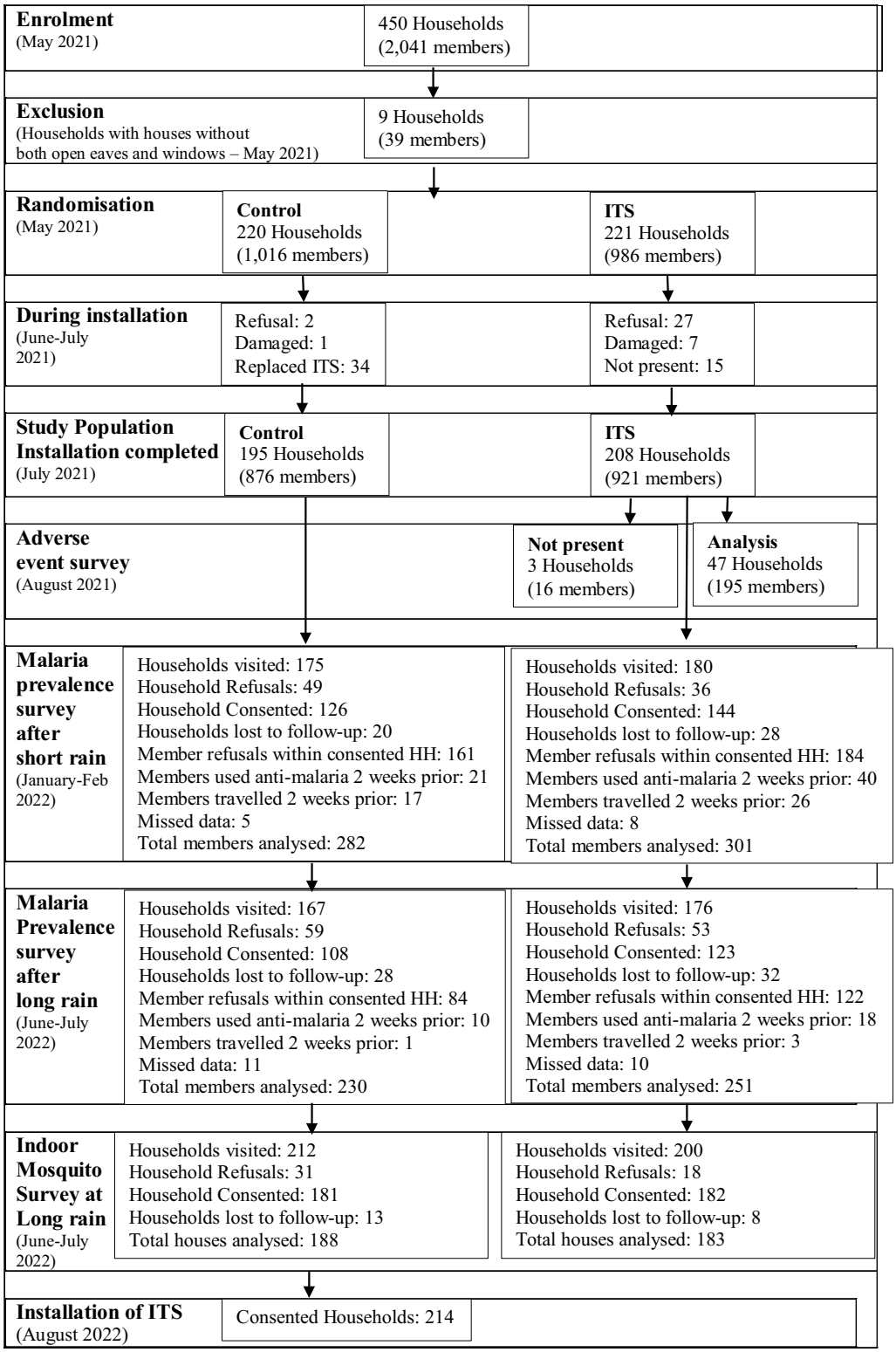

Fig. 3 (See legend on previous page.)

