Efficacy of a spatial repellent for control of malaria in Indonesia:
a cluster-randomized controlled trial

Din Syafruddin¹, Puji BS Asih¹, Ismail Ekoprayitno Rozi¹, Dendi Hadi Permana¹, Anggi Puspa
Nur Hidayati¹, Lepa Syahrani¹, Siti Zubaidah¹, Dian Sidik², Michael J. Bangs³, Claus Bøgh⁴,
Fang Liu⁵, Evercita C. Eugenio⁵, Jared Hendrickson⁶, Timothy Burton⁷, JK Baird⁸, Frank
Collins⁷, John P. Grieco⁷, Neil F. Lobo⁷± and Nicole L. Achee⁷±*

¹Eijkman Institute for Molecular Biology, Jakarta, Indonesia; Department of
Parasitology, Faculty of Medicine, Universitas Hasanuddin, Makassar,
Indonesia;
²Department of Epidemiology, Faculty of Public Health, Universitas
Hasanuddin, Makassar, Indonesia
³Public Health and Malaria Control, International SOS, Kuala Kencana,
Papua Indonesia;
⁴The Sumba Foundation, Public Health and Malaria Control, Bali,
Indonesia;
⁵Department of Applied Mathematics, University of Notre Dame, Indiana;
⁶Center for Computer Research, University of Notre Dame, Notre Dame,
Indiana;
⁷Department of Biological Sciences, Eck Institute for Global Health,
University of Notre Dame, Notre Dame, Indiana;
⁸Eijkman-Oxford Clinical Research Unit, Jakarta, Indonesia, and the Centre
for Tropical Medicine, Nuffield Department of Medicine, University of
Oxford, Oxford, United Kingdom;

±Indicates equal seniority

*Corresponding author:

Nicole L. Achee, PhD
Research Professor
Eck Institute for Global Health
Department of Biological Sciences
239 Galvin Life Science Center
Notre Dame, Indiana 46556
Ph: 574.631.1561
Email: nachee@nd.edu
Abstract. A randomized cluster, double-blinded, placebo-controlled trial was conducted to estimate protective efficacy of a spatial repellent against malaria infection at Sumba, Indonesia. Following radical cure in 1,332 children aged 6mo-5yrs in 24 clusters, households were given transfluthrin or placebo passive emanators. Monthly blood screening and biweekly human-landing mosquito catches (HLC) were performed during 10-months baseline (June 2015 to March 2016) and a 24-month intervention period (April 2016 to April 2018). Screening detected 164 first-time malaria infections and an accumulative total of 459 infections in 667 subjects in placebo-control households; and 134 first-time and 253 accumulative total infections among 665 subjects in active intervention households. The 24-cluster protective effect of 27.7% and 31.3%, for time to first-event and overall (total new) infections, respectively, was not statistically significant. Purportedly, this due in part to zero to low incidence in some clusters during intervention undermining the ability to detect an effect. Subgroup analysis of 19 clusters where at least one malaria infection occurred showed 36.0% and 40.9% (statistically significant at 1-sided 5% significance level; p=0.0236) protective effect for time to first-event and overall infections, respectively. Primary entomological analysis of impact proved not statistically significant, with indoor and outdoor anopheline HLC reduced by 16.4% and 11.3%, respectively. Among 12 high-risk clusters, a significant impact on infection was detected (about 60% protective efficacy). While this study suggests protective effects of the intervention, additional evidence is required to demonstrate that spatial repellents provide a practical and effective means in reducing malaria transmission.
It has been nearly 75 years since the role of spatial repellency (deterrence or avoidance) was first described as a potentially beneficial attribute in malaria control, showing chemicals could effectively disrupt normal mosquito behavior and interrupt contact with humans, thus preventing disease transmission without actually killing the insect. Spatial repellency is used here as a general term to refer to a range of insect behaviors induced by airborne chemicals that ultimately result in a reduction in human-vector contact. This can include movement away from a chemical stimulus, and interference with host detection (attraction-inhibition) and/or feeding response. Spatial repellency action can be measured and distinguished from other chemical actions, primarily contact irritancy and toxicity, in the laboratory and in semi-field evaluations. A repellent action drives mosquitoes away from a treated space. Toxics chemical properties can ultimately kill mosquitoes while irritants rely on brief physical contact between mosquito (tarsal segments) and a treated surface to promote excitation and avert prolonged resting. Many compounds can exhibit two or more modes of action and can be distinguished by the concentration or dose exposure required to achieve a specific outcome. Currently, the majority of commercial spatial repellent products utilize either low concentrations of short-duration USEPA registered synthetic pyrethroids (pyrethrin, metofluthrin or most most recently transfluthrin) or botanical-based compounds.

The World Health Organization (WHO) Vector Control Advisory Group (VCAG) is the WHO advisory group that assesses evidence on the epidemiological effectiveness of new vector control interventions and by doing so supports WHO’s development of policy recommendations, including the potential use of spatial repellents as a public health vector control strategy. Reviewed by VCAG since 2014, the assessment of rigorous epidemiological evidence for
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endorse a policy recommendation of a spatial repellent intervention class remains limited and
deemed insufficient. A malaria prevalence study in China evaluating mosquito coils containing
0.03% transfluthrin demonstrated a 77% reduction in human Plasmodium falciparum cases\(^{17}\)
and the use of coils containing 0.00975% metofluthrin provided 52% protective efficacy (PE)
against new (incident) malaria infections in Indonesia.\(^{18}\) Although primary outcomes from both
studies were encouraging, neither met the VCAG requirements of rigor due to study design\(^{19}\)
(non-RCT for China) and/or being underpowered. The importance of a WHO policy for
implementation of spatial repellents in malaria control programs could dramatically increase
investments and efforts by private industry to develop chemicals that operate through different
modes of actions other than acute kill. This would potentially introduce a new generation of
effective active ingredients and product formulations into the disease control/eradication
arsenal.\(^{20,21}\) In combination with existing WHO-recommended malaria control interventions,
spatial repellents have the potential to add significant protective benefit, especially where
traditional long-lasting insecticidal nets (LLINs) or indoor residual spraying (IRS) may not be
protective, be unavailable, or are impractical and/or feasible. Control or elimination of malaria in
these circumstances will require innovative approaches, as example spatial repellents providing a
highly protective role against transmission and this may be where spatial repellents may be most
beneficial.\(^{22,23}\)

The current claim of a spatial repellent intervention class for public health value is
‘deployment of a spatial repellent will prevent human-vector contact to reduce pathogen
transmission’. With this epidemiological primary endpoint in mind, the current large-scale study
aimed to build upon previous epidemiological findings and provide rigorous evidence of a spatial
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repellent to provide a sufficient protective effect against malaria infection risk by decreasing the mean anopheline human landing (biting) rates in endemic communities.

MATERIALS AND METHODS

The study was registered in clinicaltrials.gov (Identifier: NCT02294188) and performed according to Good Laboratory Practice (GLP) and Good Clinical Practice (GCP) guidelines.

