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Evaluation of Attractive Targeted Sugar Baits, a new outdoor vector control strategy against malaria: Results from a cluster randomised open-label parallel arm controlled trial in Southwestern Mali

Sophie Sarrassat [a,*,1], Mahamoudou Toure [b,1], Ayouba Diarra [b], Makan Keita [b], Hamady Coulibaly [b], Abdoul Zamilou Arou [b], Mohamed Traore [b], Cheick Oumar Tangara [b], John Bradley [a], Gunter Muller [b], Silas Majambere [c], John C. Beier [d], John Vontas [e,f], Sekou F. Traore [b], Samba Diop [b], Immo Kleinschmidt [a,g,2], Seydou Doumbia [b,2]

[a] Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine (LSHTM), London, United Kingdom
[b] University Clinical Research Centre, University of Sciences, Techniques and Technologies of Bamako, Bamako, Mali
[c] Pan-African Mosquito Control Association, Nairobi, Kenya
[d] School of Medicine, University of Miami Miller, Miami, FL, United States
[e] Institute of Molecular Biology & Biotechnology, Foundation for Research and Technology, Heraklion, Greece
[f] Agricultural University of Athens, Athens, Greece
[g] Wits Research Institute for Malaria, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

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SUMMARY

Background: The Attractive Targeted Sugar Bait (ATSB) is a new malaria outdoor vector control tool targeting sugar-feeding behaviours of vector mosquitoes. In Mali, a two-year open-label two-arm cluster randomised controlled trial compared the efficacy and safety of ATSB plus insecticide treated mosquito nets (ITN) versus ITN alone on malaria burden.

Methods: 76 clusters were formed, of which 38 were randomly allocated to the intervention. Cohort studies and household surveys were performed to assess clinical malaria incidence (primary outcome) in children aged 5 to 14 years and malaria infection prevalence in individuals aged 6 months or older, respectively. Primary analyses were performed on an intention-to-treat basis. The trial was designed to detect a minimum 30% reduction in the two outcomes over a two-year period with a power of at least 80%. The trial is registered at ClinicalTrial.gov (NCT04149119).

Findings: The proportion of sleeping buildings with at least 2 ATSB ranged between 70% and 80%. Coverage of ATSB in good condition was lower (50% or less). Over the two-year trial period, the clinical malaria incidence rate in the control and intervention arm was 0.726 and 0.660 cases per person-year, respectively, with no statistical evidence for an intervention effect (Incidence Rate Ratio (IRR) = 0.90; 95%CI 0.77, 1.05; p = 0.188). Malaria infection prevalence was approximately 37% in both arms (Odds Ratio (OR) = 0.96; 95%CI 0.76, 1.21; p = 0.729). In clusters with coverage of stations in good condition above 80%, there was evidence for a 26% reduction in malaria incidence compared to control clusters after controlling for confounders (adjusted IRR = 0.74; 95%CI 0.61, 0.90; p = 0.002).

Interpretation: Overall, the trial did not demonstrate evidence of additional protection against malaria of ATSB compared to using ITN alone. Suboptimal coverage and maintenance of ATSB in good condition in the field may explain the lack of an intervention effect.

Background

Insecticide-treated mosquito nets (ITN) and indoor residual spraying of insecticides (IRS) have made major contributions to the reduction of malaria disease and mortality.[1] However, resistance to insecticides and behavioural plasticity of malaria vectors such as

* Correspondence to: London School of Hygiene and Tropical Medicine, Keppel Street, WC1E 7HT London, United Kingdom.
E-mail address: sophie.sarrassat@lshtm.ac.uk (S. Sarrassat).
[1] Joint 1st authors.
[2] Joint senior authors.

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outdoor and daytime biting have resulted in ongoing transmission even when these interventions are deployed to scale.[2–5] In recent years, the challenge of pyrethroid resistance has been countered by the development of effective new technologies in the form of dual active ingredient nets and new classes of insecticides for IRS.[6–8] Nevertheless, it is widely accepted that these tools alone will not be sufficient to further reduce malaria burden, and interventions that can complement ITN and IRS by killing mosquitoes outdoors using other biologic mechanisms need to be developed and evaluated.[9,10]

The Attractive Targeted Sugar Bait (ATSB) is a new class of outdoor malaria vector control tool targeting the sugar-feeding behaviours of vector mosquitoes. Bait stations, which are hung on the outside walls of houses, contain a plant-based mosquito attractant, sugar (as a feeding stimulant), and an active ingredient that kills mosquitoes upon ingestion. It is designed to provide population-wide protection that results from killing mosquitoes on a large scale. Modelling estimates have suggested that ATSB could markedly reduce mosquito populations across a range of different transmission intensities and should have potential for significant transmission reduction when used in combination with indoor vector control tools.[11,12] ATSB could also help mitigate insecticide resistance because there are numerous alternative ingestion toxicants from different chemical classes that could be used in a bait station.

In Mali, annual malaria cases have steadily increased since 2000 to over 8 million in 2023,[13] despite the widespread implementation of WHO recommended interventions, including high coverage and use of ITN as well as complementary interventions such as seasonal malaria chemoprevention (SMC) for children under five and Intermittent Preventative Treatment in pregnancy (IPTp). In Mali, as well as other endemic countries, people are exposed to high numbers of mosquito bites outdoors in the early evening and indoors when not using a net.[4,5] ATSB are intended to reduce the malaria burden by addressing this residual exposure to mosquito bites. In 2017 and 2018 entomological field studies established an optimal deployment pattern for outdoor use of ATSB in the local setting and reported a daily mosquito feeding rate of 25%.[14,15] These studies found a large reduction in mosquito longevity, suggesting ATSB may prevent female *Anopheles* mosquitoes from surviving long enough for the maturation of ingested malaria gametocytes to sporozoites, thereby resulting in more than 85% reductions in outdoor entomological inoculation rates (EIR).[14]

Between January 2021 and December 2023, a phase III open-label two-arm cluster randomised controlled trial (CRCT) was conducted in Mali to assess the efficacy and safety of ATSB on epidemiological and entomological malaria outcomes. The objective was to evaluate the efficacy of ATSB plus universal coverage of ITN, over two transmission seasons, on a minimum 30% reduction in cohort-based incidence of blood confirmed malaria cases (primary outcome) in children aged 5 to 14 years, and a similar reduction in cross-sectional community-based malaria infection prevalence, compared with universal coverage of ITNs alone. A reduction of 30% was deemed the minimum to be of public health relevance and smaller effect sizes would require a prohibitively large trial. Independently powered stand-alone CRCT were also conducted in Kenya and Zambia which are being reported separately.[16] Here, we report on the epidemiological outcomes of the Mali trial. Entomological results will be reported separately.

Methods

The trial design, methods and Statistical Analysis Plan (SAP) were standardised across Mali, Kenya and Zambia and have been previously described.[17,18] Fig. 1 gives an overview of data collection and timelines of the Mali trial. A summary of the trial design and methods is given below.

