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Uses of mosquito-stage transmission-blocking vaccines against *Plasmodium falciparum*

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A quantitative framework is used to explore the potential applications and probable effects of sexual stage or mosquito stage transmission blocking vaccines (TBVs) against malaria. The combination of TBVs with biocides or other malaria vaccines will increase chances of interrupting transmission, whereas the value of TBVs for morbidity control will be limited. Vaccine combination will also protect against selection of insensitive parasites. Simulations indicate that TBVs will reduce risks of reestablishment of transmission when vector control is withdrawn. Simple mathematical analysis shows that efficacy and coverage are equally important, implying that a vaccine that requires a small number of doses (ideally one) is preferable to one that is difficult to deliver, even if this entails accepting a lower efficacy.

Renewed interest in the potential of mosquito stage TBVs

Recent international commitments to malaria elimination and eradication have increased interest in the development of new tools for reducing malaria transmission, even ones with limited potential for the immediate control of disease or mortality. These include vaccines aimed at parasite stages that are exposed only in the mosquito, referred to as transmission-blocking vaccines* (TBVs). Several mosquito-stage antigens, including Pfs 48/45, Pfs 230, Pfs 25, and Pfs 28 of *Plasmodium falciparum*, (and some of their *Plasmodium vivax* analogs) have undergone pre-clinical development, some proving highly immunogenic [1]. None have yet passed early stages of clinical testing [2].

Possible uses of TBVs extend beyond immediate interruption of transmission. This article illustrates this using simple mathematical analyses and simulations. It discusses: (i) how the efficacy of a TBV can be quantified, and what effect TBV deployment can have on the reproduction number and the entomological inoculation rate (EIR); (ii) the potential use of TBVs to control morbidity and mortality in high transmission areas; (iii) the potential use of TBVs in elimination programs; and (iv), the potential use of TBVs to protect other vaccines from the evolution of parasite insensitivity to vaccine-induced responses.

Measures of the efficacy and effect of TBVs

The efficacy of a TBV can be expressed in terms of c, the probability that an infectious host transmits when a mosquito feeds on it (Box 1). Unfortunately, it is extremely challenging to estimate this probability from field data because gametocytemia is often cryptic, making precise determination of which humans are infectious impossible. This is the case even when molecular methods that detect and quantify gametocyte-specific RNA are used [3], because only a small proportion of blood is sampled. It is probable that the best way of making definitive estimates of the efficacy of a TBV is through a field trial, measuring the efficacy in terms of κ, the probability that a mosquito becomes infected during a single feed on an individual with unknown infection status, but known vaccination status (Box 2). When both vaccine and placebo recipients are from the same population, the proportion of infectious humans is the same, and κ can be estimated by letting mosquitoes feed on the blood of trial participants from either arm of the trial [4,5]. Membrane feeding assays can be well standardized and avoid ethical issues associated with direct feeding of mosquitoes, but will not necessarily provide unbiased estimates of the true field values of κ. However, if bias affects both arms of the trial equally, estimates of the efficacy will be unbiased. High-throughput processing of batches of mosquitoes will be required to obtain adequate statistical power. In some trials it might be preferred to infer protection from antibody titers [6]. These can be calibrated against the gold standard of entomologically demonstrated protection [6].

In principle, a TBV program might aim either to interrupt or merely reduce transmission. When the aim is to interrupt transmission, the efficacy relates directly to the effect size that can be achieved by a TBV in reducing the reproduction number (Box 1). When the aim is to reduce transmission, estimates of the efficacy will be useful in predicting the effects of TBV deployment on the EIR (Box 2). In high transmission settings, the effect on the EIR will be very similar to that on the reproduction number, whereas in low transmission settings the effect on the EIR is expected to be greater than that on the reproduction number.

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* All vaccines that interfere with the life cycle of the parasite have effects on transmission.