Ethics statement. Ethical review and approval for this study was granted by the Ethics Committee (EC) of the Faculty of Medicine, Universitas Hasanuddin (Protocol #UH14070385), the University of Notre Dame (Protocol #14-01-1448) and endorsed by the Eijkman Institute Research Ethics Committee, Jakarta, Indonesia. Consent was obtained from parents or guardians of child recruits following EC guidelines. During the consenting process, the study was described and the consent form read. The consent form detailed the design of the study including the purpose of collecting and storage of blood samples (towards laboratory-based malaria diagnosis), descriptions of the study risks, benefits, and procedures of therapeutic radical cure and follow-up. All households were provided with a signed copy of the consent form after agreeing to participate in the study. For sentinel households participating in entomological collections, a script was read that explained the mosquito collection techniques and signed consent was sought from the head-of-household. No data on individuals was collected during the entomological collections. Adverse events were captured during participant follow-up and reported to monitoring authorities in accordance with the approved protocol.
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**Study site.** The study was conducted in Southwest and West Sumba Districts, East Nusa Tenggara Province, Indonesia (Figure 1). The ca. 400,000 residents of the two districts occupy 175 village ‘groups’ (Desas) and several small-sized towns. Thirteen village groups, with populations ranging 1,067 to 3,904 (avg. 2,132), served as study locations for the final selection of the 24 study clusters. Organized bed net campaigns were recently introduced with the last mass distribution occurring in February-March 2018 across the study area. A mass distribution of long-lasting insecticidal nets (LLINs) occurred in October-December 2014 in all districts (Olyset Net® (Olyset Net®, permethrin 2.0% w/w) with > 95% coverage (1-3 nets per household). A second round of LLIN mass distribution occurred February-March 2018 (PermaNet® 3.0, deltamethrin 180mg/m² + PBO). The last round of focal IRS occurred in 2003 (pyrethroid-based). The malaria prevalence based on microscopically diagnosed parasitemia drawn from a random sampling (50% of residents) in 13 villages conducted in 2015, 10 months before the start of the trial intervention, averaged 15.5% (2.5% to 37.3%) (Table 1).

Although very little is known about the malaria vector distribution and bionomics in this study area, an earlier (August 2007) entomologic survey documented 13 species of anophelines occurring in West Sumba District: *Anopheles sundaicus* s.l. (Rodenwaldt), *Anopheles subpictus* s.l. (Grassi), *Anopheles barbirostris* s.l. (Van der Wulp), *Anopheles hycranus* group species (Pallas), *Anopheles aconitus* (Doenitz), *Anopheles flavirostris* (Ludlow), *Anopheles annularis* (Van der Wulp), *Anopheles maculatus* (Theobald), *Anopheles tesselatus* (Theobald), *Anopheles vagus* (Doenitz), *Anopheles leucosphyrus* group species (Doenitz), *Anopheles maculatus* s.l. (Theobald) and *Anopheles kochi* (Doenitz). These species vary spatially in relative abundance in accordance with their respective preferred habitats ranging from coastal brackish and freshwater marshes and ponds, seasonally productive rice paddies, to...
forested hillsides with perennial running streams and small rivers. At the time of the survey, human-landing collections revealed *An. subpictus* and *An. vagus* the predominant species in the upland interior locations and *An. sundaicus* the most common species along the coastal plain. The majority of residents in study villages work in small-holder agriculturalist pursuits, lack public electricity or articulated water supply, and reside predominately in traditional large, thatched-roof homes raised ~1m above ground and averaging 80 m$^3$ in size with bamboo walls and floor that offer little protection from mosquito entry (Figure 2).

**Sample size.** Previous malaria incidence rates collected in a portion of the study area in coastal Kodi Subdistrict \(^{18}\) were used to estimate the likely malaria attack rate in the current study villages at 0.3 infections/person-year. Sample size determination was based on the hazard rate comparison in the proportional hazards regression model. \(^{28}\) The required number of first-time infections was estimated at 417 to permit detection of a 30% protective effect by the spatial repellent intervention compared to placebo with 80% power at the type-I error rate, assuming a between-cluster coefficient of variance (CV) of 30% and a baseline hazard rate of 0.3 per person-year. With 12 clusters per treatment arm (active or placebo), with 2-month accrual and 22 months follow-up, and an estimated 20% loss-to-follow-up (LTFU) during intervention, a total of 54 subjects per cluster was required (n=1,296).

**Study design.** The study was a cluster-randomized, double-blind, placebo-controlled, clinical trial with a total of 24 clusters divided into 12 clusters per intervention arm. Clusters were based on housing type, focusing on traditional houses (with or without thatch roofing) (Figure 2), mitigation of potential chemical dispersion 'spillover' effect (i.e., distance between nearest homes...
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in different clusters ~500 m apart), and logistical considerations (i.e., position of field-based satellite laboratories). About fifty-four households were recruited within each cluster based on human sample size requirements. Households from 13 villages were stratified into 24 clusters before randomization: Gaura (pop. 2,831; houses 432), Kahale (pop. 3,904, houses 253), Karang Indah (pop. 1,169, houses 139), Manutoghi (pop. 1,067; houses 165), Matakapore (pop. 2,805; houses 209), Panenggo Ede (pop. 1,271; houses 90), Rada Malando (pop. 1,842; houses 133), Tana Mete (pop. 2,124; houses 213), Waikarara (pop. 2,709; houses 446), Wailangira (pop. 1,878; houses 226), Waimakaha (pop. 2,334; houses 211), Waimaringi (pop. 2,598; houses 116), and Weetana (pop. 2,670; houses 275) (Figure 3).

Clusters were allocated to receive either active or placebo treatment using a random number generator (https://www.random.org). The cluster allocation code was made available from the intervention manufacturer to the Data Safety Monitoring Board (DSMB) for use in safety assessments. The site database manager assigned a unique identification number to each household (HIN) and the site intervention administrator coordinated distribution of blinded active or placebo to enrolled households within each cluster corresponding to the pre-labeled package code. Unblinded assignments were shared with a site administrator in a sealed envelope placed in a secure location within the managing center of the research project (Jakarta) for purposes of emergency unblinding related to adverse and/or serious adverse events. Thus, the investigators, research team, study subjects, and residents were blinded as to which cluster received active versus placebo devices until after completion of the study.

The primary endpoint for estimating the protective efficacy (PE) of the spatial repellent intervention was malaria incidence (time to first infection) among the enrolled sentinel residents study participants in each of the 24 clusters, i.e., 1,332 subjects in all. These sentinel subjects,
each living in separate households, received a directly observed, presumptive radical cure to

clear them of any standing (patent, sub-patent, or latent) malaria infections. Biweekly blood film

exams were conducted from subjects reporting fever within the previous 48hrs (passive case
detection) with active screening for malaria infection in all subjects occurring every 4 weeks for
the duration of the study.

