Estimating the potential impact of Attractive Targeted Sugar Baits (ATSBs) as a new vector control tool for *Plasmodium falciparum* malaria

Keith James Fraser (keith.fraser@imperial.ac.uk)
Imperial College London https://orcid.org/0000-0002-8933-9750
Lazaro Mwandigha
Imperial College London
Sekou Traore
Universite des Sciences des Techniques et des Technologies de Bamako
Mohamed Traore
Universite des Sciences des Techniques et des Technologies de Bamako
Seydou Doumbia
Universite des Sciences des Techniques et des Technologies de Bamako
Amy Junnila
Universite des Sciences des Techniques et des Technologies de Bamako
Edita Revay
Universite des Sciences des Techniques et des Technologies de Bamako
John Beier
University of Miami
John Marshall
University of California Berkeley
Azra Ghani
Imperial College London
Gunter Muller
Universite des Sciences des Techniques et des Technologies de Bamako

Research

Keywords: malaria, mosquito, vector control

Posted Date: September 8th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-72317/v1

Version of Record: A version of this preprint was published at Malaria Journal on March 17th, 2021. See the published version at https://doi.org/10.1186/s12936-021-03684-4.

Abstract

Background: Attractive targeted sugar baits (ATSBs) are a promising new tool for malaria control as they can target outdoor-feeding mosquito populations, in contrast to current vector control tools which predominantly target indoor-feeding mosquitoes.

Methods: We sought to estimate the potential impact of these new tools on *Plasmodium falciparum* malaria prevalence in African settings by combining data from a recent entomological field trial of ATSBs undertaken in Mali with mathematical models of malaria transmission.

Results: The entomological study showed a significant reduction of 55.4% (95 % CI 33.7-77.1%) in mosquito catch numbers, and a larger reduction of ~91% (95% CI 75-100%) in the entomological inoculation rate due to the fact that, in the presence of ATSBs, most mosquitoes do not live long enough to transmit malaria. The key parameter determining impact on the mosquito population is the excess mortality due to ATSBs, which we estimate from the observed reduction in mosquito catch numbers to be lower (mean 0.13 per mosquito per day, seasonal range 0.10-0.16 per day) than the bait feeding rate obtained from one-day staining tests (mean 0.34 per mosquito per day, seasonal range 0.28-0.38 per day). Using a mathematical model capturing the lifecycle of *P. falciparum* malaria in mosquitoes and humans and incorporating the excess mortality, we predict that ATSBs could result in large reductions (>30% annually) in prevalence and clinical incidence of malaria, even in regions with an existing high malaria burden.

Conclusions: Results suggest that this new tool could provide a promising addition to existing vector control tools and result in significant reductions in malaria burden across a range of malaria-endemic settings.

1. Background

Nearly half the world's population is at risk of contracting malaria [1]. Since the year 2000, the prevalence of its most common and dangerous causative parasite, *Plasmodium falciparum*, has more than halved, leading to the prevention of an estimated 500 million clinical cases of malaria between 2000 and 2016 [2]. This progress has been largely attributed to the scaling-up of vector control tools (VCTs), predominantly Long-Lasting Insecticide Treated Nets (LLINs) and Indoor Residual Spraying (IRS), both of which are now used in malaria-endemic regions across the globe [3]. However, there has been growing concern that an increase in resistance among mosquito vectors to the pyrethroid-based insecticides used in LLINs and IRS is hampering further progress [2]. In addition, it has been suggested that in response to the scaling up of LLINs and IRS which target mosquitoes attempting to feed on humans indoors, the mosquito vectors may be modifying their feeding behaviour to outdoor feeding between dawn and dusk, thus reducing the efficacy of LLINs and IRS [4]. This has led to calls for the development of non-pyrethroid-based insecticides [3] as well as the development and adoption of more effective VCTs specifically targeting outdoor biting [5].

Plant sugars are an essential dietary component for female and male mosquitoes, with female mosquitoes combining this with protein obtained from blood meals to metabolize egg development [6]. Targeting this aspect of the mosquito lifecycle using Attractive Targeted Sugar Baits (ATSB) [7, 8] (also referred to in the literature as Attractive Toxic Sugar Baits) has therefore been proposed as a potential strategy that may complement LLINs and IRS in suppressing mosquito vector populations [9–11]. ATSBs provide a manufactured sugar-based alternative to plant sugars for female and male mosquitoes with the addition of a toxin that rapidly kills on ingestion or contact. Previous studies have found the effect of ATSBs to be two-fold. First, ATSBs suppress the overall mosquito population by reducing the numbers of female and male mosquitoes available for reproduction. Second, ATSBs diminish the number of mosquitoes living long enough to pass on the malaria parasite, since many are killed before completing the extrinsic incubation period [12–14] – the length of time between a mosquito biting an infectious human and becoming infectious themselves, typically of the order of ~ 10 days [15]. The suppression of the mosquito population (entomological endpoint) due to the use of ATSBs is expected to result in the reduction of malaria prevalence and clinical incidence (epidemiological endpoints). Although several VCTs have confirmed the entomological endpoint (reduction in catch numbers of several mosquito vector species) attributable to ATSBs [10–14], there is currently no empirical data demonstrating a link between the entomological and epidemiological endpoints for this tool.

A previous study [8] by Marshall *et al* used a mathematical modelling approach, parameterised using data from a previous ATSB entomological field study in Mali [7], to understand the entomological impact of ATSBs in a West African setting. Mosquito catch number reductions of ~ 80% were projected over a timescale of a few weeks during a time period when the mosquito catch numbers at a control location remained approximately constant. One of the key parameters determining the efficacy of ATSBs on entomological endpoints was the bait feeding rate parameter, which describes the probability of a particular mosquito ingesting the bait on a given day. This was estimated to be ~ 0.4–0.5 per day – a rate substantially higher than the baseline mosquito death rate of ~ 0.1 per day in this

setting. Given that mosquito lifetime following ATSB consumption was estimated to be just a few hours [8], this implies a very large reduction in average mosquito lifespan in the presence of ATSBs.

