DENGUE

# Modeling the impact on virus transmission of *Wolbachia*-mediated blocking of dengue virus infection of *Aedes aegypti*

**Neil M. Ferguson,[1]* Duong Thi Hue Kien,[2] Hannah Clapham,[1] Ricardo Aguas,[1] Vu Tuan Trung,[2] Tran Nguyen Bich Chau,[2] Jean Popovici,[3] Peter A. Ryan,[3] Scott L. O'Neill,[3] Elizabeth A. McGraw,[3] Vo Thi Long,[2] Le Thi Dui,[2] Hoa L. Nguyen,[2] Nguyen Van Vinh Chau,[4] Bridget Wills,[2,5] Cameron P. Simmons[2,5,6]**

**Dengue is the most common arboviral infection of humans and is a public health burden in more than 100 countries. *Aedes aegypti* mosquitoes stably infected with strains of the intracellular bacterium *Wolbachia* are resistant to dengue virus (DENV) infection and are being tested in field trials. To mimic field conditions, we experimentally assessed the vector competence of *A. aegypti* carrying the *Wolbachia* strains *w*Mel and *w*MelPop after challenge with viremic blood from dengue patients. We found that *w*MelPop conferred strong resistance to DENV infection of mosquito abdomen tissue and largely prevented disseminated infection. *w*Mel conferred less resistance to infection of mosquito abdomen tissue, but it did reduce the prevalence of mosquitoes with infectious saliva. A mathematical model of DENV transmission incorporating the dynamics of viral infection in humans and mosquitoes was fitted to the data collected. Model predictions suggested that *w*Mel would reduce the basic reproduction number, $R_0$, of DENV transmission by 66 to 75%. Our results suggest that establishment of *w*MelPop-infected *A. aegypti* at a high frequency in a dengue-endemic setting would result in the complete abatement of DENV transmission. Establishment of *w*Mel-infected *A. aegypti* is also predicted to have a substantial effect on transmission that would be sufficient to eliminate dengue in low or moderate transmission settings but may be insufficient to achieve complete control in settings where $R_0$ is high. These findings develop a framework for selecting *Wolbachia* strains for field releases and for calculating their likely impact.**

## INTRODUCTION

Dengue is an acute systemic viral infection (*1*). In 2010, there were an estimated 100 million apparent infections globally (*2*). The etiological agents of dengue are four dengue viruses (DENV1 to DENV4), with transmission from human to human primarily by *Aedes aegypti* mosquitoes. Existing disease prevention strategies are based on reducing the mosquito vector population, yet this has been largely unsuccessful in halting dengue transmission in endemic countries.

A new entomologically based control method uses the phenotype of *A. aegypti* experimentally infected with strains (*w*Mel and *w*MelPop) of the bacterial symbiont *Wolbachia* (*3*, *4*). The heritable *w*MelPop infection of *A. aegypti* is characterized by widely disseminated and dense infection of mosquito tissues (*3*). *w*MelPop infection confers numerous phenotypic traits on *A. aegypti*, including refractoriness to DENV infection (*5*), reduced life span (*3*), reduced viability of desiccated eggs (*6*), and reduced blood feeding success (*7*). The heritable *w*Mel infection of *A. aegypti* is associated with a relatively lower intensity of tissue infection and can also confer complete resistance to disseminated DENV infection after laboratory challenge (*4*). The mechanism of virus interference is unknown, but could potentially be mediated by *Wolbachia*-triggered changes in immunoregulatory microRNA expression, elevation of reactive oxygen species, or competition between DENV and *Wolbachia* for critical metabolic resources (*8*–*10*). Successful field releases of *w*Mel-*A. aegypti* have occurred in the northern Australian city of Cairns (*11*), providing proof of concept that stable, long-term establishment of *Wolbachia* in mosquito populations can be achieved.

The cost of developing a new operationalized vector control measure and testing its effectiveness in the field makes it a priority to try to predict the likely impact of the introduction of *Wolbachia* into *A. aegypti* populations on dengue transmission. However, previous vector competence studies of *Wolbachia*-infected *A. aegypti* had significant limitations in that they used a single serotype of laboratory-passaged DENV that was spiked into animal or human blood to create infectious blood meals (*4*, *5*). This model system probably does not accurately mimic a human DENV infection in that DENVs have evolved to be efficiently transmitted to mosquitoes through fresh blood meals from infected human hosts. We describe here vector competence studies that use viremic blood from dengue patients to blood-feed field-derived *Wolbachia*-infected *A. aegypti* and thus provide "real-world" measures of vector competence.

More generally, translating laboratory studies of vector competence into an assessment of the potential effectiveness of *Wolbachia* in reducing dengue transmission to human populations requires an understanding of multiple interacting aspects of mosquito ecology and the biology of DENV infection. In addition to characterizing the invasion dynamics of *Wolbachia* into *A. aegypti* populations

[1]Medical Research Council Centre for Outbreak Analysis and Modelling, School of Public Health, Imperial College London, Norfolk Place, London W2 1PG, UK. [2]Oxford University Clinical Research Unit, Hospital for Tropical Diseases, 764 Võ Văn Kiệt, District 5, Ho Chi Minh City 748010, Vietnam. [3]School of Biological Sciences, Monash University, Clayton, Victoria 3800, Australia. [4]Hospital for Tropical Diseases, 190 Ben Hám Tú, District 5, Ho Chi Minh City 748010, Vietnam. [5]Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford OX1 7FZ, UK. [6]Department of Microbiology and Immunology and Nossal Institute of Global Health, University of Melbourne, Carlton, Victoria 3010, Australia.
*Corresponding author. E-mail: neil.ferguson@imperial.ac.uk

(the goal of field trials currently under way), we require better understanding of (i) the development of DENV infection in mosquitoes (and how this is modified by *Wolbachia*), (ii) the within-host dynamics of DENV infection in humans, and (iii) DENV transmission from mosquitoes to humans and from humans to mosquitoes (and how this is modified by *Wolbachia*). Here, we begin to address these data needs by combining experimental characterization of the impact of *Wolbachia* infection on vector competence with mathematical modeling of the natural history of DENV infection in humans and vectors. By using more biologically realistic experimental and mathematical models than hitherto possible, we have generated estimates of the impact of *Wolbachia* strains on dengue transmission that can be used with greater confidence to inform future field trials in dengue-endemic areas and to guide the development of additional *Wolbachia* strains in *A. aegypti*.