Enrollment for incidence cohort and radical cure. The average number of children <5 y.o. in

each cluster was 68 (57-79). From individual households in study clusters, one child, aged 6 to

59 months old at time of recruitment, were provided the opportunity to enroll as subjects in the
study. Following informed consent, screening consisted of physical examination by a study

physician and a qualitative NADPH spot test for G6PD deficiency (Trinity Biotech qualitative

G6PD assayTM, ref 345-UV, Trinity Biotech, St. Louis, MO) 29. Additional to G6PD normal

status, eligibility requirements included a bodyweight 40 kg, hemoglobin > 5mg/dL (Hb201+,

HemoCue AB, Angelholm, Sweden) 30, no severe acute illness/infection on the day of inclusion,
temperature ≤ 38°C, participant acknowledging sleeping in the village > 90% of nights during

any given month, not participating in another clinical trial, and no plans for extended travel
during the study period. A total of 1,451 subjects were presumptively radically treated using a

fixed combination formulation of dihydroartemisinin (DHA)-piperaquine (P) (containing 40 mg
dihydroartemisinin and 320 mg piperaquine; Zhejiang Holley Nanhu, Beijing Holley Cotec)
administered as a weight per dose regimen of 2.25 and 18 mg/kg per dose of dihydroartemisinin
and piperaquine, once daily for 3 days, and primaquine 0.25 mg/kg body weight (PT, Pharos
Tbk, Semarang, Indonesia) for the 14 days immediately before implementing intervention. The
DHA+P combination is currently the first-line antimalarial drug for malaria treatment in Indonesia.

All subjects were examined for *Plasmodium* spp. infection by expert microscopy, and polymerase chain reaction (PCR) from matched blood spot samples on filter paper. New malaria infections among the participants were monitored every 4 weeks using microscopic examination of Giemsa-stained blood films according to WHO guidelines based on 200 high magnification (1000x oil-immersion) thick blood film fields. Two certified expert microscopists independently (blinded) examined slides on-site at project-dedicated field laboratories. PCR detection of parasites was conducted at the Eijkman Institute for Molecular Biology (EIMB) central laboratory in Jakarta using PCR techniques for detection of DNA of all four *Plasmodium* species. A blood sample was defined as ‘positive’ for inclusion in incidence analyses if meeting criteria of having two diagnostic outcomes indicating presence of parasite (e.g., 2x microscopy; 1x microscopy + 1x PCR; or 2x PCR). All positive and 10% randomly selected negative samples diagnosed at EIMB were re-tested at the University of Notre Dame. Discordant microscopy and PCR results prompted reexamination of both initial findings. Participants found malaria infected at point-of-care were immediately treated with DHA+P and were removed from contributing to further person-time at risk of first-time infection; however, remained in the study to monitor cumulative incidence for analyses of overall (total new) infections and estimate number of cases averted.

**Intervention.** Immediately after completion of radical cure, intervention was simultaneously initiated in all subject households and non-subject households that consented to receive intervention. The spatial repellent intervention is a transfluthrin-based passive emanator.
produced by S.C. Johnson & Son, Inc (SCJ) designed to release a volatile chemical into the air and prevent human–vector contact within the treated space. Transfluthrin is a registered compound commonly found in commercially available mosquito coils globally based on WHO specifications. Emanators (active and placebo) of identical packaging and color were distributed by study personnel every 2 weeks at the individual household application rate of 2 units / 9m² according to SCJ specifications. There was a median of 1 room (range 1,9) and 10 emanators (range 4,56) per household. Interventions were positioned indoors of houses by hanging on two metal hooks attached to walls. Each position remained static throughout the study. The hooks facilitated stabilization of the interventions so the chemical-treated surface was consistently exposed facing the interior space. Research staff placed, removed and replaced emanators in households at set intervals and recorded attrition during each replacement period to ascertain application rate for use in estimating cluster coverage. Potential adverse health effects (AEs), possibly related to transfluthrin exposure, in subjects and other household members, were reported by the study team during both active and passive blood sampling using a standard survey according to protocol. Investigation of study-related AEs was performed by the on-site study clinician. Severe adverse health events (SAE) were also recorded according to protocol, regardless of possible relationship to intervention. Health clinic data were compiled on a quarterly basis starting in Dec 2017 for DSMB safety assessment of the study population. During the period of the trial, quality control analysis was performed by Ross Laboratories, India on used (after 2-week deployment) and unused (in storage) emanators representing each cluster to check for appropriate amounts of transfluthrin in actives and the absence of transfluthrin in placebos. At the end of follow-up period (April 2018), used, unused and expired emanators
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(approximately >2 tons of material) were incinerated by PT. Wahana Pamunah Limba Industri, Jakarta according to Indonesian regulations.

Entomologic surveys. Adult mosquito diversity and densities were measured using human-landing catches (HLC) every two weeks from start of baseline through end of follow-up period in a subset of 12 clusters. Clusters for entomological sampling were hierarchically stratified based on human landing rate and blindly allocated to treatment arm to ensure a balanced recruitment (6 clusters in each treatment group). Four neighboring sentinel houses within each of the 12 clusters were randomly selected for mosquito collections (n=48). Collections were conducted at sentinel houses for 1 night every 2 weeks from paired active/placebo clusters (e.g., 3 pairs on Monday night and 3 pairs on Wednesday night) during intervention. All mosquito collectors were trained and assessed as competent to perform duties required. Each collector provided informed consent at beginning of the study. Teams of two collectors were assigned per house, one positioned indoors near the center of the house and one located outside on the house verandah, approximately ~1 m from the exterior wall. Collectors removed all mosquitoes landing on their exposed lower legs using a mouth aspirator. Collections were conducted from 1800 to 0600 h for 50 min every hour. Paired collectors rotated the indoor and outdoor position every hour. Samples were placed into individual holding containers labeled by collection hour interval, unique house code (linked to blinded treatment code), and collection location (indoor or outside). Captured mosquitoes were immediately killed by organic compound vapor in the field and initially identified to species (or species complex) using morphological characters. All specimens were transported to an on-site study base laboratory upon completion of the 12 h collection and a random sample of representative anopheline species (up to 30 per cluster and
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...hour of collection) were dissected for parity and scored as either gravid/parous or nulliparous.  

Partial (head-thorax for those dissected for parity) or whole anopheline specimens were placed singly into individual Eppendorf® 1.5ml vials and stored over silica gel desiccant until further processing at EIMB, Jakarta, for detection of malaria sporozoites and molecular-based species identification,  

Where applicable. Mosquito samples were evaluated for Plasmodium spp. infection using polymerase chain reaction (PCR) methodologies to derive corresponding malaria sporozoite infection rates by parasite species and vector species. Together with time-adjusted HLC densities (anophelines/person-night), matched sporozoite rates were used to derive the entomological inoculation rates (EIRs) for each treatment arm.  

Anopheline species identification was verified at the University of Notre Dame following previous protocols.  

Insecticide susceptibility assays. Permethrin was evaluated using WHO tube and CDC bottle assay during baseline, intervention and post-intervention periods at the WHO recommended diagnostic concentration for anophelines 0.75% and CDC recommended dose 21.5µg/ml active ingredient. Both WHO tube assays and CDC bottle assays were performed on F0 mixed anopheline species collected as immatures from 13 clusters (23 locations) during baseline, intervention and 6-mo post-intervention period (ended Oct 2018). Assays were conducted using non-bloodfed, 3-5 day old females according to established guidelines. After each test period, all chemical and control specimens were stored individually over silica gel for analysis at EIMB to confirm species identification and for detection of target-site mechanisms (e.g., kdr mutations) of resistance.
**Data management and verification.** Data collection was designed around a tablet-based survey platform linked to a custom-built database and web portal. CommCare (Dimagi Inc, MA) was selected as the frontend form application, providing critical capabilities, such as: a parent-child case structure, the ability to store forms when offline, update form versions after deployment, build forms with complex logic in a web browser, and export form data to other tools. Data was cleaned according to rules specified in the study protocol to ensure data integrity. Study data related to participating subjects, participating households, intervention (placement/replacement), collected mosquitos, and lab analyses was cross checked, identifying missing, incomplete, or suspect data submissions. These were sent back to the site data manager to be resubmitted or corrected. Once data correction was complete, data was verified and requested for analyses.