We combine data from the first cluster-randomised entomological study [16] of ATSBs in Africa with mathematical modelling to explore the potential utility of this new tool to reduce *P.falciparum* malaria prevalence and clinical incidence in humans. The cluster-randomised entomological study was undertaken in southern Mali between April 2016 and December 2017, with the efficacy measured through one-day tests using non-toxic stained bait (to estimate the bait feeding rate), monthly mosquito catches in intervention and control villages and monthly estimates of the entomological inoculation rate (EIR) – the number of infective bites received per person per unit time (to estimate onward infectivity to humans). For further details of the study, see Sect. 5.1. To estimate the subsequent impact on human endpoints, we adapted a mathematical model [17–19] of the transmission of *P.falciparum* malaria to incorporate the presence of ATSBs and used the field study data to estimate key parameters for the model. The model was applied across a range of malaria transmission settings capturing different transmission intensity and seasonality to evaluate the potential utility of ATSBs as an additional VCT.

2. Methods

2.1 Entomological study of ATSB

A Phase II entomological study (previously reported in this journal [16]) was undertaken in 14 villages in central Mali. The climate in this region is highly seasonal, with high rainfall in the rainy season (peaking in September) and very dry conditions in the dry season (December-March). Full details of the study and outcomes are reported elsewhere and are summarised here for completeness.

Fourteen villages were selected to participate in the study. In the first year of the study (April 2016 to May 2017), baseline entomological data were collected in all 14 villages. They were randomly sorted into two groups of seven, with one group designated as the intervention (ATSBs + standard of care) group and one as the control (standard of care) group. ATSBs were then deployed in the intervention villages in June 2017, and entomological data collected through to December 2017. To estimate the feeding rate on the ATSBs, 1-day tests using stained bait were carried out at monthly intervals in the control villages. As a means of estimating the bait-feeding rate, simple tests were carried out in which attractive sugar baits without toxic additives (ASBs) were temporarily introduced to villages where ATSBs were not used. These baits contained a harmless dye which allowed captured mosquitoes in the relevant villages to be separated into those which had fed on the ASBs and those which had not.

Mosquitoes were collected monthly in each village using Centre for Disease Control (CDC) UV light traps, Malaise traps and pyrethroid spray catch (PSC) inside houses. Here we use the data from CDC traps as a measure of mosquito density. In addition, human landing catch (HLC) measurements were carried out indoors and outdoors. A random sample of the captured mosquitoes were examined to determine the proportion containing viable sporozoites and therefore onwardly infectious; these data were combined with the number caught per human in HLC experiments to estimate the entomological inoculation rate (EIR).

2.2 Estimating the impact of ATSBs on mosquito density and EIR

To estimate the impact of the ATSBs on the two entomological endpoints – mosquito count and EIR – we formulated a non-linear model to capture the seasonal variation in outcomes in addition to the effect of the intervention whilst accounting for the village cluster-level variability. The non-linear model with mosquito count outcomes from the CDC light traps had convergence difficulties. To improve convergence, the mosquito counts were divided by 1,000. The resultant outcome, a continuous variable that captured the mosquito density (mosquito count per thousands), was then modelled by a normal distribution with its parameters captured by the model shown in Equation 1. We let M_{ij} denote the mosquito population density in village i where $i = 1, 2...14$ (7 treatment and 7 control villages) at time t (indexing days as proportion of year in which count of mosquito outcome was obtained, this proportion being calculated by dividing the reported day of the year by 365). The mean of the normal distribution was defined by the term

$(1 - R\delta_T)(a\sin(bt - c) + d) + r_i$ and the residual error variance by ε_{ij}.

$$M_i(t) = (1 - R\delta_T)(a\sin(bt - c) + d) + r_i + \varepsilon_{ij} \quad (1)$$

The fixed effect part of Equation 1 containing the term $a \sin(bt - c)$ is a trigonometric function that captures the seasonal variation in the mosquito population density where a, b, c, d are parameters to be estimated. The term R denotes the treatment effect coefficient for ATSB (the fractional decrease in population density) while δ_T is the predictor that identifies treatment assignment at village level (coded as 1 and 0 for treatment and control villages respectively). The terms r_i and ε_{ij} denote the random intercept that captures the correlation of mosquito population density at village level and the residual variability respectively. These are assumed to be normally distributed as $r_i \sim N(0,\sigma_r^2)$ and $\varepsilon_{ij} \sim N(0,\sigma_e^2)$ where σ_r^2 and σ_e^2 are variance components to be estimated.

The EIR is modelled in a similar manner (Equation 2). Due to the nature of the data, the time t was measured in months in which the EIR value was obtained. The months were coded as t= 1 to 7 representing the months from June to December.

$$EIR_i(t) = (1 - R\delta_T)(a \sin(b \times t - c) + d) + r_i + \varepsilon_{ij} \quad \textbf{(2)}$$

In both cases, the model was fitted to the mosquito catch and EIR data using PROC NLMIXED with the adaptive Gauss-Hermite quadrature method [20] in Statistical Analysis System (SAS) software version 9.4 [21] to obtain the point estimate for the parameter R together with its corresponding 95% confidence interval based on a two-sided p-value for the null hypothesis $H_0 : R = 0$ versus the alternative $H_A : R \neq 0$. The parameter values extrapolated from population and EIR data are shown in the supplementary information.

2.3 Estimating the excess mortality

Equation 3 expresses the rate of change of the mosquito catch M following the introduction of ATSBs. This expression, based on the approach taken by Marshall *et al* [8], is a simplified version of the more detailed mosquito population model (see section 2.4 and supplementary information), used here as a means of relating the function fitted to the observed data (Equation 1) to mosquito mortality parameters.

$$\frac{dM(t)}{dt} = \mu_{BASE} M_{EQ}(t) - (\mu_{BASE} + \mu_{ATSB}) M(t) \quad \textbf{(3)}$$

Here M_{EQ} is the equilibrium mosquito catch rate (which may be constant or vary seasonally). μ_{BASE} is the baseline mosquito birth and death rate in the absence of ATSBs (such that the catch rate will equal M_{EQ} under control conditions), and μ_{ATSB} is the excess mortality due to ATSBs. μ_{BASE} is given by the natural mosquito death rate μ_{NAT} added to any additional mortality due to vector control interventions present in both control and ATSB villages. This is discussed in more detail in section 3.2.