Table 1 Demographic and socio-economic characteristics of study households

	Baseline		6 months after installation		12 months after installation	
Factor	**Control arm**	**ITS arm**	**Control arm**	**ITS arm**	**Control arm**	**ITS arm**
Number of HH	195	208	126	144	108	123
HH Members	876	921	282	301	230	251
Age group of the head of household						
18–24	10 (5.1%)	10 (4.8%)	6 (4.8%)	4 (2.8%)	3 (2.8%)	3 (2.4%)
25–49	105 (53.9%)	96 (46.2%)	58 (46.0%)	65 (45.1%)	53 (49.1%)	53 (43.1%)
50-above	80 (41.0%)	102 (49.0%)	62 (49.2%)	75 (52.1%)	52 (48.2%)	67 (54.5%)
Sex of head of HH						
Male	148 (75.9%)	148 (71.2%)	91 (72.2%)	103 (71.5%)	78 (72.2%)	88 (71.5%)
Female	47 (24.1%)	60 (28.9%)	35 (27.8%)	41 (28.5%)	30 (27.8%)	35 (28.5%)
Education of the head of HH						
No formal education/pre-primary	42 (21.5%)	41 (19.7%)	32 (25.4%)	28 (19.4%)	24 (22.2%)	24 (19.5%)
Primary	140 (71.8%)	151 (72.6%)	87 (69.1%)	105 (72.9%)	79 (73.2%)	88 (71.5%)
Secondary/tertiary	13 (6.7%)	16 (7.7%)	7 (5.6%)	11 (7.6%)	5 (4.6%)	11 (8.9%)
HH size						
1–2 members	42 (21.5%)	49 (23.6%)	26 (20.6%)	33 (22.9%)	23 (21.3%)	23 (18.7%)
3–5 members	98 (50.3%)	104 (50.0%)	66 (52.4%)	74 (51.4%)	56 (51.9%)	64 (52.0%)
6 and above	55 (28.2%)	55 (26.4%)	34 (27.0%)	37 (25.7%)	29 (26.9%)	36 (29.3%)
HH access* to nets						
No	106 (54.4%)	110 (52.9%)	69 (54.8%)	76 (52.8%)	54 (50.0%)	61 (49.6%)
Yes	89 (45.6%)	98 (47.1%)	57 (45.2%)	68 (47.2%)	54 (50.0%)	62 (50.4%)
Primary material of house's roof						
Grass/banana leaves/Thatch Dung/mud/soil	18 (9.2%)	6 (2.9%)	8 (6.4%)	3 (2.1%)	7 (6.5%)	3 (2.4%)
Iron sheets	177 (90.8%)	202 (97.1%)	118 (93.7%)	141 (97.9%)	101 (93.5%)	120 (97.6%)
Primary material of house's wall						
Sticks/Iron sheet/Mud	168 (86.2%)	179 (86.1%)	104 (82.5%)	121 (84.0%)	92 (85.2%)	103 (83.7%)
Burnt/cement bricks	27 (13.9%)	29 (13.9%)	22 (17.5%)	23 (16.0%)	16 (14.8%)	20 (16.3%)
Primary material of house's floor						
Earth/sand/mud	139 (71.3%)	136 (65.4%)	90 (71.4%)	93 (64.6%)	78 (72.2%)	80 (65.0%)
Cement	56 (28.7%)	72 (34.6%)	36 (28.6%)	51 (35.4%)	30 (27.8%)	43 (35.0%)
HH socioeconomic status						
Lowest	48 (24.6%)	38 (18.3%)	24 (19.1%)	24 (16.7%)	24 (22.2%)	19 (15.5%)
Low	43 (22.1%)	40 (19.2%)	31 (24.6%)	29 (20.1%)	22 (20.4%)	23 (18.7%)
Middle	35 (18.0%)	44 (21.2%)	26 (20.6%)	30 (20.8%)	23 (21.3%)	26 (21.1%)
High	34 (17.4%)	51 (24.5%)	17 (13.5%)	35 (24.3%)	18 (16.7%)	33 (26.8%)
Highest	35 (18.0%)	35 (16.8%)	28 (22.2%)	26 (18.1%)	21 (19.4%)	22 (17.9%)
Hamlets						
Miembe Saba	20 (10.3%)	30 (14.4%)	13 (10.3%)	19 (13.2%)	13 (12.0%)	18 (14.6%)
Mswelezi	10 (5.1%)	12 (5.8%)	7 (5.6%)	9 (6.3%)	7 (6.5%)	5 (4.1%)
Mikuyuni	10 (5.1%)	10 (4.8%)	8 (6.4%)	8 (5.6%)	5 (4.6%)	6 (4.9%)
Tobora	13 (6.7%)	17 (8.2%)	8 (6.4%)	10 (6.9%)	9 (8,3%)	9 (7.3%)
Kibonkho	58 (29.7%)	66 (31.7%)	36 (28.6%)	38 (26.4%)	28 (25.9%)	33 (26.8%)
Mbuyu	23 (11.8%)	18 (8.7%)	11 (8.7%)	15 (10.4%)	9 (8.3%)	11 (8.9%)
Mkwazu	19 (9.7%)	19 (9.1%)	17 (13.5%)	15 (10.4%)	15 (13.9%)	14 (11.4%)
Mitindi	10 (5.1%)	6 (2.9%)	7 (5.6%)	5 (3.5%)	7 (6.5%)	4 (3.3%)
Kihangaiko	32 (16.4%)	30 (14.4%)	19 (15.1%)	25 (17.4%)	15 (13.9%)	23 (18.7%)

* access is defined as having at least one ITN for two household members that slept in the households the previous night of the survey

HH indicates household

CI 0.33–1.25), p = 0.191] and adjusted [OR 0.67 (95% CI 0.35–1.28), p = 0.227] analyses (Table 2).

A total of 282 eligible participants in the control and 301 in the ITS arm were included in the analysis of the secondary outcome after the short rain survey. In this survey, there was also no significant difference between the members in the control arm versus those in the ITS arm, both in the unadjusted [18.9% vs 18.7%, OR 1.04 (95% CI 0.58–1.85), p = 0.191] and adjusted [OR 1.27 (95% CI 0.68–2.38), p = 0.452] analyses (Table 2).

In the stratified analysis, the protection of ITS was seen over the control arm only among the school-age children [15.5% vs. 38.3%, aOR 0.11 (0.02–0.73), p = 0.022], while there was no significant difference in malaria infection prevalence between the control and ITS arms restricted within households with houses categorized into: traditional walls, modern walls, modern roof, use and non-use of ITN the night before the survey, in either the surveys conducted after the long and short rainy seasons (p > 0.05) (Table 3).

Reported adverse effects

Of the 50 households (222 participants) selected for recording AES, only 47 households (with 195 members available and 11 members not available) were reached. One household had moved away, and two households were unavailable during the visit. AEs were captured for all 20 ITS installation technicians. No serious adverse effect was recorded in the study that warranted treatment. All effects recorded were those known to occur with the use of ITNs, and the most common of all the adverse effects recorded was sneezing (65% for technicians and 16% for household members) (Table 4).

