# Efficacy of indoor residual spraying with dichlorodiphenyltrichloroethane against malaria in Gambian communities with high usage of long-lasting insecticidal mosquito nets: a cluster-randomised controlled trial

*Margaret Pinder, Musa Jawara, Lamin B S Jarju, Kolawole Salami, David Jeffries, Majidah Adiamoh, Kalifa Bojang, Simon Correa, Balla Kandeh, Harparkash Kaur, David J Conway, Umberto D'Alessandro, Steve W Lindsay*

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**Medical Research Council Unit, Banjul, The Gambia** (M Pinder PhD, M Jawara MSc, K Salami MD, D Jeffries PhD, K Bojang PhD, M Adiamoh MSc, S Correa MSc, Prof U D'Alessandro PhD, Prof D J Conway PhD); **National Malaria Control Programme, Banjul, The Gambia** (L B S Jarju MSc, B Kandeh MSc); **London School of Hygiene and Tropical Medicine, London, UK** (M Pinder, H Kaur PhD, Prof S W Lindsay PhD, Prof D J Conway); **Institute of Tropical Medicine, Antwerp, Belgium** (Prof U D'Alessandro); **and Durham University, Durham, UK** (M Pinder, Prof S W Lindsay)

Correspondence to: Prof Steve W Lindsay, School of Biological and Biomedical Sciences, Durham University, Durham DH1 3LE, UK **s.w.lindsay@durham.ac.uk**

## Summary

**Background** Although many malaria control programmes in sub-Saharan Africa use indoor residual spraying with long-lasting insecticidal nets (LLINs), the two studies assessing the benefit of the combination of these two interventions gave conflicting results. We aimed to assess whether the addition of indoor residual spraying to LLINs provided a significantly different level of protection against clinical malaria in children or against house entry by vector mosquitoes.

**Methods** In this two-arm cluster, randomised, controlled efficacy trial we randomly allocated clusters of Gambian villages using a computerised algorithm to LLINs alone (n=35) or indoor residual spraying with dichlorodiphenyltrichloroethane plus LLINs (n=35). In each cluster, 65–213 children, aged 6 months to 14 years, were surveyed at the start of the 2010 transmission season and followed in 2010 and 2011 by passive case detection for clinical malaria. Exposure to parasite transmission was assessed by collection of vector mosquitoes with both light and exit traps indoors. Primary endpoints were the incidence of clinical malaria assessed by passive case detection and number of *Anopheles gambiae sensu lato* mosquitoes collected per light trap per night. Intervention teams had no role in data collection and the data collection teams were not informed of the spray status of villages. The trial is registered at the ISRCTN registry, number ISRCTN01738840.

**Findings** LLIN coverage in 2011 was 3510 (93%) of 3777 children in the indoor residual spraying plus LLIN group and 3622 (95·5%) of 3791 in the LLIN group. In 2010, 7845 children were enrolled, 7829 completed passive case detection, and 7697 (98%) had complete clinical and covariate data. In 2011, 7009 children remained in the study, 648 more were enrolled, 7657 completed passive case detection, and 7545 (98·5%) had complete data. Indoor residual spraying coverage per cluster was more than 80% for both years in the indoor residual spraying plus LLIN group. Incidence of clinical malaria was 0·047 per child-month at risk in the LLIN group and 0·044 per child-month at risk in the indoor residual spraying plus LLIN group in 2010, and 0·032 per child-month at risk in the LLIN group and 0·034 per child-month at risk in the indoor residual spraying plus LLIN group in 2011. The incident rate ratio was 1·08 (95% CI 0·80–1·46) controlling for confounders and cluster by mixed-effect negative binomial regression on all malaria attacks for both years. No significant difference was recorded in the density of vector mosquitoes caught in light traps in houses over the two transmission seasons; the mean number of *A gambiae sensu lato* mosquitoes per trap per night was 6·7 (4·0–10·1) in the LLIN group and 4·5 (2·4–7·4) in the indoor residual spraying plus LLIN group (p=0·281 in the random-effects linear regression model).

**Interpretation** We identified no significant difference in clinical malaria or vector density between study groups. In this area with high LLIN coverage, moderate seasonal transmission, and susceptible vectors, indoor residual spraying did not provide additional benefit.

**Funding** UK Medical Research Council.

## Introduction

In the past 10 years there have been unprecedented reductions in malaria in many parts of sub-Saharan Africa. Scale-up of the use of long-lasting insecticidal nets (LLINs), indoor residual spraying,[1] and prompt treatment of clinical cases with artemisinin combination therapies[2] have resulted in at least eight countries in the region meeting the Millennium Development Goal of reducing the incidence of malaria. Despite this major public health achievement, in 2012 there were an estimated 207 million cases of malaria and 627 000 deaths due to malaria worldwide, with an estimated 90% of these deaths occurring in sub-Saharan Africa. The number of LLINs delivered in sub-Saharan Africa increased from 6 million in 2004 to 145 million in 2010, with 54% of households having at least one net in 2013 and about 36% of the population sleeping under a LLIN.[2] Today, universal coverage with either LLINs or indoor

residual spraying is the major malaria prevention strategy and in many settings where indoor residual spraying is used, LLINs are already deployed. Although the protection afforded by LLINs[3] and indoor residual spraying[4] alone is well known, the joint effect of these interventions is poorly understood.[5,6]

Some models suggest that LLINs and indoor residual spraying combined would interrupt transmission in areas of moderate transmission.[7,8] Others suggest that the effects could be antagonistic against the major African vectors *Anopheles gambiae sensu stricto*[9] and *A arabiensis*.[10] The argument for an antagonistic effect centres on the mode of action of dichlorodiphenyltrichloroethane (DDT) used for indoor residual spraying and the pyrethroids used in LLINs. DDT, the most persistent insecticide used for spraying,[11] is regarded as both a spatial and contact repellent.[12,13] Therefore, the repellent effect of DDT might reduce the contact of mosquitoes on LLINs and because LLINs reduce blood-feeding, fewer blood-fed mosquitoes might rest on sprayed surfaces.