Experimental data suggest that the rate of death after ingestion of the toxin in the ATSB is so high as to be effectively instantaneous [8]. In this case, we expect μ_{ATSB} to be equal to the bait feeding rate. From Equation 1, the average mosquito catch rate in the control and ATSB arms can be written as shown in Equations 4a-b.

$$M_{CON}(t) = a \sin(bt - c) + d \quad \textbf{(4a)}$$

$$M_{EXP}(t) = (1 - R)[a \sin(bt - c) + d] \quad \textbf{(4b)}$$

The expression for catch rate changes in terms of the bait feeding rate μ_{ATSB} (Equation 3) can be re-arranged to express μ_{ATSB} as a function of M_{EXP} and M_{EQ}:

$$\frac{\delta M_{EXP}}{\delta t} = \mu_{BASE} M_{EQ} - (\mu_{BASE} + \mu_{ATSB}) M_{EXP} \quad \textbf{(5a)}$$

$$\frac{\delta M_{EXP}}{\delta t} - \mu_{BASE}(M_{EQ} - M_{EXP}) = -\mu_{ATSB} M_{EXP} \quad \textbf{(5b)}$$

$$\mu_{ATSB} = \frac{1}{M_{EXP}}\left(\mu_{BASE}(M_{EQ} - M_{EXP}) - \frac{\delta M_{EXP}}{\delta t}\right) \quad \textbf{(5c)}$$

If the approximation $M_{EQ} = M_{CON}$ is used (i.e. it is assumed that when $\mu_{ATSB} = 0$, the population remains at equilibrium levels) and Equations 4a-b used to substitute for M_{CON} and M_{EXP}, Equation 5c can be rewritten as follows to give an estimate of μ_{ATSB} in terms of the estimated parameters *a*, *b*, *c*, *d*, *R* and the natural death rate:

$$\mu_{ATSB} = \frac{1}{(1-R)M_{CON}}\left(\mu_{BASE}(M_{CON} - (1-R)M_{CON}) - \frac{\delta}{\delta t}((1-R)M_{CON})\right) \quad \textbf{(6a)}$$

$$\mu_{ATSB} = \mu_{BASE}\frac{R}{(1-R)} - \frac{1}{M_{CON}}\frac{\delta}{\delta t}(a\sin(bt-c)+d) \quad \textbf{(6b)}$$

$$\mu_{ATSB} = \mu_{BASE}\frac{R}{(1-R)} - \frac{ab\cos(bt-c)}{a\sin(bt-c)+d} \quad \textbf{(6c)}$$

2.4 Estimating the impact of ATSBs on malaria prevalence and incidence

An existing detailed model [17–19] of malaria was used for simulations of the effects of ATSBs on malaria infection levels in human populations. In the model, individuals begin life susceptible to *P.falciparum* infection and are exposed to infectious bites at a rate that depends on local mosquito density and infectivity. Newborn infants passively acquire maternal immunity, which decays in the first 6 months of life. After exposure, individuals are susceptible to clinical disease and may progress through a range of infection categories (clinical infection, asymptomatic infection, subpatent infection, treated and prophylaxis). As they age, the risk of developing disease declines through natural acquisition of immunity, at a rate that depends on the rate of continued exposure. At older ages, parasitaemia levels fall so that a high proportion of asymptomatic infections become sub-microscopic. Full mosquito-population dynamics were included in the model to capture the effects of vector control in preventing transmission, killing adult female mosquitoes, and the resulting reduction in egg-laying. The model has previously been fitted to existing data on the relationship between rainfall, mosquito abundance, entomological inoculation rate (the rate at which people receive infectious bites), parasite prevalence and clinical disease incidence in order to establish parameter values. Full mathematical details of the model and a complete parameter list are included in the supplementary information.

The effect of ATSBs was included in the model by modifying the death rate of mosquitoes from μ_{BASE} to $\mu_{BASE} + \mu_{ATSB}$ as shown in the previous section. The initial conditions for a study were created by generating characteristics (proportions of humans in different infection categories, immunity levels, etc.) at steady state under particular levels of adult mosquito density, then after an extended period of time with particular seasonal variation in adult mosquito density. ATSBs were then introduced to modify the mosquito death rate, resulting in reduced mosquito populations due to direct death and reduced larval birth rate. As noted above, the population of infectious mosquitoes decreases more significantly than the overall population, due to increased death rates causing fewer infected mosquitoes to survive for the duration of the parasite incubation period. This in turn caused reductions in EIR which in turn reduced the number of new infections. Benchmark data values including malaria prevalence and clinical incidence were recorded at regular intervals and the results compared with the same data values under control conditions (where the mosquito death rate is simply equal to the natural value μ_{NAT}) to measure the effectiveness of ATSBs.

3. Results

3.1 Impact of ATSBs on mosquito catch numbers and EIR in Malian villages

A cluster-randomised entomological study [16] was undertaken in 14 villages in southern Mali between April 2016 and December 2017. In the first year of the study (April 2016 to May 2017) baseline entomological data were collected in all 14 villages. ATSBs were then deployed in the seven intervention villages in June 2017, and entomological data collected through to December 2017.

Figure 1A-B shows monthly mosquito catch number data (collected using CDC traps) for the two arms of the study from April to December in 2016 and 2017. Whilst there was substantial variation between villages, as illustrated by the error bars, there was no

significant difference in the average number of collected mosquitoes per village between the two arms (Groups 1 and 2) during the baseline period (Fig. 1A, average reduction factor $R = 0.017$, $p = 0.90$; see Sect. 5.2 for statistical methods). Following the intervention with ATSBs, there was a significantly lower mosquito count in the intervention villages compared to the control villages, with a 55.4% average reduction in mosquito count (Fig. 1B, R = 0.554, 95% confidence interval 33.7–77.1%, $p < 0.001$). Figure 1C-D shows the estimated EIR in the intervention and control arms for the five wettest months of 2017 (dry months were excluded due to the small number of mosquitoes caught). We estimate a substantially greater reduction in the EIR of 89% ($R = 0.890$, 95% confidence interval 75–100%, $p < 0.001$) for indoor human landing catch (HLC) and 93% ($R = 0.9208$, 95% confidence interval 75–100%, $p < 0.001$) for outdoor HLC. This indicates that, as anticipated, the effect of ATSBs on the malaria infection rates is greater than that which can be inferred from reductions in mosquito catch numbers, due to an elevated mosquito death rate reducing the population of older females and hence the average mosquito lifespan. This in turn reduces the number of infected mosquitoes which survive the extrinsic incubation period.