Indoor number of mosquitoes

A total number of 741 female mosquitoes were caught in the control houses and 728 in the ITS-installed houses. Only 85 of these mosquitoes were identified as *Anopheles*, and the majority of these (84/85, 99%) were confirmed molecularly to be *An. funestus* (Supplementary file 3). The mean number of female *Anopheles* mosquitoes caught per trap night of the survey was 2.4 in the control arm and 1.7 in the ITS arm, although this difference was not significantly different [Crude Relative Risk: 0.71 (95% CI 0.16–3.09)]. Between control and ITS households, the capture rates for female *Cx. quinquefasciatus* mosquitoes were similar [31.0 vs 31.3, Crude Relative Risk: 1.01 (95% CI 0.45–2.28)] as were capture rates of female *Ae. aegypti* mosquitoes [0.2 vs 0.1, Crude Relative Risk: 0.40 (95% CI 0.07–2.21)] (Table 5).

Cone bioassays and chemical analysis of insecticide-treated screening

Chemical bioavailability and retention assessment of net samples one year after ITS installation revealed a 67% and 88% loss of deltamethrin and PBO synergist content, respectively, and low 72-h mosquito mortality (33%) (Table 6). The mosquito mortality varied within species (Supplementary Table 2).

Discussion

This is the first trial to determine the impact of insecticide-treated screening (ITS) against malaria using epidemiological endpoints. Although overall malaria prevalence (primary endpoint) was lower in houses with ITS, the difference was not statistically significant. However, a stratified analysis of the secondary outcome indicated a protective effect for school-age children.

Prior to this trial, other studies have shown the entomological efficacy of ITS, and so their impact against malaria may be expected. A Kenyan trial demonstrated over 70% reduced indoor mosquito density in houses with combined ITS deployed as eave nets, and curtains plus ITNs versus ITNs alone [24]. Semi-field testing comparing one-year naturally aged ITS to one-year aged Olyset® Plus ITNs in Tanzania demonstrated similar mosquito mortality (51% vs 56%), but significantly higher personal protection (blood-feeding inhibition:

Table 2 The efficacy of insecticide-treated screening on malaria infection prevalence in Chalinze district, Tanzania

Outcome	Intervention		Unadjusted		Adjusted for covariates	
		Prevalence % (n/N)	Odds Ratio	p-value	Odds Ratio	p-value
Long rainy season	Control	28.3 (65/230)	1.00		1.00	
	ITS	19.9 (50/251)	0.64 (0.33–1.25)	0.191	0.67 (0.35–1.28)	0.227
Short rainy season	Control	19.2 (54/282)	1.00		1.00	
	ITS	18.9 (57/301)	1.04 (0.59–1.83)	0.899	1.27 (0.69–2.35)	0.442

Covariates adjusted for fixed effect: age groups (< 5, 5–14, and 15–above), sex, ITN use the previous night of the survey, house wall (mud & stick vs cement), and house roof (traditional vs iron sheet)

Household as a random effect

Table 3 Efficacy of insecticide-treated screening on malaria infection prevalence stratified by house walls, roofs, and individual use of ITNs in the previous night of the surveys carried out in the long and short rainy seasons

Variable	Long rainy season (one year after ITS installation)					Short rainy season (six months after ITS installation)				
	Prevalence % (n/N)	Unadjusted		Adjusted		Prevalence % (n/N)	Unadjusted		Adjusted	
		OR (95%CI)	P-value	OR (95%CI)	P-value		OR (95%CI)	P-value	OR (95%CI)	P-value
Mud wall										
Control	29.8 (56/188)	1.00		1.00[a]		18.5 (43/229)	1.00		1.00[a]	
ITS	20.5 (42/205)	0.59 (0.29–1.22)	0.155	0.62 (0.31–1.23)	0.170	18.8 (48/255)	1.04 (0.56–1.92)	0.911	1.26 (0.65–2.44)	0.496
Brick and cement wall										
Control	21.4 (9/42)	1.00		1.00[a]		20.8 (11/53)	1.00		1.00[a]	
ITS	17.4 (8/46)	1.08 (0.18–6.69)	0.932	1.17 (0.14–9.57)	0.885	19.6 (9/46)	1.07 (0.25–4.54)	0.931	1.33 (0.16–11.25)	0.795
Iron sheet roof										
Control	27.1 (58/214_	1.00		1.00[b]		19.6 (53/271)	1.00		1.00[b]	
ITS	19.5 (48/246)	0.66 (0.32–1.35)	0.255	0.65 (0.32–1.32)	0.234	18.7 (56/299)	0.98 (0.55–1.74)	0.936	1.22 (0.65 2.27)	0.533
No net use										
Control	42.4 (14/33)	1.00		1.00[c]		29.2 (14/48)	1.00		1.00[c]	
ITS	34.4 (11/32)	0.80 (0.18–3.48)	0.763	1.10 (0.11–11.16)	0.937	18.0 (11/61)	0.72 (0.15–3.33)	0.671	0.87 (0.19–3.97)	0.854
Net used										
Control	26.2 (51/195)	1.00		1.00[c]		15.6 (33/212)	1.00		1.00[c]	
ITS	17.6 (38/216)	0.60 (0.29–1.26)	0.178	0.62 (0.30–1.29)	0.203	19.5 (39/200)	1.35 (0.70–2.60)	0.365	1.44 (0.76–2.76)	0.267
Age groups										
Young children (0–4)										
Control	19.1 (4/21)	1.00		1.00[d]		6.1 (2/33)	1.00		1.00[d]	
ITS	12.9 (4/31)	0.63 (0.14–2.86)	0.549	0.66 (0.13–3.40)	0.623	19.5 (8/41)	10.97 (0.23–514.34)	0.223	15.30 (0.18–1291.99)	0.228
School-age (5–14)										
Control	38.3 (23/60)	1.00		1.00[d]		23.2 (16/69)	1.00		1.00[d]	
ITS	15.5 (11/71)	0.08 (0.01–0.65)	0.018	0.11 (0.02–0.73)	0.022	13.0 9/69)	0.22 (0.02–2.14)	0.191	0.76 (0.09–6.74)	0.806
Adult (15-above)										
Control	25.5 (38/149)	1.00		1.00[d]		20.0 (36/180)	1.00		1.00[d]	
ITS	23.5 (35/149)	0.92 (0.44–1.89)	0.813	0.86 (0.42–1.76)		20.9 (40/191)	1.07 (0.62–1.85)	0.813	1.21 (0.68–2.16)	0.522

