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ORIGINAL ARTICLE

Targeted Bacillus sphaericus larval in integrated environmental management and Anopheles larval density in southern Ghana: a cluster-randomised controlled trial

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ABSTRACT

BACKGROUND:
Larviciding is recommended where aquatic habitats are scarce, fixed and findable, but controlled evidence from south-eastern Ghana, where outdoor resting and pyrethroid resistance limit indoor tools, is scarce.

OBJECTIVE:
To evaluate the effect of targeted Bacillus sphaericus larviciding, delivered within an integrated environmental management package, on Anopheles larval density and habitat detection.

METHODS:
Randomised controlled trial with Two-arms in Ada West (intervention) and North Tongu (control), randomly selected at district level. Anopheles-positive habitats within 500 m of sampled households (44 per cluster; 132 per arm; three clusters per arm) were surveyed by standard dipping in six rounds between June and December 2024. Intervention habitats received fortnightly B. sphaericus (Vectolex® WDG) from trained community resource persons, with habitat modification, manipulation, and health education. The change in larval density was analysed at the cluster level as a ratio of ratios; habitat detection was modelled with generalised estimation equations.

RESULTS:
The mean number of Anopheles larvae per habitat decreased from 28.6 to 0.5 (98.3%) in intervention clusters and from 125.9 to 68.9 (45.3%) in control clusters; cluster-level reductions were 97.7–99.3% and 43.5–48.4%, respectively. The ratio of ratios was 0.026 (95% CI 0.010–0.067; exact one-sided permutation p = 0.05, the smallest attainable). Detection fell from 24.2% to 6.8% in intervention habitats and from 53.0% to 42.4% in control habitats. Detection odds declined by 19% per round in the intervention arm and 3.5% in the control arm (interaction odds ratio 1.19, 95% CI 1.04–1.37). Baseline larval density was 4.4 times higher in the control arm.

CONCLUSION:
Targeted microbial larvae within integrated environmental management were associated with near-complete suppression of Anopheles larvae in treated habitats. With two randomised districts and co-delivered components, the effect cannot be attributed to larviciding alone; larger trials measuring transmission are needed.

KEYWORDS:
Anopheles; Bacillus sphaericus; randomised trial in clusters; Ghana; integrated vector management; larval source management; larviciding; malaria; mosquito control

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INTRODUCTION

Malaria caused an estimated 282 million cases and 610,000 deaths in 2024, and about 94% of cases and 95% of deaths occurred in the World Health Organization (WHO) African Region.[1] Insecticide-treated nets and indoor residual spraying remain the core vector control tools, but both act mainly on mosquitoes that bite and rest indoors. Pyrethroid resistance has been confirmed in 48 of the 53 countries that reported monitoring data between 2020 and 2024,[1] and a growing share of bites is taken outside the hours when people are under nets, which sets a ceiling on what indoor tools can achieve.[2]

The management of larval sources targets the aquatic stages of the vector and is therefore unaffected by the location where adult mosquitoes bite or rest and their resistance to pyrethroids. WHO conditionally recommends larviciding as a supplementary intervention where aquatic habitats are few, fixed and findable, and makes no recommendation on habitat modification or manipulation because the evidence is insufficient.[3,4] A Cochrane review found low-certainty evidence that larviciding reduces malaria incidence and parasite prevalence where habitats are less extensive, but few trials met its inclusion criteria.[5] Microbial larvicides based on *Bacillus thuringiensis* var. *israelensis* (Bti) and *Bacillus sphaericus* are effective at low application rates, safe for non-target organisms and acceptable to communities.[6] Large field trials have since reported marked reductions in adult *Anopheles* density after Bti larviciding in Burkina Faso,[7,8] a 79% reduction in the entomological inoculation rate after combined Bti and *B. sphaericus* larviciding in a cluster-randomised trial in Yaoundé, Cameroon,[9] and large reductions in larval density after drone-delivered larviciding in Rwandan rice fields.[10] In contrast, community-driven larval source management added no measurable benefit to high bed net coverage in a cluster-randomised trial in Malawi.[11] These mixed results have prompted calls to evaluate larval source management in more settings, with attention to local ecology and delivery.[12,13]

The coastal savannah and the lower Volta of South-East Ghana are a strong candidate environment. *Anopheles gambiae* s.l. dominates, productive larval habitats are often created by human activity, such as dug-out wells and irrigation furrows, and habitat presence depends on rainfall and proximity to settlements.[14,15] About four in five resting *An. gambiae* s.l. in Ghanaian surveys were collected outdoors,[16] island communities on the Volta lake have annual entomological inoculation rates of 37 to 100 infective bites per person,[17] and high-intensity pyrethroid resistance has reduced the efficacy of bed nets in southern Ghana.[18,19] Malaria prevalence also rises steeply from Greater Accra to the Volta Region,[20] and the 2023 spillage of the Akosombo Dam flooded communities along the Lower Volta.[21] A digital larviciding programme in Obuasi, Ghana, reduced mosquito numbers, but its effect on malaria could not be determined.[22]

We conducted a randomised trial of a comprehensive environmental management package in the Ada West and North Tongu districts. This report addresses the entomological objective of the trial: to evaluate the effect of targeted larviciding with B. sphaericus, delivered within the package, on the larval density of Anopheles and on the proportion of aquatic habitats with Anopheles larvae in the intervention district relative to the control district. The null hypothesis was that the change in larval density would not differ between arms. The effect on human malaria parasitemia, the primary outcome of the trial, is reported separately.

