

METHODOLOGY

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A novel model fitted to multiple life stages of malaria for assessing efficacy of transmission-blocking interventions

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Abstract

Background: Transmission-blocking interventions (TBIs) aim to eliminate malaria by reducing transmission of the parasite between the host and the invertebrate vector. TBIs include transmission-blocking drugs and vaccines that, when given to humans, are taken up by mosquitoes and inhibit parasitic development within the vector. Accurate methodologies are key to assess TBI efficacy to ensure that only the most potent candidates progress to expensive and time-consuming clinical trials. Measuring intervention efficacy can be problematic because there is substantial variation in the number of parasites in both the host and vector populations, which can impact transmission even in laboratory settings.

Methods: A statistically robust empirical method is introduced for estimating intervention efficacy from standardised population assay experiments. This method will be more reliable than simple summary statistics as it captures changes in parasite density in different life-stages. It also allows efficacy estimates at a finer resolution than previous methods enabling the impact of the intervention over successive generations to be tracked. A major advantage of the new methodology is that it makes no assumptions on the population dynamics of infection. This enables both host-to-vector and vector-to-host transmission to be density-dependent (or other) processes and generates easy-to-understand estimates of intervention efficacy.

Results: This method increases the precision of intervention efficacy estimates and demonstrates that relying on changes in infection prevalence (the proportion of infected hosts) alone may be insufficient to capture the impact of TBIs, which also suppress parasite density in secondarily infected hosts.

Conclusions: The method indicates that potentially useful, partially effective TBIs may require multiple infection cycles before substantial reductions in prevalence are observed, despite more rapidly suppressing parasite density. Accurate models to quantify efficacy will have important implications for understanding how TBI candidates might perform in field situations and how they should be evaluated in clinical trials.

Keywords: Atovaquone, *Plasmodium berghei*, Transmission-blocking drugs, Transmission-blocking vaccines

Background

Malaria remains a serious public health concern; in 2015, an estimated 214 million (range 149–303 million) new cases and 438,000 (236,000–635,000) deaths resulted

from the infection [1]. Malaria is transmitted between vertebrate hosts by anopheline mosquitoes. Circulating male and female gametocytes are ingested by mosquitoes during blood feeding where they undergo sexual reproduction before developing into oocysts on the wall of the midgut. On rupturing, an average 1250 (interquartile range 313–2400) salivary gland sporozoites are released from a single *Plasmodium falciparum* oocyst [2]. Sporozoites are then injected onward to the vertebrate host

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during a subsequent feed. Within the host, sporozoites travel to the liver and invade cells, divide and proliferate to produce tens of thousands of haploid forms, merozoites, per liver cell. Merozoites go on to reproduce asexually, resulting in blood-stage parasites. These can differentiate into gametocytes that complete the life cycle when transmitted back to the insect vector.

There are multiple transmission-blocking interventions (TBIs) currently under development that aim to reduce transmission by inhibiting the development of oocysts in the mosquito midgut or by targeting sexual, sporogonic and/or mosquito antigens (SSM-VIMT) [3]. Different assays have been developed to capture the data required to evaluate the efficacy of different TBIs and triage the most promising candidates [4]. The current gold standard is the standard membrane feeding assay (SMFA) [5, 6]. Here, mosquitoes are fed on cultured, infected blood through a membrane and oocysts can develop. Additionally, multiple derivative assays such as the direct membrane feeding assay (DMFA) and the direct (skin) feeding assay (DFA) are used [5]. The efficacy of drugs and vaccines against malaria have been assessed using data from these assays by measuring the relative reduction in the mean number or prevalence of oocysts in the vector, or prevalence for treated host populations in comparison to control groups [7, 8]. The highly aggregated (overdispersed) distribution of oocysts means that analysis of the reduction in the mean count of oocysts is inherently uncertain [9] and large sample sizes are needed to accurately capture infections [5]. One approach to overcome this is the use of the zero-inflated negative binomial distribution [10] of parasitic life stages that has been used to assess vaccines [11], and suggested as an improvement to capture the influence of aggregated infections on efficacy estimates [9].