## RESULTS

### Vector competence assessments of *w*MelPop-*A. aegypti*

We measured the susceptibility of *w*MelPop-*A. aegypti* to DENV infection after human viremic blood feeding ($n = 27$ independent feeds). *w*MelPop-*A. aegypti* were highly resistant to acquiring DENV compared with their wild-type counterparts as assessed by assaying their abdomen tissues (Fig. 1). In a subset of mosquitoes with detectable virus in their abdomen, salivary glands were assayed for the presence of DENV infection. For wild-type mosquitoes, 90% [95% credible interval (CI), 87 to 94%] of salivary glands contained DENV, whereas for *w*MelPop-infected mosquitoes, virus was detected in only 2.6% (95% CI, 0.5 to 7.6%) of the salivary glands tested (Fig. 1). We did not explicitly fit mathematical models to the *w*MelPop data because, under the highly plausible assumption that infection of salivary glands is required for DENV transmission, the salivary gland data suggested at least 90% blocking of transmission.

### Vector competence assessments of *w*Mel-*A. aegypti*

We postulated that *w*Mel infection would confer lower levels of resistance to DENV infection in *A. aegypti* than *w*MelPop on the basis that *w*Mel is present at lower tissue densities (*11*). To test this hypothesis, we measured the prevalence of DENV-infected mosquito abdomens and saliva in wild-type and *w*Mel-*A. aegypti* after 42 independent human viremic blood feeds. Groups of mosquitoes were assessed at multiple time points after viremic blood feeding to assess whether the phenotype of *w*Mel-*A. aegypti* had a temporal component. The results, stratified by serotype, plasma viremia, time since blood meal, and mosquito tissue type, are shown in Fig. 2.

We used a nonparametric sign test (see Materials and Methods) to assess differences in infection rates between *w*Mel and wild-type mosquitoes (Table 1). Note that for all data subsets examined, the number of paired observations for which infection rates in wild-type mosquitoes exceeded those in *w*Mel-infected mosquitos was always greater or equal to the number of pairs where the converse was true. Overall, the proportion of mosquitoes with DENV-infectious saliva was significantly lower in *w*Mel-*A. aegypti* than in wild-type mosquitoes 10 and 14 days after blood meal ($P < 0.005$; Table 1), these being the two most data-rich time points. Abdomen infections were significantly lower in *w*Mel-*A. aegypti* compared to wild-type at day 14 after blood meal ($P = 0.0044$) and close to significant at day 10 ($P =$

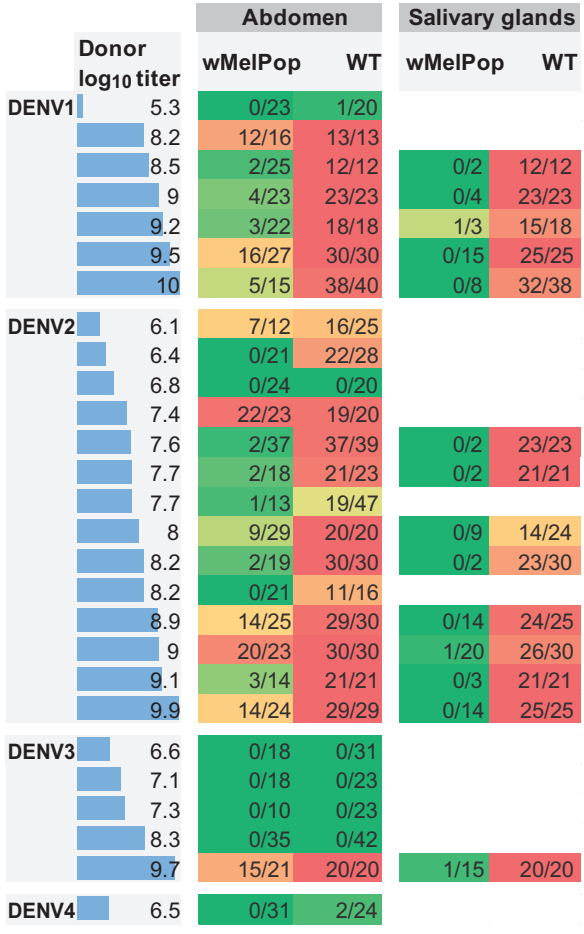


**Fig. 1. Susceptibility of wild-type and *w*MelPop-infected mosquitoes to DENV infection.** Each row represents the results of feeding cohorts of wild-type (WT) and *w*MelPop-infected mosquitoes on viremic blood collected from human dengue cases. The $\log_{10}$ viral titer (RNA copies/ml) in plasma of the donor blood is given in the first column (also indicated by the horizontal bars). Other columns indicate the numbers of mosquitoes with detectable abdomen or salivary gland infection over the total numbers fed on blood from that donor. Only mosquitoes with detectable abdominal infection, a prerequisite for disseminated infection, were tested for salivary gland infection. Background color of table cells indicates the proportion of mosquitoes with detectable infection [0% (dark green) to 100% (red)].