**Statistical analyses (S1. Statistical Analyses Plan).** Subjects were excluded from analyses when a subject had no blood sample taken during the intervention period or when a household might have contributed more than two subjects (rarely) when there was LTFU on the first recruited subject and a 2nd subject was recruited as a replacement. In the latter case, only the subject with a longer follow-up period was used based on per-protocol allowing only one subject per household.

The primary hypothesis on PE against first-time malaria infection was tested by comparing the hazard rates of the first-time malaria infection between active and placebo (blank) intervention. The complementary log-log (cloglog) regression model

\[
\log \left( -\log \left( 1 - \theta_{ijt} \right) \right) = \beta_0 t + \chi_{ijt} \beta + z_i
\]

was. \( \theta_{ijt} \) is the discrete time hazard rate of subject \( i \) in cluster \( I \) at time \( t \), \( \chi_{ijt} \) contains visit (as a categorical predictor), the individual-level (age, gender), household-level (number of doors, open eaves Y or N, wall type), and cluster-level (baseline incidence rate, cluster population size, intervention group) covariates, and \( z_i \sim N(0, \sigma^2) \) is the cluster-level random effect. PE was
estimated by \((1 - \exp(\beta)) \times 100\%\) with a 90% confidence interval (CI) based on the Wald test, where \(\hat{\beta}\) is the estimated regression coefficient associated with the intervention group, and \(\exp(\hat{\beta})\) is the estimated hazard ratio (HR) between active and placebo. The null hypothesis of PE is 0 was tested by the Wald’s test \(z = \hat{\beta}/s\), where \(s\) is the estimated standard error of \(\hat{\beta}\). It was concluded that active intervention reduces the first-time malaria hazard rate compared to placebo if \(z < z_{0.05} = -1.645\); otherwise, the study would not have enough evidence to suggest that active intervention reduces the first-time malaria hazard rate compared to placebo at a one-sided significance level of 5%. The Kaplan-Meier (KM) curves on first-time malaria infection per cluster was provided for the active and placebo arms, respectively.

The statistical method for analyzing the secondary endpoint of the overall (total new) malaria infections detected in study subjects was similar to that used for analyzing the primary endpoint of the first-time malaria infection except that the above cloglog model has additional term \(z_{\chi^2}\) the random effect at the individual level to account for the dependence among multiple malaria infections per individual. The same set of analyses as the above were performed in a subgroup analysis in clusters with non-zero baseline incidence rates in clusters used for entomology data collection.

The effects of active intervention on the secondary entomological endpoints were estimated by applying the negative binomial regression model, if applicable. Specifically, the anopheline landing rate (surrogate ‘bite’ based on HLC and indicator of human-vector contact) is defined as the number of mosquitoes caught during the 12-hr interval overnight. The covariates in the model for analyzing the landing rate includes the fixed effects of intervention group, the interaction between treatment and location of collection (inside or outside), visit (as categorical), baseline incidence rate, baseline vector count, cluster population, and random effects for household...
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nested within cluster and for cluster. The % change in landing rate by intervention was estimated from the model. The frequencies and percentages of the captured anophelines were also summarized by species. The set of the covariates in the model for analyzing the parity rate are similar to the model for analyzing the landing rate, with an additional offset term of the daily landing rate. Due to the data sparsity issue in the sporozoite positivity rate (>99% of the collected mosquitos were uninfected), no model-based analysis was performed and only summary statistics are provided for the sporozoite positivity rate data. The entomological inoculation rate (EIR) is defined as the number of infective mosquito bites a person receives per a unit time (typically annually) and is calculated as \[ \text{EIR} = \text{sporozoite positivity rate} \times \text{human biting rate}. \] The data sparsity issue experienced by the sporozoite positivity rate data also exists in the EIR data, and thus only summary statistics are provided on EIR.

RESULTS

Figure 4 summarizes screening, enrollment, and follow-up of the 1,332 subjects of the incidence density cohorts among the 24 clusters. All subjects completed radical cure and were followed 24 months.

Protective effect against malaria infection among 24 clusters (pre-planned).

Trial outcomes show baseline covariates regarding subject, house construction, population and baseline malaria incidence between the active and placebo arms were balanced at the individual-, household-, and cluster-level (Table 2). The intervention coverage rate, defined as the proportion of actually placed emanators over the total number required per household, ranged from 82.2%
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to 98.6% by cluster over the entire intervention period, with a mean application rate of 93.2% and 92.3% for the active and placebo arms, respectively.

There were 134 and 164 first-time infections in active and placebo treatment arms, respectively, with a hazard ratio of 0.72 (90% CI: 0.43, 1.21) (Table 3). A 27.7% decrease in the first-time malaria hazard risk was estimated using active compared with placebo (90% CI: 21.3%, 56.9%). The 27.7% PE was not statistically significant at the 5% one-sided significance level. The estimated PE of active intervention against overall (total) malaria infections (first and subsequent) was 31.3% (90% CI: -10.8, 57.4%) with a one-sided p-value = 0.098 (Table 3).

Investigation of potential shift in parasite species infection frequency (P. falciparum vs. P. vivax) between active and placebo will be reported in subsequent publications.

A total of 164 first-time malaria infections occurred during approximately 1032 person-years at risk in study participants whose households were given placebo, with a calculated incidence density of 0.159 infections/person-year (Table 3, Figure 5). In contrast, 134 total malaria attacks occurred in transfluthrin-active households with approximately 1079 person-years at risk resulting in a calculated 0.124 infections/person-year. A cumulative 439 malaria infections (first-time and subsequent) during approximately 1216 person-years at risk were detected in participants whose households were given placebo, with an incidence density of 0.361 infections/person-year. Contrastingly, 253 total malaria attacks occurred among participants living in active households with approximately 1216 person-years at risk equaling 0.209 infections/person-year (Table 3).

Subgroup analyses of protective effect against malaria infection among non-zero baseline incidence and entomology clusters (not pre-planned).
Among the 24 clusters, there were five clusters with zero baseline incidence which had zero to very low incidence rates during intervention. The first subgroup analysis used the 19 clusters with non-zero baseline incidence rate as the remaining five clusters would have been excluded from the intervention if baseline analysis had been performed before the randomization. Excluding these five clusters, the estimated PE for overall (total new) infections in the remaining subgroup of 19 clusters with non-zero baseline incidence rates was 40.9% (90% CI: 8.61, 61.8%) resulting in a one-sided p-value of 0.0236 (Table 3). The second subgroup analyses included incidence from the 12 clusters having entomology collection data (i.e., mean anopheline landing rate), and where the average baseline incidence was ~ 4 folds greater than the other clusters. The PE using active intervention against time to first-event and overall (total new) malaria infections in this subgroup was 55.3% and 66%, respectively (Table 3). The data indicate the baseline covariates (subject, house construction, population and baseline malaria incidence) between the active and placebo arms were balanced at the individual-, household-, and cluster-level in both of the subgroup analyses (not shown).