Covariates adjusted for fixed effect: age groups (< 5, 5–14, and 15-above), sex, house wall (mud & stick vs cement), house roof (traditional vs iron sheet), and ITN use
Household is adjusted for as a random effect
1.00—reference
a—wall structure was excluded from the covariates
b—roof was excluded from the covariates
c—net use was excluded from the covariates
d—age and roof (due to collinearity) were excluded from the covariates

Table 4 Adverse effects reported by the trial technicians and participants

Type of Adverse Effects	20 Technicians	47 Households (195 members)
	N (%)	N (%)
Skin itching	5 (25)	4 (2.1)
Facial burning	5 (25)	0 (0)
Sneezing	13 (65)	31 (15.9)
Nasal discharge	4 (20)	16 (8.2)
Headache	2 (10)	11 (5.6)
Nausea	0 (0)	3 (1.5)
Eye irritation	4 (20)	0 (0)
Experience bad smell	7 (35)	5 (2.6)
Other symptoms	0 (0)	0 (0)
Report to a physician	0 (0)	11 (5.6)

84% vs 54%, and reduced hut entry: 2 vs 13) [Odufuwa, in press]. There are several potential reasons why this current trial did not show a statistically significant reduction in malaria prevalence. Firstly, the trial took place during the coronavirus disease 2019 pandemic. Tanzania's response to the pandemic was characterized by limited lockdowns and an emphasis on local intervention [42]. Many participants feared that the trial was related to the pandemic and were reluctant to participate in tests that required blood samples [30]. This resulted in a high rate of household refusals that reduced the sample size and limited study power, but could also have resulted in selection bias. Secondly, during the surveys after the short rainy season, participants were tested with RDT, and those who were positive were treated. This may have led to prevalence being lower in the long rainy season than it would have been, and so further reduced study power. Thirdly, chemical bioavailability and retention assessment at the end of the trial showed poor performance of ITS in mosquito bioassays and low concentrations of the insecticide. The degradation may be attributable to evaporative loss of insecticide since ITS were neither washed nor handled much [43]. The combination of these factors may have contributed to the lack of evidence of a protective effect of ITS against malaria, despite previous entomological studies giving reasons to expect one.

This trial was randomized at the household level [19]. This design captures the direct protection of household members by preventing mosquito entry into and exposure within their houses. If ITS were deployed at a sufficient scale, we would also expect further protection from a *community effect*. This effect results from mosquito population-wide impact (wide-scale reduction in mosquito population size and longevity), which protects not only those households with ITS but also those without. The ideal study design to capture both direct and community protection would be a cluster randomized trial, in which larger units, such as villages, are randomized to ITS or control arms, as used for a trial of screened ceilings [44].

The trial showed that malaria infection prevalence was the same in both the ITS and control arms (19% each) after the short rainy season (6 months post-ITS deployment). As we would expect a difference in the prevalence between the control and ITS arms. This may reflect a delayed impact of ITS [45], as asymptomatic infections were likely present in both groups.

It was hypothesized that ITS might exhibit greater protective efficacy in certain age groups, house structures, or ITN use. Gaps in access to malaria control tools, most especially ITNs among school-age children have been reported [46]. Low household population access to ITNs influences decisions on who uses the limited ITNs available within the household, in which school-age children are often not prioritized and thus a reservoir for the continuous malaria transmission [16, 47, 48]. In response, continuous distribution campaigns including the school nets programme in Tanzania have been distributing ITNs to increase access among this group [14, 49]. ITS might have likely protected this group given that at the time of the survey, access to ITN was low (50%), and the group likely had low access

Table 5 Efficacy of insecticide-treated screening on indoor female mosquito density

Species	Intervention	Number of trapping nights	Total female mosquitoes	Female Mosquito/night (95% CI)	Crude IRR (95% CI)	P-value
All species combined	Control	22	741	33.6 (17.7–49.6)	1.00	
	ITS		728	33.1 (19.1–47.1)	0.98 (0.43–2.24)	0.969
Anopheles funestus	Control		52	2.4 (0–5.7)	1.00	
	ITS		37	1.7 (0–3.8)	0.71 (0.16–3.09)	0.650
Culex quinquefasciatus	Control		683	31.0 (16.6–45.5)	1.00	
	ITS		689	31.3 (18.1–44.5)	1.01 (0.45–2.28)	0.983
Aedes aegypti	Control		6	0.2 (0–0.5)	1.00	
	ITS		2	0.1 (0–0.2)	0.40 (0.07–2.21)	0.293

Table 6 The chemical bioavailability (72-h mosquito mortality) and retention of insecticide-treated screening one year after ITS installation in the community