1471-4922/$ – see front matter doi:10.1016/j.pt.2010.12.011 Trends in Parasitology, May 2011, Vol. 27, No. 5

Glossary

Field measurable quantities needed for estimating efficacy of TBVs

Quantity	*Notation*
Average probability that a mosquito becomes infected during a single feed in a completely unvaccinated population	$\bar{\kappa}_n$
Average probability that a mosquito becomes infected during a single feed in a (partially) vaccinated population	$\bar{\kappa}_v$
Probability that a mosquito becomes infected during a single feed on an unvaccinated individual in a partially vaccinated population	κ_n
Probability that a mosquito becomes infected during a single feed on a vaccinated individual in a (partially) vaccinated population	κ_v

Additional quantities relevant to the feasibility of maintaining interruption of malaria transmission

Quantity	*Explanation*	*Notation*
Basic reproduction number	Number of secondary cases from one primary case in a naive population	R_0
TBV efficacy	Proportionate reduction in the probability that a mosquito vector acquires an infection in any given feed, associated with the host being vaccinated	ξ
Vaccine coverage	Proportion of the local human population vaccinated	θ
Effective coverage of the (post-vaccination) surveillance system	Proportion of transmission from introduced infections that is eliminated by treating actively and passively detected cases	η
Controlled reproduction number (vector control)	Reproduction number allowing for effects of concomitant vector control interventions	$R_c^{(VC)}$
Naturally acquired immunity	Effect of previous exposure on transmission (which we expect to decline with time)	$\alpha(t)$

Use of TBVs to control morbidity and mortality in high transmission areas

Vaccines are among the most powerful tools for preventing morbidity and mortality caused by infectious diseases, and the most obvious setting for deploying a malaria vaccine is in areas with a high disease burden. Unfortunately, simulations of TBV deployment [7] suggest that they would generally be of little benefit in combating disease in areas of initially stable endemicity because transmission reduction results in a less than proportionate reduction in disease incidence. The utility of TBVs for morbidity control in such settings could be enhanced by combining them with pre-erythrocytic (PEVs) or blood-stage vaccines (BSVs), but to be of substantial value they will need to be deployed at high coverage in combination with very highly efficacious PEVs [7]. In the best cases, a TBV would augment herd immunity effects of the PEV, leading to substantial reductions in transmission with consequent health benefits. The probable health effects of TBV programs in settings where transmission is naturally low or has been lowered through vector control, but not interrupted, remain to be studied.

Use of TBVs in elimination programs

The mathematics of eliminating malaria is more complicated than that of eliminating viruses which lead to lifelong immunity, for which a simple relation between the basic reproduction number, R_0 (Box 1), and the required vaccination coverage applies [8]. Prior immunity reduces malaria infectiousness, but immunity does not prevent infection, which complicates full analysis of the system. Achievement of interruption of transmission is equivalent to keeping the effective reproduction number, R_e (Box 1), which is the average number of secondary infections per infection, below one until there are no more infections. Once transmission has been interrupted, R_e measures the receptivity of the site to introduced cases and depends on quantities listed in the second part of the Glossary. Assuming these different factors act independently, R_e can be expressed as the product: (1.13 in Box 1)

$$R_e = R_c^{(VC)}\alpha(1-\eta)(1-\theta\xi).$$

This equation does not imply equivalence in the effort required to achieve effects by increasing different parameters, but the multiplication of vaccine efficacy, ξ, and coverage, θ, does imply: (i) an equipoise between vaccine efficacy and coverage in terms of the importance of optimizing them; and (ii) an equipoise between the terms $(1-\eta)$ and $(1-\theta\xi)$, corresponding respectively to the probability that a transmission event is missed by the surveillance system, and to the probability that it evades the vaccine effect.

Although useful for mathematically understanding the effects of TBVs on transmission, reproduction numbers are problematical to measure. They are not single uniform quantities, but vary spatially and temporally, raising both conceptual and practical challenges in predicting the feasibility of elimination. The probability of interrupting transmission is difficult to quantify, both because of the challenge of measuring transmission when it is low, and because the outcome is sensitive to model assumptions. In low transmission settings, the dynamics of malaria also depend on the vulnerability, that is, the challenge to the population by imported infections, which in turn depends on the degree of connectedness among human populations, and the extent of mosquito migration. The prospects of success and the probable size of epidemics that might arise when transmission is not prevented also depend stochastically on the size of the human population. Other relevant factors are heterogeneities[†] over time in vaccine efficacy and in natural immunity, and possible changes in the effects of vector control.

