Adjunctive ivermectin mass drug administration for malaria control on the Bijagos Archipelago of Guinea-Bissau (MATAMAL): a quadruple-blinded, cluster-randomised, placebo-controlled trial

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Summary

Background Arthropod vectors feeding on the blood of individuals treated with ivermectin have substantially increased mortality. Whether this effect will translate into a useful tool for reducing malaria burden at scale is not clear. Our trial aimed to assess whether using ivermectin as an adjunct to mass drug administration (MDA) with dihydroartemisinin–piperaquine would further reduce malaria prevalence.

Methods MATAMAL was a quadruple-blinded, cluster-randomised, placebo-controlled trial, conducted on the Bijagos Archipelago, Guinea-Bissau, an area of seasonal malaria transmission. All residents were invited to participate, with exclusions for drug safety. 24 clusters were randomised in a 1:1 ratio, using restriction randomisation, to either MDA with three daily oral doses of dihydroartemisinin–piperaquine and ivermectin (300 µg/kg per day) in three sequential months during the transmission season in 2021 and 2022, or MDA with dihydroartemisinin–piperaquine and placebo in the same schedule. The primary outcome was quantitative PCR prevalence of *Plasmodium falciparum* parasitaemia in all age groups, during peak transmission, after the second year of intervention. The primary entomological outcome was anopheline parity rate. The trial is registered with ClinicalTrials.gov (NCT04844905).

Findings Participants were recruited between June 7, 2021 and Sept 21, 2022. The baseline population was 25 882 (12 634 [50·6%] were female individuals and 12 317 [49·4%] were male individuals): 13 832 were in the intervention group and 12 050 in the control group. Cluster-level coverage for dihydroartemisinin–piperaquine ranged from 60·4% to 78·7%, and for ivermectin or ivermectin–placebo from 58·1 to 77·1%. Following the intervention, the prevalence of *P falciparum* infection was 118 (5·05%) of 2300 in the control group and 141 (6·64%) of 2083 in the intervention group. The adjusted risk difference was 1·67% (95% CI –1·44 to 4·78; p=0·28). There were 124 adverse events in the control group (1·0% of participants) and 267 in the intervention group (1·9% of participants). Two serious adverse events were reported, neither related to the intervention, and no treatment-related deaths. The anopheline parity rate was 1679 (67·8%) of 2475 in control clusters and 1740 (72·3%) of 2414 in intervention clusters. The adjusted risk difference was –1·32 (95% CI –14·77 to 12·12; p=0·84).

Interpretation Adding ivermectin to dihydroartemisinin–piperaquine MDA had no additional effect on reducing malaria prevalence or vector parity in this setting. The intervention was well tolerated. To our knowledge, this trial is the first to be designed to assess whether ivermectin has an additive effect on malaria when coadministered with dihydroartemisinin–piperaquine MDA.

Funding The National Institute for Health and Care Research, Medical Research Council, Wellcome, and Foreign, Commonwealth & Development Office.

Lancet Infect Dis 2024

Published **Online** November 14, 2024 https://doi.org/10.1016/S1473-3099(24)00580-2

See **Online/Comment** https://doi.org/10.1016/S1473-3099(24)00652-2

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Introduction

Progress towards malaria elimination has stalled and key vector-control tools, such as insecticide-treated nets, face challenges from insecticide resistance and changing vector behaviours.[1] Innovative approaches are required.

Ivermectin is a potent endectocide, lethal to endoparasites and ectoparasites feeding on dosed blood, including malaria vectors.[2] This property has led to the proposal that ivermectin could be used as a malaria control tool. There are few reports of ivermectin resistance and, as a systemic treatment for humans, its

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Research in context

Evidence before this study

We searched MEDLINE and PubMed on July 3, 2024, without language or date restrictions using the terms "ivermectin," "malaria," "anophel*", and "trial." Laboratory studies have consistently shown that ivermectin-dosed blood, in vitro and in vivo, is toxic to malaria vectors at a variety of doses for at least 7 days after treatment, and it has long been suggested that this toxicity might make it an effective tool to reduce transmission. The IVERMAL trial in Kenya reported lethality in vectors 28 days after dosing with 300 µg/kg for 3 days, with few adverse events. The safety data for ivermectin is robust, even at the very high doses used for ectoparasites. The safety and efficacy of ivermectin have also been demonstrated in combination with artemisinin-based combination therapies including dihydroartemisinin-piperaquine. The single-blind, parallel-assignment, cluster-randomised RIMDAMAL trial reported that mass drug administration (MDA) of ivermectin to communities in Burkina Faso reduced clinical malaria incidence in young children; however, a Cochrane review assessed that this trial was inadequately powered. The open-label, cluster-randomised MASSIV trial in The Gambia compared ivermectin-dihydroartemisinin-piperaquine MDA against no trial intervention, reporting a significant reduction in malaria prevalence and vector density, but no effect on vector parity; furthermore, assessing the effect of ivermectin was not possible because the study did not have a control group that made use of using dihydroartemisinin-piperaquine or ivermectin monotherapy.

Added value of this study

To our knowledge, MATAMAL is the first trial designed and powered to identify the additive effect of ivermectin when given in combination with dihydroartemisinin-piperaquine MDA, and the first trial to assess the effect of the drugs on epidemiological outcomes. In this large, cluster-randomised, quadruple-blinded, placebo-controlled trial, we present robust evidence that despite high treatment coverage, adjunctive ivermectin MDA provides no additional benefit to dihydroartemisinin-piperaquine MDA in either clinical malaria or entomological outcomes in this seasonal transmission setting. The results do not support data from smaller phase 2 and phase 3 studies that suggested that ivermectin has an effect as an endectocide beyond efficacious malaria control measures, such as insecticide-treated nets. Further evaluation of alternative formulations or regimens of ivermectin in different settings is needed to establish whether ivermectin has a role in malaria control. This study also strengthens the safety profile of ivermectin.