ITS	Cone bioassay				Chemical retention			
	No of pieces tested	No. of mosquitoes	No. of 72 h dead mosquitoes	% M72hr (95% CI)	Content	Number	AI content (g/kg) Arithmetic Mean (SD)	% AI content retained after trial
East eave	20	1520	457	30.1 (26.9–33.2)	Deltamethrin	6	0.9 (0.2)	35.7 (31.0–40.4)
					PBO		1.4 (0.7)	13.9 (8.1–19.7)
West eave	20	1520	479	31.5 (28.3–34.7)	Deltamethrin	6	1.1 (0.2)	42.2 (34.2–50.2)
					PBO		2.1 (1.2)	20.2 (10.3–30.0)
East window	14	1120	373	33.3 (29.6–37.0)	Deltamethrin	2	0.4 (0.3)	16.4 (4.0–28.8)
					PBO		0.3 (0.4)	3.1 (0–7.3)
West window	16	1280	427	33.4 (29.8–36.9)	Deltamethrin	4	0.7 (0.7)	26.6 (0–70.1)
					PBO		1.4 (2.0)	13.5 (0—42.0)
Untreated (negative control)	4	1520	11	0.7 (0–10)	–	–	–	–
Overall without untreated net	70	5440	1736	31.9 (30.2–33.6)	Deltamethrin	18	0.8 (0.4)	32.6 (25.4–39.8)
					PBO		1.4 (1.1)	13.5 (8.2–18.9)

Note: Proportion of mortality is for combined pyrethroid-resistant strains (*An. arabiensis* (Kingani), *An. funestus* (FUMOZ), *Cx. quinquefasciatus* (Bagamoyo)) and susceptible strain *Ae. aegypti* (Bagamoyo)

Total mosquitoes tested per strain was 380 for east eave ITS, 380 for west eave ITS, 280 for east window ITS, 320 for west window ITS, and 380 for untreated nets

Not all houses had windows in the east or west

For chemical analysis, deltamethrin and PBO content were 2.5 g/kg and 10.2 g/kg, respectively, at baseline (Estimates were calculated using estimates from individual pieces of net to account for variation within net)

to ITNs, thereby bridging the gaps in protection existing in the use of ITNs. House structures have been previously associated with increased malaria transmission; for example, cracks in mud walls and holes in traditional roofs facilitate mosquito entry [8]. However, there was no evidence to indicate this; analyses within the investigated groups of wall type, roof type, and prior ITN usage showed no difference between control and ITS arms.

Despite fewer *Anopheles* mosquitoes being caught per trap per night in ITS households, this was not significantly different from those households without ITS. Whilst the lower catch in ITS households could be attributed to their impact against the more predominant indoor feeding and resting mosquitoes, such as *An. funestus* [50], the screening may have been less efficacious against those mosquitoes with more exophilic behaviours, such as *An. arabiensis*. Furthermore, the current ITS may not have comprised the optimal and balanced concentrations of pyrethroid and PBO to induce sufficient lethal effects against pyrethroid-resistant *An. funestus* as demonstrated in a semi-field study [Odufuwa, in press]. This finding could also be attributable to insufficient statistical power, to detect a difference of 10% between ITS and control households at 80% power, 500 households per arm would have been needed to be sampled. To compound this, the study period coincided with weather anomalies in Tanzania that year, characterized by higher temperatures and reduced rainfall [51], factors that negatively impact the emergence and survival of *Anopheles* mosquitoes [52], and which may have contributed to a lower overall mosquito abundance during the survey. Conversely, almost no difference in the average daily densities of *Cx. quinquefasciatus* was observed in houses with ITS compared to those without. This observation, as previously described [Odufuwa, in press], may be due to the specific ITS incorporation technique of the pyrethroid and PBO, resulting in limited impact against

culicines. Additionally, unlike anophelines, *Culex* mosquitoes could have gained entrance through the door as found in the Kenyan trial [24] and in entomological studies in Tanzania [53]. Screening doorways could be impractical and increase occupant exposure to the insecticide. *Anopheles* mosquitoes enter mainly through the eaves [54], and applying the intervention at scale would likely have the best impact on mosquito density as the modelling based on data from the SFS showed [Odufuwa, in press].

The adverse effects reported by both technicians and participants are consistent with known reactions to pyrethroid exposure [55]. Notably, the majority of all reported adverse effects resolved within 72 h of exposure since very few (6%) of the household members reported to the physician, made available to all participants at no cost. The high prevalence of sneezing observed among 13 technicians (65%) was attributed to the underutilization of face masks provided due to perceived discomfort and possible association with COVID-19. Future studies may consider mandating the use of face masks.

Despite the limitations of this trial, including an insufficient sample size and potential design flaws of household instead of cluster, the effect size of the efficacy of insecticide-treated screening (ITS) after the long rains on malaria infection prevalence is noteworthy. ITS remains a promising method for modifying house structure to reduce indoor mosquito exposure due to its adaptability to various housing types and minimal impact on indoor airflow, as shown in the semi-field study where airflow was measured [Odufuwa, in press]. As ITNs are a cornerstone of vector control with demonstrated efficacy in reducing malaria infection [56], and considering the observed > 5% difference in malaria prevalence between households with ITS regardless of net use, further investigation is warranted to assess the potential of ITS as both a standalone intervention and as a supplementary component. Future studies should focus on ITS incorporated with insecticides with enhanced efficacy against pyrethroid-resistant malaria mosquitoes and other relevant vectors and nuisance biters for insecticide resistance management. Moreover, these insecticides should exhibit prolonged residual activity beyond four years to ensure cost-effectiveness compared to other vector control tools such as ITNs, IRS, and eave tubes [30]. Although the physical integrity of ITS was inspected within one year, it was not informative given that > 95% of the nets were still intact. Therefore, future studies should investigate ITS physical durability over three years of field use [30]. Given the emerging limitations of conventional vector control tools in providing adequate protection, optimizing the use of ITS as an additional layer of defense against mosquito entry warrants further investigation.

