Vector-borne disease control: antiviral *Wolbachia* limits dengue in Malaysia

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Summary

Vector-borne viral infections represent a pressing public health challenge, particularly in the tropics. Field releases of mosquitoes carrying bacterial symbionts that reduce vector competence are ongoing in Kuala Lumpur, Malaysia. Early results show that *w*AlbB *Wolbachia* can persist in mosquitoes in urban settings and decrease dengue fever incidence in humans.

Main text

The control of vector-borne diseases is a challenging task, as proven by their increasing morbidity burden worldwide. Traditional means of control typically provide only short-term benefits and have a range of other issues. The use of insecticides, for example, requires a very high coverage of target areas and selects for resistance that degrades ongoing effectiveness. Urban settings make the control of mosquito breeding sites, like water-filled trash or containers, logistically difficult. Other interventions, such as the use of bed nets, is largely ineffective for mosquito species that bite during daytime, including *Aedes aegypti*, the vector of dengue, Zika and chikungunya viruses. The laborious nature of control, combined with global warming that is expanding the range of the mosquito, help explain the 30-fold increase in the number of dengue fever cases in the last 50 years [1]. The World Health Organization has hence declared dengue "the most important mosquito-borne viral disease in the world" [1].

Wolbachia, a widespread maternally-inherited bacterial symbiont of insects, has emerged as a viable tool to control vector-borne diseases. Insects harbouring this microbe have lower viral loads than their uninfected counterparts, a trait known as virus blocking [2,3]. *Aedes aegypti* mosquitoes (Figure 1) are naturally uninfected with *Wolbachia*, but have been artificially transinfected with multiple *Wolbachia* strains that form stably inherited infections. These infections render the insects less susceptible to contracting and transmitting arboviruses [4].

Since these advances, a few teams have focused on leveraging the antiviral properties of *Wolbachia* for human disease prevention. WHO has recommended carefully planned pilot *Wolbachia* deployments under field conditions to test for efficacy with respect to reducing human disease incidence [5]. Field interventions include rearing of *Wolbachia*-infected mosquitos in specialised facilities and release into the environment in dengue-affected areas. *Wolbachia* is then passed from female mosquitos to their offspring every generation, and can establish in wild populations due cytoplasmic incompatibility – the mechanism facilitating its own spread. Once established, *Wolbachia* blocks replication of arboviruses in mosquitoes, which become unable to spread disease-causing viruses to humans [6].

The first pilot releases of *Wolbachia*-infected *Aedes aegypti* to block dengue virus spread were pioneered by the largest *Wolbachia*-based initiative, The World Mosquito Program [6]. First deployed in 2011, the releases are now ongoing in 12 countries, and all use the same *Wolbachia* strain – *w*Mel derived from the fruit fly, *Drosophila melanogaster* [7]. Despite an intense effort over the last eight years, epidemiological data showing decrease in dengue cases among humans are limited to the single area in Australia [8]. This area is historically characterised by episodic outbreaks of the disease caused by imported virus, that have now been quelled by *Wolbachia* release [8].

Dengue control in endemic tropical areas presents a greater challenge, as well as a more pressing issue for human suffering and economic losses. Now, Nazni, Hoffmann *et al.* have shown that the *w*AlbB *Wolbachia* strain can be successfully deployed for dengue control in urban, dengue endemic areas of Malaysia [9]. *Wolbachia*-infected mosquitoes were released in a number of carefully selected, differently structured and populated sites. Similar, intervention-free control sites were selected and monitored in parallel. The results show that *Wolbachia* can establish at high frequencies in most local mosquito populations in these complex urban settings. More importantly, *Wolbachia* deployment translates to a 5-65% decrease in the number of human dengue cases, with the exact reduction depending on the site. As the authors note, these numbers likely underestimate the benefit of release, as daily human movement between residential release sites and untreated areas enables import of the disease. Precise quantification of the effect size awaits data on the rate of intra-household transmission events, which provide a more focussed measure of the local benefits of the control measure [10].

The evident success of this small-scale release has inspired trust from the Malaysian Ministry of Health, which supports extension of this strategy into a larger number of locations. As such, despite being a relatively small observational study, this release programme will be important for turning *Wolbachia*-based control from a promising strategy into a validated tool. Although large scale, randomised field trials are required to prove the effectiveness of *Wolbachia*-based biocontrol, smaller releases are invaluable for planning and optimization of the these more powerful and expensive designs [10].

The work presented by Nazni, Hoffmann and colleagues is foundational for the larger scale trial designs for several reasons. The releases in variable urban environments facilitate generalisation of the results, as the number of people living in large cities is on the rise globally [11]. Additionally, the study demonstrates that the release of *Aedes aegypti* eggs (Figure 2) (rather than a combination of eggs and adults [6]) can be successful in the field, reducing intervention costs and implementation times. The most important innovation, however, is the use of a *w*AlbB *Wolbachia* strain, which has not previously been deployed in

open releases. The *w*AlbB strain of *Wolbachia* originates from *Aedes albopictus*, a close relative of *Aedes aegypti*, and has a number of characteristics that can make it favourable in comparison with the more commonly applied *w*Mel. *w*AlbB-host interactions are more robust at high environmental temperatures, maintaining higher *Wolbachia* loads, high maternal transmission and stronger cytoplasmic incompatibility than the *w*Mel-mosquito symbioses [12,13]. All these properties are crucial for the long-term stability of the intervention and its associated disease prevention. Efficient maternal transmission and cytoplasmic incompatibility ensure high numbers of dengue resistant mosquitoes in the wild, while high *Wolbachia* densities are correlated with strong antiviral protection [14,15]. Robustness in the face of elevated temperatures is key, as vector-borne diseases are most prevalent in hot climates. In areas where *w*Mel fails to establish or its effectiveness drops over time, the use of *w*AlbB or release of double infected *w*Mel-*w*AlbB mosquitoes [16] may be necessary.