3.2 Estimated bait feeding and killing rates

Figure 2A shows estimates of the bait feeding rate calculated from 1-day staining tests using non-toxic bait from the 2017 study. The proportion of female mosquitoes stained by the baits 24 hours after their introduction ranges from 0.28–0.38 per day during the period when ATSBs were in use (June-December). The proportion of mosquitoes stained is generally highest in the drier months of the year when measurements were taken (April-May) and lowest in the wetter months (August – December). We used the monthly statistical estimates of the effect size of the intervention on mosquito populations (R) from 2017 to estimate the excess mortality by transforming a birth-death model for the mosquito population (see Sect. 2.2-3). Figure 2B shows the results. The values vary in the range 0.10–0.16 per day (mean 0.13 per day) when the baseline mortality μ_{BASE} is 0.096/day (based on the value used for the natural mosquito death rate μ_{BASE} – see Sect. 2.3), as shown in the lower line in Fig. 2B. These values are notably lower than the estimated bait feeding rate shown in Fig. 2A.

If the baseline mortality μ_{BASE} is increased, higher values of μ_{ATSB} are obtained, as shown in the upper lines in Fig. 2B. This represents additional baseline mortality above the value of μ_{NAT} present in both control and intervention villages due to non-ATSB vector control interventions. Long-lasting insecticide-treated nets were present in the study region at high coverage [16], but a figure for the additional baseline mortality cannot be estimated accurately as the efficacy (which can vary depending on usage patterns and insecticide resistance) is not known. The value obtained where $\mu_{BASE} = \mu_{NAT}$ is used in the remainder of this paper as a conservative estimate of the actual excess mortality and an effective value applicable to calculations where LLINs are not incorporated.

Next, we used the simplified mathematical model of the mosquito population dynamics (see Sect. 2.3) to predict the mosquito catch rate with and without the ATSB intervention, using either the bait feeding rate or the excess mortality as estimates to parameterise the impact of ATSBs. The model outputs based on the bait feeding rates estimated from 1-day staining tests overestimate the observed reduction in the mosquito catch (Fig. 3A). In contrast, using the excess mortality estimated from catch data results in model output that closely mirrors the observed mosquito counts (Fig. 3B).

3.3 Predicted impact of ATSBs on malaria transmission

To obtain preliminary estimates of the impact of ATSBs on human endpoints, the changes in malaria prevalence and incidence in humans expected to be produced by ATSBs were calculated based on the measured impact on EIR in the field study. This was carried out using model-estimated relationships between EIR, parasite prevalence and clinical incidence previously obtained from fitting to data on these three metrics [17–19]. Figure 4 shows the equilibrium relationship (obtained by running the model for 5 years from initial values calculated for steady-state at constant rainfall) between annual EIR and all-ages parasite prevalence (Fig. 4A) or clinical incidence (Fig. 4B) averaged over the year using the seasonal rainfall variation in the study area in Mali.. The estimates of EIR from the HLC data (Fig. 3) are shown super-imposed on this profile. From this relationship, the observed reduction in EIR values corresponds to a reduction in all-ages prevalence from 43% (95% CrI 37–52%) to 26% (95% CrI 20–35%) or 44% (95% CrI 37–53%) to 31% (95% CrI 24–40%) for outdoor and indoor HLC collection respectively, and a reduction in annual all-age clinical incidence from 0.85 (95% CrI 0.48–1.30) cases per person per year to 0.62 (95% CrI 0.29–1.03) or 0.85 (95% CrI 0.49–1.31) to 0.69 (95% CrI 0.34–1.12) cases per person per year.

To obtain more detailed predictions of the impact of ATSBs across different malaria transmission levels that take into account the dynamics generated by changes in immunity in the human population, we generated outputs from our mathematical model of *P.falciparum* malaria transmission using the estimated excess mortality from the field study and with the seasonality in transmission determined by rainfall patterns in Mali (see Methods). Figure 5A-B shows predicted changes in parasite prevalence (Fig. 5A) and clinical incidence (Fig. 5B) over the course of 1 year, at a baseline malaria level corresponding to the highest annual EIR measured in the field study (~ 190/person-year). Here the excess mortality is assumed to be constant over the course of the year. In this highly seasonal

setting, clinical incidence is predicted to be concentrated during the malaria transmission season, whilst parasite prevalence is predicted to show less seasonal variation. The greatest observable impact of ATSBs is predicted to occur in clinical incidence; however, substantial reductions are also predicted to be observed in parasite prevalence. Furthermore, we predict more substantial reductions in clinical incidence than obtained from the equilibrium relationships; this is in part due to the benefit from higher levels of pre-existing immunity that we expect to decay over subsequent years if the intervention is maintained. However, the study also showed a lower impact on the HLC endpoint underlying the EIR estimates compared to the CDC trap data used for our dynamic model projections [16] .

Figure 5C-D shows the predicted reductions in parasite prevalence and clinical incidence due to ATSB for a range of excess mortality values (on the x-axis) and baseline transmission levels (on the y-axis). In all settings the predicted impact is large even for relatively low excess mortality values. Notably, we predict a greater reduction in clinical incidence compared to parasite prevalence in areas with high levels of malaria at baseline. A 30% reduction (highlighted on each graph) is predicted for clinical incidence when the excess mortality is less than 0.05, even in areas with high baseline malaria. For parasite prevalence, the excess mortality required to achieve this threshold varies more strongly with the baseline malaria transmission level, but even when the baseline year-round all-age prevalence is as high as 45%, a reduction of 30% is predicted with an excess mortality above 0.1.

We next used the model to understand whether these results would differ in areas without such strong seasonal patterns of malaria. Figure 6 shows the same outputs as Fig. 5 but for constant rainfall as opposed to the highly seasonal rainfall patterns observed in the region in which the field study was conducted. Although the dynamics of the effect vary as expected (Fig. 6A-B), the overall percentage reduction in year-round prevalence and incidence for a given baseline malaria transmission level (Fig. 6C-D) is predicted to be similar to that predicted in areas with seasonally- varying rainfall.