Evidence about this crucial issue is contradictory. Only one experimental hut study has been done to investigate this issue and showed no additional benefit of using indoor residual spraying with LLINs.[14] However, analysis of survey data from 17 African countries showed that the use of LLINs and indoor residual spraying together was associated with lower malaria prevalence than the use of LLINs alone,[15] and a review of non-randomised studies showed that addition of LLINs to indoor residual spraying was associated with lower parasite rates than indoor residual spraying alone.[6] Similarly, investigators of a non-randomised field trial in Kenya showed that use of a combination of pyrethroid indoor residual spraying and LLINs provided 61% greater protection against the incidence of infection in children than the use of LLINs alone.[16] Since indoor residual spraying and LLINs are community-level interventions, the effect of the combination needs to be assessed in trials randomised by cluster. In a cluster-randomised controlled trial carried in Benin, when LLINs were targeted to pregnant women and children younger than 6 years, indoor residual spraying had no additional benefit against malaria disease or infection.[17] By contrast, in a cluster-randomised controlled trial in Tanzania where LLINs usage was less than 50%, malaria prevalence was significantly lower in one of three surveys in the LLIN and indoor residual spraying groups.[18] Our study was designed to determine whether universal coverage with LLINs and DDT indoor residual spraying combined provided significantly different protection against clinical malaria from the use of LLINs alone.

## Methods

### Study design and participants

A detailed description of the study protocol has been reported previously.[19] In this cluster-randomised, controlled, efficacy study 70 clusters of villages, located more than 2 km from neighbouring villages to avoid spillover,[20] were randomly allocated at the start of the 2010 transmission season to either LLINs alone or LLINs plus indoor residual spraying. We sampled children aged from 6 months to 14 years according to cluster size and enrolled them into a study cohort. These children were followed during the malaria transmission seasons in 2010 and 2011. We assessed exposure to malaria vector mosquitoes and parasites indoors using standardised mosquito light and exit traps monthly from July to December and then identified *A gambiae* mosquitoes and detected sporozoite infection.

Village clusters in Upper River Region of The Gambia, consisting of one to three neighbouring villages, were enrolled with more than 110 children aged 6 months to 14 years on June 1, 2010.[19] Enrolled children were randomly selected from household survey lists prepared by MP with statistical software (STATA version 11.0), stratified by age (<5 years, 5–10 years, and >10 years), and weighted towards the younger children, who were less immune, at a ratio of 2:2:1. Informed consent was sought at the village level after sensitisation meetings attended by village community leaders and health staff, and all selected villages agreed to participate. Children were enrolled by project field staff if their carers or parents gave witnessed, written informed consent and, for children who were able to understand at least some of the issues, if they assented. Individuals and households were free to withdraw their participation at any time without giving a reason. If consent was not provided then replacement children were selected from a second enrolment list. The effect of the intervention on the density of malaria vectors and their infection rate with malaria were monitored in 32 clusters, 16 in each study group. In each cluster, six rooms in six different compounds were selected randomly (by MP) and enrolled (by MJ), and Centers for Disease Control and Prevention light traps and exit traps were placed one night each month from July to December in both years.

The study was done in accordance with the principles set forth in the International Conference on Harmonisation Tripartite Guideline for Good Clinical

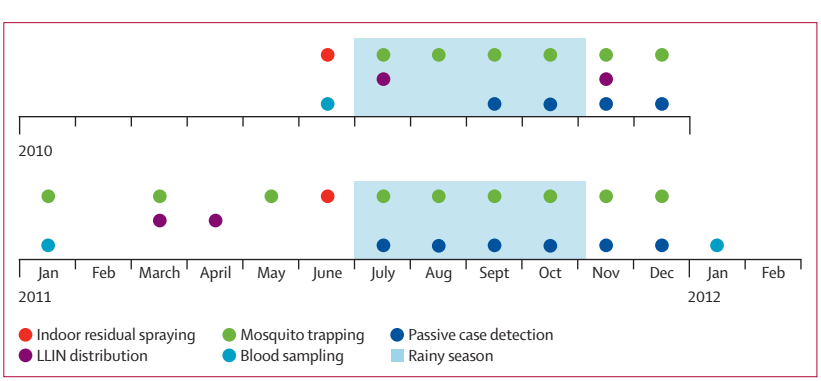


***Figure 1:*** **Trial profile**
LLIN=long-lasting insecticidal net.

Practice and the Declaration of Helsinki in its present version (2000), whichever afforded the greater protection to the participants. The trial was approved by the Gambian Government and MRC Unit Joint Ethics Committee on Aug 12, 2008 (reference L2009.15) with minor amendments approved on April 30, 2010 (L2010.19; SCC1128), and by the London School of Hygiene & Tropical Medicine Ethics Committee on Sept 16, 2009 (reference 5592). A Data Safety Monitoring Board reviewed the trial procedures and results. The only incentives given to households that participated in the trial were provision of LLINs and indoor residual spraying, treatment of study children during the study, and fares to reach referral clinics (refunded by study staff on the basis of known tariffs).

See Online for appendix

### Randomisation and masking

Villages were grouped into 70 clusters, which were initially stratified into small and large on the basis of median population size, and further divided into four geographical areas. Each of the clusters in each category of size and location was then randomly assigned using a computerised algorithm to indoor residual spraying plus LLINs or LLINs alone, assuming equal allocation to each group and constraining the allocation to 35 clusters in each group. We then used a MATLAB programme to randomly repeat this allocation 100 000 times. Balanced randomisation was used to enrol children of similar ages in each cluster with the target number enrolled increasing with village size. Selection of entomology clusters is described in the appendix. Observer bias was reduced where feasible. Slide microscopists and their supervisors were masked to the identity and intervention status of the participants. Mosquito collector bias was reduced by the use of standard traps, which do not depend on the ability of the fieldworker to collect specimens. Trap catches were examined by someone other than the trap collector and were masked to the trap location. Apart from data for indoor residual spraying, no data forms or samples included the group allocation and this was only added to the datasets after final cleaning. Further information about randomisation and masking is included in the appendix.