METHODS

Trial design

This was a parallel, two-arm cluster-randomised controlled trial in which sub-districts were the units of delivery and aquatic habitats were the units of entomological observation. A cluster design was used because larviciding and habitat management act on water bodies shared by whole communities, so households or habitats within a community could not be allocated separately without contamination. It is reported in line with the CONSORT extension for cluster randomised trials and the CONSORT 2025 statement.[23,24] Seven sub-district clusters took part: three in Ada West District (Sege, Bornikope and Anyamamam) and four in North Tongu District (Battor, Juapong, Volo, and Dorfor). To limit contamination across the Volta River and to match administrative

boundaries, randomisation was restricted and stratified by district, so that all clusters in a district received the same allocation. The number of units randomised was therefore two, and district is confounded with trial arm. The trial was not prospectively registered in a WHO-approved public trials registry and no protocol was published prior to enrolment.

Setting

The Ada West District lies in the coastal savannah of the south-eastern Greater Accra Region and is bounded by the Volta estuary, lagoons, coastal wetlands and salt pans. Fishing, irrigated vegetable farming, salt extraction, sand winning, and livestock rearing bring residents into regular contact with aquatic habitats. North Tongu District lies across the Volta River in the Volta Region, shares the bimodal rainfall and riverine hydrology of the intervention district, and was affected by the October 2023 Akosombo Dam spillage.[21] The intervention period ran from July to December 2024.

Eligibility of clusters and habitats

Sub-districts were eligible if they lay wholly within a study district, had at least one aquatic habitat with Anopheles immatures at baseline dipping, were accessible to sanitation teams throughout the rainy season and were managed by a single sub-district health team. Sub-districts sharing a contiguous water body with a sub-district in the other arm were excluded. In each group, all water bodies within 500 m of households sampled for the parasitological survey were georeferenced, and those in which Anopheles immatures were found at initial dipping were classified as productive and entered the surveillance frame. Entomological surveillance covered the three intervention clusters (AWD-SSD, AWD-BSD, and AWD-ASD) and three of the four control clusters (NTD-JPC, NTD-MPC, and NTD-BC), with 44 habitats per cluster. The four control groups were merged because, following the mapping exercise, the number of breeding sites was too small to support a separate analysis.

Interventions

The intervention is described using the TIDieR checklist.[25] Intervention groups received an integrated environmental management package with four components. Targeted larviciding applied B. sphaericus (Vectolex® WDG), now classified as Lysinibacillus sphaericus, every two weeks in habitats identified as productive by larval surveillance, at 0.20 g/m^2. Applications were made by fifteen (15) community resource persons who lived in the cluster, were 18 years or older, and completed a two-day training course on habitat identification, dosing, application, and personal protection, followed by supervised field application before working independently. Habitat modification (filling of holes and depressions, land levelling, construction and repair of drains, and permanent covering of water-holding structures) was carried out by municipal sanitation teams with community labour at the start of the intervention, with remedial work as needed. Habitat manipulation (emptying containers, clearing vegetation, de-silting canals, removing debris and flushing) was carried out fortnightly by community volunteers through existing Environmental Sanitation Day structures. District health promotion officers and volunteers delivered monthly community health education sessions with household contact. Delivery was recorded in fortnightly logs of habitats treated, larvicide used, sanitation activities, and education sessions, and a group was considered adherent if at least 80% of planned activities were delivered.

The control groups continued to receive the standard national malaria package of long-lasting insecticide nets, intermittent preventive treatment during pregnancy, case management, and routine health promotion, with no environmental management activities during the trial. The package was offered to control communities after endline data collection.

Outcomes

The two outcomes reported here were prespecified secondary outcomes of the trial. Anopheles larval density was the number of Anopheles larvae collected per habitat. The density of the Anopheles larvaein a survey, using ten dips with a standard 350 mL dipper per habitat per visit,[26] summarised as the mean per habitat in each group. Anopheles larval detection was the presence of at least one Anopheles larva in a habitat in a survey round. Surveillance consisted of six rounds between June and December 2024, and Round 1 was completed before the first application of larvicide. The larvae were identified as genus on site by body position on the water surface, presence and shape of the respiratory syphon, breathing posture, movement, and

body form; Culex and Aedes larvae were pooled as culicines. A subset was kept for laboratory confirmation. At enrolment, each habitat's type, substrate, vegetation, turbidity, sun exposure, permanence, surrounding land use, distance to the nearest house, and the presence of pupae were recorded.

Sample size

The habitat sample was based on a reduction in the proportion of larva-positive habitats from 50% to 30%, the smallest reduction considered meaningful for biolarvicides,5,6 with two-sided α of 0.05 and 90% power. This required at least 124 habitats per arm; 132 per arm (44 per cluster) were enrolled. The calculation did not include a design effect for clustering of habitats within sub-districts, which was not feasible with the clusters available. No interim analyses were planned or done.

Randomisation, allocation concealment, and blinding

An independent statistician at the University of Port Harcourt, who had no other role in the study, randomly assigned the two district strata to the trial arms using a computer-generated sequence with a prespecified seed, and kept the sequence in a sealed envelope until the trial inception meeting. Clusters had been enrolled and households enumerated before the allocation was disclosed. Because the intervention was visible, communities and implementers could not be blinded. Larval samples carried coded identifiers, laboratory technicians did not know the arm, and the primary analyses were carried out with arms labelled A and B.

Statistical analysis

The characteristics of the habitat at enrolment were described by arm as numbers and percentages, without significance tests.[23] Because only three groups per group contributed entomological data, the main analysis of the larval density was carried out at the cluster level, the approach recommended when the groups are small.[27–29] For each group we calculated the ratio of the mean number of Anopheles larvae per habitat in Round 6 to that in Round 1. The natural logarithms of these ratios were compared between arms with a two-sample t-test on four degrees of freedom, and the exponentiated difference in means gave the ratio of ratios with its 95% confidence interval (CI). One minus the ratio of ratios estimates the proportional reduction in larval density in the intervention arm beyond that in the control arm. The statistical analysis plan named negative binomial regression for larval counts; the cluster-level analysis was used instead because habitat-level models would treat the 132 habitats in each arm as independent of their clusters. An exact permutation test was run over all 20 possible allocations of the six clusters to two groups of three. The smallest attainable one-sided p value is therefore 1/20 = 0.05, and, because the actual randomisation was of districts rather than clusters, the permutation result is descriptive.