The study showed a seasonally variable excess mortality, with higher rates estimated during the drier months and lower rates during the wettest months. We therefore also explored whether seasonal variation in the excess mortality modified our predicted impact of ATSBs. Figure 7 shows the predicted parasite prevalence and incidence over time for in the absence of ATSBs, with ATSBs assuming a constant excess mortality and with ATSBs displaying a variable excess mortality based on the results shown in Fig. 2B. Overall, we predict a small (< 10%) reduction in the impact of the intervention if the ATSB excess mortality varies in the pattern observed in the field study.

4. Discussion

The results from the first cluster-randomised entomological field study of ATSBs [16] demonstrate the potential of this new tool to significantly suppress *Anopheles* mosquito catch numbers, confirming results from earlier studies[8, 11, 13]. Using this data, we estimate a statistically significant reduction in the mosquito count in the villages with ATSBs and LLINs compared to those with LLINs alone of 55% (95% CI 34–77%) over a 1-year follow-up period. Notably, this effect is most apparent in the reduction in the seasonal peak mosquito catch rate concomitant with the period of highest malaria transmission. Furthermore, we estimate a greater reduction in onward transmission as captured by the EIR of 91% (95% CI 75–100%), reflecting the impact that this intervention is likely to have in reducing the lifespan of mosquitoes and hence the potential for mosquitoes to survive the extrinsic incubation period. Our modelling results suggest that these large reductions in vector populations should translate to significant public health impacts, with > 30% reductions in both parasite prevalence and clinical incidence predicted across a wide range of transmission settings and across a range of potential values for the excess mortality.

While theoretically, reductions in entomological endpoints should lead to reductions in epidemiological endpoints, this relationship is not always clear empirically. However, it is worth noting that the reductions in mosquito catch and EIR that we observed are similar to or greater than those observed to date in cluster-randomised studies of other VCTs. For example, a large cluster-randomised trial of ITNs in Western Kenya [22] showed a 58.5% reduction in *An.gambiae* and 90% reduction in *An.funestus* captured using pyrethrum spray collection. This same trial resulted in a reduction in malaria incidence and prevalence in young children of 60% and 19% respectively [23, 24]. However, extrapolation from entomological endpoints alone cannot be made for ITNs since their efficacy will represent both direct and indirect protection. Whilst there are no cluster RCTs of IRS alone [25], in recent years the impact of LLINs and IRS on mosquito populations has been assessed in two large cluster-randomised trials comparing the benefits of combining IRS and LLINs. In the first in the Gambia (in which IRS did not show any additional epidemiological benefit in addition to LLINs), mosquito counts were 33% lower in the LLIN + IRS group compared to the LLIN-only group, but this difference was not statistically significant. In this study the EIR was low and not statistically different between intervention arms. In the second trial in Tanzania mosquito counts were reduced by 29% and EIR by 87% in the LLIN + IRS arm compared to the LLIN-only arm, although these differences were of marginal statistical significance. However, this translated to a 50% reduction in parasite prevalence in the LLIN + IRS arm compared to the LLIN-only arm. Our projected

epidemiological impact of ATSBs based on the observed entomological endpoints therefore appears plausible but requires confirmation in epidemiological randomised trials.

One of the key parameters determining the likely efficacy of ATSBs is the excess mortality, which, given the lethality of the toxin, is primarily determined by the rate at which mosquitoes feed on the bait. From the observed reduction in mosquito catch numbers, we estimate this excess mortality to be in the range ~ 0.10–0.16/day, effectively at least doubling the natural death rate of Anopheles mosquitoes. These estimates are notably lower than the estimates of the bait feeding rate obtained by Marshall *et al* [8] (0.40/day) and the values estimated here in the one-day staining experiments using dyed, non-toxic bait (0.28–0.38/day in the period of ATSB use) although they are more consistent with the bait feeding rate in the control arm of the study by Marshall et al (0.15/day for female mosquitoes).

There are a number of possible explanations for this discrepancy. Firstly, the base mosquito mortality may be higher than the natural mortality rate, as noted in Sect. 3.2. This can lead to an under-estimation of the ATSB mortality rate, as shown in Fig. 2B, where the extrapolated ATSB mortality rate increases if the base mortality rate is increased. In addition, short-term experiments may give rise to higher values than those observed over longer time periods due to variations in the bait-feeding rate between mosquitoes – if some mosquitoes are more disposed to feed on the bait than others, these will be killed early on and over time the bait-feeding rate may decline.

Another possibility is that migration of mosquitoes from areas unaffected by the ATSBs acts to mitigate the population reduction and produce a lower apparent increase in the death rate. The villages in which the data reported here was gathered are located in the flood plain of the Niger river with rice paddies and other breeding sites nearby. In contrast, the previous small-scale village studies in central Mali [8] were carried out at the end of the rainy season without significant nearby breeding sites and this difference may account for the higher apparent excess mortality. Thus, whilst the one-day staining experiments can continue to provide useful information, particularly on the variation in the bait feeding rate between different ecological locations [11, 12, 14, 26], it will be important to continue to collect entomological outcomes in future RCTs of this new intervention.

The bait feeding rate estimated here from the one-day staining experiments was found to be negatively correlated with the rainfall level, suggesting that the ATSB efficacy may vary seasonally. This seasonal effect may be due to greater availability of natural sugar sources such as flowers and fruit inside villages during the wet season, providing alternative attractant sugar sources for both male and female mosquitoes. Alternatively, the observed feeding rates of mosquitoes inside the villages may be lowered by large numbers of already sugar fed mosquitoes invading the villages from nearby breeding sites. If this is the case, it suggests that the overall efficacy of ATSBs can be expected to vary significantly between ecological and/or geographical settings, with locations where natural sugar sources are more abundant showing reduced efficacy. In this context, the impact of invasive plants flowering during the dry season also needs to be considered [26]. Previous studies of ATSB in arid environments with varying levels of natural sugar availability suggest that natural sugar sources have only a delaying effect on ATSB efficacy [13]. However, further data are needed from the range of malaria-endemic environments to confirm this.