The study was located in the Koulikoro region, approximately 60 kilometres southeast of Bamako in Southwestern Mali. In this region, malaria is seasonal, with high numbers of malaria cases occurring between July and December each year. The primary malaria vectors are *An. gambiae s.s.* and *An. coluzzii*, and *An. arabiensis* is a secondary vector. Pyrethroid resistance has been found in the primary malaria vectors.[19,20] According to the 2018 Demographic and Health Survey, 94% of households in the Koulikoro region owned at least one ITN, 63% owned at least one ITN per two persons (universal coverage), 78% of respondents reported having slept under an ITN the night prior to interview, and 34% of women who had a live birth in the past two years reported receiving 3 doses or more of Sulfadoxine-Pyrimethamin for the prevention of malaria in pregnancy. Amongst children under five, 22% tested positive by Rapid Diagnostic Test (RDT).[21]

Cluster formation and randomisation

After conducting a population census in 118 villages in the study area, 76 trial clusters were delineated, each consisting of 1340 persons on average (range approximately 500 to 4000) and with a minimum distance of 1200 m between the edges of clusters. The 76 clusters were allocated to either the intervention arm (ATSB plus universal ITN coverage) or the control arm (universal ITN coverage only) using restricted randomisation to balance the trial arms with respect to indicators measured at baseline including malaria case incidence in children 5 to 14 years old, ITN and SMC coverage, housing density, cluster population, distance to the nearest health facility and to the Niger river. The trial clusters are shown in Fig. 2.

Interventions

ATSB Westham Sarabi version 1.2 (0.11% dinotefuran w/w as active ingredient) were produced by Westham Co. (Hod-Hasharon, 4131905, Israel). During year 1, preceding the start of the first cohort follow-up, two ATSB were installed on the external walls of each sleeping building of all consenting households in intervention clusters. A total of 32,416 bait stations were installed during the first deployment (May 2022). Bait stations were then replaced every eight months. During the second (January 2023) and third deployment (August 2023), 29,544 and 27,865 bait stations in total were installed (Fig. 1). At the start of year 1 and year 2 cohort follow-up, Permanent 2.0 nets (Vestergaard, 9/253 Minh Khai Street, Hai Ba Trung District, Hanoi, Vietnam) were distributed to all households in all clusters (Fig. 1).

Data collection

Cohort studies

Cohorts of children aged 5 to 14 years were randomly selected from population census lists in each cluster at baseline (2021), year 1 (2022) and year 2 (2023). Children under 5 years were not selected because of their eligibility to receive SMC. Each year, recruitment took place at the beginning of the malaria season in May, and subject to the consent of their caregiver, selected children were given a full treatment dose of Artemether/Lumefantrine for malaria parasite clearance prior to follow-up. Children who were confirmed parasite-free by malaria RDT (Bioline™ Malaria Ag P.f/Pan) two weeks after recruitment were followed up monthly until the end of the malaria season, in January of the following year.

Clinical malaria incidence (primary outcome) was measured at each follow-up visit by testing for circulating malaria antigens using a RDT all children who had an axillary temperature ≥37.5 °C or a recent history of fever (last 24 h). Those testing positive had a blood smear taken for confirmatory microscopy. Caregivers were asked to take enroled children to the local community health worker (CHW)

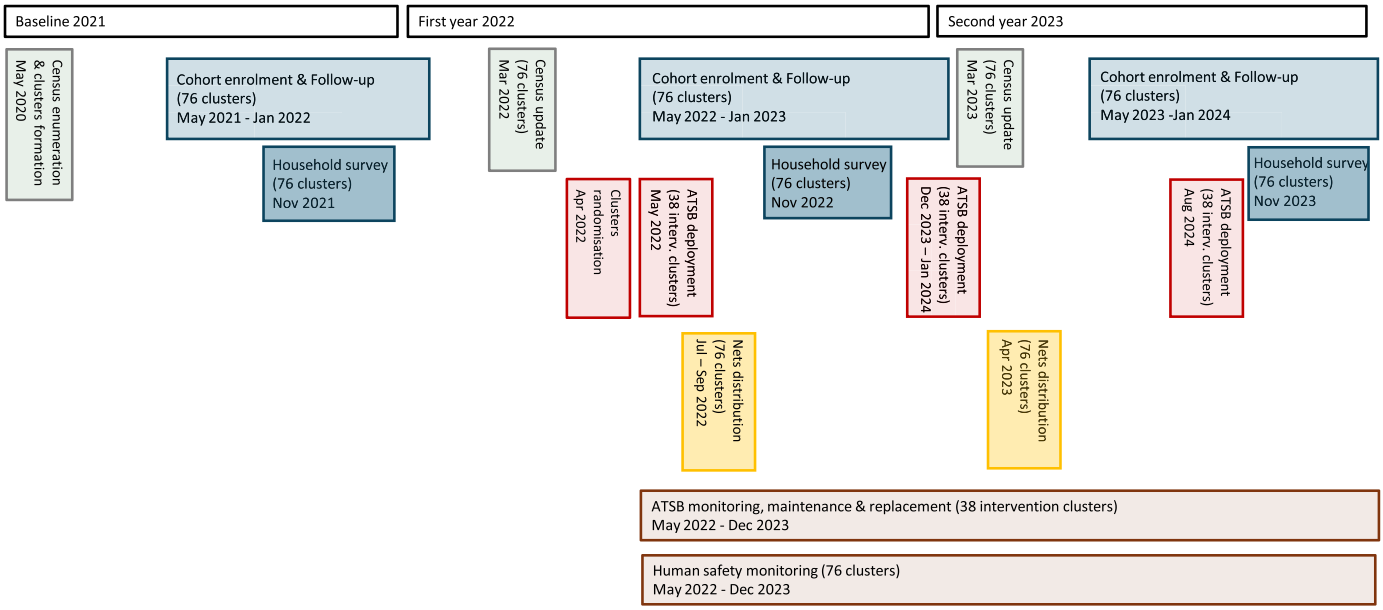

Fig. 1. Overview of data collection in intervention and control trial clusters.

or closest health facility if they had febrile symptoms between follow-up visits, and all cases of confirmed clinical malaria were recorded and combined into the final cohort dataset.