Conclusion

In Chalinze district, prevalence of malaria infection and indoor mosquito abundance were not significantly different between households fitted with insecticide-treated screening (ITS) and those without screening. This could be due to the study design, intervention insecticidal properties and residuality, and the high number of withdrawals of participants from the study. Despite differences not being significantly different, during the long rainy season lower prevalence of parasite infection was detected at the household level, and this was significant among school-age children. The indoor abundance of anophelines was also reduced, indicating that further studies would be of interest. Therefore, a cluster-randomized trial design is recommended, coupled with robust community engagement and sensitization efforts to ensure participant retention throughout the study.

List of Abbreviations

AI	Active ingredient
CI	95% Confidence interval
GLP	Good laboratory practice
IHI	Ifakara health institute
ITNs	Insecticide-treated nets
ITS	Insecticide-treated screening
IRS	Indoor residual spraying
IRR	Relative risk
M72	Mortality at 72 h
MR	Metabolic resistance
RDT	Rapid diagnostic test
OR	Odds ratio
PBO	Piperonyl butoxide
qPCR	Quantitative real-time Polymerase chain reaction
VCPTU	Vector control product testing unit
WHO	World health organization

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12936-025-05434-2.

Additional file 1.

Additional file 2.

Additional file 3.

Additional file 4.

Additional file 5.

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Author contributions
OGO conceived the trial, implemented the trial, analysed the data, and drafted the manuscript; SJM conceived the study, advised on the trial implementation and analysis of data, and substantially contributed to the manuscript's revision; ZMM was the local principal investigator, and contributed to the study concept and revision of the manuscript; RM, FM and LMH advised on blood collection, storage and analysis of samples molecularly, and revised the manuscript; IM provided guidance on mosquito trapping and physiologically identified mosquitoes; SA conducted the molecular analysis of Anopheles mosquitoes to sibling species and revised the manuscript; MAR was the clinician of the trial and revised the manuscript; RP: provided guidance on the management of the project and revised the manuscript; FK: provided guidance on the retention of study participants and revised the manuscript; JM: provided guidance on the implementation of the trial in the field and revised the manuscript; HN, RB, and OS manufactured the ITS, provided guidance on the trial implementation, and revised the manuscript; JS: critically reviewed the manuscript; JBM contributed substantially to the trial implementation in the field, managed data, and revised the manuscript; JB the principal investigator, conceived and designed the trial, supervised all activities in the trial and substantially contributed to the revision of the manuscript. All authors agreed to submit this draft for publication.

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Availability of data and materials
Data generated from this trial are in supplementary file 4.

Declarations

Ethics approval and consent to participate
Households were recruited for participation in the study, which included malaria testing and indoor mosquito collection upon informed consent obtained from the head of household or another adult member (≥ 18 years old). For malaria testing, individual informed consent was obtained: consent from all adult household members, and for children (7–12) and adolescents (13–17), both assent and parental/guardian consent were obtained. The trial commenced after obtaining relevant clearances from the Ifakara Health Institute-Institutional Review Board (IHI/IRB/No: 19–2020), National Institute for Medical Research (NIMR), Tanzania (NIMR/HQ/R.8c/Vol.I/885), Chalinze District Medical Officer (HWC/M/10/20), and London School of Hygiene and Tropical Medicine (LSHTM) Observational / Interventions Research Ethics Committee (21639-1).

Consent for publication
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Competing interests
OGO, RP, FK, JM, JBM, IM, and SJM test vector control tools for private and public companies, including Moon Netting. HN works for Biolytrics Viet Nam Co., Ltd, which analyses chemistry in insecticides, RB works for Vegro Aps, and OS consults on the design of vector control tools for private and public companies, including Vegro Aps.