| | LLIN group | Indoor residual spraying plus LLIN group |
|---|---|---|
| **All study clusters (n=70)** | | |
| Population | | |
| North bank, west | 5716 | 7272 |
| North bank, east | 3791 | 2879 |
| South bank, west | 4231 | 3763 |
| South bank, east | 4686 | 4707 |
| Total | 18 424 | 18 621 |
| Mean cluster population | 518 (407–629) | 528 (389–668) |
| Ethnic origin | | |
| Mandinka | 11 109/18 424 (60%) | 7920/18 621 (43%) |
| Fula | 5326/18 424 (29%) | 8098/18 621 (43%) |
| Serrehule | 1385/18 424 (8%) | 1578/18 621 (8%) |
| Wollof and others | 36/18 424 (<1%) | 20/18 621 (<1%) |
| House features | | |
| Thatched roof | 3236/7383 (44%) | 3664/7683 (48%) |
| Mud walls | 3689/7637 (48%) | 3862/7350 (53%) |
| Matt-painted walls | 3040/7637 (40%) | 2853/7350 (39%) |
| Gloss-painted walls | 150/7367 (2%) | 94/7350 (1%) |
| **Entomology clusters (n=32)** | | |
| Mean cluster population | 476 (345–608) | 446 (356–535) |
| Ethnic origin | | |
| Mandinka | 4766/7558 (63%) | 3554/6919 (51%) |
| Fula | 2300/7558 (30%) | 3240/6919 (47%) |
| Serrehule | 476/7558 (6%) | 125/6919 (2%) |
| Wollof | 16/7558 (<1%) | 0/6919 (0%) |

Data are n, n/N (%), or mean (95% CI). Eave status (open or closed) was recorded for children in the cohort in 2011. Open eaves were most common with 62% (2369/3820) in the long-lasting insecticidal net (LLIN) group and 59% (2269/3837) in the indoor residual spraying plus LLIN group.

***Table 1:*** **Baseline characteristics of study clusters at the beginning of the transmission in 2010**

### Procedures

In clusters randomly assigned to indoor residual spraying plus LLINs, we used Hudson X-pert sprayers (HD Hudson Manufacturung Company, Chicago, IL, USA) to apply DDT (DDT 75% wettable powder; Hindustan Insecticides, New Delhi, India) at a target dose of 2 g/m² to dwelling rooms on July 15–28, 2010, and July 20, to Aug 9, 2011, in accordance with WHO guidelines.[21] The spray teams had experience of national campaigns and included operators from the Gambian National Malaria Control Programme and team leaders from the regional health team. All internal walls were sprayed, apart from those with gloss paint, and the inside surface of thatch roofs were sprayed. Samples of DDT were analysed for compliance with WHO standards by an accredited laboratory and passed WHO/SIF/1.R 9 specifications for appearance, DDT content, wettability wet sieving, and suspensibility. During indoor residual spraying, insecticidal sprays were sampled in four to eight houses per area on Whatman No. 4 filter papers, under careful supervision to avoid over spraying, and the insecticide concentration was estimated by use of high-performance liquid chromatography (HPLC) (Dionex Ultimate 3000 systems, Hemel Hempstead, UK; software from Thermofisher, Hemel Hempstead, UK).[21] Concentrations were expressed as grams of active ingredient per square metre by reference to a standard curve. LLINs were manufactured with permethrin at 2% w/w (Olyset Nets, Sumitomo Chemicals, Japan), in a factory that met WHO specifications. Persistence of insecticides on walls and LLINs was measured by use of WHO cone tests[22,23] (appendix). Baseline surveys established the number of sleeping places per household and the number of any mosquito nets in position, and

LLINs were provided to heads of household to cover all remaining sleeping places.

Parents or carers of children enrolled in the cohort were encouraged to take their child to the nearest heath post or clinic if the child had fever. Clinical malaria was defined as a child presenting at government primary or secondary health facilities with an axillary temperature of 37·5°C or more, or a history of fever in the past 48 h, together with the presence of *Plasmodium falciparum* parasites of any density detected by microscopy or rapid diagnostic test. Severe adverse events in enrolled children were documented.

During the cross-sectional surveys (figure 1), all children in the cohort were examined clinically for obvious symptoms and signs of illness, temperature, and spleen enlargement. Then, at least 50 children per cluster were randomly selected, stratified by age, together with those who reported fever in the previous 48 h or had a temperature of 37·5°C or more, and were finger-pricked for immediate measurement of anaemia with a spectrophotometer (HemoCue, Ängelholm, Sweden), and presence of parasites by rapid diagnostic test (Paracheck Pf, Orchid Biomedical Systems, Goa, India). Only samples taken randomly were included in the analyses. Thick blood films were stained with Giemsa and examined under 1000 times magnification by trained, experienced microscopists. Parasite counts were recorded per high-power field and 100 fields were counted before a slide was declared negative. Parasite density was estimated assuming that one parasite per high power field equals 500 parasites per µL.[24] Two slides were prepared from each individual and assessed separately by two experienced microscopists, with discrepancies resolved by a third.

## Outcomes

Primary endpoints were the incidence of clinical malaria assessed by PCD and the number of *A gambiae sensu lato* collected per light trap per night. Secondary endpoints were haemoglobin concentration, the proportion of children with moderate anaemia (defined as haemoglobin <80 g/L) and severe anaemia (haemoglobin <50 g/L), presence of malaria parasites, parasite density, the proportion of children with high parasitaemia (≥5000 parasites per µL), the prevalence of children with enlarged spleens measured at the end of the transmission season each year, sporozoite rate estimates in trapped mosquitoes, and estimated entomological inoculation rate (ie, the mean number of infective mosquito bites per person per season). Children in the cohort were monitored for residence in their villages for the duration of the PCD and if they were absent more than 50% of the time their data were censored from analysis.

## Statistical analysis

For the power calculation, we assumed a range of incidence rates on the basis of findings from a previous study and an a priori estimate of a realistic number of

| | LLIN group (n=3896) | Indoor residual spraying plus LLIN group (n=3949) |
|---|---|---|
| Girls | 1918/3949 (49%) | 1929/3896 (49%) |
| Mean age (years) | 6·11 (5.99–6·23) | 6·18 (6·07–6·29) |
| Using LLINs | 2311/3949 (58%) | 1983/3896 (51%) |
| Using untreated bednets | 527/3949 (13%) | 544/3896 (14%) |
| Febrile children with positive rapid diagnostic test | 3/179 (2%) | 0/131 (0%) |
| Prevalence of mild anaemia (haemoglobin >80 to 110 g/L) | 747/2179 (34%) | 735/2086 (35%) |
| Prevalence of moderate anaemia (haemoglobin >50 to 80 g/L) | 76/2179 (4%) | 91/2086 (4%) |
| Prevalence of severe anaemia (haemoglobin ≤50 g/L) | 2/2179 (<1%) | 3/2086 (<1%) |
| Mean haemoglobin concentration (g/L) | 112·0 (111·3–112·8) | 112·5 (111·9–113·2) |
| *Plasmodium falciparum* parasite rate | 34/2163 (2%) | 35/2069 (2%) |
| *P falciparum* parasite rate (high parasitaemia, >5000 parasites per mL) | 0/2163 | 0/2069 |
| Geometric mean parasite density (per mL) | 24·9 (12·2–50·9) | 48·6 (29·6–79·5) |
| Prevalence of enlarged spleen | 190/3892 (5%) | 114/3733 (3%) |

Data are n/N (%) or mean (95% CI). 2011: LLIN group, mean age of cohort (June 2011) 6·39 years (6·27–6·50); 48% (1830/3837) girls; indoor residual spraying plus LLIN group, mean age 6·39 years (6·28–6·51); 49% (1869/3818) girls. LLIN=long-lasting insecticidal net.