Effects on entomological endpoints.

Anopheline landing rates. A total of 26 weeks of HLC were performed within a small subset of 12 clusters during the intervention period. Initial results based on morphological species identification detected 19 putative malaria vector species showing spatial and temporal variation across monitored clusters. The most common species attracted to humans included: Anopheles aconitus, An. annularis, An. barbirostris, An. flavirostris, An. kochi, An. maculatus, An. subpictus, An. sundaicus, An. tessellatus and An. vagus (Table 4). The cumulative indoor and outdoor anopheline biting rates are shown in Figure 6. There was a numerical reduction in
indoor and outdoor landing density from collections performed at sentinel households containing active intervention as compared with those assigned to placebos. This difference resulted in 16.4% and 11.3% reduction in anopheline attack rate on collectors positioned indoors and outdoors, respectively, at sentinel households with active intervention compared with placebo houses, although this reduction was not statistically significant (Table 5).

Sporozoite positivity and Entomological Inoculation Rate (EIR). The frequency of sporozoite positive anophelines is given in Table 6. Only P. falciparum and P. vivax infections were detected in captured mosquitoes. During baseline and intervention period, the sporozoite rate was less than 0.5% for both treatment and placebo arms. Data sparsity regarding comparison of sporozoite rates precluded formal statistical analyses. The EIR, defined as the number of infective mosquito bites a person receives per unit time (for example, nightly, monthly or annually) \(^3\) was <1 bite per year in both treatment arms during baseline and intervention periods.

Parity rate (age-grading). The proportion of sampled females categorized as “older,” combining parous and gravid states (mosquitoes with advanced ovarian follicle development as evidence of recent blood meal), and those “younger” as nulliparous (non-bloodfed) and recently emerged were compared between active and placebo treatment for the 12 clusters where entomological monitoring was conducted. Overall, transfluthrin-active emanators proportionally increased nulliparity in the sampled anpheline populations compared to placebo for both indoor and outdoor locations (Table 7).
Insecticide resistance. A total of 5,091 adult female anophelines (chemical and control assays) were evaluated for susceptibility: 700 samples during baseline, 1,805 during intervention and 2,586 post-intervention. *Anopheles vagus* was the most widely distributed and tested anopheline (56%), followed by *An. sundaicus* (12.6%) and *An. subpictus, An. barbirostris, An. kochi, An. aconitus, An. maculatus*, and *An. tessellatus* (proportionally, all <10%). Baseline, intervention and post-intervention tests using permethrin (21.5ng/ml) showed 100% knockdown in the CDC bottle assay between 15-30 min, and 100% 24hr mortality (permethrin 0.75% at 60 min) in the WHO tube assay. Based on the assumption of predominately pyrethroid-susceptible wild populations of *Anopheles* spp. present in trial sites, there was no conclusive evidence of development or increase in phenotypic resistance to pyrethroid class chemicals between pre-and post-intervention periods. In several clusters during the post-intervention phase, there were indications of reduced susceptibility in a few *An. barbirostris* populations to permethrin. Follow-up investigations provided inconsistent results, thus confirmation that these populations showed low levels of resistance could not be verified.

Adverse Events (AE) and Severe Adverse Events (SAE).

A total of 523 and 144 AEs were reported from non-participants (using government clinic health records) and the intervention study cohort (collected by the study team), respectively. General respiratory complaints were most common, followed by general fever. There were 6 total SAEs reported in the study cohort during follow-up: 2 deaths of unknown cause; 1 case suspected brain infection; 1 case respiratory infection; 1 case malaria with concomitant bacterial or viral infection; and 1 case of drowning.
A total of 180 emanator samples were analyzed in 2017 taken from different active and placebo clusters with manufacturing dates ranging from April to November 2016 (15mo - 8mo-old samples). All sampled placebo interventions were found to be absent for transfluthrin. The average transfluthrin quantity from all sampled active interventions was within specification range (55.00 ± 2.75 mg).

**DISCUSSION**

Malaria remains a significant global public health burden despite recent progress in reducing disease rates. The primary objective of this large-scale RCT conducted on Sumba Island, Indonesia was to demonstrate and quantify the protective efficacy (PE) of a passive emanating spatial repellent intervention (transfluthrin-treated), for reducing malaria incidence (transmission) in humans. Such evidence of human health impact is a fundamental and essential requirement in the critical path of development of new vector control intervention classes being assessed by the WHO VCAG to recommend a global public health policy. Outcomes reported here are promising for malaria control, despite the study being unable to demonstrate clear, statistical protective efficacy on primary analysis. The primary per-protocol analyses provides an estimate of 27% PE against malaria infection, which is near the efficacy of 30% selected in the power calculations. This effect, however, was not statistically significant with confidence intervals that include zero. There were 298 first-time infections, in contrast to an expected number of at least 417 assumed in the sample size calculations, thus resulting on the trial being underpowered. This study highlights three primary challenges for consideration in future spatial repellent trials as well for new vector control intervention classes more broadly. Firstly, having
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‘adaptive’ study designs, especially for evaluation in low to moderate malaria transmission settings and settings with large cluster-to-cluster variance on transmission intensity; second, defining and identification of the ‘key’ entomological correlates of protection; and third, ensuring reliability and feasibility in AE/SAE reporting for accurate safety assessment.