For larval detection, proportions in each round were reported with Wilson 95% CIs and compared with Pearson's χ^2 test. Generalised estimating equations (GEE) with a logit link, robust standard errors and an exchangeable working correlation accounted for repeated visits to the same habitat. Model 1 included arm and round (coded 0 to 5) as a continuous term; Model 2 added an arm-by-round interaction; Model 3 treated round as categorical. Because detection already differed between arms at Round 1, the interaction term, which compares the change in detection over time between arms, was taken as the estimate of intervention effect, and was also expressed as the ratio of the change in detection odds between Round 1 and Round 6 (exp[-5β]). Models were compared with the quasi-likelihood information criterion (QIC), and the arm effect was refitted under independence and first-order autoregressive working correlations, because correlation structures in longitudinal vector control trials are often complex.30 The GEE models treat the habitat, not the sub-district, as the unit of clustering, so their CIs do not reflect variation between clusters. Analyses used R version 4.4.1 and IBM SPSS Statistics version 27; cluster-level estimates and CIs were recomputed in Python 3. Secondary outcomes were not adjusted for multiplicity, and two-sided $p < 0.05$ was taken as statistically significant.

Ethical considerations

The University of Port Harcourt Research Ethics Committee (UPH/CEREMAD / REC/MM119/089) approved the trial. The district health administrations, District Assemblies and traditional leaders of every

enrolled cluster gave permission, and community meetings were held in each cluster before implementation; communities retained the right to refuse cluster-level activities. Participants in household surveys gave their informed written consent, with a witnessed thumbprint for those unable to read. The applicators were trained and supplied with protective equipment, and the communities were informed in advance about the larvicide applications. Larvicide-related harms (skin, eye, or respiratory irritation in applicators or residents, and effects on livestock, fish, or domestic water use) were recorded on a standard form at each application round. The trial followed the Declaration of Helsinki.

RESULTS

Flow of clusters and habitats

Both districts and all seven sub-district clusters were enrolled and analyzed according to allocation (Fig. 1). The 264 habitats in the entomological frame, 132 per arm, were each visited in all six rounds, giving 1584 habitat-visits (792 per arm), and no habitat was excluded from analysis.

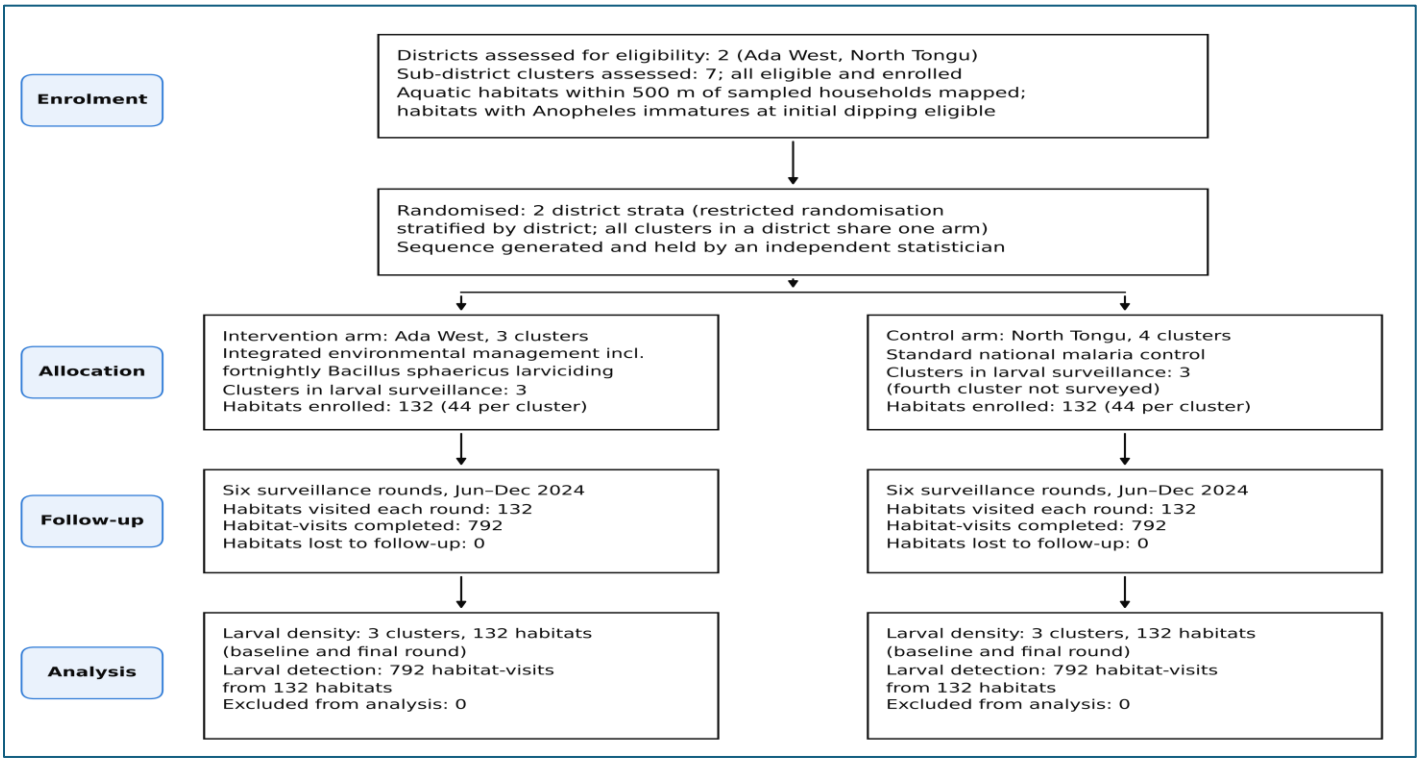


Figure 1. Flow of districts, clusters, and aquatic habitats through the trial. Randomisation was restricted and stratified by district, so the two districts were the units randomly selected. *Entomological surveillance covered three clusters in each arm.*