Transient effects of specific intervention strategies are therefore difficult to analyze mathematically, motivating stochastic simulation approaches [7]. Uncertainty can be

[†] In contrast to pre-erythrocytic vaccination, heterogeneities between humans in vaccine efficacy are not relevant to the outcome of TBV programs, as these heterogeneities are averaged out if there is a well-mixing mosquito population

Box 1. Effects of TBVs on the reproduction number

Expressions are derived for the effect of interventions on reproduction numbers based on the Ross-Macdonald model[‡]. Although this simplifies many processes of the malaria life cycle, it still allows us to analyze in a relatively easy fashion the effects of TBVs, vector control, surveillance and case management, and naturally acquired immunity on the important processes of malaria transmission. These reproduction numbers are a product of processes in the mosquito and in the human host. All the entomological parameters determining transmission are captured by the vectorial capacity, Γ.

Vectorial capacity was originally defined as ''the average number of inoculations with a specified parasite originating from one case of malaria in unit time that the population would distribute to men if all the vector females biting the case became infected'' [17],

$$\breve{\Gamma} = \frac{ma^2 \exp(-\mu\tau)}{\mu} \tag{1.1}$$

with parameters: m: number of female mosquitoes per human host, a: number of bites on humans per mosquito per unit time, τ: extrinsic incubation period (time), μ: death rate of mosquitoes (per unit time).

However, a more general definition allowing for incomplete susceptibility of mosquitoes is:

$$\Gamma = c\breve{\Gamma} = \frac{ma^2 c \exp(-\mu\tau)}{\mu} \tag{1.2}$$

where c is the probability that a bite on an infectious human with no prior immunity results in a viable infection of the mosquito (provided the latter survives τ). The basic reproduction number in the Ross-Macdonald model is the product of Γ and parameters representing the human part of the cycle:

$$R_0 = \frac{b\Gamma}{\gamma}. \tag{1.3}$$

where:

b: probability that an infectious bite from a mosquito leads to an infection in a naive human, and γ: recovery rate of humans (per unit time).

Which leads to the familiar formula:

$$R_0 = \frac{ma^2 bc \exp(-\mu\tau)}{\gamma\mu} \tag{1.4}$$

Assuming that TBVs only affect the probability that a bite on an infectious human results in a viable infection of the mosquito:

$$\tilde{c} = c_v\theta + c_n(1-\theta) \tag{1.5}$$

where c_n is the infectivity of non-vaccinated infectious humans to mosquitoes, c_v is the infectivity of vaccinated infectious humans to mosquitoes, and θ is the coverage level of the vaccine. The vaccine efficacy, ξ, is then:

$$\xi = 1 - \frac{c_v}{c_n}. \tag{1.6}$$

Some further algebraic manipulations show that the controlled reproduction number [18] for a TBV can be written as:

$$R_c^{(TBV)} = \frac{R_0(c_v\theta + c_n(1-\theta))}{c_n} = R_0(1-\theta\xi). \tag{1.7}$$

Assuming that vector control transforms m, a, and μ, to $\tilde{m}$, $\tilde{a}$ and $\tilde{\mu}$ respectively, the controlled reproduction number for vector control is:

$$R_c^{(VC)} = \frac{\tilde{m}\tilde{a}^2 bc \exp(-\tilde{\mu}\tau)}{\gamma\tilde{\mu}}. \tag{1.8}$$