***Table 2:*** **Baseline characteristics of the enrolled children at the beginning of the transmission in 2010**

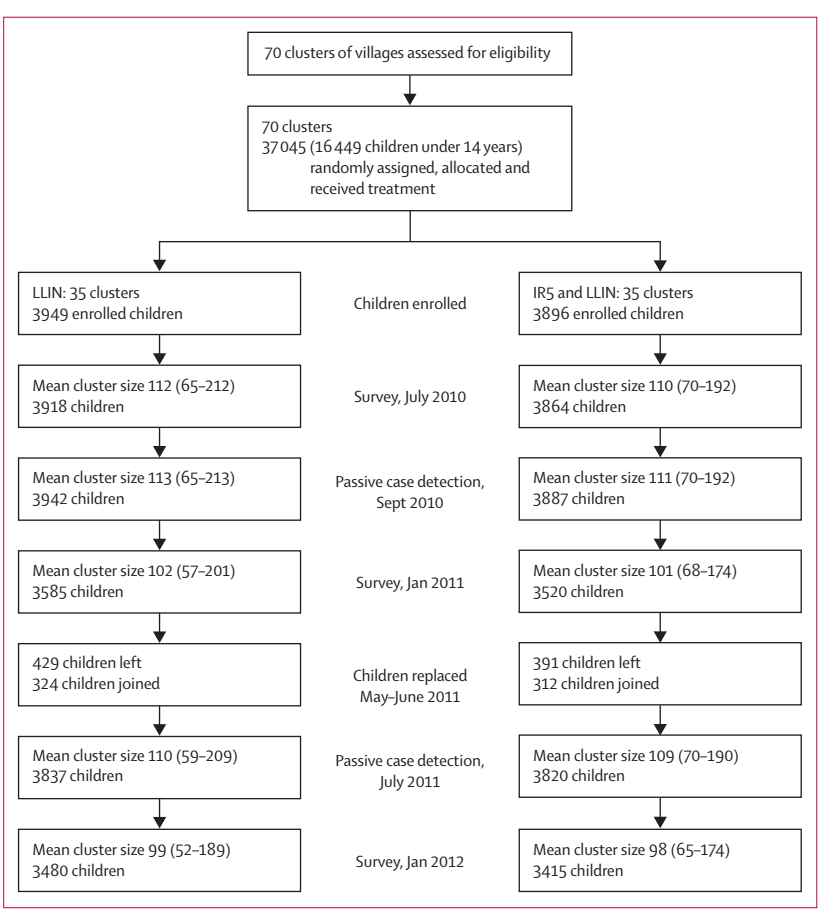


***Figure 2:*** **Flow chart of the child cohort**

clusters within each group.[19] Assuming an incidence of 0·02 cases per child-month in the control (LLIN) group, and an estimate of 888 child-months per cluster, we calculated an intervention effect size of 30% difference in incidence rate with a coefficient of variation of 0·4; for these parameters, 35 clusters per group gives 80% power to detect a significant difference at the 5% level.[25] We planned the trial for two malaria seasons to allow for yearly variation in incidence. We regarded an intervention effect size of 30% to be of public health significance, accounting for the extra costs of indoor residual spraying.[26] With slide-positive parasite prevalence treated as a proportion and haemoglobin as a concentration, 35 clusters with 110 children each would have 80% power to detect a 30% difference in parasitaemia and a 5 g/L difference in haemoglobin at the 5% level of significance if half the child cohort was sampled.

The final clean datasets were submitted to the statistician of the data safety monitoring board on Nov 6, 2012, before unmasking of the data, and analysis followed the detailed analytical plan established on March 30, 2012. We examined clinical malaria by calculating incidence rates for each cluster, including multiple attacks in children if the second or third attack occurred at least 28 days after the onset of the previous attack; we calculated unweighted mean ratios by year and study group. All subsequent analyses used incidence rates calculated over both malaria seasons. We calculated 2-year incidence rates for each cluster and calculated the mean rate ratio by study group, with CIs calculated from the approximations by Bennett and colleagues.[27] We measured time to first malaria attack by survival analysis, using Kaplan-Meier curves to compare the probability of patients in the two groups becoming infected as the malaria transmission seasons progressed. Significance was calculated with a log-rank test. We fitted a mixed-effects negative binomial regression model with a random effect for cluster, fixed effects for individual and cluster level covariate effects, and an offset for person time. Parasite rates and density and haemoglobin concentrations were estimated from community survey data averaged over clusters. Anaemia was defined with upper limits of 110 g/L for mild anaemia, 80 g/L for moderate anaemia, and 50 g/L for severe anaemia, as stipulated in the analytical plan. The trial is registered at the ISRCTN registry, number ISRCTN01738840.

| | LLIN group | Indoor residual spraying plus LLIN group |
|---|---|---|
| Indoor residual spraying | | |
| Coverage per cluster in 2010 (%) | .. | 86% (82·84–90·16) |
| Coverage per cluster in 2011 (%) | .. | 83% (79·27–86·28) |
| Mean DDT sprayed in 2010 (g/m²) | .. | 1·69 (1·39–1·99) |
| Mean DDT sprayed in 2011 (g/m²) | .. | 3·27 (2·39–3·96) |
| Long-lasting insecticidal nets | | |
| Reported bednet* coverage in June, 2010 (% of sleeping places) | 6698/10 827 (62%) | 6289/10 693 (59%) |
| Reported LLIN coverage in child cohort in January, 2011 | 3256/3543 (92%) | 3105/3492 (89%) |
| Reported LLIN coverage in child cohort in January, 2012 | 3622/3791 (96%) | 3510/3777 (93%) |

Data are n/N (%) or mean (95% CI). LLIN=long-lasting insecticidal net. DDT=dichlorodiphenyltrichloroethane.
*Includes all net types.