Data on housing characteristics and household assets, including bed net ownership, and individual demographic data on caregivers and enroled children were collected at recruitment. At each follow-

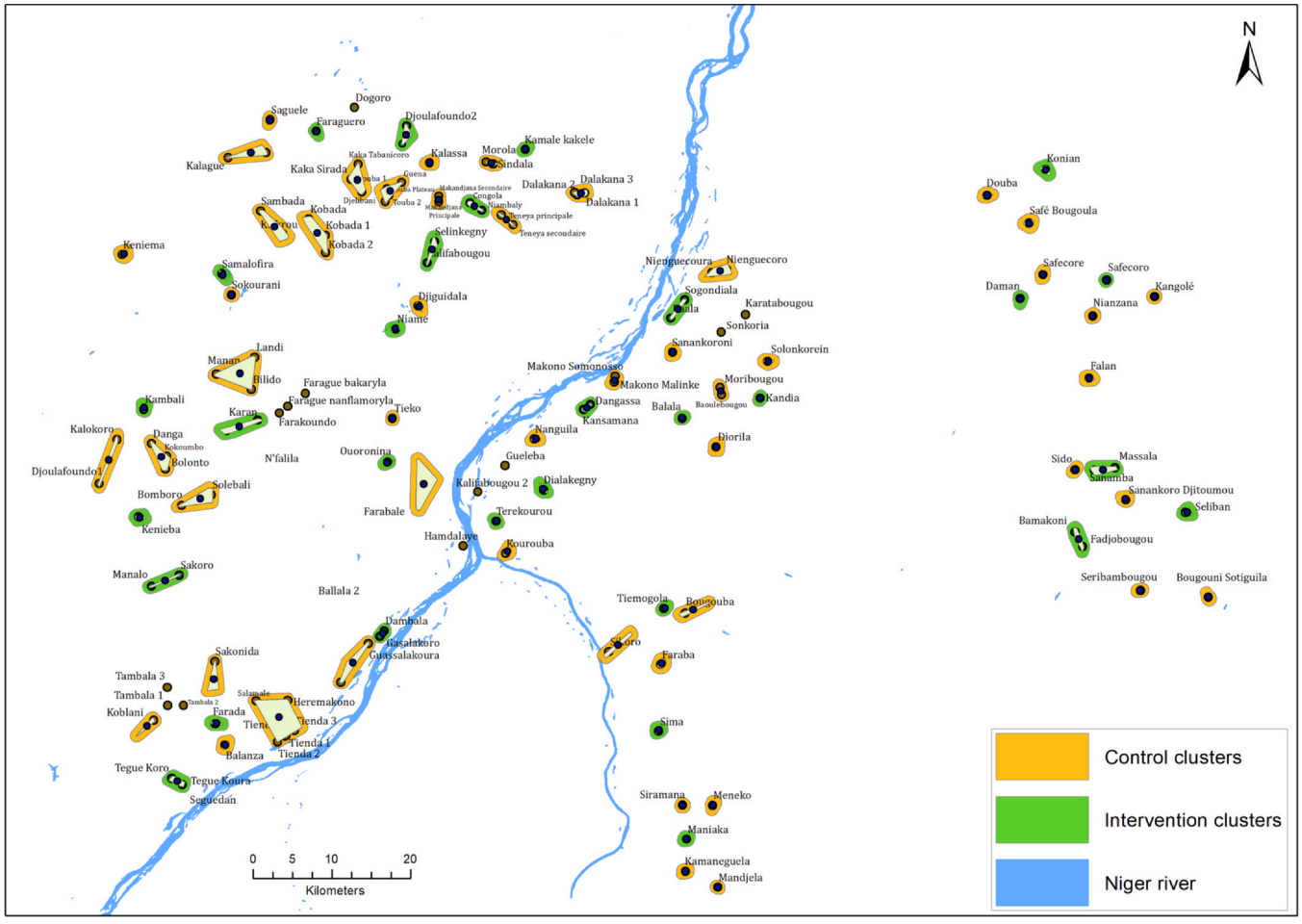

Fig. 2. Map of intervention and control clusters included in the trial.

up visit, the caregiver was asked about the child's use of a bed net the night prior to the interview, and the child's travel history during the past month. The number and condition of bait stations on the external walls of the child's sleeping building were observed at each visit. The condition of bait stations was recorded using a standardised checklist, which assessed whether bait stations were damaged with tears, leak, mould growth, depletion, or dirt (Supplementary material, Table 1).

Household surveys

Cross-sectional household surveys were conducted in November each year, during the peak of the malaria season. A sample of individuals aged 6 months or older were randomly selected in each cluster from population census lists at baseline (2021), year 1 (2022) and year 2 (2023). Malaria infection (secondary outcome) was measured by testing consenting individuals using RDT.

A structured questionnaire, adapted from standard Roll Back Malaria indicator surveys,[22] was used to collect household and individual data. Household heads were interviewed about ownership of assets and housing characteristics for calculating an asset-based socio-economic status score (SES); and were asked to provide a listing of all household members and all bed nets in the household for estimating universal coverage of bed nets. Household members were questioned on the use of a bed net the night prior to the interview; the uptake of SMC in children under-5; care seeking for fever in the two weeks prior to the interview; and their travel history during the past month. In years 1 and 2, the number and condition of bait stations on the external walls of sleeping buildings were observed at sampled houses. Condition of bait stations was recorded using a standardised checklist, and household heads were asked whether they saw insects other than mosquitoes, or rodents or birds resting or feeding on the bait stations.

Data quality assurance

All fieldworkers were trained in survey objectives, methods and standard operating procedures, including obtaining informed consent and preserving participant confidentiality and privacy during interviews. When a selected participant was absent, up to three revisits were attempted. Interviews were recorded using electronic devices, thus facilitating continuous data monitoring.

Human safety monitoring

Bait stations safety was assessed through the household surveys by asking household members in both trial arms whether they experienced a set of symptoms in the month prior to the interview, whether they sought care, and whether they were hospitalised for any reason.

Sample size

The trial was designed to detect 30% (or greater) reduction in clinical malaria incidence and malaria infection prevalence between study arms. For malaria incidence, a sample of 3648 children in 38 clusters per arm (48 per cluster) and 3890 person-years of follow-up was required to give 88% power, assuming a baseline malaria incidence of 0.4 cases per person-year, 8 months follow-up per year (16 months over 2 years), and a 20% loss to follow-up. For malaria infection prevalence, a sample of 2660 individuals (35 per cluster) per cross-sectional survey was required to give 92% power, assuming a baseline malaria infection prevalence of 50%, and a non-response of 20%. For both outcomes, a coefficient of variation between clusters of 0.40 was assumed.

Ethics

The trial was approved by the Institutional Review Boards of the University of Bamako (N°2021/124/CE/USTTB) and the London School of Hygiene and Tropical Medicine (Reference 17283-1). Participants aged 18 years or more provided informed written consent. Parental consent was sought for participants aged less than 18 years and participants aged 12 to 17 years were asked to assent. Children enroled in the cohorts and household survey participants who were found RDT positive were treated according to national guidelines. Participants safety and adherence to the trial protocol were overseen by an independent data safety monitoring board (DSMB). The trial is registered at ClinicalTrials.gov (NCT04149119).

Statistical analysis

Primary analysis was performed as intention-to-treat (ITT), and unless specified otherwise, on individual data.