Assuming that surveillance and treatment of infected humans reduces the duration of infectiousness,

$$\frac{1}{\breve{\gamma}} = \frac{(1-\eta)}{\gamma}, \tag{1.9}$$

where $\breve{\gamma}$ is the human recovery rate in the presence of surveillance and η is the effective coverage level of surveillance. The controlled reproduction number, accounting for surveillance and treatment, is then:

$$R_c^{(Surv)} = R_0(1-\eta) \tag{1.10}$$

Assuming that naturally acquired immunity only reduces the ratio of bc to γ by some factor α,

$$\frac{\tilde{b}\tilde{c}}{\tilde{\gamma}} = \alpha\frac{bc}{\gamma}, \tag{1.11}$$

where $\tilde{b}$ is the probability that an infectious bite from a mosquito leads to an infection in a population with naturally acquired immunity, and $\tilde{c}$ is the probability that a bite on an infectious human with naturally acquired immunity results in a viable infection in the mosquito, and $\tilde{\gamma}$ is the recovery rate in a human with natural immunity. The effective reproduction number (in the absence of control), which includes the effects of naturally acquired immunity, would then be:

$$R_e^{(Imm)} = R_0\alpha \tag{1.12}$$

Assuming that the TBV, vector control, surveillance and treatment, and naturally acquired immunity act independently of each other, the overall effective reproduction number can be written as:

$$R_e = R_c^{(VC)}\alpha(1-\eta)(1-\theta\xi) \tag{1.13}$$

[‡] Assuming that TBVs do not change vector behavior, and that the effect of multiple infection of the mosquito can be ignored, as most naturally infected mosquitoes harbor only a single oocyst [16], which results in a very large number of sporozoites.

quantified by using an ensemble of model structures and parameterizations. To illustrate this, the health effects associated with withdrawing an IRS program, and how these effects would be mitigated by mass vaccination of the population with a TBV at the same time as delivering the last IRS spray round, are simulated. There is a clear rebound in incidence with IRS alone (Figure 1(a)), reflecting reestablishment of transmission, but the timing of this is highly unpredictable (the uncertainty is captured by the different median lines and 95% probability intervals for sub-models). With a TBV added to the last IRS round (Figure 1(b)), the reestablishment does not generally occur until after the vaccine efficacy has fallen considerably (and the protection has been diluted by new births). When reestablishment occurs it does so more slowly.

Use of TBVs to protect other malaria vaccines

Another use of TBVs would be to protect other types of malaria vaccines against selection for vaccine-insensitive parasites (Box 3). Both naturally occurring variation in sensitivity and antigenic profile changing mutations, (which are probably less tightly constrained than mutations that alter drug resistance), will allow parasites to evade recognition by vaccine-induced antibodies. Even during a trial of a potential BSV, evidence for selection of insensitive parasites was found [9]. Evolution of insensitivity might also be an immediate threat to PEVs. Many PEVs, including the currently most advanced candidate, RTS,S [10], target the amino acid repeat sequence asparagine–alanine–asparagine–proline (NANP) of the *P. falciparum* circumsporozoite protein. As the selective pressures maintaining this sequence can

Box 2. Effects of TBVs on the EIR

If $\kappa = cx$ is the probability that a mosquito becomes infected during a single feed, with x the proportion of infectious humans in the population and c as defined in Box 1, equation 1.6 (Box 1) for the vaccine efficacy, ξ, can be expanded:

$$\xi = 1 - \frac{c_v}{c_n} = 1 - \frac{xc_v}{xc_n} = 1 - \frac{\kappa_v}{\kappa_n}, \quad (2.1)$$

where the different infectiousness parameters are as explained in the glossary. If $\bar{\kappa}_n$ is the value of κ for a non-vaccinated population with vectorial capacity Γ and EIR E_n, then, assuming super-infection of the vector to be negligible,

$$E_n = \Gamma\bar{\kappa}_n. \quad (2.2)$$

In an otherwise equivalent partially-vaccinated population with coverage θ, the value of κ is:

$$\bar{\kappa}_v = \kappa_v\theta + \kappa_n(1-\theta), \quad (2.3)$$

and the equilibrium EIR is therefore:

$$E_v = \Gamma\bar{\kappa}_v = \Gamma(\kappa_v\theta + \kappa_n(1-\theta)). \quad (2.4)$$