There are a number of limitations to this study. Firstly, the projected impact of ATSBs made here are based on the results of a single field study in a single ecological zone. Our results should therefore be interpreted as indicative rather than predictive. Secondly, as noted above, the excess mortality can be expected to vary between different ecological environments and at different times of the year, depending on the availability of alternative sugar sources. Thus, whilst we predict a major impact on both malaria and clinical incidence across a range of potential excess mortality value, if the feeding rate on the bait is very low then we would predict a sharp reduction in efficacy. Thirdly, trials have not yet been carried out to assess the effect of ATSBs on epidemiological outcomes in the field. Evidence from past VCT trials has demonstrated that it can be difficult to extrapolate epidemiological outcomes based on entomological outcomes alone given that many other factors could vary between settings. Finally, the large impact of ATSBs on mosquito catch numbers observed in the study in Mali, if replicated elsewhere, could be expected to exert a strong selective pressure. Adaptation of mosquito behaviour in response to the presence of ATSBs could reduce efficacy in the longer-term, making it difficult to predict the sustainability of this new tool, although it may be possible to mitigate this through the use of multiple toxins.

5. Conclusions

The results from the first cluster-randomised study of ATSBs suggest that this new tool could provide a promising addition to existing VCTs and result in significant reductions in malaria burden across a range of malaria-endemic settings. These estimates can help to

inform the design of future cluster randomized field studies in different ecological settings and/or incorporating epidemiological outcomes that will be required to confirm the efficacy of ATSBs as a public health intervention.

Abbreviations

ATSB – Attractive targeted sugar bait (sometimes referred to as attractive toxic sugar bait); CDC – Centre for Disease Control; EIR – Entomological inoculation rate; HLC – Human landing catch; IRS – Interior residual spraying; ITN – Insecticide-treated net; LLIN – Long-lasting insecticide-treated net; PSC – Pyrethroid spray catch; RCT – Randomized clinical trial; SAS – Statistical Analysis System; VCT – Vector control tool

Declarations

Ethics approval and consent to participate

This study did not involve human participants.

Consent for publication

No personal data was collected in this study.

Availability of data and materials

We will make available, on request and via official databases where possible, all field data contributing to the work presented here along with all code and output data relating to data extrapolation.

Competing Interests

We declare no competing interests.

Acknowledgements

We would like to thank Mathias Mondy, Jason Richardson, Tom McClean and Steve Kern for helpful discussions.

Authors' Contributions

KF drafted the manuscript and carried out entomological and epidemiological modelling. LM carried out statistical analysis. AG, LM assisted in drafting the manuscript. GM and JB were involved in conceiving and designing the field study. GM, MT, ST, SD, and ER supervised and carried out the field work. AJ contributed in assessing data and writing the entomological trial paper. JM contributed to the design of the bait station feeding experiments and data interpretation. All authors read and approved the final manuscript.

Funding

This work was financially supported by the Integrated Vector Control Consortium (IVCC). We additionally acknowledge support from the Bill and Melinda Gates Foundation and Centre funding from the UK Medical Research Council and Department for International Development

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Figures

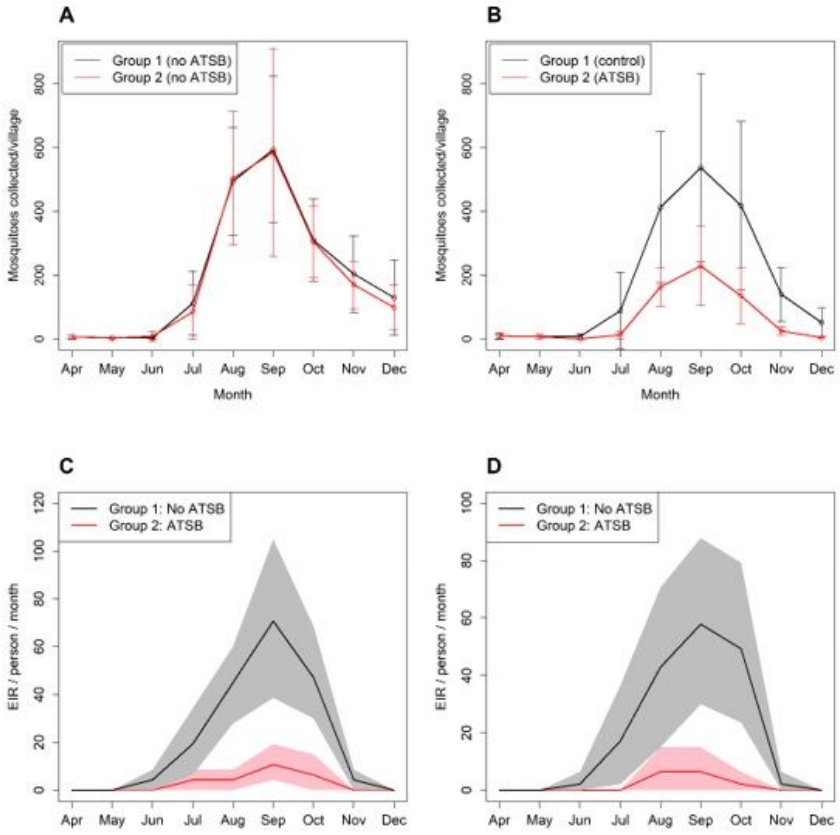

Figure 1

A-B: Number of female mosquitoes caught per village using CDC traps in 2016 (panel A) and 2017 (panel B) for each group of 7 villages[16]. ATSBs were introduced in Group 2 in 2017. Error bars show the standard deviation between villages. C-D: Estimated EIR in ATSB and control villages calculated from the fraction of mosquitoes infected among those caught using the human landing catch method, split into indoor (C) and outdoor (D) collection[16]. Shaded regions show 95% bootstrap percentile interval based on 5000 bootstrap samples.