Habitat characteristics at Enrolment

Substrate, presence of vegetation, turbidity, sun exposure, permanence, and surrounding land use were broadly similar between arms, but habitat types differed (Table 1). Sand-winning pits (38.6% vs 17.4%), swampy areas (22.7% vs 15.2%) and stream fringes (13.6% vs 3.8%) were more common in the intervention arm, and drains or ditches (3.0% vs 22.0%) and tyre tracks (6.1% vs 13.6%) in the control arm. Submersed vegetation predominated in vegetated intervention habitats (63.2%) and emergent vegetation in control habitats (75.9%). At the baseline survey, 23 701 larvae were collected, of which 20 398 (86.1%) were *Anopheles*. Control habitats contributed 79.9% of all larvae, and mean *Anopheles* density was 125.9 larvae per habitat in the control arm against 28.6 in the intervention arm, a 4.4-fold difference.

Table 1. Characteristics of the aquatic habitats surveyed at enrolment, by trial arm

Characteristic	Intervention (n = 132)	Control (n = 132)
Habitat type		
Sand-Winner Pit	51 (38.6)	23 (17.4)
Swampy area	30 (22.7)	20 (15.2)
Stream fringe	18 (13.6)	5 (3.8)
Pond	12 (9.1)	20 (15.2)
Pool	9 (6.8)	17 (12.9)
Tyre track	8 (6.1)	18 (13.6)
Drain or ditch	4 (3.0)	29 (22.0)
Substrate		
Muddy	67 (50.8)	65 (49.2)
Sandy	65 (49.2)	67 (50.8)
Aquatic Vegetation	87 (65.9)	87 (65.9)
Vegetation type[a]		
Submerged	55 (63.2)	21 (24.1)
Emergent	32 (36.8)	66 (75.9)
Turbid water	81 (61.4)	70 (53.0)
Direct sun exposure	61 (46.2)	69 (52.3)
Permanent water body	58 (43.9)	66 (50.0)
Surrounding land use		
Cultivated land or roadside	76 (57.6)	65 (49.2)
Grassland	56 (42.4)	67 (50.8)
Within 200 m of the nearest house	52 (39.4)	63 (47.7)
Pupae present	88 (66.7)	96 (72.7)
Larvae collected at the baseline surveyb		
Anopheles, n (% of larvae)	3780 (79.4)	16 618 (87.7)
Culicines, n (% of larvae)	983 (20.6)	2320 (12.3)
Mean *Anopheles* larvae per habitat	28.6	125.9
Habitats with *Anopheles* larvae	32 (24.2)	70 (53.0)

Values are the numbers of habitats (%) unless stated otherwise. No significance tests were performed, in line with CONSORT guidance for randomized trials.

[a]Percentages of the 87 habitats with aquatic vegetation in each arm.

[b] Round 1 survey. The culicines are Culex and Aedes larvae, which were not counted separately.

Anopheles larval density

Between Round 1 and Round 6, the total number of Anopheles larvae in the intervention groups fell from 3780 to 63, and the mean density from 28.64 to 0.48 larvae per habitat, a reduction of 98.3% (Table 2; Fig. 2). Reductions in the three intervention clusters were 97.7%, 98.3% and 99.3%. In control clusters, total larvae fell from 16 618 to 9097 and mean density from 125.89 to 68.92 larvae per habitat (45.3%), with cluster reductions of 48.4%, 43.5% and 43.8%. The cluster-level ranges did not overlap. The ratio of ratios was 0.026 (95% CI 0.010–0.067; t = −10.56, 4 degrees of freedom, $p < 0.001$), corresponding to a 97.4% (95% CI 93.3–99.0%) greater proportional reduction in the intervention arm than in the control arm. Pooling all habitats in each arm gave a similar ratio of ratios of 0.030. In the permutation analysis, the observed allocation was the most extreme of the 20 possible, giving an exact one-sided p value of 0.05, the smallest attainable with six clusters (two-sided p = 0.10).

Table 2. *Anopheles* larval counts and density at baseline (Round 1) and at the final survey (Round 6), by cluster, with the cluster-level estimate of intervention effect

Arm and cluster	Habitats	Total larvae, baseline	Total larvae, final	Mean per habitat, baseline	Mean per habitat, final	Final/ baseline ratio	Logarithmic ratio	Reduction (%)
Intervention arm								
AWD-SSD	44	1505	34	34.20	0.77	0.023	−3.79	97.7
AWD-BSD	44	1296	22	29.45	0.50	0.017	−4.08	98.3
AWD-ASD	44	979	7	22.25	0.16	0.007	−4.94	99.3
Arm total	132	3780	63	28.64	0.48	0.017		98.3
Control arm								
NTD-JPC	44	5516	2844	125.36	64.64	0.516	−0.66	48.4
NTD-MPC	44	5701	3220	129.57	73.18	0.565	−0.57	43.5
NTD-BC	44	5401	3033	122.75	68.93	0.562	−0.58	43.8
Arm total	132	16 618	9097	125.89	68.92	0.547		45.3

Cluster-level effect: ratio of ratios (geometric mean final:baseline ratio, intervention ÷ control) 0.026 (95% CI 0.010–0.067); two-sample t-test on log ratios, t = −10.56, 4 degrees of freedom, p < 0.001; exact permutation test over 20 allocations, one-sided p = 0.05 (two-sided p = 0.10). Pooled arm-level ratio of ratios 0.030.