From the above it follows that:

$$E_v = \frac{\kappa_n}{\bar{\kappa}_n}(1-\theta\xi)E_n. \quad (2.5)$$

Introduction of TBV will reduce the infectivity of the vaccinated individuals and through (2.3) the whole population. As the vectorial capacity does not change, infection in the mosquitoes would decrease and the EIR will be reduced. The reduction in EIR, in turn, will affect κ (Figure I). Depending on the initial EIR, this reduction in EIR could possibly:

- Have no further reduction in κ, in which case this reduced EIR would remain the EIR of the vaccinated population. This is because at high exposure levels (to the right of the arrow on Figure I) all parasitological indices saturate as a result of the immune control of blood stage parasites. The estimates of $\bar{\kappa}_n$ are then more or less independent of E_n. In such settings, reduction in EIR has thus little effect on κ so $\kappa_n \approx \bar{\kappa}_n$ and the effect on EIR is very similar to the effect on the reproduction number i.e:

$$E_v \approx (1-\theta\xi)E_n. \quad (2.7)$$

- Decrease κ even further (if the EIR is to the left of the arrow on Figure I), in which case there would be positive feedback and the EIR would be reduced even further.

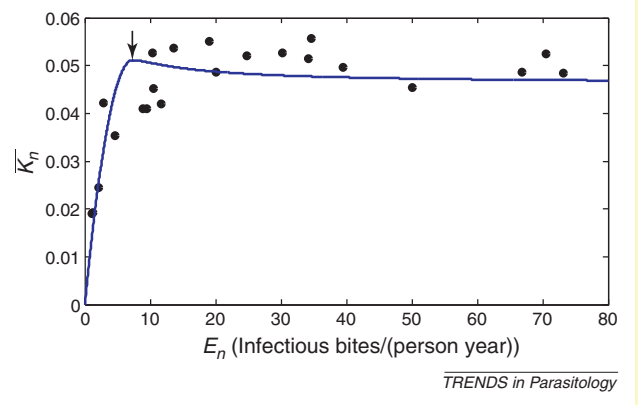

Figure I. Equilibrium infectiousness in relation to EIR. This figure presents an estimate of how the probability that a mosquito becomes infected during a single feed on a person from a non-vaccinated population $\bar{\kappa}_n$ varies with the entomological inoculation rate E_n. This figure (reproduced, with permission, from [19]) shows a smooth curve (blue line) fitted to simulation results for a variety of different patterns of seasonality (black points) [20]. The arrow delimits the region where $\bar{\kappa}_n$ is largely independent of EIR.

be weak [11], averting the evolution of insensitivity will be crucial once such vaccines are deployed on a large scale.

Drugs that block transmission through gametocytocidal properties have a slightly increased risk of selecting for resistant parasites compared to drugs that act only against asexual stages [12], because with the latter group, sensitive parasites have a longer effective infective lifespan. However, TBVs do not have this disadvantage compared to BSVs or PEVs with similar efficacies, because with all vaccines, sensitive parasites have an effective infective lifespan of zero.

Implications for target product profiles and vaccine deployment

The simple mathematical analysis presented here leads to the following important practical conclusion: for the interruption of transmission, efficacy and coverage are equally important. Complete protection of the entire susceptible population is not essential, nor do all gametocytes need to be made noninfective. Clearly, a high coverage delivery via a single dose through mass vaccination that achieves less than perfect efficacy might be preferred to a logistically more complicated delivery schedule, which would reach higher efficacy, but at a lower coverage because of storage problems or complex dosing schedules. Also, a gradual delivery, for instance through making use of the Expanded Programme on Immunization, would have, at least initially, negligible effect on transmission because of the low proportion of the population vaccinated [7]. Apart from efficacy and coverage, the other factors determining whether interruption occurs, discussed in Box 1, are contextual. A TBV that interrupts transmission in one specific context will not necessarily do so elsewhere. For licensure it therefore makes no sense to require demonstration of interruption of transmission.