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References

1. WHO. World malaria report. addressing inequity in the global malaria response. Geneva: World Health Organization; 2024. p. 2024.
2. Okumu F, Finda M. Key Characteristics of residual malaria transmission in two districts in South-Eastern Tanzania - implications for improved control. J Infect Dis. 2021;223(12 Suppl 2):S143–54.
3. Tusting LS, Bottomley C, Gibson H, Kleinschmidt I, Tatem AJ, Lindsay SW, et al. Housing improvements and malaria risk in sub-Saharan Africa: a multi-country analysis of survey data. PLoS Med. 2017;14:e1002234.
4. Fox T, Furnival-Adams J, Chaplin M, Napier M, Olanga EA. House modifications for preventing malaria. Cochrane Database Syst Rev. 2022;10:CD013398.
5. Sternberg ED, Cook J, Alou LPA, Assi SB, Koffi AA, Doudou DT, et al. Impact and cost-effectiveness of a lethal house lure against malaria transmission in central Côte d'Ivoire: a two-arm, cluster-randomised controlled trial. Lancet. 2021;397:805–15.
6. Tusting LS, Ippolito MM, Willey BA, Kleinschmidt I, Dorsey G, Gosling RD, et al. The evidence for improving housing to reduce malaria: a systematic review and meta-analysis. Malar J. 2015;14:209.
7. Ippolito MM, Searle KM, Hamapumbu H, Shields TM, Stevenson JC, Thuma PE, et al. House structure is associated with *Plasmodium falciparum* infection in a low-transmission setting in Southern Zambia. Am J Trop Med Hyg. 2017;97:1561–7.
8. Ngadjeu CS, Doumbe-Belisse P, Talipouo A, Djamouko-Djonkam L, Awono-Ambene P, Kekeunou S, et al. Influence of house characteristics on mosquito distribution and malaria transmission in the city of Yaounde Cameroon. Malar J. 2020;19:53.
9. Kaindoa EW, Finda M, Kiplagat J, Mkandawile G, Nyoni A, Coetzee M, et al. Housing gaps, mosquitoes and public viewpoints: a mixed methods assessment of relationships between house characteristics, malaria vector biting risk and community perspectives in rural Tanzania. Malar J. 2018;17:298.
10. Degarege A, Fennie K, Degarege D, Chennupati S, Madhivanan P. Improving socioeconomic status may reduce the burden of malaria in sub Saharan Africa: a systematic review and meta-analysis. PLoS ONE. 2019;14:e0211205.
11. Sarfo JO, Amoadu M, Kordorwu PY, Adams AK, Gyan TB, Osman AG, et al. Malaria amongst children under five in sub-Saharan Africa: a scoping review of prevalence, risk factors and preventive interventions. Eur J Med Res. 2023;28:80.
12. Odufuwa OG, Ross A, Mlacha YP, Juma O, Mmbaga S, Msellemu D, et al. Household factors associated with access to insecticide-treated nets and house modification in Bagamoyo and Ulanga districts. Tanzania Malar J. 2020;19:220.
13. Tanzania National Malaria Control Programme. National Malaria Strategic Plan 2021–2025: transitioning to malaria elimination in phases. Mainland Tanzania, Dodoma, 2020.
14. The United Republic of Tanzania Ministry of Health CD, Gender, Elderly and Children. The 2019 school malaria and nutrition survey (SMNS) Report. Mainland Tanzania, Dodoma, 2021.
15. Renggli S, Mandike R, Kramer K, Patrick F, Brown NJ, McElroy PD, et al. Design, implementation and evaluation of a national campaign to deliver 18 million free long-lasting insecticidal nets to uncovered sleeping spaces in Tanzania. Malar J. 2013;12:85.
16. Tairou F, Gaye I, Herrera S, Nawaz S, Sarr L, Cisse B, et al. Malaria prevalence and use of control measures in an area with persistent transmission in Senegal. PLoS ONE. 2024;19:e0303794.
17. WHO. Guidelines for malaria. Geneva, World Health Organization, 2024.
18 Pryce J, Medley N, Choi L. Indoor residual spraying for preventing malaria in communities using insecticide-treated nets. Cochrane Database Syst Rev. 2022;2022:CD012688.
19. Kirby MJ, Ameh D, Bottomley C, Green C, Jawara M, Milligan PJ, et al. Effect of two different house screening interventions on exposure to