Implications of all the available evidence

Further research is required to understand why this widely available, safe, and cheap intervention did not have the anticipated effect. Areas of focus should include pharmacology, MDA schedules, and delivery methods, to optimise the proportion of the population with endectocidal blood concentrations, because this proportion might have reduced intervention efficacy in this setting. Differing vector behaviour and ecology might also modulate results. Despite these caveats, the results of this trial remain valid, and provide important context to trials in progress, to future studies considering the use of ivermectin or other endectocides in a package of malaria control interventions, and to policy makers considering the intervention for programmatic use. This important phase 3 evaluation of a promising malaria control intervention suggests that further data will be required before it can be fully supported as an effective public health intervention.

mechanism of action captures *Anopheles* mosquitoes regardless of their biting behaviour. Billions of doses have been safely administered by control programmes for neglected tropical diseases (NTDs).[3]

In three west African sites, one dose of ivermectin at 150 µg/kg significantly reduced *Anopheles gambiae* survival, parity rates, and sporozoite rates,[4] and modelling projections that use 150 µg/kg for 3 days predict infectious vector populations would drop by 68% for 60 days.[5] For the IVERMAL trial in Kenya, ivermectin was administered at 300 µg/kg per day or 600 µg/kg per day alongside dihydroartemisinin–piperaquine for three days, reporting a significant reduction in survival rates of blood-fed mosquitoes up to 28 days after treatment, with fewer adverse events at the lower dose.[2]

Mass drug administration (MDA) with artemisinin-based combination therapy (ACT) reduces malaria prevalence by targeting the human reservoir of *Plasmodium falciparum*, including asymptomatic infections.[6] Dihydroartemisinin–piperaquine is an attractive MDA agent given its safety, efficacy, and the long half-life of piperaquine.[7] Furthermore, there is little published evidence of resistance to dihydroartemisinin-piperaquine in Africa.[8]

Randomised controlled trials (RCTs) of dihydroartemisinin–piperaquine MDA in Zambia[9] and The Gambia[10] showed reduced *P falciparum* prevalence and clinical incidence, although these effects were short-lived, especially in areas of high transmission.

Trials have demonstrated the safety of combined ivermectin and dihydroartemisinin–piperaquine MDA[2] and a pharmacokinetic–pharmacodynamic study showed increased vector mortality compared with ivermectin alone.[11] Population-level modelling predicts that coadministration would boost reductions in *P falciparum* prevalence in both high-transmission and low-transmission settings.[5]

RIMDAMAL,[12] a single-blind, cluster-randomised trial in Burkina Faso, compared ivermectin-only MDA (five doses of 150–200 µg/kg, every 3 weeks) after a single

dose of ivermectin-albendazole, against the single dose of ivermectin-albendazole only. The trial reported reduced clinical incidence of malaria in children in the intervention cluster group.[12] The statistical methods and small number of clusters have, however, raised uncertainty in these conclusions.[13] MASSIV,[14] an open-label, cluster-randomised trial done in The Gambia, compared 3 days of ivermectin (300–400 µg/kg) and dihydroartemisinin–piperaquine MDA, given in three sequential months in 2018 and 2019, against no trial intervention; both groups received standard national malaria control programme measures. Intervention clusters reported 70% lower odds of prevalence and a 79% lower incidence of clinical malaria than control. Mosquito parity, the primary entomological outcome, did not differ between groups, and the trial design prevented isolation of the additive effect of ivermectin.[14]

The Bijagos Archipelago lies 50 km from the coast of mainland Guinea-Bissau. A largely rural population of approximately 26 000 lives across 18 permanently inhabited islands. Travel to the mainland is restricted by cost and availability. There is seasonal migration within the archipelago, particularly for farming and traditional ceremonies.[15]

Local malaria transmission is highly seasonal; prevalence peaks in November after the rainy season between July and October. *A gambiae* sensu stricto is the main local malaria vector.[16] All-age population-based quantitative PCR (qPCR) prevalence across the archipelago was 10·0% in November, 2019 (95% CI 8·7–11·1; unpublished, n=2336). Insecticide-treated nets are distributed every 3 years, most recently in 2023 (Permanent; Vestergaard, Lausanne, Switzerland); reported coverage (92%) and usage (86%) are high,[17] although Anopheles pyrethroid resistance is present.[18] 11 islands have health centres and every village has minimally trained community health-care workers (CHWs). Intermittent preventive therapy in pregnancy is used, whereas indoor residual spraying is not. One pilot round of seasonal malaria chemoprophylaxis was delivered in August, 2020, but not continued. Ivermectin-sensitive NTDs are coendemic.[19]

MATAMAL is the first trial to assess the specific effect of ivermectin on malaria transmission when combined with dihydroartemisinin–piperaquine MDA, providing crucial context and data with direct comparability to the small number of existing and ongoing studies. The aim was to determine whether combined ivermectin and dihydroartemisinin–piperaquine MDA significantly reduces the population-based prevalence of *P falciparum* parasitaemia during peak malaria transmission season compared with dihydroartemisinin–piperaquine MDA alone.

Methods

Study design

MATAMAL was a quadruple-blinded (participant, intervention provider, investigator, and analyst), cluster-randomised, placebo-controlled trial. Community-wide MDA with either combined ivermectin and dihydroartemisinin–piperaquine or ivermectin–placebo and dihydroartemisinin–piperaquine was delivered on the Bijagos Archipelago, Guinea-Bissau. Ethical approval was obtained from the London School of Hygiene & Tropical Medicine, London, UK, and the Comité Nacional de Ética para a Saúde, Bissau, Guinea-Bissau. The protocol has been published.[20] This trial has been reported according to CONSORT guidance.[21]

Participants

24 clusters were defined (appendix p 2): 15 individual islands and three larger islands subdivided into three clusters each. Villages in separate clusters were separated by a minimum distance of 2·2 km. One island was excluded before randomisation; its consistently high malaria prevalence made it an outlier and would imbalance the groups. This island received dihydroartemisinin–piperaquine only and was excluded from analysis.

See Online for appendix

A sensitisation campaign was delivered by CHWs before the intervention. CHWs obtained informed written consent according to protocol. Independent witnesses signed on behalf of non-literate participants and guardians signed on behalf of children younger than 18 years. Informed assent was obtained from children aged 12–17 years.

All consenting residents, defined as anyone sleeping in the cluster for the majority of a given month, were offered MDA, with the following exclusion criteria: severe illness; age younger than 6 months (dihydroartemisinin–piperaquine); height lower than 90 cm or weight lower than 15 kg (ivermectin or ivermectin–placebo); pregnancy or breastfeeding (ivermectin or ivermectin–placebo) or first-trimester pregnancy (dihydroartemisinin–piperaquine); known hypersensitivity to either medication; concomitant use of drugs affecting cardiac function or the QT interval (dihydroartemisinin–piperaquine); and travel to a Loa loa-endemic country (ivermectin or ivermectin–placebo).

All residents were eligible for participation in surveys. Gender was reported by participants. Urinary pregnancy testing was offered to women aged 15–49 years.

Randomisation and masking

An independent statistician assigned the 24 clusters to one of the two groups (intervention and control) in a 1:1 ratio using restricted randomisation.[22] Restriction variables were population, baseline *P falciparum* prevalence (qPCR and rapid diagnostic test [RDT]), vector density, seasonal malaria chemoprophylaxis coverage, and presence of a health centre. Approximately 10% of 100 000 computerised randomisations satisfied the criteria of balancing restriction variables between groups; the final randomisation was selected from this subset.

Only the independent statistician and independent pharmacist were unmasked. All ivermectin and ivermectin–placebo tablets were identical in appearance. Before delivery to the study site, the independent pharmacist applied new labels to all bottles, according to protocol and approved procedure. These new labels were identical, with identifying codes generated by the independent statistician to ensure delivery to the correct cluster. There were no accidental instances of unmasking.

Procedures

The interventions comprised entire-community MDA using dihydroartemisinin–piperaquine (20 mg and 160 mg, and 40 mg and 320 mg tablets; Alfasigma, Bologna, Italy) and ivermectin or ivermectin–placebo (6 mg tablets; Laboratorio Elea Phoenix, Buenos Aires, Argentina).

Details of all procedures are presented in the protocol.[20] The intervention group received dihydroartemisinin–piperaquine (according to the dose by bodyweight set by the manufacturer) and ivermectin (300 µg/kg per day, nearest whole tablet) taken orally with water. The control group received dihydroartemisinin–piperaquine and ivermectin–placebo at the same doses. All doses were directly observed and participants were advised not to eat for 3 h before or after. Dihydroartemisinin–piperaquine could be crushed and mixed with water and readministered if vomited within 30 min (half-dose within 60 min).

A full course of MDA comprised three sequential daily doses of both medications, given monthly in July, August, and September, 2021 (year 1) and 2022 (year 2; figure 1). Monthly MDA commenced simultaneously in all clusters 28 days after the start of the previous round and was delivered by CHWs in their own villages, supervised by trial staff. Participant age, sex, weight, pregnancy, eligibility, and doses received were recorded by household. Standard National Malaria Control Programme interventions (insecticide-treated nets distribution, intermittent preventive therapy in pregnancy, case detection, and treatment with artemether-lumefantrine) continued in both groups.

CHWs were trained to actively identify and report adverse events during MDA, and for 48 h afterwards. Participants and health centres were advised to report adverse events to CHWs. Daily reports were reviewed by the trial doctor for relatedness and severity; referrals were made to health centres as appropriate, and all adverse events were followed up until resolution. Post-hoc adverse-event data were collected during cohort and cross-sectional surveys. Cluster-level births, hospital admissions, miscarriages, and deaths were recorded every month between July 1 and Nov 30, 2022. Clinical incidence data were collected every month from all health centres from July 1 to Nov 30, in both 2021 and 2022; age, sex, and cluster were recorded for all RDT-confirmed malaria cases.

Cross-sectional surveys were done across all clusters beginning 4 weeks after completion of the third round of MDA in both 2021 and 2022. 200 participants were randomly selected from each cluster using a two-step procedure (household and individual) within a core group of villages, purposively defined to capture sufficiently populated villages far from other clusters, but logistically feasible to reach: so-called yolk villages in a modified fried-egg design.[22] Geotagged data were collected including demography, bed-net usage, history of fever, location during MDA, and adverse events. Fingerprick dried blood spots were collected onto Whatman 3 mm filter paper (Cytiva; Marlborough, MA, USA), firstly for *var* gene acidic terminal sequence qPCR molecular analysis, capable of detecting 0·03–0·15 parasites per µL blood,[23] and secondly for serological analysis using a multiplex bead assay on the Luminex MagPix platform (Luminex; Austin, TX, USA).[24] Serological responses to a panel of *P falciparum* exposure markers were measured as median fluorescent intensity (MFI) and variability between plates was accounted for by using loess normalisation.[24] Seropositivity was defined as any response in excess of three SDs above the mean response seen in malaria-naive controls (Public Health

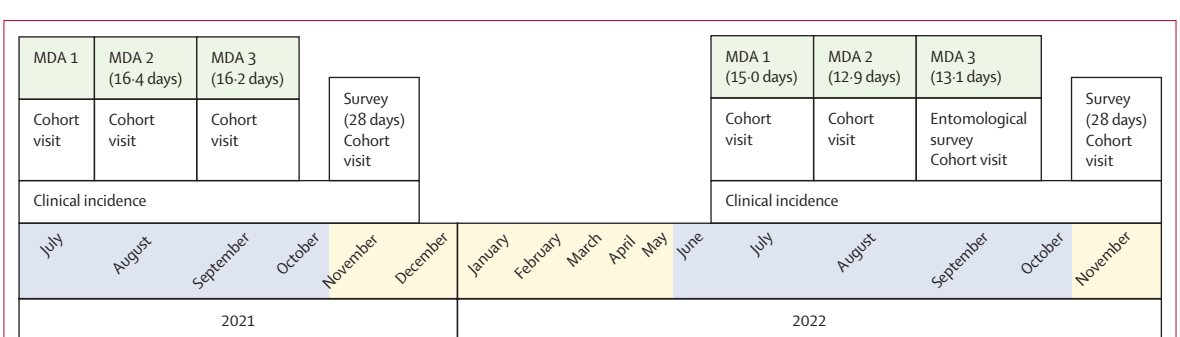

Figure 1: **Trial timeline**

MDA with mean days to completion (appendix p 3). Cohort visit indicates the monthly cohort visit. Survey indicates the cross-sectional survey, with days to completion. Clinical incidence is the passive collection of malaria case incidence from health centres. Blue months indicate the rainy season. MDA=mass drug administration.

England, London, UK). In this analysis we report the serological responses to early-transcribed membrane protein 5 (Etramp5.Ag1), which is associated with exposure to *P falciparum* in the preceding 6–12 months and therefore a marker of seroincidence, allowing assessment of community exposure to *P falciparum* over longer time periods than qPCR or RDT.[25]

A cohort of 50 children per cluster aged 5–14 years was recruited each year in 18 clusters using the randomisation process described for the cross-sectional surveys. Visits were made 7–14 days after each round of MDA concluded, and again during the cross-sectional survey. At each visit, geotagged data were collected including demography, bed-net usage, history of fever, compliance, and adverse events, and dried blood spots were collected for molecular and serological testing for malaria. Tympanic temperature was recorded; if higher than 38°C, an RDT (CareStart pLDH PAN; Access Bio, Somerset, NJ, USA) was done to record clinical incidence and treatment was provided.

Mosquito trapping for the principal entomological outcomes was done in every cluster, 7–14 days after the final round of MDA in 2022. Indoor US Centers for Disease Control light traps were used over three consecutive nights, between 19:00 and 07:00.[26] 15 households were randomly sampled in one so-called yolk village per cluster. Mosquitoes were counted and dry-preserved in the field before transport to the field laboratory for morphological identification.[27] A sample of 200 rehydrated female anopheline mosquitoes from each cluster was assessed for parity, a surrogate for vector population age structure, using Detinova's ovarian tracheation method.[28] For quality control, 70% of assessments were made by several assessors, and interobserver reliability was calculated.[29,30]

Outcomes

The primary outcome was population-based qPCR prevalence of *P falciparum* parasitaemia in all age groups, measured during the peak-prevalence malaria season, after 2 years of intervention.

Secondary outcomes included population-based all-age *P falciparum* qPCR prevalence after 1 year of MDA, incidence of passively detected clinical malaria throughout the transmission season, incidence of clinical malaria (RDT) and *P falciparum* infection (qPCR and serology) in the cohort throughout the transmission season, seroprevalence of markers of *P falciparum* exposure at peak transmission season, vector parity and density after the final MDA round, adverse events, and coverage.

All protocol outcomes have been assessed. Serological and entomological outcomes will be presented in separate publications.

Statistical analysis

Sampling 200 participants in each of 12 clusters per arm has 82% power (with a two-sided α level of 5%) to detect a difference in malaria qPCR prevalence between arms if control-arm prevalence is 10% and intervention-arm prevalence 5% after two years, a reduction informed by mathematical modelling. This assumes a co-efficient of variation of 0·46, informed by baseline variation, and three-dose coverage of 70% of the eligible population.

Dissecting 200 mosquitoes per cluster 7–14 days after the final round of MDA gives 83% power to detect a difference between arms if control-arm parity was 80% and intervention-arm parity 50%, assuming a co-efficient of variation of 0·30. Parity is defined as the proportion of parous females identified in the sample. *Anopheles* density is defined as the total number of trapped *Anopheles* mosquitoes divided by the total number of trap nights.

The primary outcome was assessed by comparing cluster-level mean *P falciparum* qPCR prevalence in all ages. A risk difference or ratio was calculated with 95% CIs, and a p value generated using a t test on cluster-level means. This analysis was also used to estimate prevalence after one year of MDA, vector parity, vector density, Etramp5.Ag1 seroprevalence, and adverse events (per 1000 people). Cluster-level mean clinical incidence (from July to November) and infection incidence in the cohort (cases per 1000 person-months) were compared similarly. Human data were adjusted for age group (<5 years, 5–14 years, >14 years), bed-net use, presence of a health centre on the island, and individuals who were absent throughout all MDA rounds. Cluster-adjusted entomological data were adjusted for temperature, humidity, and rainfall. All analyses were done by intention to treat. Adjustments were made using a standard two-step process for cluster-level analysis.[22]

Monthly coverage was defined as the proportion of the eligible population receiving three doses of MDA that month. Coverage as a proportion of the total population, and coverage of at least one dose were also calculated.

All analysis was done using STATA software, version 17.0. The Data Safety Monitoring Board reviewed trial data annually. The trial was registered on ClinicalTrials.gov (NCT04844905).

Role of the funding source

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

Results

MDA recruitment ran from June 7, 2021 to Sept 21, 2022. Recruitment for the primary outcome survey ran from Oct 31 to Nov 22, 2022 (figure 2). 25 clusters were assessed for eligibility, and 24 were enrolled and randomised to the trial groups. No clusters were excluded or dropped out after randomisation.

Baseline population (June, 2021) was 25 882 (table 1): 12 634 (50·6%) were female individuals and 12 317 (49·4%) were male individuals, and median age was 20 years

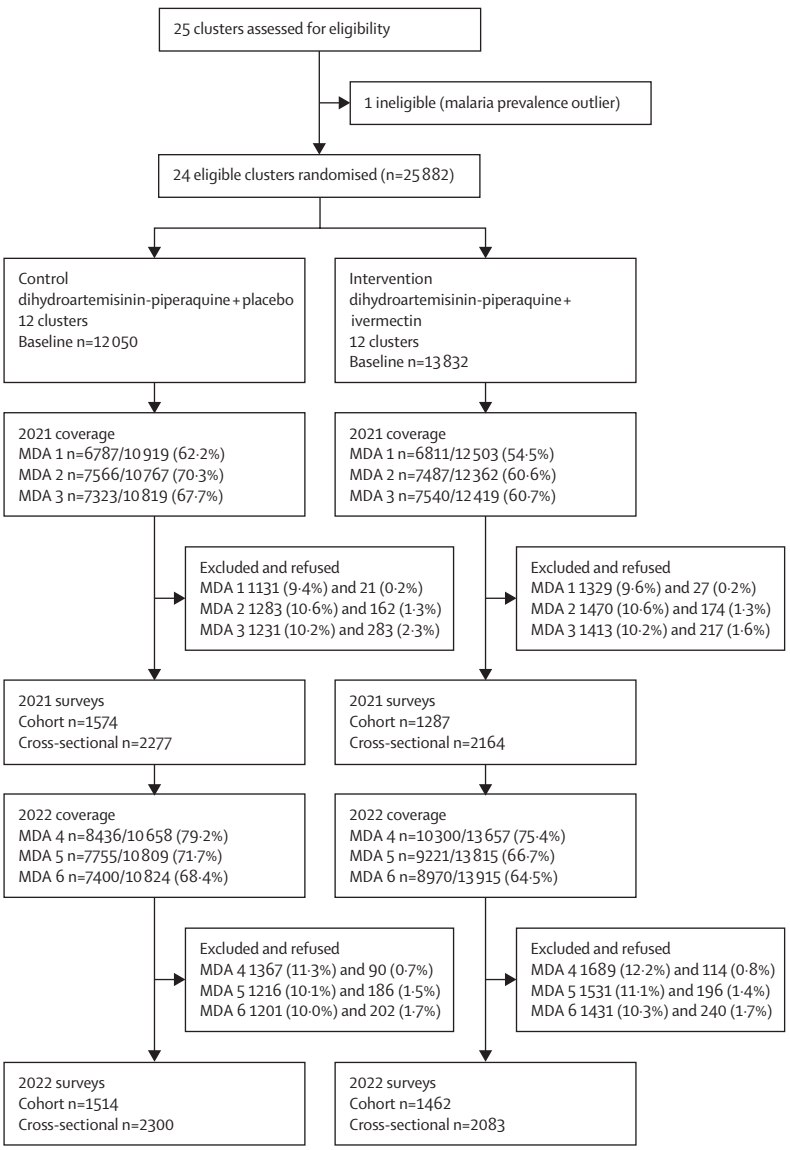

Figure 2: **MATAMAL trial profile**
Coverage indicates the proportion of eligible participants receiving three doses of ivermectin or placebo per round of MDA. Exclusion and refusal statistics relate only to ivermectin or placebo.

	Control	Intervention
Clusters	12	12
Population	12 050 (46·6%)	13 832 (53·4%)
Household size	5·99 (0·9)	6·43 (0·9)
Age		
Median (IQR)	19 (9–36)	20 (9–38)
0–4 years	1336 (11·8%)	1437 (10·8%)
5–14 years	3146 (27·7%)	4009 (30·2%)
>14 years	6887 (60·6%)	7842 (59·0%)
Sex		
Male	5883 (50·2%)	6434 (48·6%)
Female	5839 (49·8%)	6795 (51·4%)

Data are n (%) or mean (SD) unless otherwise stated. Subgroup numbers might not equal totals because of missing census data.

Table 1: **Participant baseline characteristics by trial group**

(IQR 9–37). 13 832 residents were assigned to the intervention group and 12 050 to the control. The baseline peak-season qPCR prevalence of *P falciparum* infection (November, 2019) was similar between groups. Baseline characteristics for 2022, the primary outcome survey and cohort participants, are presented in the appendix (pp 5–6).

Monthly coverage ranged from 60·4% to 78·7% for dihydroartemisinin–piperaquine (mean 68·8%) and 58·1 to 77·1% for ivermectin or ivermectin–placebo (mean 66·6%; appendix p 7). Monthly coverage with at least one dose ranged from 71·8% to 84·4% for dihydroartemisinin–piperaquine and 74·1% to 86·0% for ivermectin or ivermectinplacebo. Monthly refusals ranged from 48 to 500 of 25 882 participants (0·19% to 1·93%). Combining all six MDA rounds, the mean proportion of the population ineligible for each drug was 1·48% (dihydroartemisinin–piperaquine) and 10·20% (ivermectin or ivermectin–placebo).

Days required to complete MDA by cluster ranged from 4 days to 24 days (appendix p 3); however, most delivery was completed early in the month; times appear inflated by lengthy searches for absentees (mop-up). To distribute 80% of directly observed MDA, delivery times ranged from 3 days to 18 days, the mean time being 9·1 days (SD 2·0) in the control group, and 9·4 days (2·3) in the intervention group (appendix p 4).

In November, 2022, after 2 years of MDA, the qPCR prevalence of *P falciparum* infection was 118 (5·05%) of 2300 (between-cluster SD 3·25) in the control group, and 141 (6·64%) of 2083 (4·11) in the intervention group. The risk difference was 1·59% (95% CI –1·55 to 4·72; p=0·30) and risk ratio 1·39 (95% CI 0·70 to 2·75; p=0·33). After adjusting for sex, age group, bed-net use, presence of a health centre, and participant absence throughout MDA, the difference was 1·67% (–1·44 to 4·78; p=0·28), and the risk ratio 1·41 (0·71 to 2·82; p=0·31; figure 3). Prevalence did not differ significantly between sexes or age groups, nor by bed-net usage or living on an island with a health centre (appendix p 7).

In November, 2021, after 1 year of MDA, the qPCR prevalence of *P falciparum* infection was 64 (2·44%) of 2265 (between-cluster SD 16·6) in the control group and 38 (1·75%) of 2159 (13·2) in the intervention group. The risk difference was –0·69% (95% CI –2·23 to 0·86; p=0·37). The adjusted risk difference was –0·61% (–2·01 to 0·79; p=0·38; figure 3).

There were 1476 RDT-confirmed cases of malaria recorded through passive surveillance at health centres between July and November; 827 in 2021 and 649 in 2022 (table 2). 672 (45·5%) of 1476 cases were in female

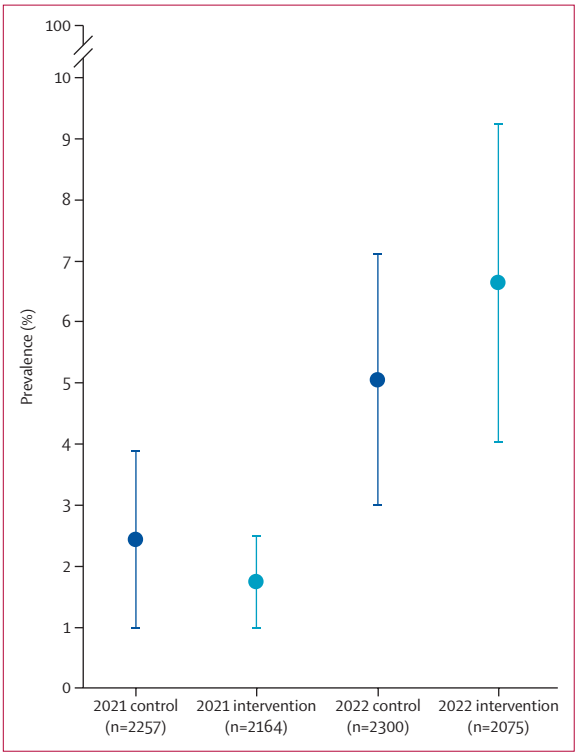

Figure 3: **Cluster-level mean quantitative PCR prevalence in each trial group after 1 year (2021) and 2 years (2022) of intervention**
Data are mean (95% CI).

	Control	Intervention	Rate difference (95% CI)	p value
Cases				
2021	411 (49·7%)	416 (50·3%)	..	..
2022	396 (61·0%)	253 (39·0%)	..	..
Total	807 (54·7%)	669 (45·3%)	..	..
Rate per 1000 person-months (95% CI)				
2021	7·13 (0·15 to 14·11)	3·64 (1·19 to 6·08)	−3·49 (−10·17 to 3·19)	0·29
2022	5·29 (−0·37 to 10·94)	4·05 (1·90 to 6·19)	−1·24 (−6·94 to 4·46)	0·66
Total	5·91 (0·59 to 11·23)	3·84 (2·35 to 5·33)	−2·07 (−7·27 to 3·13)	0·42

Data are n (%) unless otherwise stated.

Table 2: **Clinical cases of malaria, confirmed by rapid diagnostic test, recorded through passive surveillance at health centres between July 1 and Nov 30, 2021 and 2022**

individuals and 804 (54·5%) were in male individuals, 228 (15·4%) of 1476 were in infants younger than 5 years, 413 (28·0%) in children aged 5–14 years, and 835 (56·6%) in adolescents and adults older than 14 years. Overall, there were 5·91 cases per 1000 person-months (95% CI 0·59 to 11·23) in the control group and 3·84 cases per 1000 person-months (2·35 to 5·33) in the intervention group, giving a risk difference of −2·07 (95% CI −7·27 to 3·13; p=0·42). The risk differences between groups in 2021 and 2022 were also not statistically significant. The incidence of fever in the cohort of children was so low that it precluded analysis of clinical incidence.

In 2021, the qPCR incidence rate of *P falciparum* infection in the cohort was 23·4 cases per 1000 person-months (95% CI 14·0 to 32·8) in the control group and 28·5 (8·2 to 48·8) in the intervention group, a rate difference of 5·11 (95% CI −15·5 to 25·7; p=0·61). This difference was similar after adjustment for the covariates described above (p=0·55). In 2022, there were 24·3 cases per 1000 person-months (3·0 to 45·7) in the control group and 14·8 (5·7 to 24·0) in the intervention group, a risk difference of −9·52 (−30·9 to 11·8; p=0·36). Again, this difference was similar after adjustment (p=0·38).

The difference in serological responses to Etramp5.Ag1 between groups was not statistically significant (appendix pp 8–9). In 2021, seroprevalence in control clusters was 5·50% (95% CI 3·39 to 7·61), and was 4·31% in intervention clusters (2·54 to 6·0), a risk difference of −1·19 (−3·79 to 1·41; p=0·35). In 2022, the seroprevalence in the control group was 10·24% (6·42 to 14·07) and was 8·29% in the intervention group (5·07 to 11·51), a risk difference of −1·96 (−6·67 to 2·75; p=0·40). MFI data were log transformed. The difference in mean logMFI between groups was not statistically significant (2021, p=0·69; 2022, p=0·66; appendix p 9).

In the cohort study, seroprevalence in control clusters increased through the rainy season. However, in intervention clusters, seroprevalence was stable throughout 2021 and decreased during 2022. The adjusted difference between groups was not statistically significant. Mean logMFI decreased in both groups throughout both years, but was significantly lower in intervention clusters in September (mean difference −0·62; p=0·033) and November, 2021 (mean difference −0·88; p=0·037).

Mean cluster-level *Anopheles* density after the final round of MDA was 14749 mosquitoes per 525 trap nights (28·1) in the control group, and 23 445 mosquitoes per 516 trap nights (44·8) in the intervention group (appendix p 9). Data were log transformed for positive skew. The adjusted risk ratio was 1·29 (95% CI 0·57 to 2·90; p=0·53). Mean cluster-level *Anopheles* parity was 1679 (67·8%) of 2475 in control clusters and 1740 (72·3%) of 2414 in intervention clusters, giving an adjusted risk difference of −1·32 (95% CI −14·77 to 12·12; p=0·84). The inter-rater reliability score was 0·91, indicating almost perfect agreement between assessors.[30] Species composition, which did not differ between groups, is presented in the appendix (p 10). *Anopheles melas* predominates and probably has a role in perpetuating year-round transmission;[16] however *Anopheles gambiae* sensu strictu is probably the primary vector. Cluster-level mean sporozoite rates were low and the difference between groups was not statistically significant (control 0·2, intervention <0·1, adjusted rate difference −1·83, 95% CI −2·86 to 6·53; p=0·43). Differences in cluster-level mean entomological

	Control	Intervention	Rate difference (95% CI)	p value
Adverse events	124 (31·7%)	267 (68·3%)	..	..
Rate per 1000 population (95% CI)	11·6 (6·3 to 17·0)	15·3 (9·0 to 21·6)	3·67 (–4·14 to 11·47)	0·34
Severity				
Mild	122 (98·4%)	250 (93·6%)	..	..
Moderate	2 (1·6%)	17 (6·4%)	..	..
Relatedness				
Unrelated	2 (1·6%)	9 (3·4%)	..	..
Unlikely	14 (11·3%)	74 (27·7%)	..	..
Possible	76 (61·3%)	135 (50·6%)	..	..
Probable	32 (25·8%)	46 (17·2%)	..	..
Certain	0	3 (1·1%)	..	..
Vital rates per 1000 population (95% CI)				
Births	4·63 (2·78 to 6·48)	2·76 (1·07 to 4·45)	–1·87 (–4·23 to 0·49)	0·11
Deaths	1·99 (0·67 to 3·30)	1·51 (0·09 to 2·93)	–0·47 (–2·30 to 1·35)	0·60
Spontaneous miscarriages	1·31 (0·22 to 2·42)	1·27 (0·45 to 2·13)	–0·05 (–1·77 to 1·68)	0·96
Hospital admissions	5·97 (0 to 12·40)	5·63 (0 to 14·29)	–0·34 (–10·00 to 9·32)	0·94

Data are n (%) unless otherwise stated. Vital rates are for 2022 only. 19 hospitalisations could not be assigned to a trial group and were excluded from analysis.

Table 3: **Summary of adverse event data and vital rates**

inoculation rates were similarly not statistically significant (control 5·1; 95% CI –1·2 to 11·4; intervention 6·5, –7·9 to 21·0; rate difference 1·45, –13·3 to 16·3; p=0·84). A detailed analysis of entomological outcomes will be published separately.

There were 392 adverse events reported in 391 participants: 124 in the control group (1·03% of participants) and 267 in the intervention group (1·9%; table 3). 271 (69·3%) of 391 participants were female individuals and 120 (30·7%) were male individuals. 20 (5·1%) of 392 adverse events were in children younger than 5 years, 55 (14·0%) in children aged 5–14 years, and 317 (80·9%) in participants older than 14 years. Cluster-level mean adverse events were 11·64 per 1000 population in the control group (95% CI 6·26 to 17·03) and 15·31 adverse events per 1000 population in the intervention group (9·02 to 21·61). The cluster-level rate difference was 3·67 (–4·14 to 11·47; p=0·34). 373 (95·2%) adverse events were classified as mild, all others were moderate. There were two serious adverse events, both in children requiring hospital admission for malaria. The relatedness of adverse events to MDA is outlined in table 3, the majority (54·0%) being possibly related to the intervention. All adverse events resolved within the reporting timeframe. The most common recorded symptoms were dizziness (n=96), diarrhoea (n=92), and vomiting (n=86). All 17 reports of transient visual disturbance were in intervention clusters. Rates of births, deaths, miscarriages, and hospital admissions did not vary between trial groups. During the 2022 cross-sectional survey, 378 (16·4%) of 2300 participants in control clusters reported experiencing adverse events during MDA. In intervention clusters, the figure was 406 (19·6%) of 2076. The cluster-level risk difference was 2·36 (95% CI –9·05 to 4·32; p=0·47).

Discussion

Contrary to our hypothesis, the addition of ivermectin to dihydroartemisinin–piperaquine MDA had no additional effect on community prevalence of *P falciparum* infection after 1 year or 2 years of distribution in this setting. Furthermore, there was no effect on the incidence of clinical disease or *P falciparum* infection, on serological responses to Etramp5.Ag1, or on entomological outcomes. The intervention was well tolerated.

As regions approach malaria elimination, it becomes increasingly difficult to reduce transmission, and novel approaches are needed.[1] This is especially true in regions with high coverage of programmatic interventions, such as the Bijagos Archipelago.[17] Ivermectin and MDA using ACT have both been suggested as potential solutions, with promising phase 2 and field trial data upon which MATAMAL aimed to build.[2,12,14]

Pretrial modelling, based on baseline and published data, projected a significant effect from the addition of ivermectin to dihydroartemisinin–piperaquine MDA; however, this finding was not observed. After 1 year, prevalence and incidence were reduced compared to baseline. Prevalence and incidence were lower in the intervention group, especially clinical incidence. However, the risk–rate differences were small, and after 2 years, prevalence was higher in the intervention group, and the incidence difference was reduced. Such small differences between groups are unlikely to signify public health benefit and were not statistically significant in any of the analyses.

The decline from baseline in both groups indicates good effect from dihydroartemisinin–piperaquine MDA, but no augmentation from ivermectin. This effect was sustained, but not improved, in 2022, reinforcing data from The Gambia[10] that dihydroartemisinin–piperaquine MDA alone is sufficient to reduce but not interrupt transmission, probably because it can only target the human reservoir of infection, and only drug recipients. Reinforcing this finding, baseline trends, such as higher prevalence in male individuals and the 5–14-year age group were preserved, but clinical incidence and cohort incidence (molecular and serological), now declines throughout the rainy season, with only a modest end-of-season peak. The only identified difference between groups was a small decrease in cohort Etramp5.Ag1 MFI in intervention clusters during 2 months of 2021, the significance of which is probably slight. Seroprevalence increased throughout the season in control clusters, but decreased in intervention clusters implying waning exposure, as in the only published serological analysis of a malaria RCT.[31] There was no difference between groups at any timepoint,

but this trend should encourage serological analysis of future trials, especially using this antigen.

Potential reasons for the absence of ivermectin MDA effect include slightly lower coverage and slower distribution than modelled, although this finding reflects a realistic approach that would be feasible for programmatic implementation. Prevalence after 1 year was also lower than expected, probably as a result of effective dihydroartemisinin–piperaquine MDA. Prevalence may also have been reduced by bed-net distribution and seasonal malaria chemoprophylaxis campaigns in 2020; however their effect is unquantified, and new public health interventions should be expected to perform well in the context of other measures. Other variables, including the physiology or behaviour of human and vector populations, ecological and environmental conditions, or differences between archipelago and mainland geography, both physical and human, might also have had an effect. Notably, nearby coastal mainland mosquito populations exhibit increased zoophagy compared with inland populations.[32] Although there have been no vector behavioural studies in this setting, if indeed zoophagy is exhibited by local vectors, it could partially explain the absence of ivermectin effect in this setting. These divergences from modelling might reduce power to detect differences smaller than the 50% modelled; however, the agreement between all measures, including separately powered entomological outcomes, validates the conclusion that ivermectin did not have the expected effect on malaria transmission in this setting.

Although coverage was good and refusals low, effective coverage, the proportion of residents whose blood is dosed and endectocidal, leaves parasite reservoirs for ongoing transmission. More than 10% of the population were necessarily excluded from receiving ivermectin, including young children and pregnant or nursing mothers at high risk. Absentees, especially mobile groups of young men working outdoors, often perpetuate community transmission.[33] Movement between the mainland also remains a route for importing parasites. Speed of MDA delivery might also have affected effective coverage in some clusters.

Smit and colleagues[2] showed that the doses of ivermectin used in MATAMAL caused increased mortality in anopheline vectors when fed blood taken 28 days after treatment, which influenced the choice of dosing regimen in our trial. However, they also showed that median survival exceeded the extrinsic cycle length, and did not differ significantly from placebo after 14 days. MASSIV demonstrated significantly increased vector mortality up to 21 days.[14] Bioefficacy studies were not done at the trial site. There are otherwise few pharmacokinetic and pharmacodynamic data from field trials over such long timeframes. Combined with the issues surrounding effective coverage, ivermectin was possibly not circulating in high enough concentrations for long enough duration in the blood of a sufficient proportion of the population to translate efficacy into effectiveness in the field. The ivermectin dosing of MATAMAL was based on safety and efficacy data in combination with dihydroartemisinin–piperaquine, and increasing MDA frequency in this setting would be impractical in a programmatic context. Pharmacokinetic and pharmacodynamic analysis was not included in our protocol, but could prove enlightening. The dosing of MATAMAL was nonetheless based on the best-available evidence, and increasing MDA frequency would be logistically challenging.

If any effect on vector populations exists, it is too subtle to detect, challenging the proposed mechanism of ivermectin in reducing transmission. This finding is in contrast to previous studies, although MASSIV also found no effect on parity, the primary metric for assessment. Contributing factors might include the vulnerability of sampling to external variables, including weather and fragile traps, and high net coverage might increase exophagous behaviour, meaning vectors avoided indoor sampling, but this parameter would be equal across groups and the large number of trapping nights reduces bias. Indoor trapping theoretically preferentially captures human-seeking vectors, and although vector plasticity is observed, anophelines caught inside the household are still most likely to have been human seeking.[34] Other behavioural variables might be influential, but to succeed as a public health intervention, the effect of ivermectin MDA must be able to overcome a variety of local vector characteristics. Parity is a crude tool for assessing vector age but remains the gold standard. Additional work characterising local vectors and their behaviours will be presented elsewhere, and further study can be informative.

We believe these results to be robust. There was agreement between all metrics that there was no difference between groups in clinical, laboratory, and entomological outcomes. Coverage and acceptability were good. The trial was appropriately designed and powered, with a placebo control, successful blinding, and sufficiently separated clusters to reduce contamination.

These results are likely to be generalisable to seasonal and low-transmission regions of west Africa, where it seems unlikely that ivermectin and dihydroartemisinin–piperaquine MDA would be beneficial despite local differences in human, vector, and environmental variables. Results should be applied cautiously to areas of stable high transmission, different vector ecology, or widespread ACT resistance. Future research could focus on alternative formulations or dosing schedules, aiming to increase the proportion of participants with mosquitocidal blood concentrations without impeding feasibility or cost, which are already challenging for programmatic implementation. Further data on local vector behaviours and ivermectin pharmacokinetics and pharmacodynamics could be useful, and consideration

could be given to a unified one-health approach to improve effect. MATAMAL data will inform models and contextualise ongoing trials.

Limitations include population mixing between clusters, limited here by geographical isolation, but unavoidable in any trial. Refusals were rare but tended to cluster in households, potentially creating hotspots for ongoing transmission. CHWs improved acceptability, but additional targeted sensitisation might have improved coverage, albeit at increased cost. Baseline malaria prevalence might have been too low here to demonstrate an effect, which was discrepant with the modelled scenarios. Although groups were similar at baseline, and randomisation minimises random confounding, it is possible that confounding factors remained. As MATAMAL was powered to detect a 50% difference, it is possible that a smaller difference might not have been detected, although the public health benefit of such small reductions would require further evaluation.

Overall, the interventions were safe and well tolerated, with the majority of adverse events being mild, with no difference between groups. The increased reporting of adverse events post hoc suggests real-time reporting might require strengthening in the future.

In conclusion, we observed that adding ivermectin to dihydroartemisinin–piperaquine MDA is no more effective at reducing community *P falciparum* prevalence than dihydroartemisinin–piperaquine MDA alone. This absence of effect was seen in all clinical and entomological endpoints. MATAMAL is the first trial to isolate the effect of ivermectin when coadministered with dihydroartemisinin–piperaquine MDA. These results provide crucial context for ongoing and future trials, and will inform discussion regarding the future of ivermectin in malaria control programmes.

Contributors

AL, JB, CD, UD, HH, EP, HV, RTJ, DM, JGL, and AR designed the trial. AL and AR obtained ethical approval in the UK and Guinea Bissau. EP, RTJ, and JGL provided entomological expertise. HH, ETdS, and EP led the field work and conducted the data collection. HH and EP conducted the statistical analysis supported by statistical expertise from JB. HH prepared this manuscript. HV, KT, and CD provided additional expertise regarding the serological analysis. HV conducted lab work with assistance from FC, HN, AP, SS, and SM. MON, HdMS, and SC supervised laboratory processes at MRC The Gambia. PD, JEN, CM, and AR were responsible for local implementation and regulatory procedures. HS developed predictive models to inform trial design. AL as principal investigator (PI), with AR as local PI, were responsible for the oversight and implementation of all trial activity. The trial was funded by a grant obtained by AL. All authors had full access to study data, reviewed and provided input into this manuscript and share final responsibility for the decision to submit for publication. HH and JB directly accessed and verified all underlying data.

Declaration of interests

JGL declares that he is founder and Chief Executive Officer of Arctech Innovation, a company which aims to design mosquito lures and malaria diagnostics. All other authors declare no competing interests.

Data sharing

The statistical analysis plan and de-identified participant-level data will be stored, with dictionaries, for a minimum of 10 years from publication in secure London School of Hygiene & Tropical Medicine repositories (https://datacompass.lshtm.ac.uk/), with access on reasonable request. The published protocol includes examples of case record and informed consent forms.[20]

Acknowledgments

This work was supported by a Joint Global Health Trials award (National Institute for Health and Care Research, MRC, Wellcome, and the Foreign, Commonwealth and Development Office), funder reference MR/S005013/1. Electronic data solutions were provided by the London School of Hygiene & Tropical Medicine (LSHTM) Global Health Analytics (odk.lshtm.ac.uk). We acknowledge the help and support of the Bandim Health Project, University of Southern Denmark, in conducting field operations, and the MRC Unit The Gambia, LSTHM, for hosting our laboratory work. We thank the members of the Trial Steering Committee and Data and Safety Monitoring Board. We are grateful for the contributions of our LSHTM students and volunteers, and many members of the local community in supporting the trial. We thank the Regional Health Directorate and local health workers, especially the community health-care workers and activists who delivered the intervention. Lastly, our profound thanks to our field assistants, who worked tirelessly to ensure distribution was a success.

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