The larvae were collected with ten 350 mL dips per habitat per visit. Cluster codes: AWD, Ada West District; NTD, North Tongu District. The same 44 habitats per cluster were surveyed at both time points.

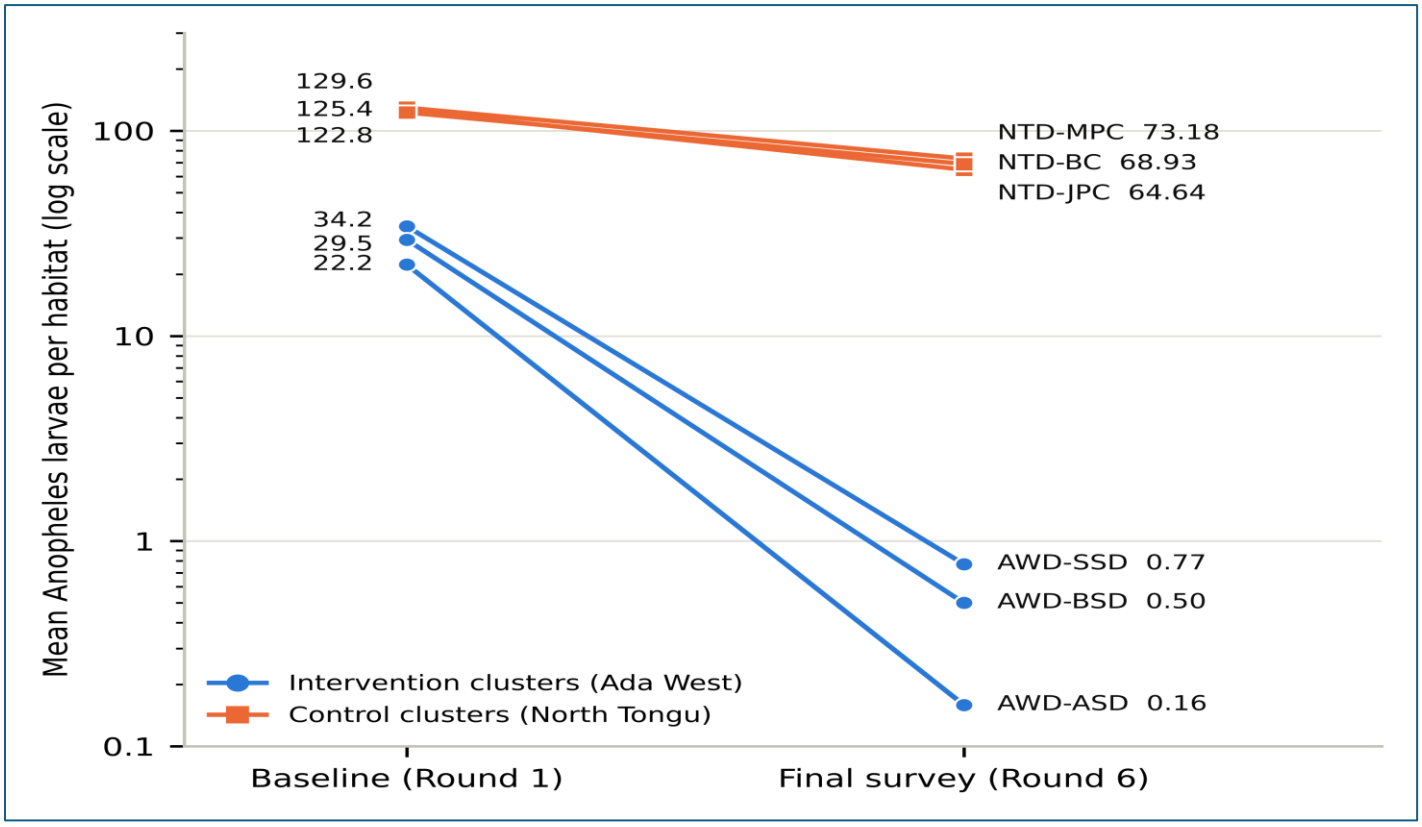


Figure 2. Mean larvae «Anopheles» per habitat at baseline and in the final survey, by cluster. Each line joins the mean for one cluster of 44 habitats in Round 1 and Round 6. *The vertical axis is logarithmic, so equal slopes indicate equal proportional change*.

***Anopheles* larval detection**

The proportion of habitats with *Anopheles* larvae was lower in the intervention arm in every round (Table 3; Fig. 3). It dropped from 24.2% (95% CI 17.7–32.2) in Round 1 to 6.8% (3.6–12.5) in Round 6 in the intervention arm, and from 53.0% (44.6–61.3) to 42.4% (34.3–51.0) in the control arm, so that the between-arm difference widened from 28.8 to 35.6 percentage points. Across all rounds, detection was 16.4% in intervention habitats and 46.2% in control habitats.