*Table 3:* **Coverage and quality of the interventions by study group**

### Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

## Results

97 villages met the enrolment criteria[19] and after community-level meetings to discuss the nature of the study and what would be required during the interventions and investigations all 97 agreed to take part in the study.

The population of 37 045 residents was evenly distributed between the two study groups and across the four geographical study areas (table 1). Mean cluster sizes were also similar between the two groups (table 1), as was baseline bednet coverage (table 2), but ethnic origin varied with more Mandinka and lower Fula individuals in the LLIN group than in the indoor residual spraying plus LLIN group (table 1). These characteristics had a similar distribution in the entomology clusters as in the entire study (table 1). House designs were similar in the two groups, but with slightly more study children living in houses with thatched roofs in the indoor residual spraying plus LLIN group than in the LLIN group.

In July 2010, a total of 7845 with an average of 111 per cluster (range 65–213) were enrolled (figure 2). In June 2011, 330 children aged more than 14 years on June 1, 2011, were excluded from the cohort, and 490 children left the study (422 moved, 56 died, and 12 withdrew consent). These children were replaced by 636 children born in 2010, selected and stratified as in the first year of study. The cohort had 7657 children in 2011 (figure 2). In 2010, outcome data were available for 7782 (99%) of 7845 children at baseline, 7829 (99·8%) during the PCD and 7105 (91%) at the end of season, whereas in 2011 outcome data were available for 7657 (100%) during the PCD and 6895 (90%) at the last survey. Enrolled children were evenly distributed by age and sex across the intervention groups (table 2). LLIN use was lower at baseline in the LLIN group compared with the indoor residual spraying plus LLIN group, but parasite prevalence and density and anaemia prevalence were similar (table 2).

In the indoor residual spraying plus LLIN group, indoor residual spraying coverage per cluster was more

| | 2010 | | 2011 | | Unadjusted rate ratio or unadjusted risk ratio (95% CI)* | |
|---|---|---|---|---|---|---|
| | LLIN group (n=3942) | Indoor residual spraying plus LLIN group (n=3887) | LLIN group (n=3837) | Indoor residual spraying plus LLIN group (n=3820) | 2010 | 2011 |
| **Passive case detection†** | | | | | | |
| Children in passive case detection with complete data | 3868/3942 (98%) | 3829/3887 (99%) | 3786/3837 (99%) | 3763/3820 (99%) | .. | .. |
| Children with one malaria attack | 450/3942 (11%) | 409/3887 (11%) | 543/3837 (14%) | 520/3820 (14%) | 0·92 (0·81–1·05) | 0·96 (0·86–1·08) |
| Children with more than one malaria attack | 33/3942 (1%) | 23/3887 (<1%) | 58/3837 (2%) | 50/3820 (1%)‡ | 0·71 (0·42–1·20) | 0·87 (0·60–1·26) |
| Incidence of malaria per child-month at risk | 0·0468 (0·0336–0·0653) | 0·0442 (0·0333–0·0587) | 0·0321 (0·0255–0·0404) | 0·0341 (0·0259–0·0452) | 0·94 (0·61–1·46) | 1·06 (0·74–1·46) |
| **Cross-sectional surveys§** | | | | | | |
| *Plasmodium falciparum* parasite prevalence | 282/1979 (15%) | 334/1997 (17%) | 360/2083 (17%) | 345/2141 (16%) | 1·17 (1·01–1·36), p= 0·031 | 0·93 (0·82–1·07) |
| Parasite prevalence (>5000 parasites per mL) | 18/1979 (<1%) | 12/1997 (<1%) | 27/2083 (1%) | 22/2141 (1%) | 0·66 (0·32–1·37) | 0·79 (0·45–1·39) |
| Geometric mean parasite density (per mL)) | 34·56 (4·52) | 46·14 (2·96) | 62·46 (4·10) | 67·46 (3·71) | –11·58 (3·82) | –5·00 (3·91) |
| Prevalence of mild anaemia (haemoglobin >80–110 g/L) | 810/1981 (41%) | 825/2003 (41%) | 881/2068 (43%) | 940/2118 (43%) | 1·01 (0·94–1·09) | 1·04 (0·97–1·12) |
| Prevalence of moderate anaemia (haemoglobin >50–80 g/L) | 108/1981 (5%) | 114/2003 (6%) | 92/2068 (4%) | 115/2118 (6%) | 1·04 (0·81–1·35) | 1·22 (0·93–1·60) |
| Prevalence of severe anaemia (haemoglobin ≤50 g/L) | 3/1981 (<1%) | 4/2003 (<1%) | 4/2068 (<1%) | 4/2118 (<1%) | 1·32 (0·30–5·88) | 0·98 (0·25–3·90) |
| Mean haemoglobin concentration (g/L) | 112·7 (5·34) | 112·5 (4·03) | 111·3 (5·86) | 110·9 (5·02) | –0·20 (4·73) | –0·40 (5·46) |
| Prevalence of enlarged spleen | 115/3534 (3%) | 83/3400 (3%) | 11/3409 (<1%) | 19/3342 (<1%) | 0·75 (0·57–0·99), p=0·042 | 1·76 (0·84–3·97) |

Data are n/N (%), mean (SD), or mean (95% CI). p values are >0·05 unless otherwise stated (arithmetic mean and 95% CI for continuous variables and number of children [%] for categorical variables unless otherwise stated). LLIN=long-lasting insecticidal net. *Values are unadjusted rate ratio for passive case detection and unadjusted risk ratio for cross-sectional surveys; the measure of effect for geometric mean parasite density and mean haemoglobin concentration is the difference between the two groups and the SD of the difference. †Measurements in 2010 were made only during the peak transmission season, whereas in 2011 children were followed for the entire season. ‡In 2011, three children in the indoor residual spraying plus LLIN group had three malaria attacks. §Overall totals and prevalence percentages (calculated using the means of the clusters) are shown.