Regarding adaptive study designs, our assumptions for power and sample size calculations were based on a previous proof-of-concept study that was spatially very near (border) of the current study area and study villages were selected with the assumption that all clusters would experience malaria transmission during the intervention period, which, unfortunately was not the case. Baseline data indicated zero-incidence for five of the 24 selected study clusters with a 5-folds higher between-cluster variability than assumed in the original sample size calculation. The 5 clusters with zero baseline incidence (two allocated to receive active and three allocated to receive placebo) also had either zero or low incidence rates during intervention and ranked in the top 8 among all 24 clusters in terms of lowness in intervention incidence (data not shown). In retrospect, a pre-planned analysis of the baseline data would have allowed adjustment of the study design by filtering out the zero-incidence clusters, or increasing the study duration (time of follow-up), the number of subjects recruited per cluster, or the number of clusters to better satisfy requirements of power and capture the necessary number of outcome events (infections). However, given the updated baseline incidence rate of 0.131 per person-year and 97.1% CV, to maintain 80% power with the originally assumed 30% PE, 100 clusters per arm with 144 households (HHs; i.e. subjects) would have been required to be recruited to collect 5,550 first-time malaria events – an unmanageable scale that could not have been supported due to geographical, logistical and funding constraints. Moreover, increasing the follow-up time to collect more first-time malaria events would have neither counterbalanced
the large variability nor the longer duration required to collect the additional events due to the seasonally-influenced low to moderate malaria transmission among clusters. For this reason, when planning future trials in low or moderate endemic transmission settings, investigators should consider a greater number of clusters per arm and/or building into the statistical analyses plan an interim and final analyses of baseline incidence with pre-determined study design adaptations, to include down-selection of clusters with predetermined incidence thresholds. Early exercises of the impact of a lower than assumed incidence (or greater CV) during study planning should be explored amongst investigators, industry and funding partners to ensure that the study area context, intervention manufacturing, program period and/or funding can sufficiently meet the demands of power requirements if adjustments are to be made once the study begins. Stakeholders should also discuss supporting and adopting adaptive designs so to allow decision-making after planned interim analysis of intervention data, to either stop the trial for futility or to continue the trial with adjusted design parameters, such as sample size.\textsuperscript{48, 49} Perhaps just as important in the context of vector control, although RCTs are rated as high-quality evidence,\textsuperscript{50} considerations to RCT alternatives are prudent in the trial planning phase as these may offer assurances of adequate data rigor while balancing cost and time constraints of traditional RCTs.\textsuperscript{51, 52} Alternatives study designs include large observational studies for detecting population-level effects using analytical cross-sectional studies, or operational program-based evidence; the latter perhaps especially for interventions containing an existing registered chemical active ingredient (i.e., meets human safety thresholds) and where the intervention is implemented in pilot trials and impact monitored through case reports, as compared to a contemporaneous control group.\textsuperscript{52}
The inclusion of entomological endpoints in the Sumba RCT was, in part, to help understand and validate the intervention’s mode of action for the VCAG claim of a health impact through a reduction in human-vector contact. Spatial repellent chemicals may cause initial knockdown and direct kill by exposure to toxic doses at close range to the active ingredient, or a delayed kill or behavioral avoidance response, through exposure to sub-lethal doses at distances further away from the stimulus source. Therefore, entomological measurements for detecting reductions in human landing density with active intervention, and a possible change in anopheline age structure (parity rate) indicating older populations not surviving as long as in placebo areas, was built into this study. Additionally, a reduction in sporozoite infection rates because of lower blood feeding success, and/or an inability to survive the required time interval (parasite incubation period) to become infective to a susceptible host represent other endpoint measures of impact on the vector. A causal relationship with one, several or all, of these endpoints would allow future trials evaluating non-inferiority of a ‘second-in-class’ spatial repellent to integrate entomological measures only to predict protective efficacy and provide assurances of meeting minimum thresholds of acceptance for public health use.

Sporozoite positivity rates and EIR estimates from the current trial were low, with a total of 42 of 11,650 (0.36%) anophelines tested during baseline (10mo) and 19 of 17,971 (0.11%) found infected during intervention (24mo). These findings are not unusual or unexpected in low to moderate malaria transmission settings. As example, the previous Sumba study (using metofluthrin coils) reported just 15 out of 1,825 (0.82%) HLC anopheline samples sporozoite infected. The challenges for assessing sporozoite infectivity and using EIR as a measure of intervention effect in such settings should be factored into study pre-planning to carefully balance cost of sample processing with potential information gained.
These study findings showed a 16.4% reduction in indoor landing mosquitoes at sentinel collection houses with active treatment compared to placebo-control. This result differs from the previous study on Sumba evaluating a metofluthrin-active coil where a significant 32% reduction in *An. sundaicus* indoor landing rates was found compared to blank coil control.\(^1\) In the previous and smaller study, *An. sundaicus* was the overwhelmingly predominate human-feeding anopheline; whereas in the current study this species was relatively uncommon during the intervention trial. The greater complexity of anopheline diversity in the current study area may have contributed to the discordant findings between the two studies. Specifically, the range of ecologies (coastal plains to upland forested hills) varied greatly across the 12 clusters used for entomology collections in this trial compared to that of the earlier Sumba study where collections were confined to only 4 adjoining clusters along similar coastal habitats. This variability in ecology witnessed in the current study resulted in a broad range of species diversity (19 species, group species captured), each with unique habitat requirements, bionomic and behavioral characteristics (e.g., biting habits, host preferences). Similar results may occur in future trials that are conducted in settings with diverse vector populations and/or as cluster size increases thereby increasing the probability of greater habitat diversity. Other potential reasons for differences in the effect on anopheline landing rates between the two studies on Sumba include intervention formulation (i.e., coil vs. passive emanator) as well as species-specific effects by active ingredients (i.e., metofluthrin vs. transfluthrin). Perhaps just as important as measuring a reduction in indoor landing rates was the ability to demonstrate an effect on outdoor landing rates (11% reduction) – on the exposed verandah of houses. Measuring an apparent effect on outdoor landing is encouraging as many Sumba residents often sleep on the verandah without the
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protection of a bed net. Results from further data analyses on effects of this intervention on non-
anopheline populations are forthcoming.

Pertaining to monitoring of insecticide resistance, this labor-intensive activity was
integrated into the study to characterize the wild-type anopheline populations and monitor
changes in susceptibility due to continual exposure of the transfluthrin-active intervention.

Although we are confident in the data presented, there were limitations in sampling from
multiple sites with multiple species during pre-, within, and post-intervention periods to obtain a
clear (“useful”) profile of susceptibility background and shift in phenotypic (and molecular)
frequencies. Future trials requiring assessment of insecticide resistance should allow careful
planning and budgeting.

The third ‘lesson-learned’ from the current study relates to AE and SAE monitoring.

Reports of intervention safety in this study should take into account possible limitations of the
data collection. The DSMB was provided with clinic attendance data from January 2015 to
December 2018, for upper respiratory infection (URI), pneumonia, and malaria, as classified by
month, health center, and village within health center coverage. Although not gathered as part of
the study, the DSMB was keenly interested in these data because of the surprisingly low number
of illnesses/deaths reported from the study population during the entire 24-month intervention
period. For some periods, particularly for malaria infection, the case data were missing or the
health center totals were not available by village. Of the 13 villages represented in the dataset,
all but four comprised clusters (or parts of clusters) from both study intervention arms (i.e., some
allocated to active, and others to placebo); therefore, it was not possible to infer the allocation
status of the corresponding cases. For this reason, the DSMB was unable to make a reliable
assessment of any possible association between active intervention and clinic attendance for
those health conditions reported. The respiratory illnesses in the clinic data are more reflective
of the magnitude of numbers of cases one would expect from this population; however, the
DSMB was again unable to parse them into test and control clusters due to reasons stated above,
and therefore the data was of little use for monitoring purposes. The number of SAEs reported, a
total of only six, is well below the expected in a study population of this size. The WHO
estimates the crude death rate in Indonesia to be approximately 6.2 per thousand per year. 53
Based on this, for a population of 1,296 children enrolled (the sample size calculation in the
protocol), the probability of having zero deaths in a year is less than one in 1,000. Although open
commemoration of deaths is commonly practiced in the study area, it seems apparent that they
were not completely reported/recorded as SAEs.
Overall, the point to apply for future spatial repellent trials (and perhaps other trials of
new vector control interventions) is to improve the mechanism for capturing adverse events
before the initiation of the study such that all deaths and hospitalizations are reliably reported
and regular monitoring in place for unusual numbers of complaints. If these trials are regarded as
a clinical trial with a placebo that requires comparative AE an SAE data, then almost anything at
this scale could fail simply on the mass of data, however imperfectly collected. Any safety
signal detected would most likely be due to bias in reporting or collection and would have little
to do with the investigational intervention. Trials evaluating a spatial repellent intervention with
a currently registered active ingredient (i.e., a chemical meets regulatory approvals for
acceptable levels of human risk) such as transfluthrin, could focus on a small number of
complaints that might be expected due to inhalation (i.e., volatilization) and develop a
monitoring scheme that collects consistent data across the population. Clinic-based complaints
of respiratory illness such as pneumonia and asthma might be possible, but the variability in their collection (clinic or survey) will likely be highly dependent on study site infrastructure. In conclusion, while more evidence will be required to determine whether spatial repellents can serve as a viable malaria control intervention, both the primary and secondary results of this Sumba Island trial have generated valuable data and observations that can contribute to the overall assessment and improvement of testing protocols of a spatial repellent intervention class. The VCAG has recommended that data from at least one additional trial evaluating the spatial repellent be generated, and once available, the panel will be able to assess the available evidence for judging public health value. If the crude estimate of PE shown here, near 30%, is replicated in future statistically robust cluster-randomized trials, the intervention prototype evaluated in this RCT would approximate the benefit associated with LLINs. Perhaps just as important for endemic countries, the difference in the overall (total new) infection rate between transfluthrin-active and placebo-control – the number of cases averted - indicates 361 expected cases per 1000 persons per year \((0.361 \times 1000 \times 1)\) without the spatial repellent and 152 less cases expected when using the active intervention \((0.361 - 0.209) \times 1000 \times 1\). The cost-savings of averting these 152 less cases per 1000 person-years is an important consideration for health-systems strengthening. These results have encouraged further and substantial investment to validate spatial repellent efficacy through larger RCTs, including an investigation of possible diversionary effects on human health (i.e., greater than expected malaria incidence in households near intervention not receiving product), and evaluation of the optimal delivery systems for humanitarian assistance use-case scenarios.
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**Disclaimer:** The contents are the responsibility of the authors.
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FIGURES