The incidence rate (IR) of clinical malaria was calculated in each trial arm using cohort data, and for comparison between trial arms an Incidence Rate Ratio (IRR) was computed using random effect Poisson regression, accounting for correlated observations at the cluster level. Children with 2 or more consecutive missing visits were excluded from the analysis (82 children in year 1 and 30 in year 2), and 4 weeks person-time were deducted when a single follow-up visit was missed (10 person-years in year 1 and 4 in year 2). Cohort members were regarded as not at risk for two weeks after treatment with an anti-malarial drug, resulting in the loss of 51 and 46 person-years in years 1 and 2, respectively. A participant testing positive on consecutive occasions less than 2 weeks apart was counted as one case (15 occurrences in year 1 and 12 in year 2).

Malaria infection prevalence was calculated in each trial arm using cross-sectional data, and for comparison between arms, an Odds Ratio (OR) was computed using random-effect logistic regression, accounting for correlated observations at the cluster level.

Subgroup analyses were performed to test for an association between bait station coverage or bait station density and malaria incidence or malaria infection prevalence. Coverage and density estimates were computed as explained below, and were fitted as ordered categorical variables in regression models (instead of trial arm), keeping control clusters as the comparison group. Departure from linearity was tested using the Likelihood Ratio Test. Using cohort data, ATSB coverage was computed for each cluster for each follow-up visit during the malaria season as the proportion of child sleeping buildings with at least 2 bait stations (in any or in good condition). Using household cross-sectional survey data, cluster-level coverage estimates were computed as the proportion of sleeping buildings with at least 2 bait stations (in any or in good condition) in each cluster. Using geographic information systems (GIS) the number of bait stations deployed in a 1 hectare circle around each sleeping structure was calculated as a measure of the local density of bait stations around a building. The average bait station density for each cluster was calculated as the total number of bait stations deployed in a cluster divided by the area of the cluster, estimated by GIS. Bait station density estimates were based on ATSB deployments in May 2022 for year 1 and August 2023 for year 2.

All secondary analyses of incidence and prevalence were adjusted for baseline cluster-level malaria incidence rates or prevalence respectively and for potential confounding factors (household SES, individual age and sex, bed net use the night prior to the interview and distance to the Niger river). The SES was computed using Principal Component Analysis of 26 household ownership and housing characteristics, and scores were grouped into quintiles for inclusion in regression models.[23]

Results

The average proportion of sleeping buildings with at least 2 bait stations (in any condition) was estimated as 72.8% in year 1% and 83.9% in year 2 household surveys, and 73.1% in year 1% and 78.4% in year 2 cohort monitoring (Table 1a). Coverage of bait stations in good condition was 20.2% and 44.4% in years 1 and 2 from household

Table 1a
Bait station coverage: proportion of sleeping buildings with at least two bait stations in any condition & in good condition (household surveys & cohorts data).

	Two bait stations in any condition			Two bait stations in good condition*		
	N	%	Range between clusters	N	%	Range between clusters
Cohort May 2022–Jan 2023**	1678	**73.1**	37.4–93.2	1638	**38.0**	2.9–88.5
Household survey Nov 2022	2909	**72.8**	19.0–98.7	2378	**20.2**	0–91.7
Cohort May 2023–Jan 2024**	1630	**78.4**	52.2–90.0	1605	**50.7**	4.7–84.8
Household survey Nov 2023	2918	**83.9**	37.9–100	2473	**44.4**	0–89.8

* Bait stations in good condition were observed without any of the following: 1+ cells completely torn; leak; 5+ cells with mould or 1+ cell with mould larger than the end of a pencil; 8+ cells depleted; 8+ cells dirty.
** Average across all follow-up visits.

Table 1b
Damages observed on bait stations deemed as not in good condition (household surveys & cohorts data).

% of bait stations observed with:	Cohort year 1 (N = 22,802) %	Household survey year 1 (N = 3882) %	Cohort year 2 (N = 23,338) %	Household survey year 2 (N = 4152) %
1+ cell(s) completely torn	14.1	8.9	2.6	2.3
A leak	31.3	36.8	21.3	26.3
5+ cells with mould growth	10.5	17.8	1.3	5.1
1+ spot(s) of mould growth larger than the end of a pencil	10.1	14.7	3.0	7.4
8+ cells depleted	12.3	25.8	7.1	8.8
8+ cells dirty	13.0	27.6	6.3	6.8

Table 1c
Proportion of household heads who reported observing other insects, birds or rodents feeding or resting on bait stations (household surveys data).

% of household heads who reported observing:	Household survey year 1 (N = 1108) %	Household survey year 2 (N = 1098) %
Other insects resting/feeding on the bait stations	**29.6**	**14.3**
Bees	3.6	2.9
Ants	2.0	1.9
Butterflies/moths	19.5	4.1
Cockroaches	1.7	0.9
Houseflies	17.9	10.5
Rodents or birds resting/ feeding on the bait stations	**0.1**	**0.0**
Rats/mice	0.1	0.0
Birds	0.1	0.0

Table 2
Bait station density (deployment data).

	N	Mean	SD	Min	Max	Median
Year 1 (May 2022)						
Building-level density[1]	15,242	**46.8**	29.3	0.0	183.0	42.0
Cluster-level density[2]	38	**30.8**	23.6	6.0	66.6	23.6
Year 2 (Aug 2023)						
Building-level density[1]	15,242	**40.5**	25.5	0.0	145.0	36.0
Cluster-level density[2]	38	**27.4**	14.5	7.2	68.7	23.9

[1] Number of bait stations per hectare around sleeping buildings.
[2] Number of bait stations per hectare of cluster.

surveys, and 38.0% and 50.7% in years 1 and 2 from cohort monitoring. Variation across months of cohort follow-up is reported in Supplementary material (Fig. 1). For about a third of bait stations surveyed, a leak was observed (Table 1b). Other damage such as mould growth, tear, depletion or dirt was less frequent, particularly in year 2 (Supplementary material, Fig. 2). Household heads reported other insects (mainly houseflies, but also butterflies and moths) resting or feeding on bait stations, and only 1 resident 1 reported seeing a rodent attempting to feed on a bait station (Table 1c).

The mean bait station density around sleeping buildings in year 1 was 47 bait stations per hectare, and 41 per hectare in year 2, ranging from 0 to 183 in year 1 and 0 to 145 in year 2. Mean cluster-level density of bait stations was 31 per hectare in year 1 (range 6 to 67) and 28 per hectare in year 2 (range 7 to 69) (Table 2).

Use of bed nets was consistently high across both study arms and study years, with on average 95% of respondents reporting to have used a net the night before the interviews (Supplementary material, Table 6).

There was no evidence of ATSB implementation in control clusters, with the exception of one cluster in which bait stations were observed on 18/86 sleeping buildings during the household survey in year 1. Fewer than 1% of individuals reported having spent at least one night away from their villages the month prior to the household survey (Supplementary material, Table 6), suggesting a very low risk of spillover between study arms due to human movement.

Clinical malaria incidence

Over two years of deployment of bait stations, the mean clinical malaria incidence rate was 0.726 cases per person-year in the control arm and 0.660 cases per person-year in the intervention arm with no statistical evidence for a difference between arms (IRR = 0.90; 95%CI 0.77, 1.05; p = 0.188) (Table 3). Findings were similar in years 1 and 2 of the trial. Adjusting for baseline incidence, household wealth quintiles, child's sex and age, and other malaria risk factors (bed net use and distance to the Niger river) did not change the overall effect estimate.

There was evidence that the intervention was effective for coverage of bait stations in good condition above 80% (Table 4). For both years combined and controlling for month of follow-up, baseline cluster level incidence, household SES, child's sex and age, and other malaria risk factors, there was a 26% reduction in malaria incidence in clusters with coverage above 80% of bait stations in good condition compared to control clusters (adjusted IRR = 0.74; 95%CI 0.61, 0.90; p = 0.002). The incidence rate decreased by 5% per 20% increase in coverage of bait stations in good condition (adjusted linear trend per 20% coverage IRR = 0.95; 95%CI 0.92, 0.98; p = 0.003). Results by trial year are shown in Supplementary material (Table 2).

There was weak evidence that clinical malaria incidence was 15% lower in children sleeping in buildings with 45 or more bait stations deployed in their one-hectare vicinity (local density) compared to control clusters (adjusted IRR = 0.85, 95%CI 0.71, 1.02, p = 0.086) (Table 4). There was no statistical evidence of a trend of lower

Table 3
Clinical malaria incidence rate (cohorts data).

					Unadjusted analysis				Adjusted for baseline cluster level incidence				Adjusted for baseline cluster level incidence & malaria risk factors*			
	N	PY	Cases	IR/PY	95%CI		IRR	95%CI		P-value	aIRR	95%CI		P-value	aIRR	95%CI		P-value
Year 1 (Jul 2022–Jan 2023)																		
Control arm	1493	869	697	**0.802**	0.700	0.923	**1.00**	-	-	-	**1.00**	-	-	-	**1.00**	-	-	-
Intervention arm	1491	866	643	**0.742**	0.657	0.841	**0.92**	0.77	1.10	0.359	**0.92**	0.77	1.10	0.358	**0.92**	0.78	1.10	0.370
Year 2 (Jun 2023–Jan 2024)																		
Control arm	1474	984	649	**0.659**	0.577	0.756	**1.00**	-	-	-	**1.00**	-	-	-	**1.00**	-	-	-
Intervention arm	1475	982	577	**0.588**	0.510	0.681	**0.89**	0.74	1.08	0.246	**0.89**	0.73	1.08	0.234	**0.89**	0.74	1.07	0.218
Years 1 & 2																		
Control arm	2967	1854	1346	**0.726**	0.645	0.820	**1.00**	-	-	-	**1.00**	-	-	-	**1.00**	-	-	-
Intervention arm	2966	1848	1220	**0.660**	0.595	0.735	**0.90**	0.77	1.05	0.188	**0.90**	0.77	1.05	0.183	**0.91**	0.78	1.05	0.197

N: Number of children included in the analysis; PY: Person Years; IR: Incidence Rate; IRR: Incidence Rate Ratio; aIRR: IRR adjusted for baseline incidence.
* Household wealth quintiles, sex, age, bed net use the night prior to the interview, household distance to the Niger river.

malaria incidence with higher mean cluster density of bait stations, although point estimates are compatible with a trend. Results by trial year are shown in Supplementary material (Table 3).

Malaria infection prevalence

Combining data from both cross-sectional surveys, there was no evidence that malaria infection prevalence differed between study arms (OR = 0.96; 95%CI 0.76, 1.21; p = 0.729) (Table 5). Findings were similar in years 1 and 2 of the trial, and were unchanged after adjusting for baseline prevalence, household wealth quintiles, child's sex and age, and other malaria risk factors. There was no evidence that malaria infection prevalence was associated with bait station coverage or density (Table 6). Results by trial year are shown in Supplementary material (Tables 4 & 5).

Bait station safety

The proportions of household members who reported one or more symptoms that were screened for in the survey and sought care for them were balanced between trial arms: around 20% or less reported having experienced one or more symptoms in the month prior to the interview, around 10% or less reported having sought care as a result, and less than 1% reported having been hospitalised for any reason (Table 7, Supplementary material, Fig. 3). Twenty-one deaths (by any cause) were recorded across communities living in trial clusters, 9 in the control arm (53,115 inhabitants) versus 12 deaths in the intervention arm (48,695 inhabitants) (p = 0.404). Among cohort members, 3 children died, all in the intervention arm (causes of death recorded at the health facility were heart failure, nasal and oral bleeding, and unknown). All cohort deaths were investigated and reported to the Mali ethics committee and the trial DSMB. None were deemed related to the intervention.

Discussion

This cluster randomised trial, conducted over two transmission seasons in Southwestern Mali, evaluated the efficacy and safety of ATSB plus universal coverage of ITN, compared with universal coverage of ITNs alone. The overall finding from ITT analysis showed no evidence that ATSB provided additional protection against malaria in addition to using ITNs, assessed from malaria incidence in cohort follow-up, and from infection prevalence in cross-sectional surveys. The trial was powered to detect reductions of 30% (or more) in the intervention arm for both these outcomes. Despite a 10% reduction in the point estimate of clinical malaria incidence in the intervention arm compared to the control arm (IRR = 0.90; 95%CI 0.77, 1.05; P = 0.188), there was no evidence of an effect large enough that the trial could detect. There was no indication that people reduced their use of bed nets as a result of ATSB deployment in the intervention arm, with reported use remaining very high in both trial arms. Safety monitoring suggested no safety concerns related to bait stations. Similar findings were found from the CRCT conducted in Zambia (IRR = 0.91; 95%CI 0.72, 1.15; P = 0.42).[16]

There was strong evidence of a reduction in malaria incidence when ATSB coverage was above 80% and bait stations were in good condition (adjusted IRR = 0.74; 95%CI 0.61, 0.90; P = 0.002). This evidence is based on a subgroup that had not been randomly selected and may therefore be the result of residual confounding due to factors not fully adjusted for. It is nevertheless possible and plausible that the intervention may be effective at high levels of coverage of well-maintained bait stations. The deployment strategy of two ATSB per structure in which people sleep was based on earlier entomological studies.[15] Three stations per building were associated with the highest feeding rate (39% of females dye marked by ASB), but did not significantly differ from feeding rates for two stations

Table 4
Associations of clinical malaria incidence rate with bait stations coverage (4a & 4b) and bait stations density (4c & 4d) (cohorts data).