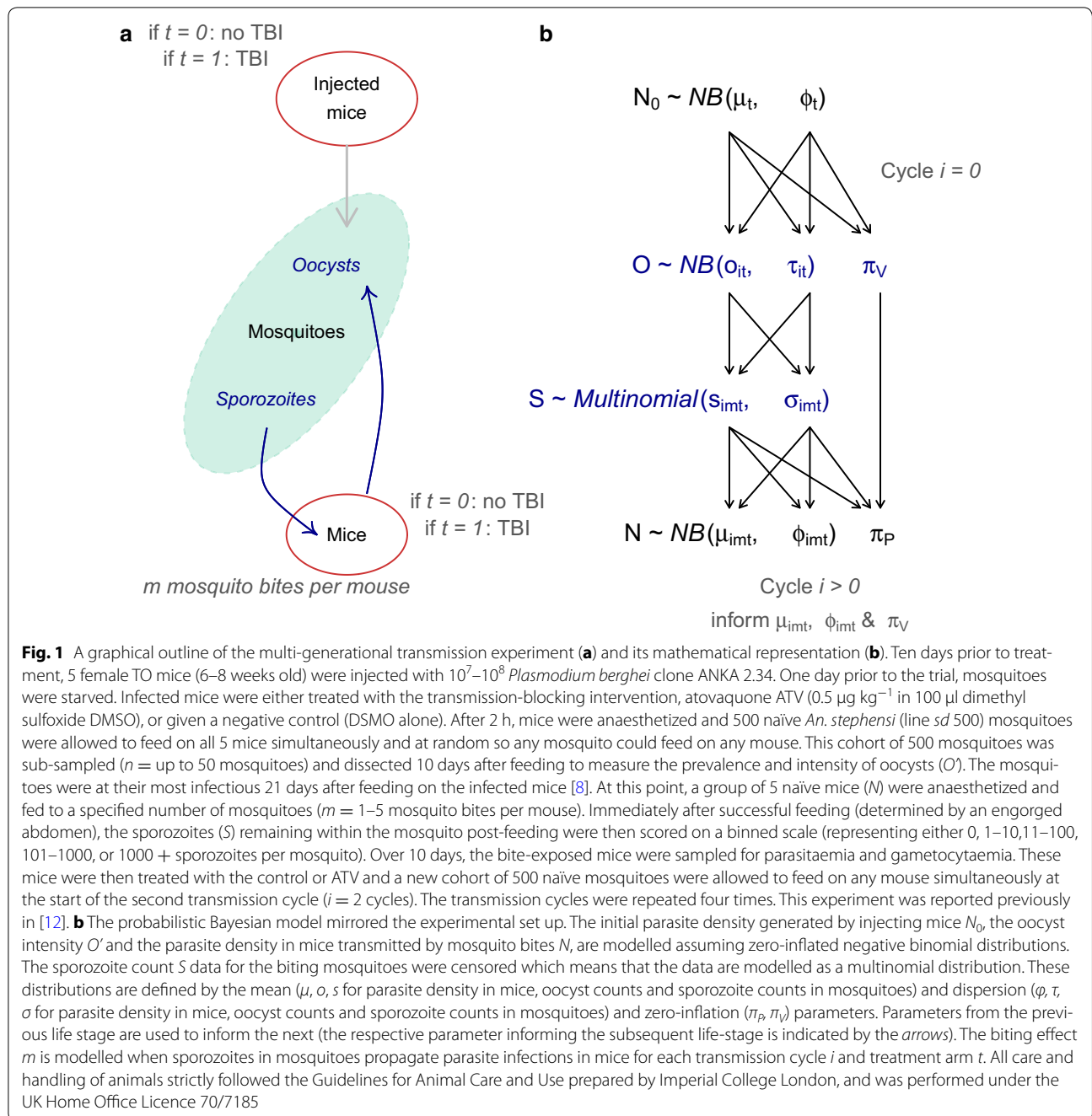
A second concern for TBI assessment has been that although SMFA (or its derivatives) capture reductions in infections to mosquitoes, they fail to capture the subsequent onward infectivity to the human host or the onward transmission over multiple cycles. To do this in laboratory studies, multi-generational experiments have been developed that simulate the passage of *Plasmodium berghei* between populations of mice and *Anopheles stephensi* mosquitoes [12, 13]. Using this approach, the transmission-blocking drug atovaquone (ATV), titrated to give a 32% reduction in oocyst intensity (ATV-32%) in a DFA, was found to have an estimated effect size of 20.4% [12]. The effect size measures the ability of a treatment to reduce the basic reproduction number of the parasite (the average number of secondary infections caused by an infected host). These estimates were made using a chain-binomial model [14, 15], but the assumptions inherent in the model affect estimation and need

further validation. Firstly, it assumes that all infectious mosquitoes and mice are equally infectious [15]. (In fact, the probability of infection from vertebrates-to-vectors or vice versa increases when hosts have higher infection intensities [16].) Secondly, the number of parasites in successive life stages is highly variable; for example, mosquitoes biting on the same blood source are likely to have widely differing oocyst or sporozoite counts [17]. The importance of parasite intensity on transmission means that it is necessary to account for stochastic variability. Thirdly, the chain binomial model assumes that the probabilities of infection from multiple infectious bites are independent. Finally, although the reproduction number and effect size calculations are widely understood by mathematical modellers, these terms are not generally used by those working in TBI development who tend to measure efficacy as either the transmission-blocking activity ((TBA), the reduction in the number of infected hosts or vectors) or transmission-reduction activity [(TRA), the reduction in mean parasite intensity of the host or vector population]. To overcome these issues, a probabilistic Bayesian model is developed that captures the generative structure of the complex data. This makes it possible to capture the relationship between successive stages of malaria across multiple vertebrate-to-vertebrate transmission cycles and generate TBA and TRA estimates. The zero-inflated negatively binomially distributed data for each parasitic life stage is used to inform the transmission probabilities between vector and host or host and vector. In this way, the transmission probability of a more heavily infected vector or host population (where each individual, on average, harbours a relatively high number of parasitic stages) could be higher than one with lower density infections even where prevalence (the proportion of individuals in the population with any infection) is matched. The Bayesian approach incorporates the variability between transmission events, which is much harder to incorporate into frequentist approaches without relying on approximations. This allows the uncertainty in the system to be captured in the model and enables relatively precise estimates of efficacy (the percentage reduction in parasite prevalence or infection intensity achieved by the TBI) to be broken down by transmission cycle and biting group (the number of potentially infectious mosquitoes biting each mouse during a transmission cycle) to provide a more detailed analysis of data.