0.053). Two versions of saliva results are presented in Table 1. The "saliva conditional" rows show results for the actual saliva samples tested, that is, conditional on detected abdominal infection. However, saliva was only tested in mosquitoes with dengue infection detected in abdominal tissue because abdominal infection is a prerequisite of more disseminated infection. The "saliva unconditional" rows in Table 1 show results for saliva infection assuming that all mosquitoes with no detectable abdominal infection also had no detectable infection in saliva. This best summarizes all available data on the impact of *w*Mel infection on the probability of detecting infectious virus in saliva. The saliva unconditional results in Table 1 show the most marked difference between DENV infection rates in the *w*Mel and wild-type groups, with significant differences ($P < 0.02$) between the groups for each serotype-specific data subset, even including DENV3—the least

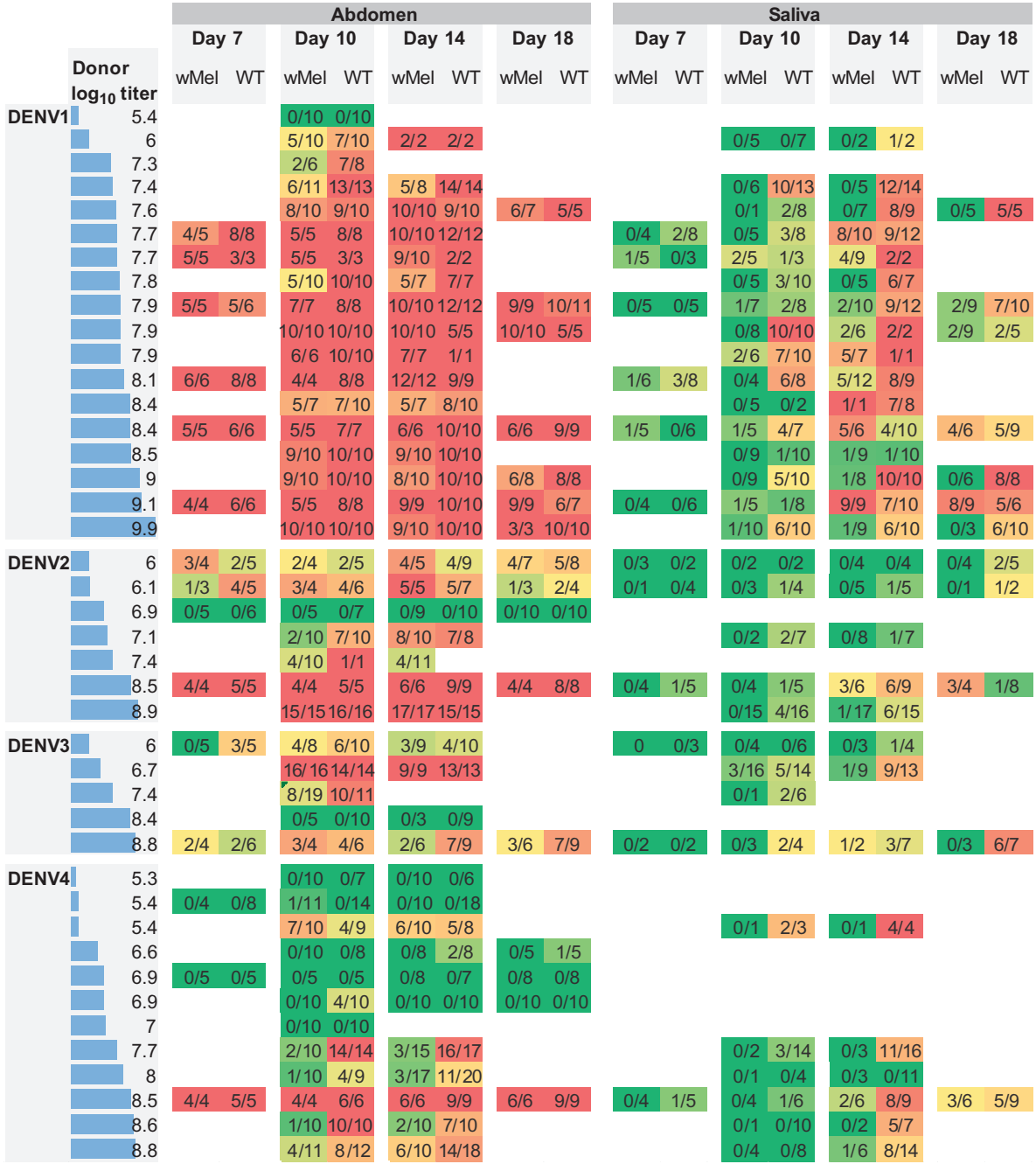


**Fig. 2. Susceptibility of WT and *w*Mel-infected mosquitoes to DENV infection.** Each row represents the results of feeding cohorts of WT and *w*Mel-infected mosquitoes on viremic blood collected from human dengue cases. The $\log_{10}$ viral titer (RNA copies/ml) in plasma of the donor blood is given in the first column (also indicated by the horizontal bars). Results indicate the numbers of mosquitoes with detectable abdomen or saliva infection over the total numbers fed on blood from that donor at four time points after feeding (days 7, 10, 14, and 18). Background color of table cells indicates the proportion of mosquitoes with detectable infection [0% (dark green) to 100% (red)].

represented serotype in our data set. This reflects the combined impact of *w*Mel on both the establishment of abdominal infection and the dissemination of that infection to saliva.

In addition, the concentration of DENV RNA in *w*Mel-*A. aegypti* abdominal tissues for all serotypes was generally at least 10-fold lower than that in wild-type mosquitoes (Fig. 3), indicating that *w*Mel conferred partial protection against fulminant DENV infection that was typical in wild-type mosquitoes. Collectively, these data, generated using physiologically relevant viremic blood meals, demonstrated significant but imperfect blocking of DENV infection by *w*Mel.

We also tested for an effect of time since blood meal in the data presented in Fig. 2. For the abdominal data, sign tests revealed no

**Table 1. Assessment of the differences in tissue infection rates in *w*Mel versus wild-type mosquitoes using the sign test.** The experimental data were treated as pairs of binomial observations corresponding to the proportions infected of the *w*Mel and wild-type mosquito groups fed on a particular blood sample, which were sampled on a specific day. The table rows present the number of observation pairs for which the proportion of *w*Mel mosquitoes infected was less than, equal to, or greater than the proportion of wild-type mosquitoes infected for different data subsets. Subsets are shown that stratify the observations by tissue type, DENV serotype, and the day after infection that mosquitoes were assayed. The two-sided *P* value is given, with *P* values <0.05 shown in italics.