	Total cluster-months	Cases	PY	IR/PY	95%CI		IRR	95%CI		P-value	Adjusted IRR*	95%CI		P-value
4a. Cluster-month level coverage: % of child's sleeping buildings w/ 2+ bait stations per cluster & month of follow-up (average over years 1 & 2)														
Control clusters	570	1346	1854	**0.726**	0.645	0.820	**1.00**	-	-	-	**1.00**	-	-	-
Less than 80%	194	412	612	**0.674**	0.549	0.838	**0.90**	0.75	1.07	0.234	**0.96**	0.81	1.15	0.676
At least 80%	376	808	1236	**0.653**	0.586	0.731	**0.90**	0.77	1.06	0.216	**0.88**	0.75	1.02	0.088
Change in IR per 20% increase in coverage ✦							**1.00**	0.97	1.03	0.995	**0.98**	0.96	1.01	0.223
4b. Cluster-month level coverage: % of child's sleeping buildings w/ 2+ bait stations *in good condition* per cluster & month of follow-up (average over years 1 & 2)														
Control clusters	570	1346	1854	**0.726**	0.645	0.820	**1.00**	-	-	-	**1.00**	-	-	-
Less than 80%	433	972	1399	**0.695**	0.614	0.790	**0.94**	0.80	1.09	0.410	**0.96**	0.83	1.11	0.563
At least 80%	136	243	446	**0.545**	0.470	0.638	**0.78**	0.64	0.94	0.011	**0.74**	0.61	0.90	0.002
Change in IR per 20% increase in coverage ✦							**0.96**	0.93	0.99	0.014	**0.95**	0.92	0.98	0.003
	Total clusters	Cases	PY	IR/PY	95%CI		IRR	95%CI		P-value	Adjusted IRR*	95%CI		P-value
4c. Local density - average number of bait stations per hectare around sleeping building (average over years 1 & 2)‡														
Control clusters	-	1346	1854	**0.726**	0.645	0.820	**1.00**	-	-	-	**1.00**	-	-	-
< 30 bait stations/ hectare	-	246	353	**0.697**	0.599	0.815	**0.94**	0.78	1.14	0.540	**0.93**	0.77	1.13	0.460
[30–45] bait stations/hectare	-	176	243	**0.726**	0.580	0.919	**1.03**	0.84	1.27	0.787	**1.03**	0.84	1.27	0.773
45+ bait stations/ hectare	-	289	466	**0.621**	0.547	0.707	**0.86**	0.71	1.03	0.102	**0.85**	0.71	1.02	0.086
Change in IR per additional 15 bait stations/hectare ✦							**0.97**	0.93	1.01	0.149	**0.97**	0.93	1.01	0.157
4d. Cluster-level density - average number of bait stations per hectare within cluster (average over years 1 & 2)														
Control clusters	76	1346	1854	**0.726**	0.645	0.820	**1.00**	-	-	-	**1.00**	-	-	-
< 30 bait stations/ hectare	47	778	1142	**0.681**	0.593	0.787	**0.93**	0.79	1.11	0.427	**0.93**	0.79	1.09	0.374
[30–45] bait stations/hectare	17	251	421	**0.596**	0.491	0.726	**0.87**	0.68	1.09	0.227	**0.90**	0.71	1.12	0.340
45+ bait stations/ hectare	12	191	284	**0.672**	0.544	0.836	**0.84**	0.65	1.08	0.167	**0.83**	0.65	1.06	0.129
Change in IR per additional 15 bait stations/hectare ✦							**0.97**	0.92	1.02	0.248	**0.97**	0.92	1.02	0.263

Panels a and b: *Adjusted for month of follow-up, baseline cluster level incidence, household wealth quintiles, sex, age, bed net use the night prior to the interview, household distance to the Niger river.
Panel a: ✦ Departure from linearity P-value = 0.029.
Panel b: ✦ Departure from linearity P-value = 0.124.
Panels c and d: *Adjusted for month of follow-up, baseline cluster level incidence, household wealth quintiles, sex, age, bed net use the night prior to the interview, household distance to the Niger river.
Panel c: ‡Density data is missing for 39.6% (590/1491) of children in year 1; 45.2% (666/1475) of children in year 2.
Panel c: ✦ Departure from linearity P-value = 0.418.
Panel d: ✦ Departure from linearity P-value = 0.089.

Table 5
Malaria infection prevalence (household surveys data).

					Unadjusted analysis				Adjusted for baseline cluster-level prevalence				Adjusted for baseline cluster-level prevalence & malaria risk factors*			
	N	P (%)	95%CI		OR	95%CI		P-value	aOR	95%CI		P-value	aOR	95%CI		P-value
Year 1 (Nov 2022)																
Control arm	1102	**34.2**	30.2	38.4	**1.00**	-	-	-	**1.00**	-	-	-	**1.00**	-	-	-
Intervention arm	1103	**34.5**	30.8	38.3	**1.00**	0.78	1.29	0.973	**1.05**	0.84	1.30	0.684	**1.03**	0.82	1.31	0.785
Year 2 (Nov 2023)																
Control arm	1120	**40.0**	35.5	43.0	**1.00**	-	-	-	**1.00**	-	-	-	**1.00**	-	-	-
Intervention arm	1155	**38.4**	32.8	44.2	**0.91**	0.65	1.28	0.604	**0.96**	0.71	1.29	0.776	**0.96**	0.69	1.34	0.810
Years 1 & 2																
Control arm	2222	**37.1**	33.7	40.7	**1.00**	-	-	-	**1.00**	-	-	-	**1.00**	-	-	-
Intervention arm	2258	**36.5**	32.7	40.4	**0.96**	0.76	1.21	0.729	**1.00**	0.83	1.21	0.987	**1.00**	0.82	1.23	0.993

N: Number of individuals aged 6 months old and above tested by RDT; P: Malaria infection prevalence; OR: Odds Ratio; aOR: OR adjusted for baseline prevalence.
* Household wealth quintiles, sex, age, bed net use the night prior to the interview, household distance to the Niger river.

(34%). Whether a strategy based on larger numbers of ATSB per building would have been more effective is unknown. More bait stations per house could provide some buffer against low coverage, but the higher costs of such an approach are likely to render it less affordable in the context of the constrained resources that are available for malaria control in most endemic countries.

There was a wide range in the density of deployment of ATSB, reflective of the variation in density of housing in the study area. The data showed no or only very weak evidence that a higher local density of ATSB (≥45 bait stations/hectare) was associated with lower malaria incidence or infection prevalence, despite the plausibility that ATSB may be more likely to be effective at high deployment density. Given the implementation strategy of two ATSB per building in which people sleep at night, it is likely that ATSB density is a proxy for human population density. Higher concentrations of people may attract more blood-seeking vectors, and hence, any benefit of higher ATSB density may be cancelled out by higher population density. Higher dwelling density may also be associated

Table 6
Associations of malaria infection prevalence with bait stations coverage (6a & 6b) & bait stations density (6c & 6d) (household surveys data).

	Total clusters	N	P (%)	95%CI		OR	95%CI		P-value	Adjusted OR*	95%CI		P-value
6a. Cluster level coverage - % of sleeping buildings w/ 2+ bait stations at the time of interview (average over years 1 & 2)													
Control clusters	76	2222	**37.1**	33.7	40.7	**1.00**	-	-	-	**1.00**	-	-	-
Less than 80%	32	916	**34.0**	29.7	38.5	**0.79**	0.60	1.04	0.094	**0.83**	0.65	1.07	0.149
At least 80%	44	1342	**38.2**	32.5	44.1	**1.10**	0.85	1.41	0.481	**1.14**	0.90	1.43	0.275
Change in odds of malaria infection per 20% increase in coverage ✦						**1.01**	0.96	1.06	0.594	**1.02**	0.97	1.06	0.478
6b. Cluster level coverage - % of sleeping buildings w/ 2+ bait stations *in good condition* at the time of interview (average over years 1 & 2)													
Control clusters	76	2222	**37.1**	33.7	40.7	**1.00**	-	-	-	**1.00**	-	-	-
Less than 80%	71	2102	**36.6**	33.1	40.2	**0.96**	0.76	1.21	0.704	**0.99**	0.81	1.22	0.960
At least 80%	4	123	**34.1**	12.4	65.5	**1.04**	0.62	1.74	0.894	**1.02**	0.61	1.70	0.937
Change in odds of malaria infection per 20% increase in coverage ✦						**1.07**	0.99	1.14	0.074	**1.04**	0.98	1.11	0.201
6c. Local density - average number of bait stations per hectare around sleeping building (average over years 1 & 2)‡													
Control clusters	-	2222	**37.1**	33.7	40.7	**1.00**	-	-	-	**1.00**	-	-	-
< 30 bait stations/hectare	-	382	**36.6**	31.3	42.4	**0.96**	0.71	1.29	0.772	**0.97**	0.73	1.28	0.826
[30–45] bait stations/ hectare	-	233	**35.6**	29.3	42.4	**0.95**	0.67	1.34	0.767	**1.07**	0.77	1.49	0.687
45+ bait stations/hectare	-	516	**34.1**	29.0	39.6	**0.86**	0.65	1.14	0.306	**1.02**	0.78	1.32	0.909
Change in odds of malaria per additional 15 bait stations/hectare ✦						**0.97**	0.91	1.04	0.372	**1.01**	0.95	1.08	0.747
6d. Cluster-level density - average number of bait stations per hectare within cluster (average over years 1 & 2)													
Control clusters	76	2222	**37.1**	33.7	40.7	**1.00**	-	-	-	**1.00**	-	-	-
< 30 bait stations/hectare	47	1383	**35.1**	30.6	39.8	**0.95**	0.73	1.22	0.666	**0.90**	0.72	1.12	0.334
[30–45] bait stations/ hectare	17	521	**43.4**	37.3	49.6	**1.22**	0.87	1.71	0.245	**1.51**	1.11	2.04	0.008
45+ bait stations/hectare	12	354	**31.6**	23.0	41.8	**0.72**	0.48	1.06	0.094	**0.86**	0.61	1.23	0.413
Change in odds of malaria per additional 15 bait stations/hectare ✦						**0.97**	0.89	1.06	0.520	**1.01**	0.94	1.09	0.791

Panel a: ✦ Departure from linearity P-value = 0.043.
Panel b: ✦ Departure from linearity P-value = 0.357.
Panel c: ‡Density data is missing for 46.5% (513/1103) of children in year 1; for 53.2% (614/1155) of children in year 2.
Panel c: ✦ Departure from linearity P-value = 0.863.
Panel d: ✦ Departure from linearity P-value = 0.011.
* Adjusted for month, baseline cluster level prevalence, household wealth quintiles, sex, age, bed net use the night prior to the interview, household distance to the Niger river.

Table 7
Bait stations safety (household surveys data).

	Control arm		Intervention arm	
	N	%	N	%
One or more symptoms self-reported in the past month*				
Year 1 (Nov 2022)	7709	**22.0**	7563	**24.6**
Year 2 (Nov 2023)	8001	**15.1**	7789	**12.4**
Care seeking in a health facility self-reported in the past month				
Year 1 (Nov 2022)	7709	**10.8**	7563	**10.5**
Year 2 (Nov 2023)	8001	**8.1**	7789	**6.1**

* Includes fever, convulsions, loss of consciousness, diarrhoea, vomiting, dizziness, headaches, conjunctiva, sore mouth or nose, cough, facial oedema, body swelling, rash, body blisters, skin detachment or skin multiple lesions, trauma (e.g. road accident, fall, …).

with lower household wealth, and it is therefore possible that there are residual confounders that we have not been able to adequately control for, that have eclipsed any relationship between ATSB density and malaria incidence and prevalence. Density estimates were computed using deployment data, and were not updated during the deployment period. Thus, density estimates did not necessarily reflect what was in place at the time of household visits, which may have biased estimates of the effect of density towards the null. In Zambia, coverage was higher, 98% in year 1% and 90% in year 2, but due to differences in the nature of settlement patterns, the density of ATSB was lower than in Mali. Subgroup analyses did not provide evidence for an intervention effect.[16]

A question that arises is why this trial showed overall no epidemiological impact of ATSB deployment, in contrast to an earlier entomological study conducted in Mali which showed very high impact of ATSB on entomological indicators such as mosquito density and EIR.[14] One possibility is that the bait stations that were used in this phase-three trial (version 1.2) were not identical to the hand-manufactured prototype devices used in the much smaller earlier trial. A challenge in the design specification of bait stations is the need for striking a balance between using a membrane that securely protects the content of the bait station (active ingredient, attractant and feeding stimulant) in the presence of harsh weather conditions, whilst being sufficiently porous and thin to attract mosquitoes and facilitate feeding. The trial did not include regular testing of the bait stations to assess their potency before replacement. Non-inferiority comparison of the version of ATSB used in this trial with the prototype of the initial studies concluded that the mass-produced version was as efficacious as the initial ones (personal communication).

Another possibility is that suboptimal coverage may have limited the impact of ATSB on malaria burden and that better maintenance of high coverage of ATSB in good condition could have led to a positive result, as our findings of impact above 80% coverage hint at. Scaling up ATSB can, however, be logistically challenging. Effective deployment may require monitoring and replacement of stations across large and often remote areas, to ensure they remain attractive and lethal to mosquitoes. In this trial, damages were relatively frequently observed on bait stations during household visits and they likely impacted the bait stations efficacy. Successful implementation also depends on community buy-in. Only 45% and 55% of household heads interviewed in years 1 and 2 surveys said they would install or recommend to install ATSB in the future (data not tabulated).