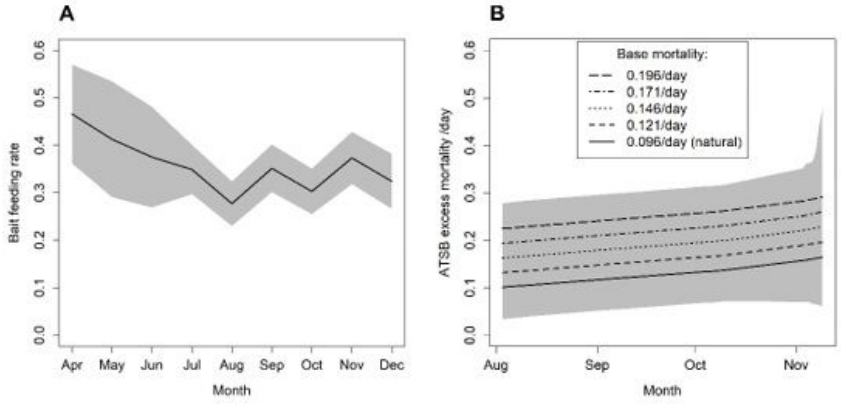

Figure 2

(A) Estimated bait feeding rate in control villages, calculated as the fraction of female mosquitoes stained in 1-day tests using non-toxic but food dye-stained bait between April and December 2017. Shaded region represents binomial confidence interval. (B) Estimated excess mortality rate μATSB in intervention villages, calculated as the additional death rate (see Equation 2) required to reproduce the observed difference in mosquito numbers between the intervention and control villages (using functions fitted to data as described in the Methods). Shaded region represents 95% confidence interval where base mortality = natural mortality rate 0.096/day.

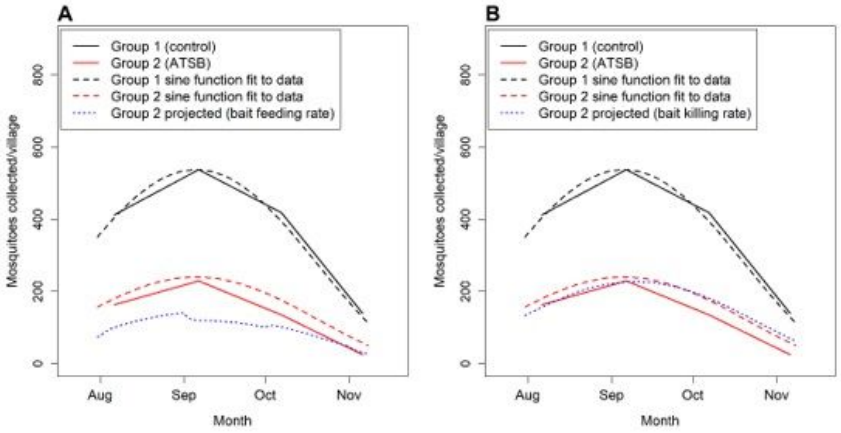

Figure 3

Sine functions fitted to 2017 mosquito collection data for Groups 1-2, compared with values projected for ATSB conditions (Group 2) using Equation 2, with equilibrium values MEQ set equal to fitting function for control conditions (Group 1). Projected values shown for (A) excess mortality given by 1-day staining tests (Figure 2A) (B) mean excess mortality extrapolated from fitting functions (Figure 2B).

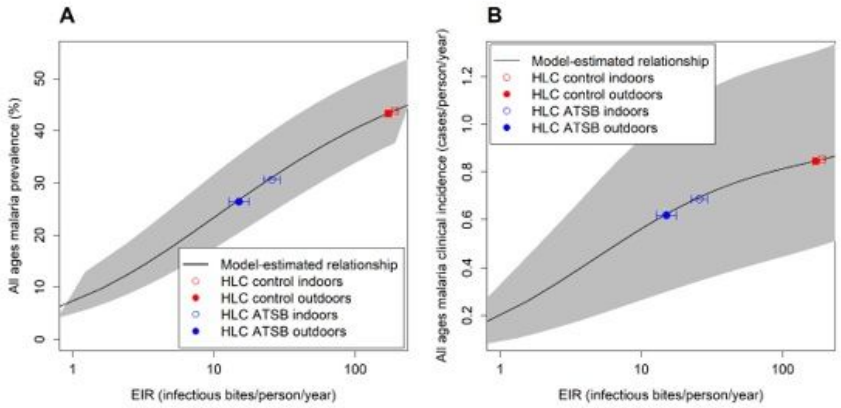

Figure 4

The lines show the equilibrium year-round average model-estimated (A) all-age parasite prevalence and (B) all-age clinical incidence (cases per person per year), plotted against annual entomological inoculation rate (EIR). The coloured points show annual EIR values calculated from field data. The shaded regions correspond to the 95% posterior credible intervals for the modelled relationship between EIR, prevalence and incidence (see Methods).