malaria vectors and on anaemia in children in The Gambia: a randomised controlled trial. Lancet. 2009;374:998–1009.
20. McCann RS, Kabaghe AN, Moraga P, Gowelo S, Mburu MM, Tizifa T, et al. The effect of community-driven larval source management and house improvement on malaria transmission when added to the standard malaria control strategies in Malawi: a cluster-randomized controlled trial. Malar J. 2021;20:232.
21. Nguela RL, Bigoga JD, Armel TN, Esther T, Line D, Boris NA, et al. The effect of improved housing on indoor mosquito density and exposure to malaria in the rural community of Minkoameyos Centre Region of Cameroon. Malar J. 2020;19:172.
22 Pinder M, Bradley J, Jawara M, Affara M, Conteh L, Correa S, et al. Improved housing versus usual practice for additional protection against clinical malaria in The Gambia (RooPfs): a household-randomised controlled trial. Lancet Planet Health. 2021. https://doi.org/10.1016/S2542-5196(21)00002-4.
23. Abong'o B, Gimnig JE, Omoke D, Ochomo E, Walker ED. Screening eaves of houses reduces indoor mosquito density in rural, western Kenya. Malar J. 2022;21:377.
24. Odhiambo MTO, Vulule JM, Afrane YA, Ombok M, Bosselmann R, Skovmand O. Supplementary effect and durability of prototype insecticide-treated eave curtains on indoor resting mosquitoes in Kadibo division Western Kenya. MalariaWorld J. 2016;7:11.
25. Odufuwa OG, Moore SJ, Mboma ZM, Mbuba E, Muganga JB, Moore J, et al. Insecticide-treated eave nets and window screens for malaria control in Chalinze district, Tanzania: a study protocol for a household randomised control trial. Trials. 2022;23:578.
26. Ntabaliba W, Vavassori L, Stica C, Makungwa N, Odufuwa OG, Swai JK, et al. Life expectancy of *Anopheles funestus* is double that of *Anopheles arabiensis* in southeast Tanzania based on mark-release-recapture method. Sci Rep. 2023;13:15775.
27. Pinda PG, Eichenberger C, Ngowo HS, Msaky DS, Abbasi S, Kihonda J, et al. Comparative assessment of insecticide resistance phenotypes in two major malaria vectors, *Anopheles funestus* and *Anopheles arabiensis* in south-eastern Tanzania. Malar J. 2020;19:408.
28. The United Republic of Tanzania (URT) MoFaP, Tanzania National Bureau of Statistics and President's Office - Finance and Planning, Office of the Chief Government Statistician, Zanzibar. The 2022 Population and Housing Census: Administrative Units Population Distribution Report; Tanzania Zanzibar. 2022.
29. National Malaria Control Programme. National Guidelines for Malaria Diagnosis, Treatment and Preventive Therapies. Mainland Tanzania, Dodoma, 2020.
30. Kihwele F, Odufuwa OG, Muganga JB, Mbuba E, Philipo R, Moore J, et al. Community perceptions and acceptability of insecticide-treated screens for mosquito proofing of unimproved houses in Chalinze district, Tanzania: a mixed-methods study. Front Malar. 2025;3:1540184.
31. Hofer LM, Kweyamba PA, Sayi RM, Chabo MS, Maitra SL, Moore SJ, et al. Malaria rapid diagnostic tests reliably detect asymptomatic *Plasmodium falciparum* infections in school-aged children that are infectious to mosquitoes. Parasit Vectors. 2023;16:217.
32. Mboera L, Kihonda J, Braks M, Knols B. Influence of Centers for Disease Control light trap position, relative to a human-baited bed net, on catches of *Anopheles gambiae* and *Culex quinquefasciatus* in Tanzania. Am J Trop Med Hyg. 1998;59:595–6.
33. Sulle E, Mkama W. A contextual analysis for village land use planning in Tanzania's Bagamoyo and Chalinze districts, Pwani region and Mvomero and Kilosa districts, Morogoro region. ILRI Project Report Nairobi, Kenya: ILRI. 2019.
34. WHO guideline for the prequalification assessment of insecticide treated nets. Geneva, World Health Organization, 2023.
35. Schindler T, Robaina T, Sax J, Bieri JR, Mpina M, Gondwe L, et al. Molecular monitoring of the diversity of human pathogenic malaria species in blood donations on Bioko Island Equatorial Guinea. Malar J. 2019;18:9.
36. Mulamba C, Odufuwa OG, Kweyamba PA, Lazaro LO, Chabo MS, Kamage JJ, et al. *Plasmodium falciparum* gametocyte burden in a Tanzanian heterogeneous transmission setting. Malar J. 2025;24:54.
37. Coetzee M. Key to the females of Afrotropical *Anopheles* mosquitoes (Diptera: Culicidae). Malar J. 2020;19:70.
38. Koekemoer LL, Kamau L, Hunt RH, Coetzee M. A cocktail polymerase chain reaction assay to identify members of the *Anopheles funestus* (Diptera: Culicidae) group. Am J Trop Med Hyg. 2002;66:804–11.
39. Scott JA, Brogdon WG, Collins FH. Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. Am J Trop Med Hyg. 1993;49:520–9.
40. WHO. Prequalification of vector control products. Bioassay methods for insecticide-treated nets: Cone test. Implementation guidance (Modules 3 and 5). Geneva, World Health Organization, 2023.
41. Vyas S, Kumaranayake L. Constructing socio-economic status indices: how to use principal components analysis. Health Policy Plan. 2006;21:459–68.
42. Mfinanga SG, Mnyambwa NP, Minja DT, Ntinginya NE, Ngadaya E, Makani J, et al. Tanzania's position on the COVID-19 pandemic. Lancet. 2021;397:1542–3.
43. Kayedi MH, Kaur H, Haghdoost AA, Lines JD. The effects of different drying methods and sun exposure on the concentrations of deltamethrin in nets treated with K-O Tab tablets. Ann Trop Med Parasitol. 2009;103:85–90.
44. Minakawa N, Kawada H, Kongere JO, Sonye GO, Lutiali PA, Awuor B, et al. Effectiveness of screened ceilings over the current best practice in reducing malaria prevalence in western Kenya: a cluster randomised controlled trial. Parasitology. 2022;149:1–39.
45. Briët OJ, Hardy D, Smith TA. Importance of factors determining the effective lifetime of a mass, long-lasting, insecticidal net distribution: a sensitivity analysis. Malar J. 2012;11:20.
46. Guglielmo F, Ranson H, Sagnon N, Jones C. The issue is not "compliance": exploring exposure to malaria vector bites through social dynamics in Burkina Faso. Anthropol Med. 2021;28:508–25.
47. Mboma ZM, Festo C, Lorenz LM, Massue DJ, Kisinza WN, Bradley J, et al. The consequences of declining population access to insecticide-treated nets (ITNs) on net use patterns and physical degradation of nets after 22 months of ownership. Malar J. 2021;20:171.
48. Andolina C, Rek JC, Briggs J, Okoth J, Musiime A, Ramjith J, et al. Sources of persistent malaria transmission in a setting with effective malaria control in eastern Uganda: a longitudinal, observational cohort study. Lancet Infect Dis. 2021;21:1568–78.
49. Koenker H, Worges M, Kamala B, Gitanya P, Chacky F, Lazaro S, et al. Annual distributions of insecticide-treated nets to schoolchildren and other key populations to maintain higher ITN access than with mass campaigns: a modelling study for mainland Tanzania. Malar J. 2022;21:246.
50. Sinka ME, Bangs MJ, Manguin S, Coetzee M, Mbogo CM, Hemingway J, et al. The dominant *Anopheles* vectors of human malaria in Africa, Europe and the Middle East: occurrence data, distribution maps and bionomic précis. Parasites Vectors. 2010;3:117.
51. Tanzania Meteorological Authority. Statement on the status of Tanzania climate in 2022. 2023.
52. Agyekum TP, Botwe PK, Arko-Mensah J, Issah I, Acquah AA, Hogarh JN, et al. A systematic review of the effects of temperature on *Anopheles* mosquito development and survival: implications for malaria control in a future warmer climate. Int J Environ Res Public Health. 2021;18:7255.
53. Ogoma SB, Kannady K, Sikulu M, Chaki PP, Govella NJ, Mukabana WR, et al. Window screening, ceilings and closed eaves as sustainable ways to control malaria in Dar es Salaam. Tanzania Malar J. 2009;8:221.
54. Spitzen J, Koelewijn T, Mukabana WR, Takken W. Visualization of house-entry behaviour of malaria mosquitoes. Malar J. 2016;15:233.
55 Hołyńska-Iwan I, Szewczyk-Golec K. Pyrethroids: how they affect human and animal health? Medicina (Kaunas). 2020;56:582.
56 Pryce J, Richardson M, Lengeler C. Insecticide-treated nets for preventing malaria. Cochrane Database Syst Rev. 2018;11:CD000363.

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