Interruption transmission is, however, by no means the only use of a TBV. An important application could be to slow epidemics or prevent reestablishment of endemic transmission following re-introduction. Abandonment of malaria transmission-reducing interventions has lead to substantial epidemics in some situations [13], notably following the cessation of Global Malaria Eradication Program efforts in São Tomé [14] and Sri Lanka [15]. Possible reasons for abandonment or program re-orientation might be the perception that the program has failed, lack of funding, changing priorities, or development of insecticide- or drug resistance or vaccine insensitivity. By reducing the effective reproduction number, deployment of a TBV in such situations might allow more time to respond to epidemics, for instance by boosting case detection and treatment, or by re-deploying vector control interventions. To understand this, there is a need for simulation models that consider the effects of TBV programs in the context of decay of natural immunity and the capacities of various surveillance systems to react to introductions.

Box 3. Selective advantage of insensitive parasites in combinations

For a vaccine with coverage θ and efficacy ξ_1, the relative contribution of sensitive parasites to the next parasite generation, i.e. the fitness $w(s, mono)$, where 'mono' denotes exposure to a single vaccine component and s denotes sensitive parasites, is reduced directly in proportion to vaccination coverage and efficacy, so that:

$$w(s, \text{mono}) = (1-\theta) + \theta(1-\xi_1). \tag{3.1}$$

The selective advantage of insensitive parasites, assuming that insensitive parasites have a similar fitness to sensitive parasites in the absence of a vaccine, is then:

$$v(\text{mono}) = \frac{w(r, \text{mono})}{w(s, \text{mono})} = \frac{1}{(1-\theta) + (1-\xi_1)}, \tag{3.2}$$

with $w(r, \text{mono})$ the fitness of insensitive parasites (denoted by r) exposed to a single vaccine component.

Similarly, if a vaccine combination is deployed (thus with both components administered together), with efficacy ξ_2 of the second component, the fitness of a parasite sensitive to both components when exposed to the combination is:

$$w(s, \text{comb}) = (1-\theta) + \theta(1-\xi_1)(1-\xi_2), \tag{3.3}$$

and the fitness of a parasite insensitive to the first component but sensitive to the second is:

$$w(r, \text{comb}) = (1-\theta) + \theta(1-\xi_2) \tag{3.4}$$

The selective advantage of a parasite insensitive to the first component but sensitive to the second is then:

$$v(\text{comb}) = \frac{w(r, \text{comb})}{w(s, \text{comb})} = \frac{(1-\theta) + \theta(1-\xi_2)}{(1-\theta) + \theta(1-\xi_1)(1-\xi_2)}. \tag{3.5}$$

The ratio $v(\text{comb})/v(\text{mono})$ expresses the selective advantage of a parasite insensitive to component 1 (but sensitive to component 2) over a sensitive parasite when exposed to a vaccine combination, relative to when exposed to a single vaccine:

$$\frac{v(\text{comb})}{v(\text{mono})} = \frac{(1-\theta) + \theta(1-\xi_2)}{(1-\theta) + \theta(1-\xi_1)(1-\xi_2)} \Big/ \frac{1}{(1-\theta) + \theta(1-\xi_1)} = \frac{1 - \theta\xi_1 - \theta\xi_2 + 1 - \theta^2\xi_1\xi_2}{1 - \theta\xi_1 - \theta\xi_2 + 1 - \theta\xi_1\xi_2} \tag{3.6}$$

We define the protective effect of component 2 as:

$$\omega = 1 - \frac{v(\text{comb})}{v(\text{mono})}. \tag{3.7}$$

For most possible parameter values, the selective advantage of parasites insensitive to a single vaccine component is lower when exposed to a combination of vaccines then when exposed to a single vaccine component (Figure I), and the protective effect increases with increases in efficacy and coverage. The stronger the efficacy of the first component, the more the second component can protect it. However, at extremely high coverage, transmission of sensitive parasites is almost completely prevented, and then addition of a second component does not protect against the evolution of insensitivity against component 1. Such high coverage rates are, however, difficult to achieve in practice.