Methods

Experimental design

Briefly, a population assay multigenerational experiment was used to cycle *P. berghei* malaria through mice and mosquitoes (Fig. 1; [12]). A statistical model was fitted



to the observed data and experimental structure using a Bayesian posterior distribution in Stan [18]. The model predictions (posterior draws) for the parasite densities of respective life stages were then used to calculate the efficacy of ATV.

For each treatment regime (ATV-32% and control), mouse-to-mouse transmission operated as described previously [12, 13], the experiment is graphically demonstrated in Fig. 1 and described in the figure’s legend. All

care and handling of animals strictly followed the Guidelines for Animal Care and Use prepared by Imperial College London, and was performed under the UK Home Office Licences 70/7185 and 70/8788.

Initial parasite density was measured by counting the number of infected red blood cells in the mice (N infected erythrocytes out of a total subsample of 1200 cells). Mosquitoes were dissected to assess the number of oocysts in the mosquito population. The sporozoite

measurement was additionally binned into specified ranges (scores of 0–4 representing 0, 1–10, 11–100, 101–1000, 1000 + sporozoites, respectively). The structure of the data (from [12] and listed in Additional file 2) resulted in four complete scenarios whereby malaria was transmitted mouse-to-mouse via mosquitoes (Fig. 2).

Statistical methods

In this experiment, it was not possible to measure the number of sporozoites reaching salivary glands from each oocyst or the number of blood-stage infections in mice resulting from injected sporozoites. These relationships are uncertain and are incorporated as nuisance parameters in the model (Additional file 1). Each of these stages is modelled sequentially, similarly to a hidden Markov model. The number of parasites in each stage is represented by a zero-inflated negative binomial distribution (NBD) to account for both infected and uninfected individuals. A bi-modal structure is fitted using a zero-inflation parameter, π , which determines the proportion of mice or mosquitoes that are uninfected and therefore cannot transmit. The shape of the relationships between different parasite life stages is unknown. This is accounted for by including a random effects component that allows the mean

number of parasites in each group to vary according to the observed data. A comprehensive description of the model is provided in Additional file 1.

Model fitting

All parameters were fitted jointly using a Bayesian posterior distribution in RStan (version 2.11, [18]). A non-centred parameterization method was employed [19, 20]. The model parameter fitting used a Hamiltonian Monte Carlo method [18], burn-in was 500 and the subsequent 500 samples from each chain ($n = 4$) were used for posterior predictive checks. The model R code is provided in Additional file 2, with the accompanying data (Additional file 2). The parameter estimates are supplied in Additional file 3.

Model output

Two measures of efficacy are presented: (1) the TBA, the percentage difference in the proportion of infected hosts between the control ($t = 0$) and treatment ($t = 1$) arms of the experiment; and, (2) the TRA, the percentage difference in parasite density between the control and treatment arms of the experiment. Efficacy estimates (denoted $TBA_{i,m}$ for prevalence, $TRA_{i,m}$ for intensity), were generated for each biting rate (m) and transmission