| Tissue | Serotype | Day | *n* pairs where $p_{wMel} < p_{WT}$ | *n* pairs where $p_{wMel} = p_{WT}$ | *n* pairs where $p_{wMel} > p_{WT}$ | *P* value (for accepting $p_{wMel} = p_{WT}$) |
|---|---|---|---|---|---|---|
| | All | All | 41 | 55 | 14 | *0.0004* |
| | All | 7 | 3 | 9 | 3 | 1 |
| | All | 10 | 16 | 20 | 6 | 0.053 |
| | All | 14 | 16 | 18 | 3 | *0.0044* |
| Abdomen | All | 18 | 6 | 8 | 2 | 0.29 |
| | DENV1 | All | 17 | 25 | 5 | *0.017* |
| | DENV2 | All | 6 | 10 | 5 | 1 |
| | DENV3 | All | 6 | 4 | 2 | 0.29 |
| | DENV4 | All | 12 | 16 | 2 | *0.013* |
| | All | All | 57 | 14 | 13 | *< 0.0001* |
| | All | 7 | 4 | 5 | 2 | 0.69 |
| | All | 10 | 22 | 7 | 2 | *< 0.0001* |
| | All | 14 | 22 | 2 | 6 | *0.0037* |
| Saliva (conditional) | All | 18 | 9 | 0 | 3 | 0.15 |
| | DENV1 | All | 30 | 4 | 11 | *0.0043* |
| | DENV2 | All | 11 | 4 | 1 | *0.0063* |
| | DENV3 | All | 6 | 2 | 1 | 0.13 |
| | DENV4 | All | 10 | 4 | 0 | *0.002* |
| | All | All | 59 | 16 | 10 | *< 0.0001* |
| | All | 7 | 4 | 6 | 2 | 0.69 |
| | All | 10 | 22 | 7 | 2 | *< 0.0001* |
| | All | 14 | 24 | 3 | 3 | *< 0.0001* |
| Saliva (unconditional) | All | 18 | 9 | 0 | 3 | 0.15 |
| | DENV1 | All | 31 | 5 | 9 | *0.0007* |
| | DENV2 | All | 11 | 4 | 1 | *0.0063* |
| | DENV3 | All | 7 | 3 | 0 | *0.0156* |
| | DENV4 | All | 10 | 4 | 0 | *0.002* |

significant difference in the proportions of mosquitoes testing positive between day 7 and day 10 ($P = 0.09$), day 10 and day 14 ($P = 0.11$), and day 14 and day 18 ($P = 0.93$). For the saliva data, there were significant differences between day 7 and day 10 ($P = 0.011$) and day 10 and day 14 ($P < 0.0001$), but not between day 14 and day 18 ($P = 0.68$).

### Model fitting to empirical data of DENV infection in wild-type and *w*Mel-*A. aegypti*

We developed mathematical models to replicate the phenotype of wild-type and *w*Mel-*A. aegypti*. Figure 4 summarizes the fit of the abdomen and saliva infection models to the experimental data, illustrating that the models capture trends by serotype (Fig. 4, A, D, and G), end time point (Fig. 4, B, E, and H), and donor plasma viral titer (Fig. 4, C, F, and I). Both the abdomen and saliva models reproduce phenotypic differences between wild-type and *w*Mel-*A. aegypti*. Model parameter estimates are listed in Table 2.

The mathematical model of abdomen infection adopted (see Materials and Methods) is a relatively simple dose-response model depending solely on $\log_{10}$ viremia of the infecting blood meal, *Wolbachia* infection status, and serotype. The impact of *w*Mel infection was found to be best represented by a simple negative offset of $\log_{10}$ viremia of the infecting blood meal, effectively meaning that the risk of DENV infection in *w*Mel-infected mosquitoes fed on a blood meal with a certain

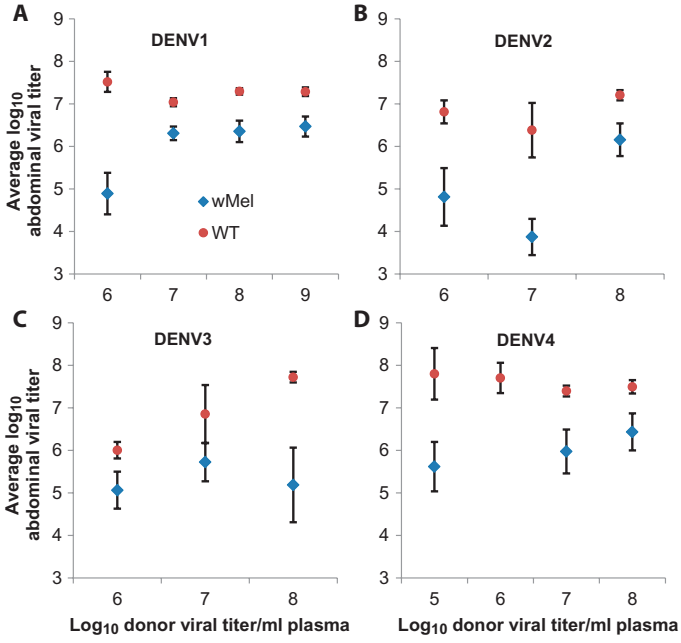


**Fig. 3. *w*Mel attenuates DENV infection of abdomen tissues.** (**A** to **D**) Shown is the mean $\log_{10}$ titer (RNA copies per abdomen) of virus measured in mosquito abdomens (average over mosquitoes with detectable virus at any time point) of WT (circles) and *w*Mel-infected (triangles) mosquitoes with DENV-infected abdomen tissues, binned by integer interval of $\log_{10}$ viral titer in the donor human blood. (A) to (D) show results for DENV1 to DENV4, respectively. Error bars show SEM.

viremia level was the same as that in wild-type mosquitoes fed on blood with a viremia about 1 $\log_{10}$ less. Figure 5A illustrates the behavior of the best-fit abdominal infection model, highlighting the major differences in infectious dose seen between serotypes and the effect of *w*Mel in partially blocking infection.