Table 3. Habitats with *Anopheles* larvae by survey round and trial arm

Survey round	Intervention n/N	Intervention % (95% CI)	Control n/N	Control % (95% CI)	Difference, percentage points	χ^2	p
Round 1	32/132	24.2 (17.7–32.2)	70/132	53.0 (44.6–61.3)	28.8	23.07	<0.001
Round 2	30/132	22.7 (16.4–30.6)	58/132	43.9 (35.8–52.5)	21.2	13.36	<0.001
Round 3	17/132	12.9 (8.2–19.7)	61/132	46.2 (37.5-54.7)	33.3	35.23	<0.001
Round 4	26/132	19.7 (13.8–27.3)	65/132	49.2 (40.9–57.7)	29.5	25.51	<0.001
Round 5	16/132	12.1 (7.6–18.8)	56/132	42.4 (34.3–51.0)	30.3	30.56	<0.001
Round 6	9/132	6.8 (3.6–12.5)	56/132	42.4 (34.3–51.0)	35.6	45.09	<0.001
All rounds	130/792	16.4 (14.0–19.2)	366/792	46.2 (42.8–49.7)	29.8	163.48	<0.001

95% CIs are Wilson intervals. Difference is control minus intervention. χ^2 is Pearson's test with one degree of freedom; the pooled test ignores repeated visits to the same habitat and is descriptive (see Table 4).

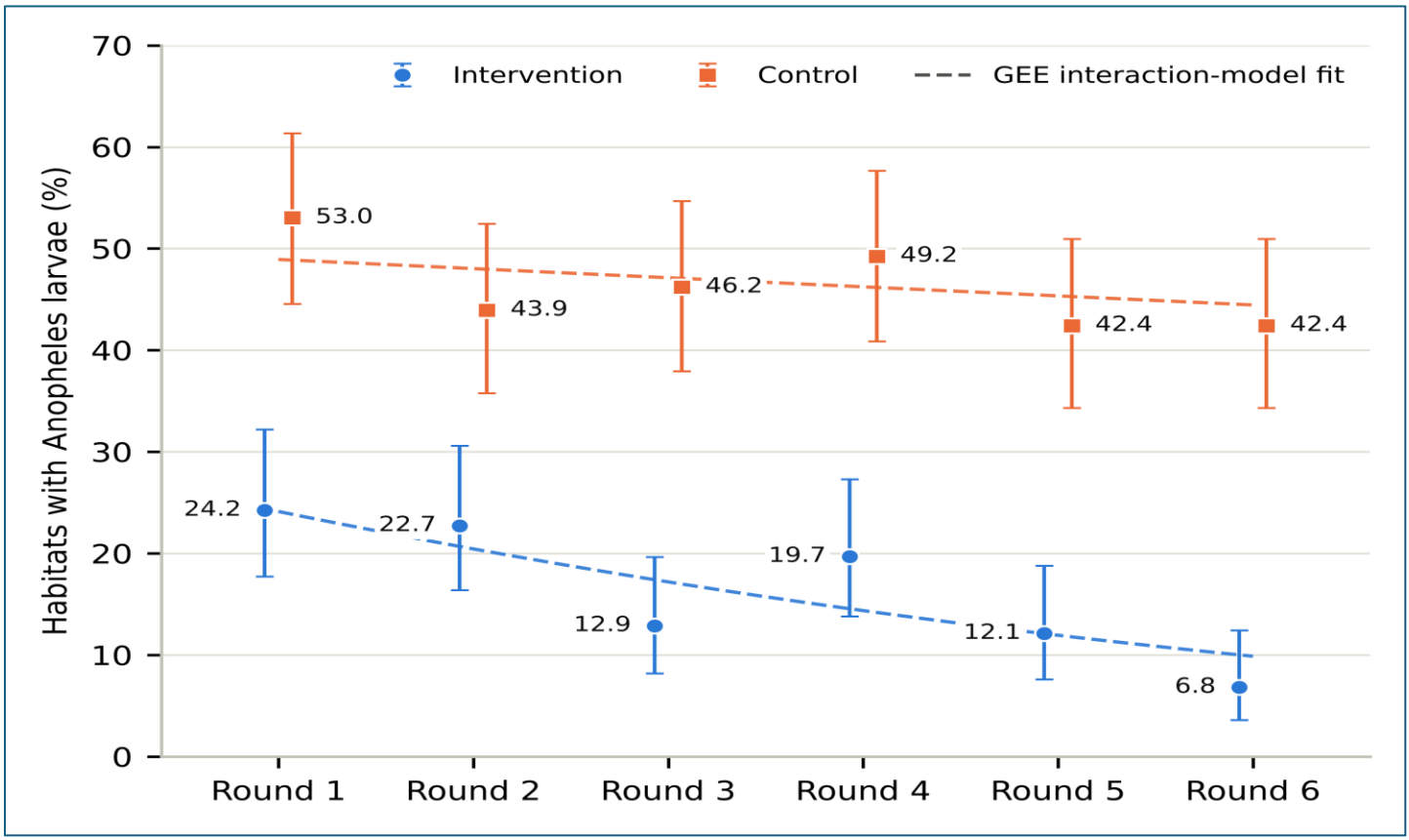


Figure 3. Proportion of habitats with «Anopheles» larvae in each survey round, by trial arm. The points show the observed percentages (n = 132 habitats per arm per round) with Wilson 95% confidence intervals. Dashed lines show fitted values from the generalised estimating equation model with an arm-by-round interaction.

In the main-effects GEE model, detection odds were 4.44 times higher (95% CI 3.52–5.59) in control habitats and declined by 11% per round in both arms (Table 4). Because this odds ratio includes the difference present at Round 1, it describes the gap between arms rather than the effect of the intervention. In the interaction model, detection odds in the intervention arm fell by 19.2% per round (odds ratio 0.81, 95% CI 0.74–0.88) and in the control arm by about 3.5% per round (0.97), and the interaction was significant (odds ratio 1.19, 95% CI 1.04–1.37; p = 0.013). Between Round 1 and Round 6, detection odds fell by 66% in the intervention arm and by 16% in the control arm, a ratio of 0.41 (95% CI 0.21–0.83). The interaction model had the lowest QIC (1782.37, compared with 1784.12 for Model 1 and 1789.46 for Model 3). The arm effect was almost unchanged under independence (odds ratio 4.43) and first-order autoregressive (4.45) working correlations, and the exchangeable within-habitat correlation was 0.14.