Approximately two-thirds of children enroled in the cohorts were reported to be attending school. Entomological studies elsewhere have shown that indoor biting continues well into the day.[24] Since children attending school are generally not protected against daytime biting, it is possible that their attendance at school diluted the protective effect of ATSB, which was limited to their home environment. A recent review highlighted the importance of incorporating vector control approaches for school-age children.[25]

Last, Muller et al. demonstrated that the presence of natural sugar sources can delay the impact of ATSB on mosquitoes.[26] The study setting of this trial is an area of scarce vegetation cover, and

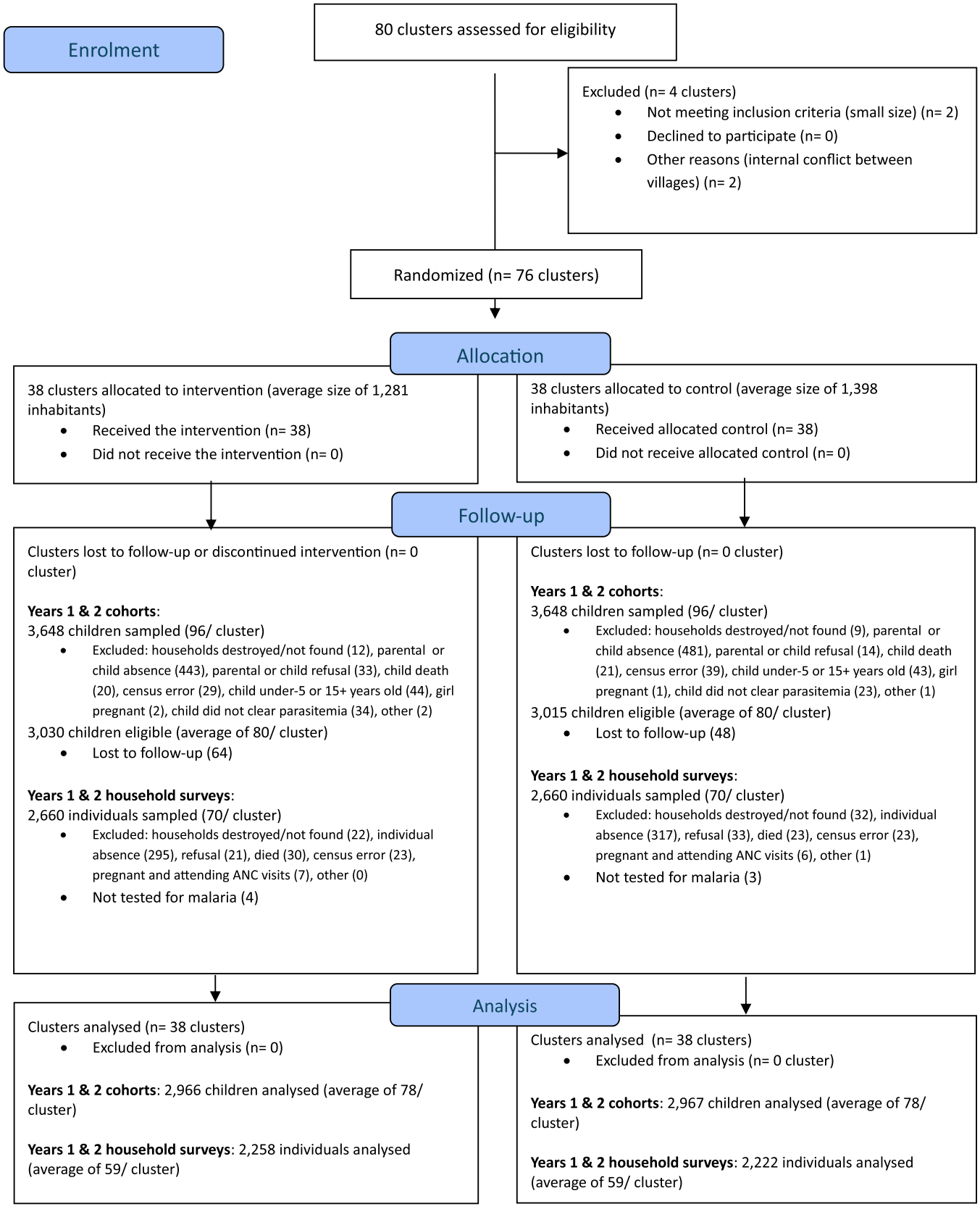

Fig. 3. CONSORT flow diagram of the progress of clusters and individuals.

the extent to which competing sugar sources may have reduced the efficacy of ATSB is unknown. However, Yalla et al. recently conducted a study in western Kenya, where mosquitoes have a wide range of sugar resources than in the Mali setting, and bait stations were found to be more attractive to local *Anopheles* mosquitoes than natural sugar sources, indicating that ATSB may be able to compete even in such environments.[27]

Study strengths and limitations

The lower than prescribed ATSB coverage and variability in implementation across clusters are significant limitations which might have led to the overall negative result of this trial. The finding of impact at higher levels of coverage may be subject to residual confounding since clusters were not randomised to different levels of coverage. The large proportion of missing data for the "local" ATSB density estimates may have limited our ability to detect a dose-response relationship with bait station density.

The study strengths, on the other hand, include validated assumptions that were used in sample size and power calculations. The between-cluster coefficient of variation was estimated between 0.30 and 0.45 in post-intervention control arm data, whereas a value of 0.4 was assumed in the trial power calculations. Since sample size requirements for cohorts and household surveys were achieved throughout, and the trial had adequate power for what it was designed to detect. It is also improbable that the negative findings were the result of contamination or treatment misallocation between study arms. The clusters were geographically well separated with an average distance of 3416 m between them (ranging from 1169 to 9495). Only a small number of buildings in one control cluster were found with bait stations (year 1 only), and participants' responses to the household surveys suggested very low human migration within the study area. Lastly, our findings can only be generalised to the specific ATSB product tested in this study and similar ecological and environmental contexts.

Conclusion

ATSB stations in sub-Saharan Africa represents an innovative tool in addressing the outdoor transmission of malaria, which remains a major challenge in many countries including Mali.[2–5] An earlier entomological trial showed a very high impact of ATSB on entomological indicators,[14] but in this large-scale epidemiological trial, we did not find evidence of an overall effect large enough that the trial could detect. Implementation challenges for maintaining high coverage might explain the lack of evidence for an effect on clinical malaria incidence and infection prevalence. Secondary analyses showed evidence for an effect on malaria incidence in clusters with coverage above 80% of buildings with at least 2 ATSB in good condition compared to control clusters. Although this finding is subject to caveats, it is possible that the intervention may be effective at high levels of coverage of well-maintained bait stations.

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Authors contributions

IK, SD, JB and MaT conceived the evaluation design. SS, MaT and AD developed the detailed plans for the fieldwork and designed the data collection instruments for the epidemiological trial outcomes. MaT, AD, HC and AZA implemented and supervised the fieldwork. MoT provided deployment data and MK produced density estimates. COT coordinated the trial, oversaw the trial's finances, human resources and logistics. IK, SS and JB developed the analysis strategy, and SS analysed the data and wrote the first draft. All authors reviewed, made inputs to and approved the final paper. IK and SD are the overall guarantors, and SS is the corresponding author.

Data availability

De-identified data are available from the corresponding author on reasonable request. Following publication of forthcoming secondary analyses of trial data, the de-identified trial dataset will be posted on data repository for long-term curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2025.106524.

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