***Table 4:*** **Malaria outcomes in the child cohort by treatment allocation**

than 80% in both years, in the whole study and in the entomology clusters. Repeated visits to clusters were needed to achieve this high coverage; 20% of clusters needed more than two visits in 2011. Mean concentrations of DDT sprayed on the walls were close to the target dose of 2 g/m² (table 3). Residual activity of DDT, estimated by WHO cone tests in 2011, was high with 99·2% mortality (95% CI 97·2–100) at 1 week post-indoor residual spraying and 94·3% (89·3–99·3) after 6 weeks. Estimates of DDT residual activity in a non-study village within the Upper River Region in 2011 that had used the same batch of DDT showed high concentrations at 5 months post-indoor residual spraying on both mud (mean mortality 92·5%) and matt painted walls (94·7%).[28]

LLINs were distributed under the auspices of the trial as recommended by the National Malaria Control Programme in The Gambia in 2010. During the household baseline survey in June 2010 householders reported that about 60% of the sleeping places in their house had a bednet (table 3). In July, 2010, LLINs were provided to those without an LLIN; 4527 were donated to the LLIN group and 4696 to the indoor residual spraying plus LLIN group. In August, 2010, however, room-to-room surveys reported only 49% (9414/19 304) of sleeping places had LLINs in use, although 71% (6558/9223) of the nets donated by the project were hung above sleeping places. In November, 2010, a further 4080 LLINs were provided to the two groups and in March–April, 2011, about 10 000 LLINs were provided by a national mass donation campaign. LLIN coverage in the child cohort at the end of 2010 was 3256 (92%) of 3543 in the LLIN group and 3105 (89%) of 3492 in the indoor residual spraying plus LLIN group (table 2). At the end of 2011, coverage rose slightly to 3622 (95·5%) of 3791 in the LLIN group and 3510 (92·9%) of 3777 in the indoor residual spraying

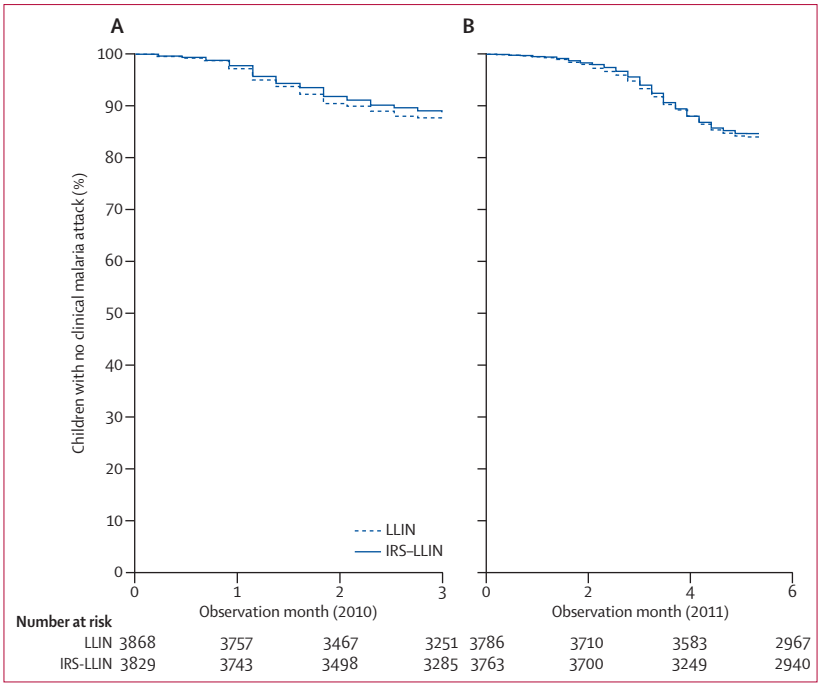


***Figure 3:*** **Survival estimates for malaria attacks in each transmission season**
Children in the cohort were passively followed for malaria attacks from Sept 14, to Dec 17, 2010 (A) and from July 15, to Dec 17, 2011 (B). Passive case detection in 2011 was for 5·3 months. LLIN=long-lasting insecticidal net group. IRS-LLIN=indoor residual spraying plus long-lasting insecticidal net group.

| | Between-cluster coefficient of variation | | |
|---|---|---|---|
| | LLIN group | Indoor residual spraying plus LLIN group | Both groups |
| **2010** | | | |
| None | 0·97 | 0·87 | 0·92 |
| Area and cluster size | 0·86 | 0·78 | 0·85 |
| **2010 (outliers removed)*** | | | |
| None | 0·80 | 0·69 | 0·74 |
| Area and cluster size | 0·63 | 0·64 | 0·63 |
| **2011** | | | |
| None | 0·63 | 0·86 | 0·74 |
| Area and cluster size | 0·58 | 0·81 | 0·72 |
| **2010 and 2011 combined** | | | |
| None | 0·71 | 0·77 | 0·73 |
| Area and cluster size | 0·63 | 0·69 | 0·70 |

LLIN= long-lasting insecticidal net.*In 2010 there were three outliers with very high malaria incidence (≥0·15 cases per month); removing these reduced the coefficients of variation for 2010.[29]

***Table 5:*** **Stratification by geographical area and cluster size**

plus LLIN group (table 3). More than 98% of the study children had complete clinical data (table 4).

Incidence of clinical malaria was 0·047 per child-month at risk in the LLIN group and 0·044 per child-month at risk in the indoor residual spraying plus LLIN group in 2010 and 0·032 per child-month at risk in the LLIN group and 0·034 per child-month at risk in the indoor residual spraying plus LLIN group in 2011 (table 4). The unadjusted incidence rate ratio for all attacks was 0·93 (95% CI 0·72–1·44, based on Bennett's approximation[27]) and the close similarity between the two groups was also apparent from the Kaplan-Meier curves (figure 3). Mixed-effects negative binomial regression controlling for study area, eave status, net use, and child of Fula ethnic origin as fixed effects, and cluster as a random effect, gave an incident rate ratio of 1·08 (95% CI 0·80–1·46) for the indoor residual spraying plus LLIN group compared with the LLIN group, for all attacks over both years. The estimated value of the coefficient of between cluster-variation (k) was high, although slightly lower when stratified by geographical area and cluster size (table 5).

Prevalence and density of parasite infection, measured at the end of both transmission seasons with surveys of children in the cohort, were similar between the study groups, although the unadjusted prevalence risk ratio shows that there were more infected children in the indoor residual spraying plus LLIN group (p=0·031, table 4). Prevalence of malaria infection increased with age and was higher in children residing in houses with open eaves and in those not using LLINs (data not shown). Adjusting for these confounders had no significant effect (odds ratio [OR] of *P falciparum* rates between study group by logistic regression: 2010, OR 1·27, 95% CI 0·79–2·03, p=0·329; 2011, 0·94, 0·60–1·47, p=0·789). Prevalence of moderate and severe anaemia was similar to the baseline values (tables 2, 4) and there was no significant difference between the study groups (OR for anaemia prevalence between study groups by logistic regression: 2010, OR 1·10, 95% CI 0·83–1·22, p=0·918; 2011, 1·12, 0·95–1·33, p=0·186).

More than 99·9% (2302/2303) of entomological collections were successful (appendix). *A gambiae sensu lato* mosquitoes were present in 839 (36%) of 2303 light traps and 207 (9%) of 2303 exit traps. Linear regression of the numbers of *A gambiae sensu lato* mosquitoes caught in light traps was not significantly different between the study groups (adjusted mean number caught per night over 2 years 6·7, 95% CI 4·0–10·1 in the LLIN group; 4·5, 2·4–7·4) indoor residual spraying plus LLIN group (p=0·281).

## Discussion

In a rural area of The Gambia with moderate seasonal malaria transmission and high coverage of LLINs, the addition of indoor residual spraying did not reduce the level of clinical malaria in study children (panel). Incidence of clinical malaria, our primary clinical outcome measure, was similar in both study groups. This finding is supported by our entomological findings (appendix) in which the number of malaria vectors entering houses and the entomological inoculation rate were similar in both study groups.

Levels of LLIN ownership, especially in 2011 were higher than those reached by many countries[2] but universal coverage is the WHO target and similarly high levels have been reported in other areas.[33] The indoor residual spraying plus LLIN group had proportionally more Fula, an ethnic group previously associated with lower susceptibility to malaria disease and infection than the LLIN group.[34] However, adjusting for ethnic origin and other possible confounders in the multivariate model did not suggest that indoor residual spraying was masked by confounders (unadjusted rate ratio 0·93 *vs* adjusted rate ratio 1·08). Importantly, the secondary clinical endpoints of anaemia, *P falciparum* infection rates, and prevalence of splenomegaly, were also similar between the two groups. Thus, there was no evidence from any of the additional malariometric variables measured during the clinical investigations that the combination of indoor residual spraying and LLINs together was different from LLINs alone for the reduction of malaria.

The original power calculation assumed a range of incidence rates and an a-priori estimate of a realistic number of clusters within each group. Actual incidence rates were close to the expected values, but even with stratification, the between-cluster coefficient of variation was higher than expected, which would have led to wider CIs and reduced the power of the study. However, both of the unadjusted incidence rate ratios are very close to unity for the two malaria seasons when analysed separately, as is the adjusted rate ratio for the two seasons together, thus supporting the conclusion that the addition of indoor residual spraying had little, if any, effect on the malaria reported in the study children.[35]

Over both study years there were slightly fewer *A gambiae sensu lato* mosquitoes entering houses in the indoor residual spraying plus LLIN group than in the LLIN alone group, but these differences were not statistically significant in either unadjusted or adjusted analyses. This finding, together with similar entomological inoculation rates, and the long-lived vector population shown by the high parity rates in both study groups (appendix) supports the clinical data and the conclusion that indoor residual spraying with DDT offered no additional protection in the presence of high LLIN coverage.

These results pose a question of major public health significance: why did the indoor residual spraying intervention have no significant effect on malaria in this population where LLIN use was high? DDT is one of the most persistent insecticides used for spraying homes, being active for more than 6 months[11] and the residual activity identified in this study and in a parallel study in the same area[28] documented effective activity for at least 5 months, sufficient to cover the main transmission season in The Gambia. Spraying teams were experienced, well trained, supervised, and achieved a high level of coverage (>80%) in both years. Additionally, the

## *Panel:* Research in context

### Systematic review

We searched PubMed with the term "malaria" and one or more of the terms: "indoor residual spray", "long-lasting insecticidal nets (LLIN)", "insecticide treated nets (ITN)", "indoor residual spraying and LLIN", "indoor residual spraying and ITN", "malaria control", "vector control", and "combined interventions", to identify articles published between Jan 1, 2009, and May 1, 2014. We restricted our search to 2009 onwards because the Cochrane review of indoor residual spraying for malaria prevention[4] had covered up to 2009, and did not identify any controlled trials comparing indoor residual spraying and LLINs to control malaria. We searched for randomised controlled trials, controlled before-and-after intervention studies, and interrupted time series of indoor residual spraying compared with LLINs. MP and SWL independently reviewed the studies for inclusion. The only published results from controlled population trials that compared the relative effects of ITNs or LLINs to ITNs and LLINs plus indoor residual spraying on key malaria indexes are those of two cluster randomised controlled trials in Benin[17] and Tanzania.[18] Both trials used a carbamate insecticide for indoor residual spraying with high coverage (>90%) and both report LLIN use by study children as less than 50%. The Benin trial[17] was a four-group study with seven village clusters per group. The baseline group was LLINs targeted to pregnant women and children younger than 6 years, and was compared with LLINs targeted to pregnant women and children younger than 6 years plus indoor residual spraying, universal coverage with LLINs, and universal coverage with LLIN plus carbamate sheeting on the interior walls of the house. None of the combinations reduced malaria infection or morbidity compared with LLINs targeted to pregnant women and children younger than 6 years. The Tanzania trial[18] studied 50 village clusters and compared malaria parasite infection prevalence in children aged 6 months to 14 years from villages given either LLINs plus indoor residual spraying or LLINs alone. Three post-intervention cross-sectional household surveys measured parasite infection in children. Intention-to-treat analysis showed lower parasite prevalence in the LLIN plus indoor residual spraying group in all three surveys, but the difference was significant only in the survey done 2 months after the second round of indoor residual spraying (OR 0·33, 95% CI 0·15–0·75, p=0·009). In a per-protocol analysis, the differences were significant for all three surveys but such analyses could be affected by confounders, especially in view of the low LLIN coverage. No significant differences in transmission intensity were reported in either trial.[17,18]

### Interpretation

To our knowledge, our results are the first to compare the relative effects of LLINs with LLINs plus indoor residual spraying according to WHO's recommendation of universal LLIN coverage (defined as ≥80%).[18] In the Benin study,[17] no group had universal LLIN coverage, and indoor residual spraying and LLIN coverage in the Tanzania study[18] was below 80%. LLIN coverage is an important difference between the present study and those in Benin and Tanzania, because the use of vector control by most residents can have a community effect on the vector populations, which can result in a substantial reduction in transmission compared with individual protection.[30] Indeed the results of the Tanzania study[18] could be interpreted to support the use of indoor residual spraying if high net usage cannot be achieved. Our study was done in the context of assessing prospects for malaria elimination because over the past decade there has been a gradual decline in malaria in The Gambia associated with the scale-up of LLIN distribution.[31,32] Thus, the study was well timed to assess whether the combination of LLINs and indoor residual spraying could contribute towards malaria elimination. However, we identified no significant difference in clinical malaria or vector density with the addition of indoor residual spraying to LLIN use. Taken together with the results of previous studies,[17,18] our findings do not support any universal recommendation for indoor residual spraying as an addition to LLINs across sub-Saharan Africa. Our advice is that high LLIN coverage is sufficient to protect people against malaria in areas of low or moderate transmission, but where LLIN coverage is low the cost-effectiveness of additional control with indoor residual spraying should be taken into account.

measured DDT concentrations were within the expected range. One possibility is that mosquitoes in the study area were resistant to DDT. Although our results suggest rising resistance, we conclude that for most of the study area resistance to DDT contact killing was low and was not the reason for the absence of an effect of the intervention (appendix).

There are possible non-operational reasons for the absence of a significant effect. The effectiveness of DDT is thought to be partly due to its insecticidal activity, but it is also a spatial repellent, reducing the entry of mosquitoes indoors, and a contact irritant, increasing the rate at which mosquitoes leave a sprayed room.[12] Although we recorded high mortality of mosquitoes exposed directly to DDT-sprayed walls during WHO cone tests, there was no reduction in house entry, suggesting poor repellence. Our results also showed no difference in exit rates of *A gambiae sensu lato* mosquitoes with and without DDT indoor residual spraying, suggesting no contact irritancy from the sprayed walls. Coverage by LLINs was high, with 83–95% coverage in children in the cohort; we note that the exit rates were lower in the survey where LLINs were directly observed (data not shown). High coverage of LLINs might reduce the number of blood-feeding mosquitoes that would normally settle on the walls.

Only two other cluster-randomised trials have examined the benefit of combining indoor residual spraying and LLINs, one in Benin[17] and one in Tanzania[18] (panel). Both trials used a carbamate insecticide for indoor residual spraying with high coverage (>90%) and both report LLINs use by study children as less than 50% (panel). In the Benin[17] trial, none of the combinations reduced malaria infection or morbidity compared with LLINs targeted to pregnant women and children younger than 6 years. The Tanzania trial[18] intention-to-treat analysis showed lower parasite prevalence in the LLIN plus indoor residual spraying group in all three surveys, but the difference was significant only in the survey done 2 months after the second round of indoor residual spraying. A non-randomised study in the western Kenyan Highlands also examined the additive benefit of indoor residual spraying to high LLIN coverage and also examined the effect of targeted larviciding[36] by post-hoc assignment of intervention and control to clusters. When LLINs coverage was high (92%), indoor residual spraying with a pyrethroid insecticide had little additional benefit (panel).

To what extent can our results be generalised to other geographical areas? The vectors found in the study area (*A gambiae sensu stricto* and *A arabiensis*) are the major malaria vectors in sub-Saharan Africa,[37] thus the results are unlikely to be restricted by species-specific considerations and could be applicable to many countries with a high malaria burden.

The vector population in the study area predominately bite indoors and at night,[38] and this low level of outside biting made the rural areas of The Gambia an excellent area to test this double intervention because areas with more outdoor biting would be less likely to show efficacy. DDT indoor residual spraying was the backbone of the Global Malaria Eradication Programme in the mid-20th century,[39] but nowadays only six of 58 countries report the use of DDT for indoor residual spraying.[2] Nonetheless, it is a persistent insecticide, and in our study showed no repellency or contact irritancy, similar to other insecticides used for indoor residual spraying.

The decline in malaria in The Gambia over the past decade suggests that combining LLINs with a persistent insecticide used for indoor residual spraying might reduce malaria to pre-elimination levels. However, our findings refute this suggestion and we would not recommend DDT indoor residual spraying in areas with high LLIN coverage and low malaria incidence. Results from the Benin study[17] also suggest that carbamate indoor residual spraying was ineffective at reducing malaria in the presence of low ITN and LLIN coverage, and high malaria incidence. By contrast, an analysis of weaker data from control programmes and non-randomised studies done in 17 countries in sub-Saharan Africa suggests that the combination of indoor residual spraying and LLINs could be more effective at reducing parasite prevalence in areas with moderate-to-low transmission.[15] More studies are needed in areas with different transmission intensities. Planned cost-benefit analysis was not done during this trial because of the lack of measured benefit but a recent systematic review of the scientific literature published from 2000 to 2010 on the cost and cost-effectiveness of malaria control interventions gives median financial costs per person per year (in 2009 US$) of $2·20 for LLINs and $6·70 for indoor residual spraying.[26]

Our trial design has potential limitations. First, the communities could not be masked to the interventions but subject bias would most probably lead to an under-reporting of clinical malaria in the group that received indoor residual spraying, and thus would bias towards an increased effect of the intervention. Second, the village clusters enrolled in the study were more than 2 km from neighbouring villages and in central Gambia 90% of *A gambiae sensu lato* mosquitoes bite within 1·36 km of their breeding sites,[20] so although the present study design would have reduced spillover, it could not totally avoid it.[40] Third, selection bias was minimised by random selection of children and allocation of the intervention, but the villages for the entomology investigation were selected for convenience, being chosen for size and location. Villages enrolled in the study were small (average population of 523, range 188–2645) with the dwelling houses close together and surrounded by their agricultural fields. Mass killing of mosquitoes would be more likely if the clusters occupied a greater geographical area because this would further restrict the spillover of mosquitoes from adjacent clusters

or villages outside the study. However, the very high survival rates of mosquitoes in our study, parity rates of 77%, suggest that insecticide killing was low. Finally, although our findings show that resistance was not pronounced near study villages, one focus of high resistance to pyrethroid and DDT was detected close to the study area in 2011,[28] and further studies are needed to investigate the distribution of insecticide resistance.

#### Contributors

SWL and MP conceived and designed the study. SWL was the trial director and MP was the trial coordinator and manager in The Gambia. MJ and LBSJ contributed to the design of the entomological collections and indoor residual spraying application. LBSJ and BK advised on the interventions and the study communities; LBSJ led the intervention teams. DJ provided statistical input to the study. KB advised on clinical aspects of the trial, and KS led these aspects. DJC and UD'A contributed to the design and management of the study. MA advised on and supervised the molecular analysis, and SC advised on and supervised the microscopy. HK advised on and did the HPLC analysis. All authors read and approved the final version of the report.

#### Declaration of interests

We declare no competing interests.

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