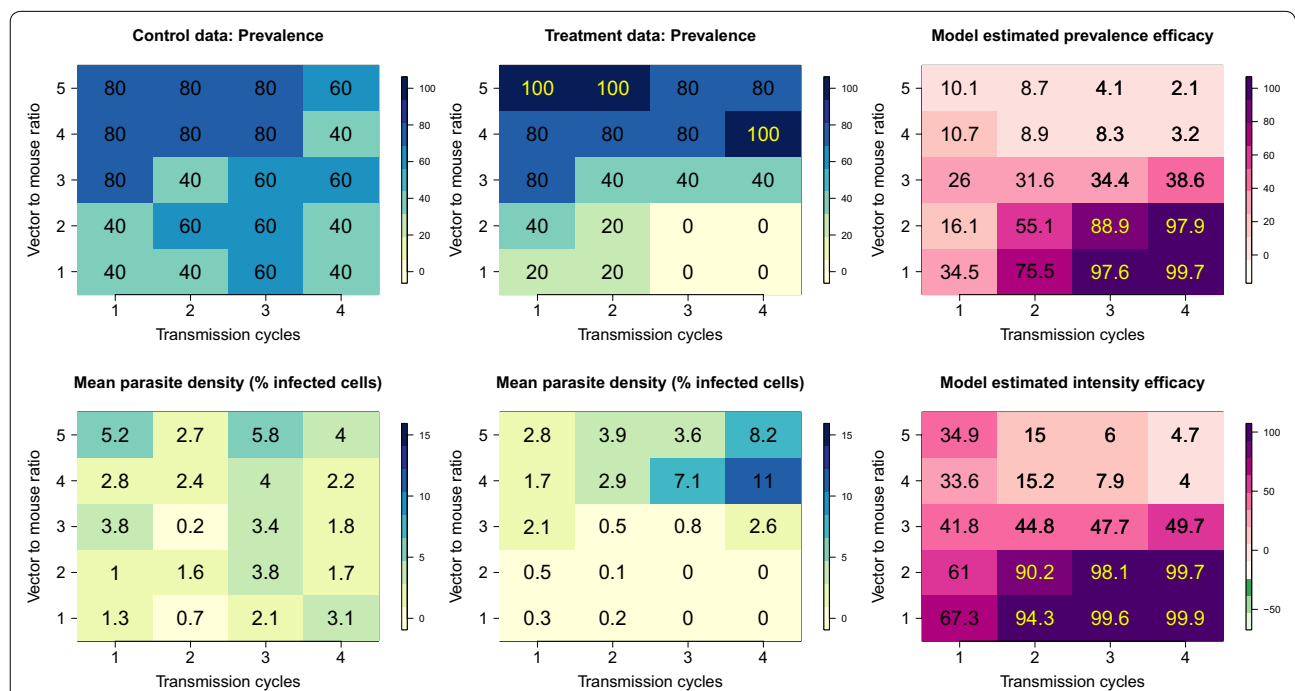


Fig. 2 Summary of the observed experimental data and efficacy estimates against prevalence and parasite intensity. Prevalence (the percentage of infected individuals) and percentage parasite densities (the mean percentage of infected erythrocytes out of 1200 cells per host) in control (column 1) and treatment (column 2) mice for transmission cycles 1–4 (x -axis) and for biting rates 1–5 (1–5 mosquito bites per mouse, y -axis). Corresponding % prevalence efficacy (top right) and % intensity efficacy (bottom right) estimated for each biting rate and transmission cycle using the probabilistic Bayesian model

cycle (*i*) from the simulated posterior predictive model outputs (*n* iterations = 2000) using the equations:

$$TBA_{i,m} = \frac{P_{0,i,m} - P_{1,i,m}}{P_{0,i,m}} \times 100$$

$$TRA_{i,m} = \frac{I_{0,i,m} - I_{1,i,m}}{I_{0,i,m}} \times 100$$

The 95% credible intervals were calculated directly from the posterior predictive outputs (Table 1).

To justify model assumptions, it was important to investigate the difference between the overall distribution of sporozoite scores in control and treatment groups, parasitaemia (%) and gametocytaemia (%). Data exploration was conducted in R, version 3.2.2 [21].

Results

Evaluating efficacy

With a full probabilistic model of the mouse-to-mouse measurements there is potential to define many possible measures of efficacy depending on exactly what elements of the model impact future interventions. Here two are presented: TBA and TRA (Table 1).

The prevalence efficacy TBA

The overall TBA of ATV-32% between the two arms of the experiment was 28.42% (28.0–28.9%: ±1.96 × standard error). There was a small increase in the impact against prevalence across transmission cycles (Table 1;

Table 1 Efficacy estimates

Group			Arithmetic mean (±1.96 × SE)		Median	
			TBA	TRA	TBA	TRA
Overall	N bites (m)	N cycles (i)	28.42 (28.0–28.9)	22.63 (21.7–23.6)	29.1	25.5
Per bite	1		28.42 (28.0–28.9)	22.63 (21.7–23.6)	29.1	25.5
	2		70.77 (69.8–71.7)	91.18 (90.7–91.6)	75.0	93.9
	3		58.60 (57.5–59.7)	88.58 (88.1–89.1)	62.5	91.2
	4		28.37 (27.2–29.5)	34.53 (32.9–36.1)	30.8	40.9
	5		5.87 (5.0–6.8)	−4.72 (−6.5 to −3.0)	6.3	−4.0
Per cycle		1	4.80 (3.9–5.7)	−8.78 (−10.6 to −7.0)	6.3	−12.2
		2	21.15 (20.3–22.0)	44.88 (43.8–45.9)	22.7	49.4
		3	30.20 (29.4–31.1)	28.63 (27.1–30.1)	31.3	35.0
		4	32.60 (31.7–33.5)	13.51 (11.7–15.3)	33.3	16.6
Per bite and cycle	1	1	28.42 (27.4–29.5)	4.16 (2.2–6.1)	28.6	1.3
	1	2	34.5 (32.0–37.0)	67.30 (65.7–68.9)	50.0	78.0
	1	3	75.53 (73.7–77.4)	94.33 (93.7–95.0)	100	100
	1	4	97.60 (97.0–98.2)	99.56 (99.3–99.8)	100	100
	2	1	99.70 (99.5–99.9)	99.9 (99.8–100)	100	100
	2	2	16.14 (13.4–18.9)	60.97 (59.3–62.6)	33.3	71.8
	2	3	55.14 (52.6–57.7)	90.18 (89.3–91.0)	66.7	96.9
	2	4	88.9 (87.6–90.2)	98.07 (97.7–98.5)	100	100
	3	1	97.93 (97.2–98.6)	99.74 (99.6–99.8)	100	100
	3	2	25.95 (23.8–28.1)	41.85 (39.8–43.9)	33.3	53.1
	3	3	31.60 (29.2–34.0)	44.80 (42.5–47.1)	33.3	57.5
	3	4	34.4 (31.7–37.2)	47.7 (45.3–50.2)	50.0	61.6
	4	1	38.60 (35.7–41.5)	49.7 (57.0–52.4)	50.0	66.3
	4	2	10.65 (8.9–12.4)	33.6 (31.6–35.6)	20.0	44.0
	4	3	8.88 (7.0–10.8)	15.18 (12.8–17.5)	0.0	22.1
4	4	8.29 (6.2–10.4)	7.87 (5.3–10.4)	0.0	14.2	
5	1	3.23 (0.6–9.5)	4.00 (1.2–6.8)	0.0	9.5	
5	2	10.15 (8.6–77.4)	34.9 (33.0–36.9)	20.0	44.1	
5	3	8.65 (6.7–10.6)	14.98 (12.5–17.4)	0.0	21.8	
5	4	4.12 (1.59–6.7)	5.99 (3.3–8.7)	0.0	11.2	

The mean and standard errors (±1.96 × SE) and median for prevalence, TBA and intensity efficacy, TRA estimates for biting rates 1–5 (the number of mosquito bites per mouse), transmission cycles 1–4, calculated from the posterior distributions of the probabilistic Bayesian model

TBA transmission-blocking activity, TRA transmission-reducing activity

Fig. 3) indicating multiple cycles were required to fully benefit from the ATV treatment.