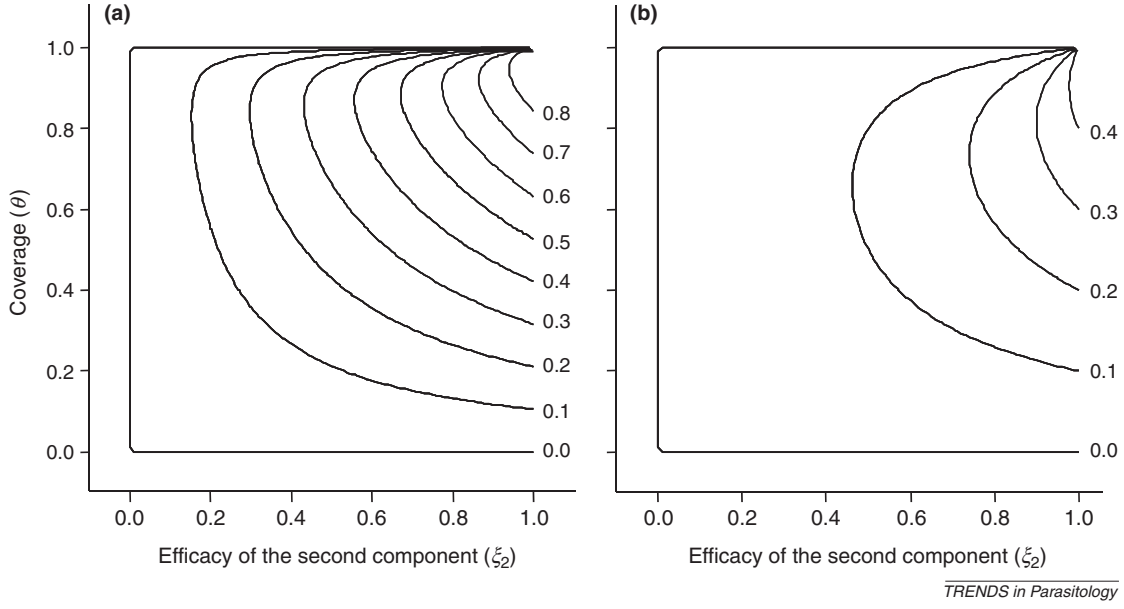

Figure I. Protective effect of a vaccine combination. The protective effect of adding a second component (with efficacy ξ_2) to a vaccine in protecting the first component (with efficacy ξ_1) from selection for insensitivity against it, depending on efficacy and coverage θ: **(a)** with $\xi_1 = 0.95$, **(b)** with $\xi_1 = 0.5$.

The most important practical consideration in terms of applicability of TBVs will be to define where they could complement other interventions. Implementation should be within the overall financial and operational strategies of the health sector and of malaria programs in particular. Combination of TBVs with other malaria vaccines will reduce transmission more than either component alone [7], increasing the chances of interrupting transmission. In endemic Africa, TBV deployment might be rational only alongside intensive use of ITNs and IRS. In settings such as SE Asia with forest malaria, where ITNs and IRS have limited effects because of exophagic, exophilic, or day-biting vectors, TBVs might be particularly suitable. Whatever the setting, high quality surveillance of clinical cases with swift and effective response mechanisms will be crucial.