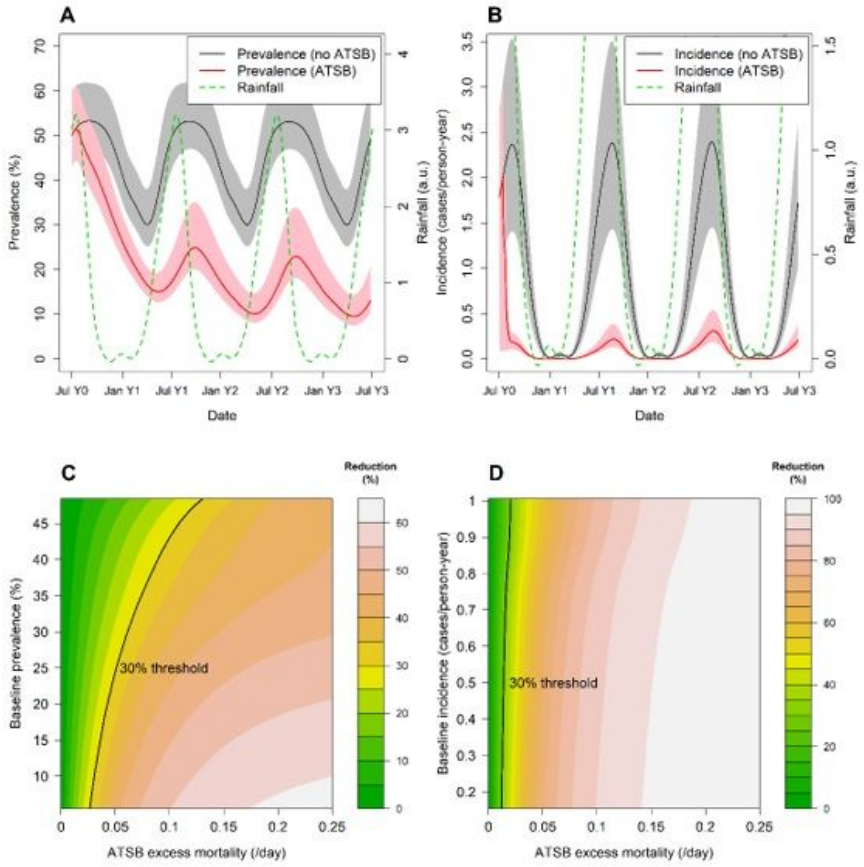

Figure 5

A-B: Model-predicted all-ages parasite prevalence (A) and clinical incidence (B) over the course of 3 years after introduction of ATSBs (red line) and without ATSBs during the same period (black line). The green dotted line shows the assumed rainfall pattern (in arbitrary units). For these runs the excess mortality μATSB is set to the average value estimated from field trial results (0.13/day). Shaded areas represent range of values obtained using model parameters in 95% credible interval. C-D: Model-predicted reduction in all-age year-round parasite prevalence (C) and clinical incidence (D) in first year of ATSB use as a function of prevalence/incidence under non-ATSB conditions and bait killing rate μATSB. All simulations use the seasonal Mali rainfall profile shown in panels A and B.

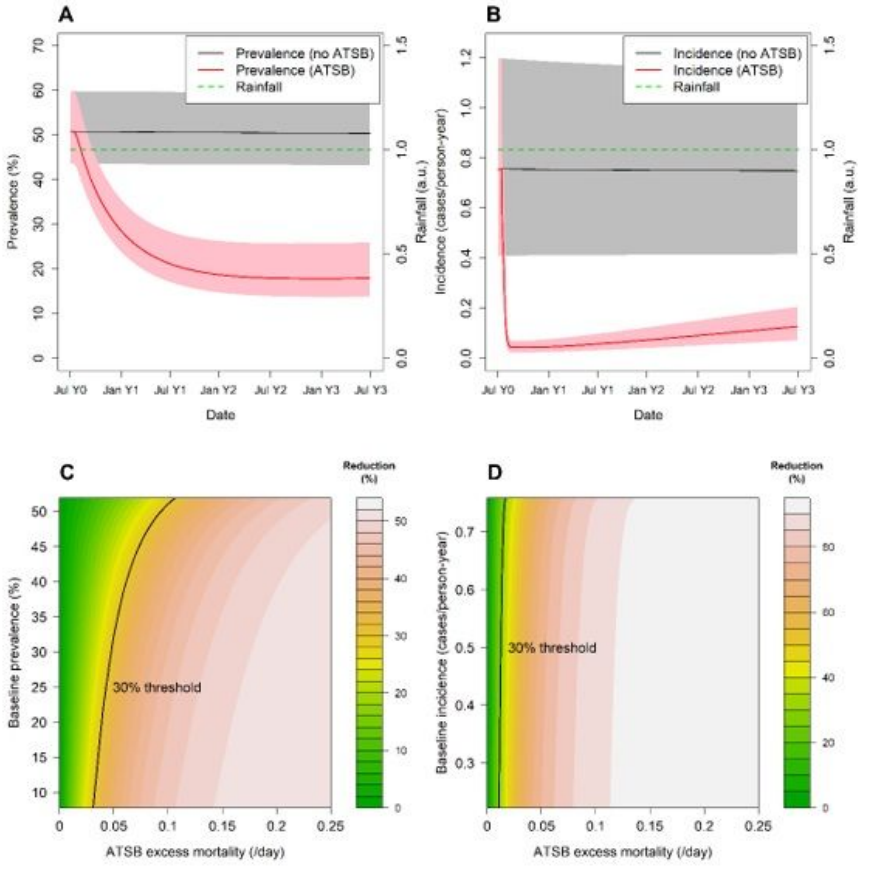

Figure 6

A-B: Model-predicted all-age parasite prevalence (A) and clinical incidence (B) over the course of 3 years after introduction of ATSBs (red line) and without ATSBs during the same period (black line) under the assumption of constant rainfall (green dotted line) For these runs the excess mortality μATSB is set to the average value estimated from field trial results (0.13/day). Shaded areas represent range of values obtained using model parameters in 95% credible interval. C-D Model-predicted reduction in all-ages year-round prevalence (C) and clinical incidence (D) as a function of prevalence/incidence under non-ATSB conditions and bait killing rate μATSB, assuming constant rainfall.

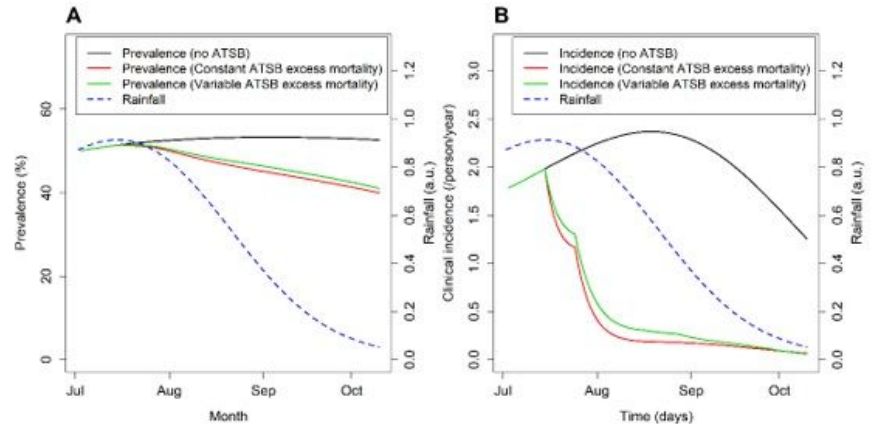

Figure 7

Predicted time progression of all-age parasite prevalence (A) and clinical incidence (B) under Mali rainfall conditions, over 100-day period representing the time period used to estimate the bait killing rate. Values are shown for an excess mortality due to ATSBs of zero

(control), for the average value estimated from field trial results (0.13/day), and for the variable values shown in Figure 2B. Credible intervals are not shown here as the red and green curves overlap.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- EstimatingthepotentialimpactofATSBsSI.pdf