Table 4. Generalised estimating equation models for Anopheles larval detection (1584 habitat-visits, 264 habitats)

Model and term	Odds ratio (95% CI)	p
Model 1: main effects (QIC 1784.12; exchangeable α = 0.142)		
Intercept (intervention arm, Round 1)	0.27 (0.20–0.36)	<0.001
Arm: control vs intervention	4.44 (3.52–5.59)	<0.001
Round (per round)	0.89 (0.85–0.94)	<0.001
Model 2: arm × round interaction (QIC 1782.37; exchangeable α = 0.138)		
Intercept (intervention arm, Round 1)	0.32 (0.22–0.46)	<0.001
Arm: control vs intervention, Round 1	3.01 (1.97–4.62)	<0.001
Round, intervention arm (per round)	0.81 (0.74–0.88)	<0.001
Arm × round	1.19 (1.04–1.37)	0.013
Derived: change in detection odds, Round 1 to Round 6, intervention relative to control[a]	0.41 (0.21–0.83)	
Sensitivity: arm effect in Model 1 under other working correlations		
Independence	4.43 (3.52–5.57)	<0.001
First-order autoregressive	4.45 (3.52–5.62)	<0.001

Logit link with robust (sandwich) standard errors; the habitat is the unit of repeated measurement. The round is coded from 0 (Round 1) to 5 (Round 6). An odds ratio greater than 1 for the arm indicates higher detection odds in control habitats. Model 3, with round as a categorical term, had a QIC of 1789.46. The models do not account for variation between sub-district clusters, so CIs are likely to be too narrow. aArm × round odds ratio increased to power −5 (change from Round 1 to Round 6); values below 1 indicate a stronger decline in the intervention arm. QIC, quasi-likelihood information criterion.

Delivery and harms: fidelity of intervention delivery

All planned intervention activities were implemented as planned, including targeted larvicide application, habitat modification and manipulation, and community education sessions. Consequently, the fidelity of the intervention delivery was 100% for each planned activity, and all intervention clusters met the predefined adherence threshold of at least 80%. Implementation was conducted in accordance with the study protocol.

Harms and adverse events

No intervention-related adverse events were recorded among larvicide applicators, residents, livestock, fish, or domestic water users during the trial.

DISCUSSION

Summary of key findings

In this randomized cluster trial in south-eastern Ghana, Anopheles larvae almost disappeared from habitats treated with B. sphaericus as part of an integrated environmental management package. The mean density fell by 98% in intervention clusters against 45% in control clusters, the cluster-level reductions did not overlap, and the proportion of habitats with Anopheles larvae fell more steeply in the intervention arm, reaching under 7% by the final round. The ratio of ratios corresponded to a 97% greater proportional reduction in larval density in the intervention arm than in the control arm, and detection odds declined significantly faster in intervention habitats than in control habitats.

The cluster-level analysis, the per-round comparisons and the interaction models all pointed in the same direction.

Strengths and limitations

The trial used concealed random allocation, coded samples and laboratory staff unaware of allocation, followed every habitat in every round, and analyzed larval density at the cluster level. The direction and size of the effect were consistent between analyses and robust to the choice of working correlation. All prespecified entomological outcomes are reported and the single departure from the statistical analysis plan is stated with its reason.

The most important limitation concerns transparency. The trial was not registered in a WHO-approved public trial registry prior to enrolment and no protocol was published in advance. Prospective registration allows readers to confirm that results and analyses were fixed before the data were seen; without it, selective reporting of results or post hoc analytical choices cannot be excluded on the basis of a public record. We have tried to limit this risk. The two outcomes reported here and the analysis plan were set out in the protocol approved by the ethics committee before enrolment, which is now provided as Supporting Information (S2 File); both prespecified entomological outcomes are reported in full; the one departure from the analysis plan, the replacement of habitat-level negative binomial regression with a cluster-level analysis, is stated in the Methods with its reason; and the cluster-level, per-round, and GEE analyses are all presented and agree in direction. These steps do not substitute for prospective registration and the findings should be regarded as preliminary evidence that needs confirmation in prospectively registered trials.

Several further limitations apply. First, only two districts were randomly selected, so the district and the arm are confounded, and any district-level factor that changed during the trial, such as flood recession, cannot be separated from the intervention. Second, larviciding was delivered with habitat modification, habitat manipulation, and health education, so the effect belongs to the package; filling and draining may have reduced larvae in some habitats independently of the larvicide. Third, with three clusters per arm, the permutation test cannot give a p value below 0.05, the t-test is based on few degrees of freedom, and the GEE CIs do not account for the variation between clusters. Fourth, the arms differed markedly at baseline in larval density and habitat type. Fifth, larvae were identified only by genus, and adult density, sporozoite rates, and the entomological inoculation rate were not measured. Sixth, surveillance was limited to habitats within 500 m of sampled households, one control group was not surveyed, and the trial covered a single six-month season. Finally, field teams were not blinded, although outcome counts were performed on coded samples.

Comparison with previous studies

The size of the effect is consistent with other trials of microbial larvicides. The combination of Bti and B. sphaericus applied fortnightly in Yaoundé reduced the density of bite of adult anopheline by 68% and the entomological inoculation rate by 79%,[9] Bti larviciding in Burkina Faso reduced adult Anopheles by 61% to 77% depending on whether all or only the most productive habitats were treated,[7,8] and drone-delivered Bti in Rwandan rice fields reduced Anopheles larval density by 95%.[10] In contrast, community-driven larval source management added no measurable benefit to high bed net coverage in a cluster-randomized trial in Malawi;[11] that trial measured transmission and infection, which, as discussed below, need not follow larval suppression.