Figure 1. Study site areas in Southwest and West Sumba districts located in Kodi, Kodi Bangedo and Lamboya sub-districts of Sumba Island, Nusa Tenggara Timur Province (eastern Lesser Sunda islands), Indonesia (map not to scale).
Figure 2. Traditional Sumba house structure (A) raised ~1m above ground and averaging 80 m³ in size with thatch roof, bamboo floors and walls (B), that offer little protection from mosquito entry.
Figure 3. Location of 24 study clusters. Clusters were selected for enrolling the incidence cohort, each consisted of ca. 100 households with an average distance of 500m between clusters. A total of 48 sentinel houses from 12 clusters were selected for routine entomological collections.
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Figure 4. Flowchart of enrollment of study volunteers. A total of 1,684 subjects were screened for G6PD deficiency and 1,488 consented to be enrolled and provided radical cure for active or presumptive malaria of which 1,451 completed the treatment and subsequently followed-up for 24 months during intervention.
Figure 5: Cumulative incidence of first-time malaria infection by cluster (12 per treatment arm).
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Figure 6. Cumulative biweekly indoor (A) and outdoor (B) anopheline landing rates by treatment group (averaged over 20 to 24 households per treatment group).
Table 1. Parasitological prevalence data of the 13 study villages on Sumba Island conducted in 10 months before cluster randomization.

| Village          | Number of samples | Number of malaria infections | Slide Positive Rate (%) |
|------------------|-------------------|------------------------------|-------------------------|
| Matakapore       | 806               | 110                          | 13.65                   |
| Manutoghi        | 622               | 217                          | 34.88                   |
| Waimakaha        | 621               | 149                          | 23.99                   |
| Wailangira       | 857               | 52                           | 6.07                    |
| Waikarara        | 1,420             | 63                           | 4.44                    |
| Panengo Ede      | 610               | 15                           | 2.46                    |
| Waimaringi       | 649               | 67                           | 10.32                   |
| Tana Mete        | 810               | 81                           | 10.00                   |
| Kahale           | 903               | 202                          | 22.37                   |
| Rada Malando     | 402               | 150                          | 37.31                   |
| Karang Indah     | 523               | 195                          | 37.28                   |
| Gaura            | 1,297             | 165                          | 12.70                   |
| Weetana          | 1,129             | 163                          | 14.40                   |
Table 2. Summary baseline covariates by treatment arm for the primary analysis.

|                  | Individual level |          |          |          |          |          |
|------------------|------------------|----------|----------|----------|----------|----------|
|                  | SR (n = 665)     | Placebo (n = 667) |
| Age in months (mean ± SD, (min, max)) | 34.0 ± 15.1 (6, 59) | 34.2 ± 14.8 (6, 59) |
| Gender (% of male subjects) | 54.1% | 51.1% |
|                  | Household level  |          |          |          |          |          |
| House wall type (Wood %) | 91.6% | 94.0% |
| Open eaves (Yes %) | 98.0% | 99.6% |
| # of doors (mean ± SD, (min, max)) | 2.06 ± 0.38 (1, 4) | 2.06 ± 0.30 (0, 4) |
|                  | Cluster level    |          |          |          |          |          |
| Cluster population (mean ± SD, (min, max)) | 694.3 ± 59.2 (624, 820) | 730.8 ± 129.0 (616,1117) |
| Baseline incidence rate per person-year (mean ± SD, (min, max)) | 0.096 ± 0.115 (0, 0.426) | 0.089 ± 0.088 (0, 0.265) |
| Baseline overall (total new) infection incidence per person-year (mean ± SD, (min, max)) | 0.094 ± 0.111 (0, 0.412) | 0.089 ± 0.087 (0, 0.261) |
Table 3. Summary first-time and overall (total) malaria incidence.

|                      | First-time infection | Overall (total new) infection |
|----------------------|---------------------|------------------------------|
|                      | All clusters | Subgroup 1# | Subgroup 2# | All clusters | Subgroup 1# | Subgroup 2# |
| Cluster              |               |             |             |               |             |             |
| Households           | 12:12         | 10:9        | 6:6         | 12:12         | 10:9        | 6:6         |
| Malaria infections   | 665: 667      | 557: 500    | 335:335     | 665: 667      | 557: 500    | 335 : 335   |
| Household person years | 134 : 164 | 130 : 158   | 93 : 140    | 253 : 439     | 249 : 433   | 196 : 408   |
| Incidence rate (ppy) | 1079 : 1032   | 873 : 722   | 506 : 444   | 1216 :1216    | 1015 : 909  | 609 : 607   |
| Hazard ratio (90% CI)| 0.124 : 0.159 | 0.149 : 0.219 | 0.184 : 0.315 | 0.208 : 0.361 | 0.245 : 0.476 | 0.322 : 0.672 |
|                      | (0.431,1.213) | (0.299, 1.367) | (0.302, 0.664) | (0.426,1.108) | (0.382, 0.914) | (0.233, 0.508) |
| PE (%) (90% CI)      | 27.7 (-21.3, 56.9) | 33.3 (-7.8, 58.7) | 55.3 (33.6, 69.9) | 31.3 (-10.8, 57.4) | 40.9 (8.61, 61.8) | 65.6 (49.2, 76.7) |
| 1-sided p-value      | 0.151          | 0.083       | 0.0004      | 0.098         | 0.0236      | <0.0001     |