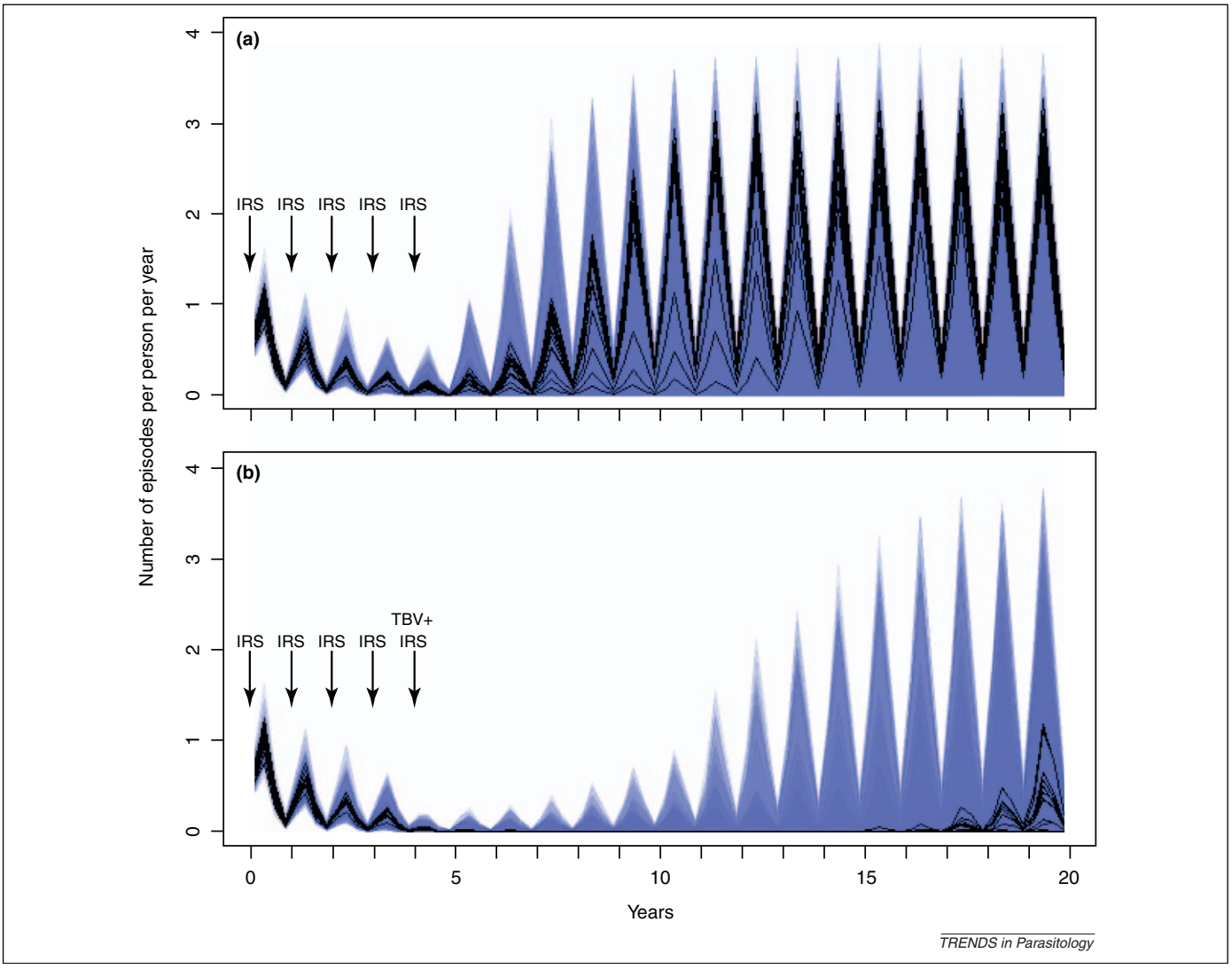

Figure 1. Simulated incidence of clinical malaria. For each scenario, simulations were carried out with 14 different stochastic models/parameterizations for malaria in humans, each fitted to the same panel of datasets, and replicated 100 times with different random seed values. All simulations used the case management model of [7] with low coverage of ACT treatment of clinical episodes, an initial EIR of two infectious bites per person per annum distributed with a typical East African pattern of seasonality based on that of Namawala, Tanzania and a rate of 0.5 imported infections per annum into a dynamic population of 1000 people. The black lines represent the median incidence in each quarter of the year for each of the 14 submodels; the (overlapping) blue areas the 95% probability intervals for each submodel. **(a)** Five annual IRS DDT spray rounds (each with 95% coverage); **(b)** five annual IRS spray rounds, the last round accompanied by delivery of a 90% efficacious TBV to 80% of the population, with exponential decay of efficacy with a five year half-life.

Acknowledgments

The authors acknowledge the contributions of many colleagues to useful discussions, in particular the valuable input to earlier drafts of Melissa Penny, Amanda Ross, Ian Hastings and Allan Saul. Diggory Hardy, Guillaume Gnaegi and Nicolas Maire programmed the simulations. This work was supported financially by Bill & Melinda Gates Foundation grant #39777.01 and by the Malaria Vaccine Initiative. The views expressed are those of the authors.

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