Within Ghana, controlled evidence on larviciding has been scarce. A digital larviciding programme in Obuasi reduced the number of mosquitoes, but its effect on malaria could not be determined[22] and studies in southern Ghana have described productive larval habitats that are often created by human activity and whose presence depends on rainfall and proximity to settlements.[14,15] The present trial adds a concurrent control and randomized allocation, and shows that larval production in such habitats can be suppressed when mapped in advance and treated fortnightly. The findings are also in line with the Cochrane review, which found that larviciding is most likely to be effective where habitats are less extensive,5 and with the WHO recommendation that restricts larviciding to settings where habitats are few, fixed, and findable.[3]

Interpretation of the findings

These estimates need careful reading because the arms differed at baseline. The control habitats had four times as many Anopheles larvae and were twice as often positive in Round 1, consistent with the regional gradient in malaria prevalence between the Greater Accra and Volta Regions[20] and with flooding along the Lower Volta after the Akosombo Dam spill.[21] For this reason, we did not use the odds ratio of the main-effects of 4.4 as the estimate of the effect, since much of it reflects the starting difference. The ratio of ratios and the interaction term compare change within each arm and so remove time-invariant differences between districts. Both still showed a large effect. The 45% decline in the control arm is itself informative. All habitats were selected because they held *Anopheles* immatures at mapping, so some decline towards the average would be expected in both arms, and seasonal drying and recession of floodwater may have added to it. The intervention arm's decline was far larger than this shared trend.

Several features may have favored the effect. B. sphaericus generally persists longer than Bti, particularly in organically enriched water[6], which suits the drains, pits, and vegetated margins of the study area. The residual activity of the microbial larvicides decreases with exposure to sunlight,[31] and about half of the habitats were in direct sunlight, so the fortnightly interval was probably important. Habitats were also mapped in advance and limited to those within 500 m of households, making them searchable, a condition that WHO sets for larviciding.[3,32] In the intervention district, sand-winning pits and swamps constituted most of the frame; both are relatively permanent and easy to revisit. These conditions will not hold everywhere, and the coverage of habitats outside the mapped frame was not measured.

The larval suppression observed here should not be read as a proportionate reduction in infection. Larval reductions are expected to exceed reductions in adults, because untreated or unmapped habitats continue to produce mosquitoes. In addition, the relationship between transmission intensity and parasite prevalence is not linear; At high rates of entomological inoculation, large reductions in transmission may produce only small changes in prevalence33 The parasitological results of the trial, reported separately, are needed to judge the effect of public health.

Implications of the findings

Larviciding is a logical addition in this ecology. Most of the resting An. gambiae s.l. in Ghana are found outdoors,[16] vectors in southern Ghana show high-intensity pyrethroid resistance,[18,19], and transmission on Volta lake islands is intense.[17] Tools that act on larvae avoid both problems.[12] For national and district malaria programmes, the findings suggest that fortnightly microbial larviciding of mapped habitats near settlements is a feasible complement to bed nets in the coastal savannah and Lower Volta.

The maintenance of larviciding depends on the delivery. In Malawi, participation was costed within a trial,[34] participation was limited by the labor and time demands of the work and the lack of incentives, [35], and the domestic and economic uses of the water bodies affect which habitats can be treated.[36] In Ghana, stakeholders see environmental sanitation structures and communal labor as a route to reducing breeding sites,[37] and digital tools and habitat mapping can help plan and supervise larviciding at scale.[22,38] Embedding applicators in existing district and Environmental Sanitation Day structures, as in this trial, is one way to keep coverage continuous.

For research, three needs follow. Trials should randomize more clusters, so that district-level factors are balanced between arms; factorial or component designs are needed to separate larvae from habitat modification, habitat manipulation, and health education; and adult vector density, the entomological inoculation rate, and infection should be measured over more than one season. Such trials should be prospectively registered and their protocols made publicly available.

CONCLUSION

Fortnightly targeted B. sphaericus larviciding, administered by community resource persons within an integrated environmental management package, was associated with almost complete suppression of Anopheles larvae in mapped habitats in south-eastern Ghana, well beyond the decline seen in the control

district. Because only two districts were randomly selected, components were delivered together and the trial was not prospectively registered, the effect cannot be attributed to larviciding alone and should be confirmed in larger registered trials measuring transmission and infection. Within these limits, larviciding mapped habitats near settlements is a promising complement to bed nets where outdoor resting and pyrethroid resistance limit indoor tools.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest. No author has a commercial relationship, consultancy, equity interest, patent, or employment relevant to this work other than the public-sector employment shown in the affiliations.

AUTHORS' CONTRIBUTIONS

OV conceived and designed the trial, coordinated the intervention and data collection, analyzed and interpreted the data, and drafted the manuscript. JBN contributed to data collection, data validation, and critical review of the manuscript. **RKE** contributed to the study methodology, interpretation of the findings, and critical revision of the manuscript. **EAO** contributed to intervention implementation, data collection, and critical revision of the manuscript. **RTM** contributed to data validation, interpretation of the findings, and critical revision of the manuscript. BA contributed to the study design, the methodological review, and the critical review of the manuscript. **PEO** contributed to data analysis, interpretation of the findings, and critical revision of the manuscript. **JKA** contributed to intervention coordination, data collection, and critical revision of the manuscript. **DS** contributed to research supervision, interpretation of the findings, and critical revision of the manuscript. CTW supervised the research, contributed to the study design and interpretation of the findings, and critically reviewed the manuscript for important intellectual content. All authors contributed substantially to the work, participated in the writing of the manuscript or critically reviewed it for important intellectual content, approved the final version for publication, and agreed to be responsible for all aspects of the work, ensuring that questions related to its accuracy or integrity are adequately investigated and resolved.

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