# Subgroup 1: clusters with non-zero baseline incidence
# Subgroup 2: clusters with entomology data
Table 4. Frequency (percentage) of anopheline species captured during sentinel human-landing catch (HLC) based on adult morphological character identification*.

| Anophelines          | Indoor SR | Placebo | Outdoor SR | Placebo | Indoor Intervention Per | Placebo | Outdoor SR | Placebo | Total SR | Placebo |
|----------------------|-----------|---------|------------|---------|--------------------------|---------|------------|---------|----------|---------|
| **Total**            | 2243 (100%) | 3327 (100%) | 2896 (100%) | 3462 (100%) | 3883 (100%) | 4897 (100%) | 4372 (100%) | 4834 (100%) |
| An. aconitus         | 467 (20.82%) | 80 (2.40%) | 768 (26.52%) | 78 (2.25%) | 2015 (51.89%) | 173 (3.53%) | 2249 (51.44%) | 162 (3.35%) |
| An. annularis        | 55 (2.45%) | 633 (19.03%) | 70 (2.42%) | 567 (16.38%) | 82 (2.11%) | 544 (11.11%) | 99 (2.26%) | 493 (10.20%) |
| An. balabacensis     | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| An. barbirostris     | 41 (1.83%) | 148 (4.45%) | 49 (1.69%) | 132 (3.81%) | 62 (1.60%) | 360 (7.35%) | 56 (1.28%) | 322 (6.66%) |
| An. barbumbrosus     | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 1 (0.03%) | 0 (0%) | 0 (0%) | 3 (0.06%) |
| An. flavirostris     | 355 (15.83%) | 417 (12.53%) | 523 (18.06%) | 504 (14.56%) | 757 (19.50%) | 430 (8.78%) | 1001 (22.90%) | 482 (9.97%) |
| An. indefinitus      | 0 (0%) | 1 (0.03%) | 0 (0%) | 1 (0.03%) | 2 (0.05%) | 12 (0.25%) | 4 (0.09%) | 20 (0.41%) |
| An. karwari          | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 1 (0.02%) | 0 (0%) |
| An. kochi            | 47 (2.10%) | 501 (15.06%) | 36 (1.24%) | 392 (11.32%) | 90 (2.32%) | 803 (16.40%) | 88 (1.71%) | 836 (17.29%) |
| Leucosphyrus Group   | 0 (0%) | 1 (0.03%) | 0 (0%) | 0 (0%) | 2 (0.05%) | 16 (0.32%) | 2 (0.04%) | 26 (0.54%) |
| An. maculatus        | 61 (2.72%) | 88 (2.65%) | 95 (3.28%) | 88 (2.54%) | 209 (5.38%) | 79 (1.61%) | 206 (4.17%) | 94 (1.94%) |
| An. montanus         | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 1 (0.02%) | 0 (0%) |
| An. parangensis      | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 1 (0.02%) | 0 (0%) | 0 (0%) |
| Hyrcanus Group       | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 4 (0.08%) | 0 (0%) |
| An. subpictus        | 15 (0.67%) | 7 (0.21%) | 13 (0.45%) | 15 (0.43%) | 3 (0.08%) | 105 (2.14%) | 10 (0.23%) | 85 (1.76%) |
| An. sundaeus         | 1001 (44.63%) | 401 (12.52%) | 1154 (39.85%) | 562 (16.23%) | 136 (3.50%) | 61 (1.25%) | 128 (2.93%) | 62 (1.28%) |
| An. tessellatus      | 94 (4.19%) | 317 (9.53%) | 89 (3.07%) | 295 (8.52%) | 205 (5.28%) | 1246 (25.44%) | 192 (4.39%) | 1215 (25.13%) |
| An. umbrosus         | 0 (0%) | 0 (0%) | 1 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) |
| An. vagus            | 107 (4.77%) | 733 (22.03%) | 98 (3.38%) | 828 (23.92%) | 315 (8.11%) | 1061 (21.67%) | 334 (7.64%) | 1031 (21.33%) |
| Unknown              | 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 1 (0.03%) | 1 (0.02%) | 2 (0.04%) | 1 (0.02%) |

*Misidentification possible depending on physical condition of specimen, molecular methods required to separate out some members in species groups and complexes.
Table 5: Intervention effect on anopheline mosquito landing rates by collection location¹.

| Location¹ | Active (mean ± SD) | Placebo (mean ± SD) | % Change (95% CI) Active vs. Placebo | p-value |
|-----------|--------------------|---------------------|------------------------------------|---------|
| Indoor    | 3.14 ± 5.84        | 3.97 ± 8.73         | -16.4 (-75.2, 182.7)               | 0.774   |
| Outdoor   | 3.54 ± 7.13        | 3.93 ± 8.64         | -11.3 (-73.7, 199.4)               | 0.847   |

¹Position at each HLC sentinel house where sample was captured (indoor = near center of house; outdoor = on verandah ~1m from edge of exterior wall.)
Table 6. Frequency of anopheline (all species) sporozoite positivity status.

| Treatment Allocation | Pf \(^1\) | Pv \(^2\) | Indeterminate \(^3\) | Noninfected | Sporozoite Positivity Rate = \frac{Pf + Pv}{Pf + Pv + \text{unclear + uninfected}} \times 100\% |
|----------------------|-----------|-----------|----------------------|-------------|--------------------------------------------------------------------------------------------------|
| **Baseline**         |           |           |                      |             |                                                                                                  |
| Active               | 12        | 9         | 0                    | 4706        | 0.44%                                                                                             |
| Placebo              | 12        | 9         | 0                    | 6244        | 0.34%                                                                                             |
| **Intervention**     |           |           |                      |             |                                                                                                  |
| Active               | 3         | 8         | 0                    | 8130        | 0.14%                                                                                             |
| Placebo              | 6         | 1         | 1                    | 9615        | 0.07%                                                                                             |

\(^1\)Pf = \textit{Plasmodium falciparum}  
\(^2\)Pv = \textit{Plasmodium vivax}  
\(^3\)Indeterminate = \textit{Plasmodium} positive sample but parasite species not identifiable.
**Table 7. Intervention effect on parity and nulliparity rates (all anopheline species).**\(^1\)

| Location   | Parous  | Nulliparous |
|------------|---------|-------------|
|            | Active  | Placebo     | % Change (95% CI)   |
|            | (mean ± SD) | (mean ± SD) | Active vs. Placebo   |
| Indoor     | 0.41 ± 0.44 | 0.41 ± 0.45 | -10.2 (-62.1, 113.2) |
| Outdoor    | 0.40 ± 0.44 | 0.43 ± 0.45 | -25.9 (-68.8, 75.6)  |
| Indoor     | 0.16 ± 0.29 | 0.12 ± 0.26 | 58.3 (-37.0, 298.0)  |
| Outdoor    | 0.17 ± 0.30 | 0.11 ± 0.25 | 54.9 (-37.6, 284.3)  |

\(^1\)Mean and SD are descriptive statistics; % change was calculated from fitting negative binomial models.

\(^2\)Position of Human Landing Catch (HLC) collector (indoor = near center of house; outdoor = on house verandah ~1m from edge of exterior wall.)