This successful use of *Wolbachia* in dengue prevention, albeit on a small scale, raises some important questions. Will *Wolbachia* in general, and *w*AlbB in particular, prove effective against dengue in the long term? As the mechanism of *Wolbachia*-mediated pathogen blocking remains elusive, it is difficult to predict how *Wolbachia*, mosquitoes and viruses will evolve in the post-release landscape. Although RNA viruses, which include dengue, Zika and chikungunya, are expected to evolve very fast, one set of recent laboratory-based experiments was unable to select for dengue virus variants resistant to *Wolbachia* inhibition [17]. The evolutionary stability of the antiviral phenotype has recently been confirmed in *w*Mel-infected *Aedes aegypti*, where artificial selection for reduced virus blocking reduced mosquito fitness [18]. Thus, there are reasons to be hopeful, at least in the short term, that the blocking phenotype will remain robust [18]. Finally, the stability of *w*Mel *Wolbachia* phenotypes in field-established *Wolbachia*-infected *Aedes* mosquitoes in Australia has been confirmed, by testing their fitness and pathogen blocking properties [19,20]. Similar data remain to be gathered for the *w*AlbB *Wolbachia* strain. In all field sites however, the growing interaction between mosquito and virus genetic diversity and *Wolbachia*-mediated blocking will need to be monitored.

To what extent will *Wolbachia* reduce the burden of vector-borne disease? *Wolbachia*-conferred protection is broad in nature, working against many double-stranded positive-sense RNA viruses and some other pathogens [2,4]. As the implementation of *Wolbachia* spreads, monitoring of dengue fever and other diseases, including Zika and chikungunya, will provide an answer. Although more work is required to confirm the degree of protection, the results so far hold a promise for a future with reduced arboviral disease burden, in Malaysia and beyond.

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Figures:

Figure 1. *Aedes aegypti* mosquito. The image shows a blood-fed female *Aedes aegypti*, vector of dengue, Zika, and chikungunya viruses. Photo credit: Steve Morton.

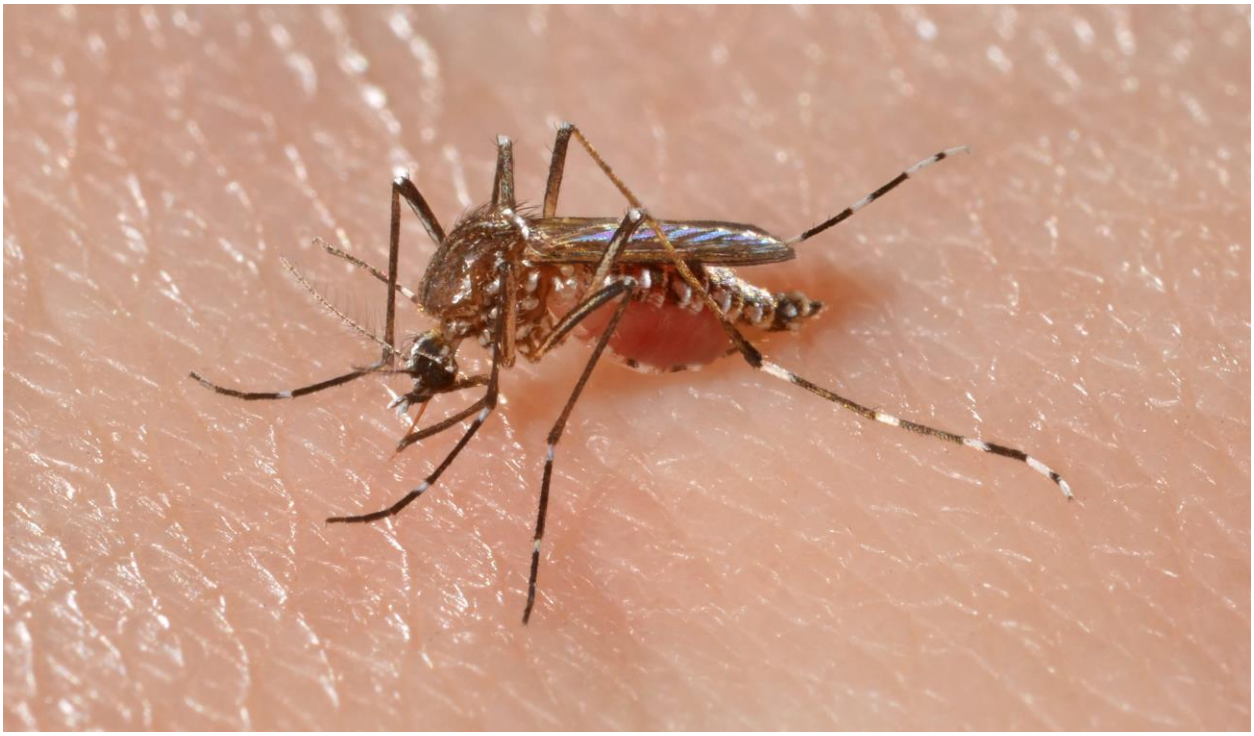

Figure 2. Mosquito eggs. The image shows *Aedes aegypti* eggs. Photo credit: Steve Morton.