The impact of ATV was greatest at low mosquito biting rates (where each mouse received a single mosquito bite, the TBA was 70.8% (69.8–71.7%); for two mosquito bites per mouse: 58.6% (57.5–59.7%); and, for three bites per mouse: 28.4% (27.2–29.5%)) whilst the impact was much reduced at higher mosquito biting rates [for four bites: 5.9% (5.0–6.8%); for five bites: 4.8% (3.9–5.7%)].

Per bite TBAs were consistent after the second completed transmission cycle (Fig. 3) suggesting that three cycles would be sufficient to capture the impact of ATV at a concentration expected to reduce transmission by 32% from vertebrate-to-vector [12].

The intensity efficacy TRA

The overall TRA was 22.63% (21.7–23.6%). The biggest impact against intensity was achieved after the first transmission cycle (Table 1; Fig. 3).

Like parasite prevalence efficacies, the impact of ATV on parasite density was greatest at low mosquito biting rates; 91.2% (90.7–91.6%), 88.6% (88.1–89.1%) and 34.5% (32.9–36.1%) for one to three mosquito bites per mouse, respectively. There was no positive impact on parasite

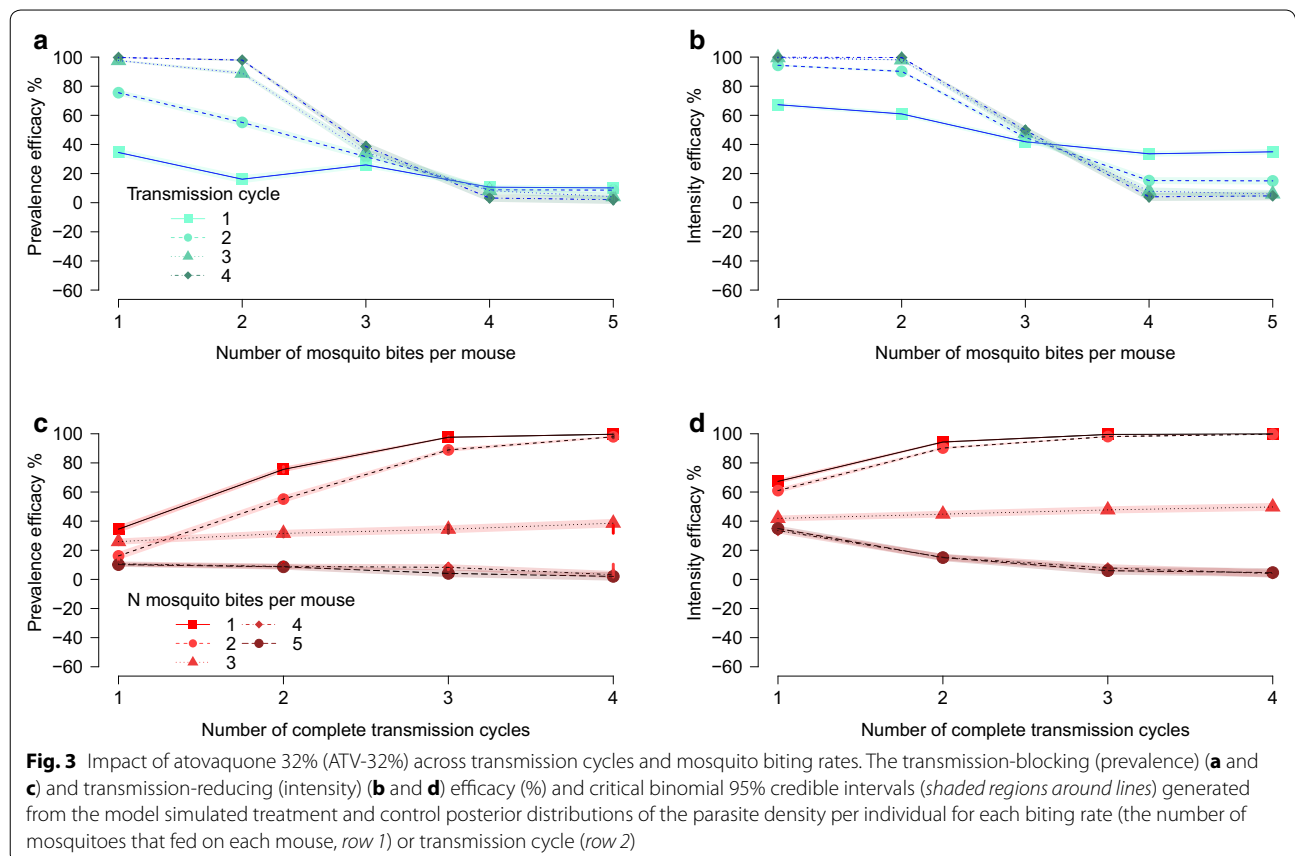
intensity at higher mosquito biting rates; -4.7% (-6.1–3.0%) and -8.8% (-10.6–7.0%) for four and five mosquito bites per mouse (Fig. 2).

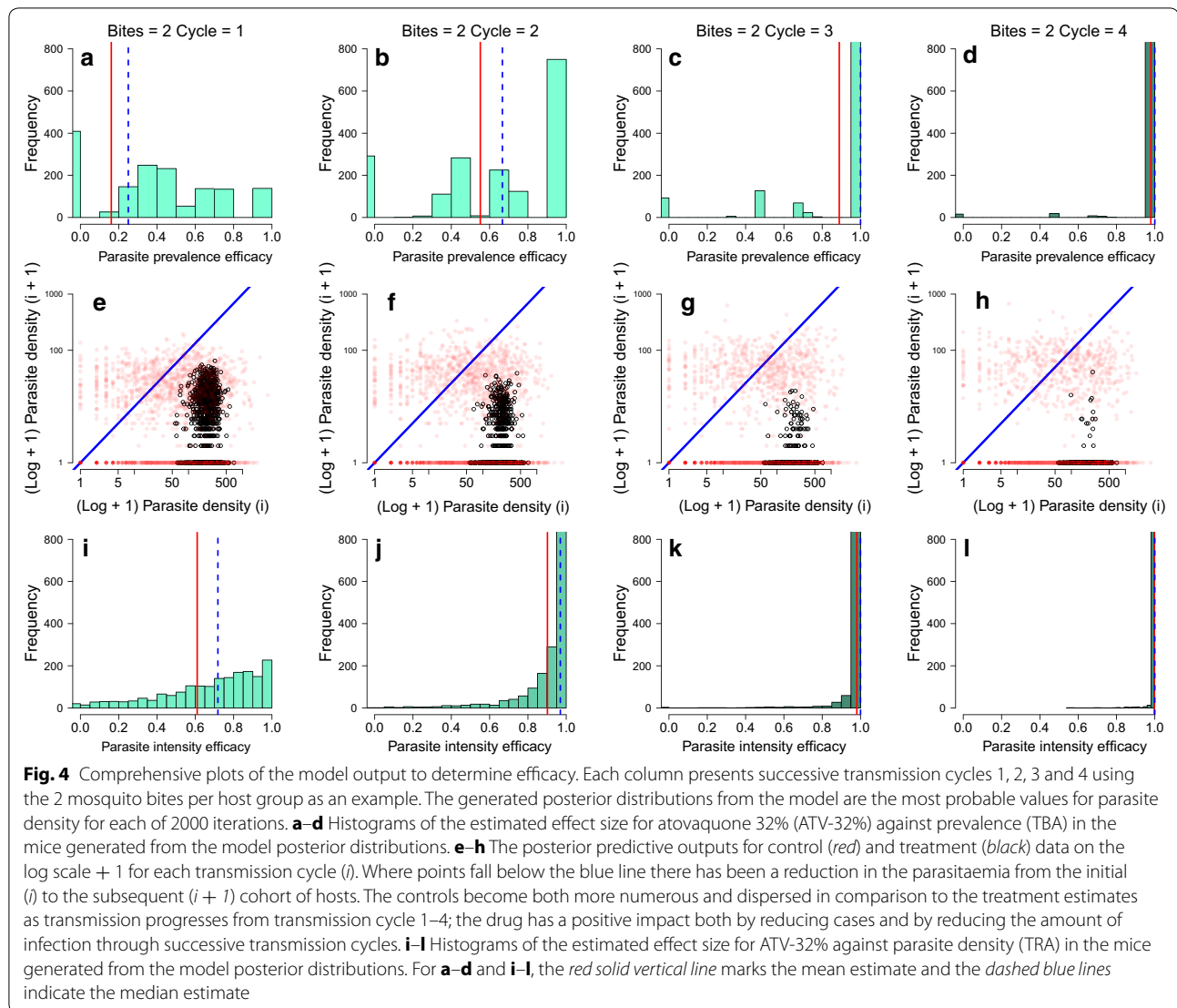
An illustration of the posterior outputs of the model is shown in Fig. 4.

Parasite intensity, transmission and heavy infections

There is a non-linear relationship between each of the different parasite life stages in the model confirming that transmission between hosts and vectors (and vice versa) are saturating (negative) density-dependent processes (Fig. 5). Capturing these relationships enables more accurate estimation of efficacy by considering the natural population dynamics of infection that are also observed in the field for human infections [22].

Assessing efficacy estimates by transmission cycle and biting rate allows more detailed examination of the intervention's impact. For example, in the treatment arm of the experiment, two of five mice in the transmission cycle 3 cohort, which received four mosquito bites each, had exceptionally high parasitaemia ($\geq 12\%$ of 1200 red blood cells were infected). Both mice had, by chance, been fed on by mosquitoes with very high post-feeding sporozoite counts (indicated by high mean sporozoite scores, >2).