The model of saliva infection describes the development of detectable virus in saliva conditional on abdominal infection having been established (see Materials and Methods) and, like the abdominal model, is also relatively simple, depending only on time elapsed since the infecting blood meal, *w*Mel infection status, and serotype. No statistically significant dependence on viremia in the infecting blood meal could be resolved [assessed by comparison of the deviance information criterion (DIC)], consistent with the patterns seen in Fig. 2. *w*Mel could have two phenotypic effects in the model: an overall reduction in the probability of detecting infectious virus (acting through a scaling of the infectious dose parameters) or a lengthening of the extrinsic incubation period (EIP) (acting through an increase in the time taken for infection to saturate in saliva). The former effect gives a level of inhibition that does not strongly depend on how much time has elapsed since the infecting blood meal, whereas the latter gives inhibition that decays. We estimated both effect sizes simultaneously in the baseline model, and the best-fit estimates predicted that the sole effect of *w*Mel infection was on scaling infectious dose, not on the lengthening of the EIP. However, because the mode of effect has a potentially substantial effect on our overall estimate of the impact of *w*Mel on DENV transmission, we also fitted an alternative model in which we forced *w*Mel infection to affect EIP only. This model fitted statistically significantly worse than the baseline model, but the qualitative quality of fit (Fig. 4, G to I) was very similar to that seen for the baseline model (Fig. 4, D to F). Figure 5 (B and C) illustrates the differences between these two models in how inhibition acts, together with the marked differences between serotypes in the probability of infectious virus being detected in saliva.

For the abdomen model, infectious dose parameters differ significantly between most pairs of serotypes; although the CIs for these parameters overlap, those for their ratios all have 95% CIs that do not include 1 (lower 95% bounds for $\theta_{DENV2}/\theta_{DENV1}$, $\theta_{DENV3}/\theta_{DENV1}$, $\theta_{DENV4}/\theta_{DENV1}$, $\theta_{DENV3}/\theta_{DENV2}$, and $\theta_{DENV4}/\theta_{DENV2}$ of 1.004, 1.20, 1.32, 1.04, and 1.17, respectively), with the exception of $\theta_{DENV4}/\theta_{DENV3}$ (95% CI, 0.94 to 1.48). For the saliva model, DENV1 has a significantly lower infectious dose parameter than the other serotypes (lower 95% bounds for $\phi_{DENV2}/\phi_{DENV1}$, $\phi_{DENV3}/\phi_{DENV1}$, and $\phi_{DENV4}/\phi_{DENV1}$ of 1.84, 1.10, and 1.24, respectively), but differences between DENV2, DENV3, and DENV4 are not statistically significant (95% CI: $\phi_{DENV3}/\phi_{DENV2}$, 0.30 to 1.28; $\phi_{DENV4}/\phi_{DENV2}$, 0.33 to 1.19; $\phi_{DENV4}/\phi_{DENV3}$, 0.53 to 2.19).

Given that the impact of *w*Mel on DENV infection in *A. aegypti* depends on viral titer in the blood meal, the expected population impact of *w*Mel will depend on the distribution of viral titers across DENV-infected human hosts, denoted $\rho_h(v|\tau)$ (see Materials and Methods). Figure S1 shows our estimates of the distribution of human plasma viremia levels, fitted using the model of $\rho_h(v|\tau)$ given in Eq. 2 in Materials and Methods. Substantial variation was seen between different patients infected with the same serotype and between serotypes. Of particular note are the higher peak viremias seen for DENV1, earlier peaks seen for DENV2, and the lower peak titers seen for DENV3 and DENV4. It should be noted that few data are available to characterize viremia around the time of peak titer because few measurements were available before day 2 of illness. This leads to considerable uncertainty in early viral kinetics. We discuss the sensitivity of our results to this uncertainty below.

### Predictions of *w*Mel impact on DENV transmission

We use Eq. 1 (see Materials and Methods) to assess the overall impact of *w*Mel infection on DENV transmission by combining the estimated posterior distributions for the dynamics of viral titer over time in infected humans, the probability that a mosquito will become infected on consuming a blood meal with a certain titer of virus, and the development of infectivity in the mosquito. We represent impact on dengue transmission by the fractional reduction of the reproduction number, $R_0$, of each serotype that would be caused by *w*Mel infection of the entire mosquito population. Figure 6 shows the resulting posterior estimates of the reduction in $R_0$ for each serotype. For the baseline scenario (which assumes that mosquito infectivity to humans is directly proportional to the probability of detecting infectious virus in saliva), a 66 to 75% reduction is predicted, varying by serotype. Although the CIs on the absolute estimates of transmission reduction overlapped across the serotypes, posterior estimates of the differences in reduction between DENV1 and DENV2/3/4 indicated that DENV1 exhibited a significantly lower level of reduction than other serotypes ($P < 0.01$).

Three other scenarios shown in Fig. 6 illustrate the sensitivity of the predictions to assumptions about how the model of saliva infectivity is translated to estimates of mosquito-to-human infectivity. The "higher dose" scenario assumed that the infectious dose parameters in the saliva infectivity model (the parameters $\phi_S$ in Eq. 4 in Materials and Methods) need to be 10-fold larger than the estimated values to

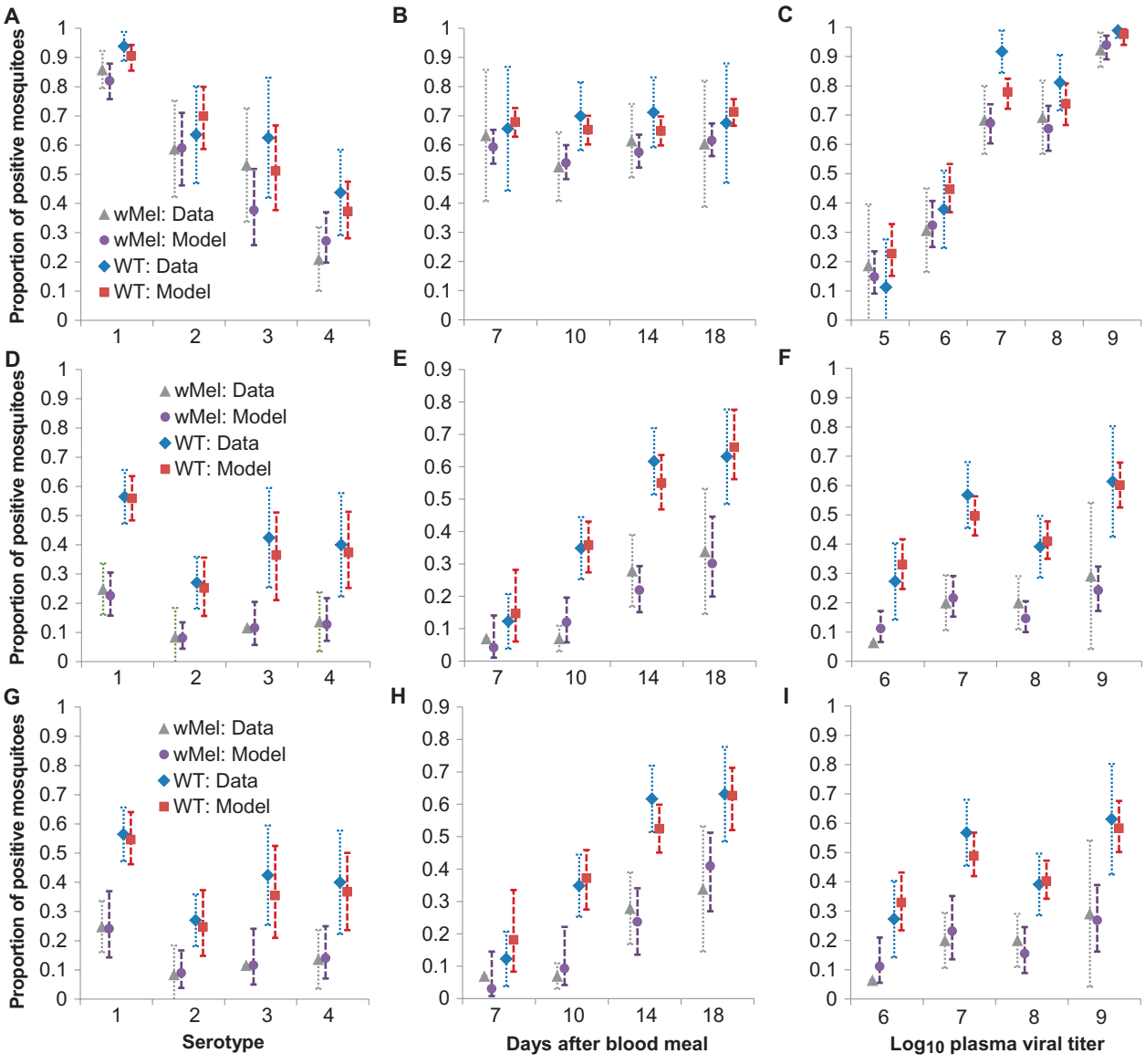


**Fig. 4. Mosquito infection model fit to the empirical evidence of *w*Mel-mediated blocking of DENV infection.** (**A** to **C**) Observed ("Data") and median posterior fitted ("Model") proportions (with exact binomial confidence intervals) of WT and *w*Mel-infected mosquitoes with detectable virus in abdomen, stratified by (A) serotype, (B) end time point, and (C) $\log_{10}$ donor plasma virus titer band. (**D** to **F**) Same as for panels (A) to (C) but showing the proportion of dengue-infected mosquitoes (that is, with detectable virus in abdomen) that also had detectable infectious virus in saliva for the baseline model. (**G** to **I**) Same as for panels (D) to (F) but for the alternative saliva model.

describe mosquito-human transmission probabilities. This scenario gave the greatest predicted reduction in transmission due to *w*Mel infection because of the predicted slower growth of viral titers in saliva of *w*Mel-infected mosquitoes. Conversely, assuming those infectious dose parameters ($\phi_S$) are 10-fold lower than for mosquito-mosquito transmission (as quantified by our assay of saliva infectivity), this resulted in substantially lower estimates of the impact of *w*Mel infection on dengue transmission compared with the baseline scenario. However, it should be noted that this scenario gives an unrealistically high per-bite probabilities of mosquito-human transmission and, thus, very high (>10 for DENV1) estimates of $R_0$ for reasonable assumptions on mosquito numbers per person and the biting rate.

The "average dose" scenario assumed that there are no serotype differences in the dose parameter for mosquito-human transmission, implemented by specifying that the saliva model dose parameter for each serotype ($\phi_S$) takes the mean of the serotype-specific estimates for each posterior distribution sample. The "same viral profile" scenario ignored the differences in human viral kinetics between serotypes shown in fig. S1 and used a single model (see Eq. 2 in Materials and Methods) of $\rho_h(v|\tau)$ for all serotypes fitted to all the patient data shown in that figure. The estimated reductions in $R_0$ due to *w*Mel in both of these scenarios were very similar to those obtained for the baseline scenario, highlighting that serotype differences in viremia kinetics do not explain the overall differences by serotype seen

**Table 2. Mathematical model parameter estimates.**

| Parameter | Description | Median estimate (95% CI)* | |
|---|---|---|---|
| Abdomen model | | | |
| $\delta_{wMel}$ | Dose-response intercept for *w*Mel-infected mosquitoes | −1.12 (−3.22, 0.33) | |
| $\theta_{DENV1}$ | Infectious dose parameter for DENV1 | 5.90 (4.53, 6.58) | |
| $\theta_{DENV2}$ | Infectious dose parameter for DENV2 | 6.78 (5.88, 7.66) | |
| $\theta_{DENV3}$ | Infectious dose parameter for DENV3 | 8.41 (7.17, 10.29) | |
| $\theta_{DENV4}$ | Infectious dose parameter for DENV4 | 9.50 (8.34, 12.27) | |
| $\gamma$ | Dose response shape parameter | 2.88 (1.66, 3.97) | |
| $\rho_{abdomen}$ | Overdispersion parameter for abdomen model | 0.46 (0.38, 0.53) | |
| Saliva model† | | Baseline | Alternative |
| $\varepsilon_{wMel}$ | Scaling of infectious dose parameters for *w*Mel-infected versus wild-type mosquitoes | 3.41 (0.66, 11.2) | Fixed at 1 |
| $\kappa$ | Power on infectivity growth with time | 3.80 (1.99, 6.59) | 3.40 (2.02, 5.04) |
| $\beta_{WT}$ | Timescale of infectivity saturation in saliva of wild-type mosquitoes | 12.3 (9.5, 30.8) | 11.6 (8.7, 19.6) |
| $\beta_{wMel}$ | Timescale of infectivity saturation in saliva of *w*Mel-infected mosquitoes | 12.8 (7.3, 32.5) | 20.7 (15.4, 40.9) |
| $\phi_{DENV1}$ | Infectious dose parameter for DENV1 | 0.52 (0.13, 0.81) | 0.60 (0.30, 0.97) |
| $\phi_{DENV2}$ | Infectious dose parameter for DENV2 | 1.57 (0.37, 2.99) | 1.79 (0.80, 3.44) |
| $\phi_{DENV3}$ | Infectious dose parameter for DENV3 | 0.94 (0.23, 2.15) | 1.11 (0.46, 2.33) |
| $\phi_{DENV4}$ | Infectious dose parameter for DENV4 | 0.99 (0.24, 1.95) | 1.13 (0.50, 2.32) |
| $\rho_{saliva}$ | Overdispersion parameter for abdomen model | 0.19 (0.13, 0.27) | 0.19 (0.13, 0.27) |

*Median estimates and 95% CIs of parameters of the mathematical models (Eqs. 3 and 4) used to fit the abdomen and saliva infection data on *w*Mel-infected and wild-type mosquitoes are shown. Time unit is days. †For the saliva model, estimates are shown for the best-fitting baseline model and an alternative model where the phenotypic effect of *w*Mel infection is forced to act on the parameter $\beta$, determining EIP.

in Fig. 6. Rather, the lower impact of *w*Mel in DENV1 is largely caused by the differences in infectious dose parameters for saliva and abdominal infection between serotypes (Fig. 1).

The last alternative model scenario of Fig. 6 shows the results when the alternative saliva infection model is used, solely representing the impact of *w*Mel as a lengthening of the EIP (Table 1 and Fig. 5C). In this model, the predicted impact of *w*Mel on transmission was about 10% lower, that is, a 57 to 66% reduction depending on serotype.

## DISCUSSION

We have experimentally characterized the phenotype of *Wolbachia*-infected *A. aegypti* mosquitoes challenged with viremic blood from symptomatic dengue patients. *w*MelPop conferred very strong resistance to DENV infection of the mosquito body and, more importantly, the salivary glands. *w*Mel conferred an intermediate phenotype in which abdomen tissues were susceptible to DENV infection, but dissemination was diminished as evidenced by a lower prevalence of mosquitoes with infectious saliva.

The profound level of virus blocking conferred by *w*MelPop infection is predicted to cause dramatic reductions in DENV transmission in settings where *w*MelPop is successfully and stably introduced. The impact of *w*Mel on DENV transmission is more nuanced and serotype-dependent; DENV1 transmission is the least affected, with a predicted 66% reduction in $R_0$ for the baseline scenario. For the other serotypes, higher estimated infectious dose parameters (compared with DENV1) for both the abdominal and saliva infection models lead to larger predicted reductions in transmission of about 75%. To put these reductions in context, estimates of the basic reproduction number ($R_0$) for dengue lie in the range of 1.3 to 6.3 (*12*), with 2 to 5 being typical of endemic settings. A reduction of 66% is sufficient to eliminate dengue in a setting where $R_0$ = 3, whereas a 75% reduction will achieve elimination for $R_0$ = 4.

Our study highlights three effects of *w*Mel infection on DENV infection in *A. aegypti* mosquitoes: an increase (compared with wild-type) in blood meal viremia required to achieve a certain probability of abdominal infection, a substantial reduction in the probability of detecting infectious virus in saliva, and a lengthening of the EIP. In our best-fit models, only the first two of these effects were found to be significant. However, an alternative saliva model that solely represented the impact of *w*Mel in terms of an increased EIP gave an adequate (though statistically poorer) fit to the data and predicted lower reductions in $R_0$ than the baseline model. Additional data, particularly if it included time points beyond 18 days, might more conclusively resolve the extent to which the impact of *w*Mel is to reduce or just delay the onset of infectiousness in saliva. This issue is important for understanding the extent to which the estimated impact of *w*Mel can be generalized to different settings: if *w*Mel reduces the probability of

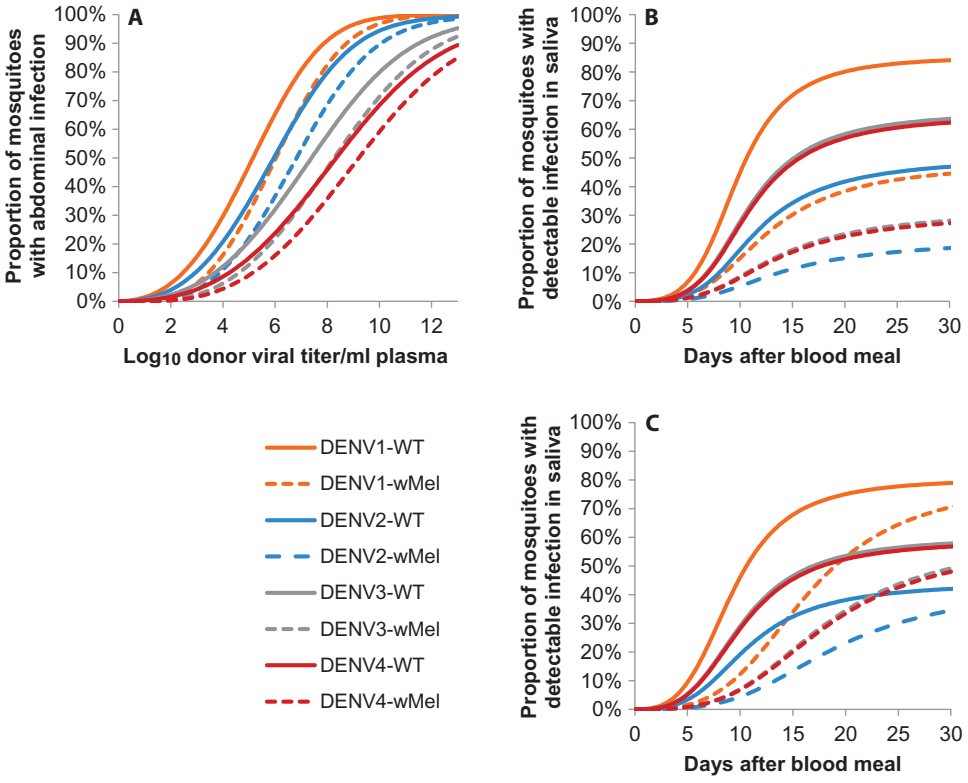


**Fig. 5. Performance of the mosquito infection model.** (**A**) Shown is the behavior of the abdominal infection model illustrating the dependence of the probability of infection on viral titer in donor blood, serotype, and *Wolbachia* infection status. (**B**) Shown is the behavior of the saliva infection model showing dependence of the probability of detectable infection in saliva (conditional on abdominal infection) as a function of the days elapsed since the infecting blood meal, serotype, and *Wolbachia* infection status. (**C**) Same as (B) but for the alternative saliva infection model where *w*Mel infection affects only the EIP. All graphs show mean posterior predictions.

mosquitoes being infectious independent of the time since infection, the reduction in $R_0$ achieved is independent of adult mosquito survival. Conversely, if the main impact of *w*Mel is to increase the EIP, this will have a larger effect on dengue transmission than that estimated here in situations where daily mosquito survival is lower than the relatively high 90% value we assumed.

Previous vector competence studies of *Wolbachia*-infected *A. aegypti* mosquitoes have used in vitro–passaged DENV strains that were spiked into animal or human blood before this mixture was presented to colony mosquitoes through membrane feeders (*4*, *5*). The current study is distinguished from previous work in using fresh viremic blood samples from hospitalized dengue cases to mimic the virological challenge that *A. aegypti* mosquitoes experience when they feed on an infectious human case. In using viremic blood from hospitalized dengue cases, in whom peak viremia levels are significantly higher than in acute ambulatory (never hospitalized) cases in the same setting (*13*), we are likely being conservative in our experimental evaluation of *w*Mel-infected *A. aegypti*. Future experimental studies could examine susceptibility to DENV infection after blood feeding on ambulatory dengue cases.

Our finding that *w*MelPop-*A. aegypti* do not develop disseminated infections with DENV is entirely consistent with the initial description of the vector competence phenotype of this strain (*5*). However, we found that *w*Mel-*A. aegypti* can develop infectious saliva after viremic blood feeding, and this contrasts with the initial description by Walker *et al.* (*4*) who detected no infectious DENV2 in the saliva of any of the 336 *w*Mel-*A. aegypti* females used in artificial feeding experiments. There are methodological reasons why our results might differ: Walker *et al.* used one laboratory strain of DENV2 at a single concentration, used cell culture to detect infectious virus in pooled saliva, and used colony-sourced mosquitoes. Of these, we speculate that the virological differences are most important and that viremic blood from a human dengue case provides the most stringent and relevant challenge of the vector competence of *Wolbachia*-infected mosquitoes. This would underscore the importance of using clinical material for robust assessments of arboviral vector competence in general. Our data also highlight the importance of assessing vector competence at multiple time points to characterize the impact on the dynamics of dengue infection in the mosquito. Whereas the *w*MelPop data presented here were all collected at a single time point (12 days) after blood meal, preliminary results from ongoing work indicate comparable levels of inhibition of DENV infection at 14 and 18 days after infection.

Our analysis suggested that *w*Mel could reduce the DENV force of infection by a degree that would have a highly significant public health impact—potentially achieving elimination in low- to moderate-transmission settings, albeit perhaps insufficient for complete control in high-transmission settings (especially for DENV1). Yet, a number of factors might lead to the field efficacy of *w*Mel on DENV transmission differing from estimates presented here. First, although we did not collect data on the concentration of infectious DENV particles in mosquito saliva, it is a reasonable hypothesis that *w*Mel reduces viral concentrations, which would lead to a larger reduction in transmission than that estimated here. Second, the effect of *w*Mel on other aspects of mosquito behavior that have an impact on transmission, such as host seeking, probing, and blood feeding success rates, has yet to be investigated in a field setting, and it is plausible that these could counteract the effect of *w*Mel-mediated interference of virus transmission to mosquitoes. Finally, here, we solely examined the impact of *Wolbachia* on the susceptibility of *A. aegypti* to DENV infection. In reality, *w*Mel may modify *A. aegypti* fitness through decreased (or, less likely, increased) fecundity or longevity. Even small reductions in the life span of *w*Mel-*A. aegypti*, as described previously (*4*), might cause reductions in dengue transmission.

A priori, that we found no statistically significant dependence on the level of viremia in the infecting blood meal in the mathematical model describing saliva infection might be viewed as surprising. However, the saliva model represents the probability of detecting infectious virus in saliva conditional on abdominal infection being detectable. The

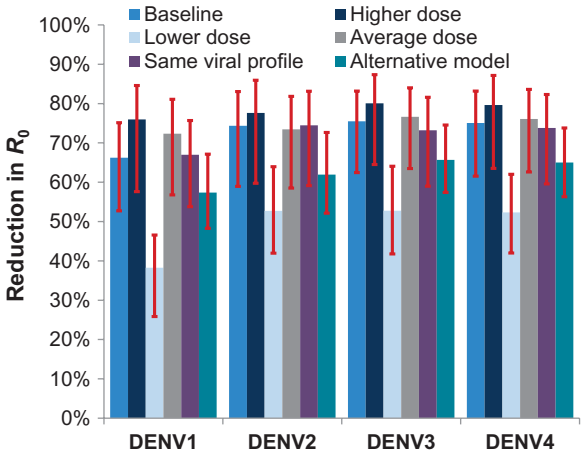


**Fig. 6. Estimated reduction in transmissibility of DENV (quantified by serotype-specific $R_0$) caused by *w*Mel infection.** Median posterior estimates and 95% CIs are shown. Baseline scenario assumes that data on infectious saliva translates directly to human infectiousness. Higher/lower dose scenarios assume a 10-fold higher/lower infectious dose for mosquito-to-human transmission than estimated using saliva infection model. