Predicting Wolbachia potential to knock down dengue virus transmission

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Abstract:

Releasing mosquitoes infected with the intracellular bacteria *Wolbachia* is a candidate strategy for dengue control that has recently advanced to field-testing. A critical next step is to evaluate the impact of this strategy on dengue epidemiology. A recent study by Ferguson and colleagues presents a mathematical framework to predict the likely effect of mosquitoes carrying *Wolbachia* on dengue virus transmission. Fitting the mathematical model to empirical data obtained with *Wolbachia*-infected mosquitoes experimentally challenged with viremic blood from dengue patients indicates that dengue virus transmission could be reduced by a degree that would have a significant impact on public health.

Keywords: *Aedes aegypti*; dengue; vector competence; *Wolbachia*.

Running title: *Wolbachia* on the test bed

The failure of traditional disease prevention methods to halt the current progression of dengue has promoted the development of novel entomological strategies. One of the most promising approaches relies on the intracellular bacterium *Wolbachia*, a bacterial symbiont commonly found in arthropods (1). The main mosquito vector of dengue viruses, *Aedes aegypti*, does not naturally carry *Wolbachia*, but can be experimentally transinfected by embryonic microinjection (2). Transinfection of *Ae. aegypti* with certain strains of *Wolbachia* results in protection against dengue virus infection (3, 4). Thus, successful establishment of *Wolbachia* in natural mosquito populations (5) supports a practical approach for dengue suppression. The next critical step is to assess the epidemiological efficacy of *Wolbachia* in reducing dengue virus transmission in the field (6).

A recent study by Ferguson and colleagues (7) lays the ground for future efficacy trials by quantitatively predicting the likely impact of *Wolbachia* on dengue virus transmission. Their study makes two significant advances. First, it provides empirical data on the vector competence of *Wolbachia*-infected *Ae. aegypti* using viremic blood from dengue patients and therefore more closely mimics field conditions than earlier studies based on laboratory challenge with cultured virus. Vector competence was evaluated by testing the presence of viral infection in the mosquito abdomen and salivary glands or saliva at different time-points after the infectious blood meal. Second, it develops a mathematical framework to describe the dynamics of dengue virus transmission between humans and mosquitoes. The model is then fitted to the empirical vector competence data to predict the effect of *Wolbachia* on the basic reproduction number (R_0) of dengue virus transmission. R_0 is the average number of subsequent infections resulting from an infected human introduced in a naïve population. Estimates of R_0 for dengue typically range from 2 to

5 (8). A pathogen will go to extinction if R_0 is less than 1 because it means that each infected individual will generate less than one new infection on average.

The study assessed the vector competence of *Ae. aegypti* mosquitoes carrying one of two *Wolbachia* strains. The first strain called *w*MelPop is characterized by high bacterial densities in mosquito tissues and results in almost complete refractoriness to dengue virus infection in laboratory challenge (4). However it also induces deleterious effects on mosquito fitness such as reduced lifespan and blood feeding success (3, 9). Experiments using viremic blood from dengue patients confirmed the strong protective effect of *w*MelPop against dengue virus, although systemic infection was not completely blocked. Only 2.6% of *Wolbachia*-infected mosquitoes had virus-positive salivary glands, compared to 90% in *Wolbachia*-free controls. The authors concluded that *w*MelPop would result in at least 90% blocking of transmission. The second *Wolbachia* strain called *w*Mel infects mosquito tissues at lower densities, and induces resistance to dengue virus infection in laboratory challenge, although to a lesser extent than *w*MelPop, and in the absence of major fitness costs (10). Consistently, there was significant but imperfect virus blocking in mosquitoes infected by *w*Mel challenged with viremic blood from dengue patients. Although viral load measured in the abdomen was at least 10-fold lower in *Wolbachia*-infected mosquitoes, most of the blocking effect was observed during viral dissemination from the abdomen to the saliva. The effect comprised a net reduction of the probability of saliva infection, and a slight lengthening of the time required for the virus to reach saliva.

Ferguson *et al*. (7) then used the empirical data generated in their vector competence assays as well as clinical records of viremia levels in patients to inform a newly developed mathematical model of dengue virus transmission (Fig. 1). The

model was designed to evaluate the effect of *Wolbachia* on dengue virus transmission based on the comparison of R_0 in a mosquito population with or without *Wolbachia*. The modeling approach only considered *w*Mel because *w*MelPop did not require mathematical modeling to predict quasi-complete blocking of transmission. The mosquito infection model consisted of a relatively simple dose-response model of abdomen infection probability as a function of viremia coupled to a model of saliva infection probability as a function of viremia as well as time elapsed since the blood meal (Fig. 1). Model fitting to the empirical data was performed separately for each of the four dengue virus serotypes. The baseline scenario predicted 66 to 75% of reduction in R_0 depending on the dengue serotype. Other scenarios were considered to account for the uncertainty in model parameters that were not directly informed by empirical data such as the minimum infectious dose for successful mosquito-to-human transmission. The percentage in R_0 reduction varied from 40 to 80% among serotypes under the alternative scenarios. Therefore, under the baseline model, a *Wolbachia* intervention using the *w*Mel strain is expected to result in two thirds to three quarters less secondary infections from an initial case. This means that the intervention would achieve elimination of dengue for initial R_0 values of 3 or 4, respectively. Thus, *Ae. aegypti* mosquitoes carrying *w*Mel could reduce dengue virus transmission by a degree that would have considerable public health impact, possibly leading to dengue elimination where transmission is low to moderate (8).

A major strength of the Ferguson *et al*. study (7) is the use of state-of-the-art methods to evaluate vector competence. Historically, methods of determining vector competence have been largely restricted to artificial infectious blood meals composed of animal blood spiked with virus grown in cell culture. These artificial methods have limited our ability to extrapolate to natural transmission and to

understand the significance of data from epidemiological studies with humans (11). Recent studies from the same group overcame this obstacle by developing vector competence assays that expose mosquitoes to the blood of naturally infected, viremic humans (12). Although in the present study viremic blood was presented to mosquitoes in an artificial feeder through a skin-simulating membrane, it is reasonable to consider this indirect mosquito feeding method as a good proxy of direct feeding through the skin of a person. Nevertheless, vector competence is only one of several parameters that influence dengue virus transmission by mosquitoes (11). It will be necessary in future studies to evaluate the effect of *w*Mel on several important entomological parameters that Ferguson *et al*. did not examine in their study such as blood feeding behavior and longevity. For instance, a shorter lifespan could act to further reduce dengue virus transmission. Conversely, increased blood feeding frequency would enhance transmission. The *w*MelPop strain confers very strong protection against dengue virus infection and further limits transmission by shortening the mosquito lifespan (3, 4, 13). But the life-shortening effect would represent a significant hurdle to establishing *w*MelPop infection in a natural *Ae. aegypti* population by reducing competitiveness against wild mosquitoes. Overall, the costs and benefits of each *Wolbachia* strain will have to be carefully balanced prior to field releases.

One limitation of the Ferguson *et al*. study (7) is that the transmission model relies on a distribution of viral titers in plasma that may not accurately reflect reality, for at least two reasons. First, the empirical distribution of plasma viremia levels that were used to develop the transmission model only included hospitalized and ambulatory patients. This distribution, therefore, did not consider inapparent (subclinical) infections that are believed to represent the majority of dengue infections

(14). People with inapparent infections are usually assumed to inefficiently infect mosquitoes because they do not reach sufficiently high viremia levels, but this assumption has not been verified (15). Second, the transmission model did not account for the epidemiological feedback. Put simply, introduction of *Wolbachia*-infected mosquitoes could affect the distribution of viremia levels in humans, and consequently modify the baseline parameters underlying the model that estimates transmission. The authors considered that modeling three distributions recapitulates the complete transmission cycle (Fig. 1): human viremia level, human-to-mosquito transmission probability (abdomen infection), and mosquito-to-human transmission probability (saliva infection). In fact, a parameter characterizing the relationship between mosquito-to-human transmission and the resulting viremia profile is missing from the cycle. One could imagine, for instance, that *Wolbachia*-infected mosquitoes inoculate smaller infectious doses that result in shorter, shallower viremia profiles. In both cases, fortunately, these shortcomings likely contributed to underestimate the impact of *Wolbachia* on dengue virus transmission. Indeed, the transmission blocking effect of *Wolbachia* would be stronger if viremia levels were reduced compared to those seen in dengue-infected people with clinical symptoms.

Taken together, this work and previous studies support the idea that *Wolbachia* has a realistic potential to knock down dengue virus transmission in the field. It is also clear, however, that *Wolbachia* alone will not be sufficient to effectively control dengue, especially in settings where transmission is high. In addition to novel vector population suppression strategies (16) and vaccines (17), *Wolbachia* may soon enrich the arsenal to effectively fight against dengue.

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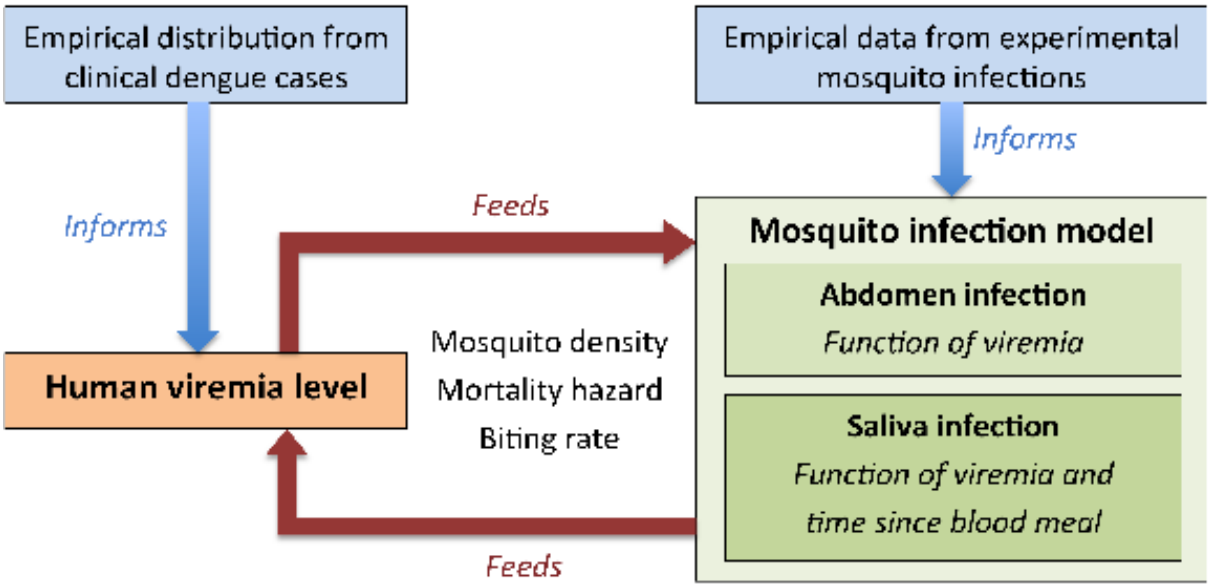

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