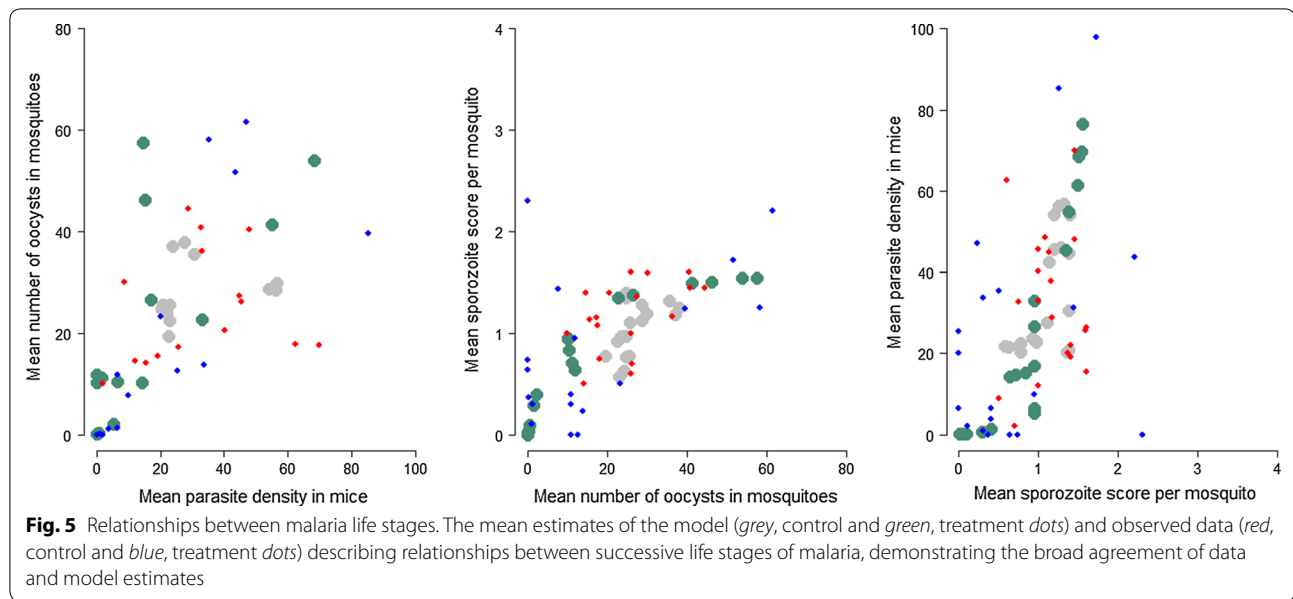


Once exposed, these mice can be fed upon by any of the 500 naïve mosquitoes in the next cohort. Consequently, higher than expected parasite densities are propagated throughout the system (Fig. 2).

Although the infectiousness of mice depends on the mature gametocytes present in the blood, rather than the asexual parasites (that are used in the model), there is less confidence in microscopic measurements of gametocyte counts. A linear relationship between mean gametocytaemia (the percentage of erythrocytes infected with sexual stage parasites) and mean parasitaemia (the percentage of erythrocytes infected with asexual stage parasites) was consistent with the data ($\text{mean gametocytaemia} \sim 0.11 \times \text{mean parasitaemia} + 0.03$, $\sigma = 0.12$: $F_{40,38} = 197.1$, $p < 0.001$, $\text{adj} R^2 = 0.83$), justifying the use of parasitaemia as a dependent variable in addition to infection status.

Discussion

Accurate methods to assess potential TBIs are important to ensure that the best candidates go forward to expensive clinical trials. Here, TBI efficacy from mouse population assays is estimated by fitting a probabilistic Bayesian model to multiple parasitic life stages with a structure that reflects the experiment structure used to collect the data. Whilst it may be more computationally expensive to fit a multistage Bayesian model such as the one presented here, the method: (i) is able to identify chance events that can occur at any point in the experiment; (ii) is statistically robust to natural fluctuations in the starting parasite density, which impacts the capacity of the treatment to reduce secondary infections; and (iii) has the flexibility to capture the density dependent (or other) relationships between parasite life stages. The



chain binomial model produced a relatively similar estimate for ATV-32% (20.4% reduction in secondary cases) when compared to the Bayesian approach (28.4% efficacy against prevalence).

Although the two metrics are not directly comparable, they provide complementary understanding of the intervention impact. Firstly, the chain binomial model presents an overall effect size that measures the reduction in secondary cases in hosts, the R_0 , that can be achieved by using vaccine. This can be used directly in mathematical models to understand the epidemiology of diseases. Conversely the Bayesian method directly calculates the reduction in the prevalence and density of infection between the different arms of the experiment. This generates more intuitive efficacy estimates which are more comparable to the results of clinical trials and are easier to understand by non-mathematical modellers. Secondly, the chain binomial model assumes that all infected mice and mosquitoes have the same infectivity irrespective of the density of infection. This can bias estimates depending on the quantity of parasite in the system. The new method allows the infectivity to vary according to the density of the parasite at the particular life-stages which provides additional information for the actions of an intervention. Finally, the chain binomial model generates a single effect size estimate whilst the Bayesian model enables intervention efficacy to be broken down by treatment round and biting rate. This finer scale of resolution allows a more detailed understanding of the dynamics of the disease, for example by showing that parasite density efficacy is higher in earlier transmission cycles compared to the efficacy against parasite prevalence. This finer resolution

also enables chance fluctuations in parasite intensity to be identified, which could bias overall estimates in populations with small sample sizes and generate misleading results. One of the advantages of the new method is that it makes no assumptions about the likelihood of infection of multiply bitten hosts unlike the chain binomial model which assumes that mice are more likely to have detectable parasites the more infected bites they receive (following a binomial distribution). This makes results from the low biting groups relatively uncertain (due to stochastic fluctuations) so these groups should be interpreted in the context of other results.

Measuring impact at different mosquito biting rates and transmission cycles shows that ATV works first to reduce parasite density and later to reduce secondary infections. Single mouse-to-mouse transmission cycle population experiments have been performed previously [13]. Although this would generate accurate predictions of efficacy for highly effective interventions (which eliminate all onward transmission), using a single cycle of mouse-to-mouse transmission could potentially underestimate the impact of partially effective TBIs. The model also indicates that, for the intervention investigated here, efficacy estimates remain relatively stable after three cycles of transmission. This may allow the length of the experiment to be shortened, lowering costs and reducing the number of mice required.

The mouse population assay outlined in [12] is a standardized experiment and will be used to address future hypotheses including proof of concept questions such as the impact of combining interventions (for example a transmission-blocking vaccines with pre-erythrocytic

vaccines) or changing levels of vaccine coverage. These types of experiments allow the consequences of different epidemiological characteristics or control interventions to be triaged in a controlled environment that would be infeasible to do in a human system or would require multiple, very expensive randomised control trials.

Given the relatively sparse data for each trial this analysis can be sensitive to systematic variation caused by fluctuations in the amount of malaria per individual in any given transmission cycle. The hierarchical structure used, however, does prevent a fluctuation in any one distribution (caused by unusually heavily infected individuals) from strongly biasing the inferences of other life-stages. Moreover, the weakly-informative priors used in the analysis limit the potential for overfitting and the predictive checks showed no indication of such pathologies. The analysis could be improved by incorporating greater biological realism into the model by increasing the number of intermediate life-stages (for example including a gametocyte life-stage or better capturing host immune responses). However, the benefit of this enhanced realism in the model depends on the applicability of the rodent system to human malaria. There is much debate about the capacity for mouse models to inform on aspects of human malaria [23–25] so extrapolating to *P. falciparum* must be done with caution.

Conclusions

Here, a standardised statistical methodology is outlined which uses the Hamiltonian Monte Carlo method (RStan, version 2.11 [18]) to fit to population assay experimental data. To the author's knowledge, this is the first time these methods have been used to examine parasitological data and their flexibility and precision makes them ideally suited for this purpose. In many parasitic infections the population dynamics of transmission and host morbidity and mortality depends on the number of parasites in a host and/or vector and not their presence/absence [26]. The distribution of parasites between hosts is typically highly aggregated [16, 27, 28]. This is particularly the case for many human parasitic infections, which are the target of elimination programmes [29, 30, 31]. As the parasite becomes increasingly rare, mean-based estimates of parasite intensity become increasingly uncertain. The range of statistical distributions available in Stan enables a more precise population estimate of parasite intensity. Many of the processes governing parasite transmission are density-dependent [27, 32]. Using a probabilistic approach enables these non-linear processes to be captured at the individual level whilst considering parasite distributions without the need for overly simplifying the experimental structure in the statistical model or running computationally expensive individual-based transmission models [33].

Additional files

Additional file 1. A comprehensive statistical description of the probabilistic Bayesian model.

Additional file 2. A collection of files containing the original data in list format (Additional file 2.1), a 'how to' R script (Additional file 2.2) to apply the Bayesian model to DFA multi-generational data in RStan (Additional file 2.1 can be used as an example). The probabilistic Bayesian model code (Additional file 2.3) and the data (Additional file 2.1) used in Blagborough et al. [12] are supplied together with functions (Additional file 2.4) to help assess the model output (Additional file 2.5).

Additional file 3. The parameter estimates and the alternative efficacy measure using infection probabilities. Table 2.1 The table of parameter estimates and estimated mean values for the posterior predictive data in the model. Table 2.2 The 'infection reduction' efficacy $E_{i,m}^R$ is calculated as the difference between the summed mean π_i and mean π_p for controls compared to treatment arms for each transmission cycle i and biting rate m . The efficacy impact that can be attributed to π_v is shown.

Abbreviations

TBI: transmission-blocking interventions; TBV: transmission-blocking vaccine; SMFA: standard membrane feeding assay; DMFA: direct membrane feeding assay; DFA: direct feeding assay; ATV: atovaquone; TBA: transmission-blocking activity; TRA: transmission-reducing activity; NBD: negative binomial distribution.

Authors' contributions

TSC and AMB secured the funding for the work. ESS, MB and TSC conceived the method. AMB, LMU, KAS, and SEZ performed the experiments and collated the data. ESS analysed and formatted the data. MB and ESS constructed the model. ESS and HSC prepared the figures. ESS, MB and TSC drafted the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data generated and analysed during the current study are included in the published article and supplementary information files.

Ethical approval

All care and handling of animals strictly followed the Guidelines for Animal Care and Use prepared by Imperial College London, and was performed under the UK Home Office Licences 70/7185 and 70/8788.

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