22

Entomological correlates of epidemiological impacts: how do we know it is working?

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Abstract

Assuming that transgenesis of malaria vector populations is feasible, it will be necessary to carry out entomological, morbidity and mortality trials to measure the degree of efficacy of this new intervention strategy. Taking into consideration the necessity to maintain reproductive fitness of a transgenic mosquito, the best strategy would be to target the sporogonic cycle and/or reduce the anthropophilic feeding behaviour of the vectors. The former would impact on the entomological inoculation rate (EIR) whereas the latter would impact on vectorial capacity. A three-phase trial would be carried out to test safety, efficacy and impact on morbidity and mortality. After confirmation of safety and efficacy, phases two and three would involve large-scale multiple site trials.
Keywords: transgenic; *Anopheles gambiae*; entomological inoculation rate; vectorial capacity; efficacy

Introduction

Assuming that it will be possible to produce a *Plasmodium*-refractory variety of *An. gambiae* through transgenesis, a number of options are available to reduce the vectorial efficiency and transmission competence. Currently, barriers to genetic transformation include, among others, gene-driving mechanisms, maintenance of gene frequency and stability of the effector constructs (Ghosh, Moreira and Jacobs-Lorena 2002).

An important property of a transgenic mosquito is its reproductive fitness compared to that of wild-type conspecifics (Moreira et al. 2004). It must be noted that natural selection has resulted in the survival of very fit varieties (inversion karyotypes) of *An. gambiae* with reference to vector habitats and bioclimatic zones.

In assessing the entomological correlates of the epidemiological impacts of transgenic forms of *An. gambiae* we must recognize that only reproductively fit transgenic individuals will have a chance to compete with the wild counterparts. Thus, while it may be possible to construct mosquito that have genes for low fecundity, this variety would be unable to compete with the wild types (Irvin et al. 2004; Moreira et al. 2004).

The current genetic modification strategies that probably have the least effect on fitness are those that focus on blocking the penetration of the midgut and salivary glands by sporozoites. The result is females that cannot support the sporogonic cycle

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(Ito et al. 2002; Moreira et al. 2000). In terms of transmission this could result in a great reduction in the entomological inoculation rates and, consequently, of malaria transmission.

A second strategy would be to change the blood-feeding preference of *An. gambiae* from man to animals. Some of the sibling species of the *An. gambiae* complex such as *An. arabiensis* and *An. quadriannulatus* can be highly zoophilic. But this would assume that animals and predominantly bovids would always be available (Pates et al. 2001).

Entomological correlates

Assuming the two strategies indicated above can be achieved, a number of impacts can be expected on the vectorial capacity and entomological inoculation rates. Vectorial capacity is the future daily sporozoite inoculation rate arising from a currently infective human case, on the assumption that all female mosquitoes biting that person become infected. It is the product of the vector density in relation to man, the proportion that bite man twice, and the expectation of the infective life span of the vector (MacDonald 1957; Garrett-Jones and Shidrawi 1969).

Vectorial capacity is mathematically expressed as:

$$VC = \frac{Ma^2 p^x}{-\ln p}$$

where
M = man-biting rate or vector density in relation to man
a = the daily man-biting rate
p = daily survival rate
x = duration of the sporogonic cycle.

Expectation of the life span of a vector:

$$\frac{1}{-\log p}$$

Expectation of the infective life span:

$$\frac{p^x}{-\log p}$$

Entomological inoculation rate (EIR):
EIR = Mas

where M and a are as defined above and s is the sporozoite rate.

Effects of reducing sporozoite rates on vectorial capacity and EIR

Sporozoite infection rate terms are not included in the vectorial capacity equation, thus a reduction in sporozoite infection rates would not be expected to have any impact on vectorial capacity. It should be noted that the vectorial capacity assumes that all female mosquitoes biting an infective human case will become infected. This assumption would be invalid for transgenic mosquitoes that are resistant to *Plasmodium* infection. A decrease in sporozoite rate would however, have a

substantial impact on the EIR. Nevertheless in areas of holoendemic malaria transmission this may not lead to a proportional decrease in disease prevalence because the level of transmission is several-fold greater than what is required to maintain prevalence at a certain (high) level. For example, a study in Tanzania showed that where the mean annual EIR was 34 infective bites per person, mean annual parasite prevalence ranged from 33% to 76%, whereas in an area where the mean EIR was 405 the prevalence ranged from 80% to 84% (Ellman et al. 1998). Consequently a 91.6% reduction in EIR led to an 8-51% reduction in malaria prevalence. In a vector control study in Kenya using permethrin-impregnated sisal curtains a 72% reduction in EIR reduced malaria prevalence by only 10% (Oloo et al. 1996).

Effects of changes in human blood-feeding behaviour and daily man-biting rates

The human-feeding rate term occurs twice in the vectorial capacity equation, thus changes in this parameter would be expected to have an impact on the ability of the vector to transmit diseases. A good example is *Anopheles arabiensis*, which, in certain areas, has a high preference for feeding on animals. This leads to a low mean annual sporozoite rate of about 1-2% compared to *An. gambiae* which preferentially feeds on humans and consequently shows a mean annual sporozoite rate of 6% (Githeko et al. 1993; 1994; Taylor et al. 1990). A reduction in human blood-feeding and the daily man-biting rate would have a substantial impact on EIR because the reduction would concurrently lead to lower sporozoite rates.

Survival rates

Classical vector control programmes using residual insecticides have two aims: killing vectors and reducing their longevity. Survival rates are a useful measure of the impacts of an insecticide on malaria transmission. However, low survival rates would be associated with a poor reproductive capacity of the transgenic mosquitoes.

Epidemiological impacts

The epidemiological impacts expected from any entomological intervention are a reduction in parasite prevalence, incidence, morbidity and mortality. Most of the published studies refer to interventions that reduce man-biting rates as a result of the application of residual insecticides or impregnated bed nets. The impact of a reduction in man-biting rates or sporozoites may be best observed by comparing transmission in areas that have similar densities of *An. arabiensis* and *An. gambiae.* Unfortunately *An. funestus* occurs frequently in habitats occupied by the two former vectors. It is therefore difficult to determine the epidemiological impact of an inefficient vector such as *An. arabiensis* versus that of *An. gambiae*, a much more efficient one.

The rate of epidemiological impact would depend upon the rate of gene drive in the wild populations. Ideally the gene would be driven to fixation. The big question, therefore, is how to know whether or not this intervention is working. It is assumed that the trial would take a similar design to malaria vaccine trials, which normally have three phases. There are two possible end points for phase-I and -II trial, namely a

significant reduction in man-biting and human blood-feeding rates and a significant reduction in EIR.

Phase I would entail a safety and efficacy trial in the laboratory to demonstrate that significant and preferably full transmission blocking occurs. In these trials it should be demonstrated that there is no increased chance of enhanced transmission of other (vector-borne) diseases, including arboviral infections, by the transgenic mosquitoes. This phase should also include life-table studies to evaluate mosquito fitness and might include competition experiments.

Phase II would be a limited field trial, possibly in a greenhouse and/or in an isolated small island where natural vector populations exist. It would be necessary to demonstrate that gene flow is occurring at an acceptable rate and stability is maximized. Phase-II studies should also evaluate the potential for horizontal gene transfer.

Phase III would involve a full field-scale trial where, after success in phase I and II, the efficacy of the intervention tool would be assessed at vector population level and subsequently in terms of human disease outcome. Ideally this would be a multiple-site trial where all climatic and other environmental parameters would be taken into account. The end point of this trial would be a reduction in parasite prevalence and disease incidence. This stage would be challenged by many ethical and political issues.

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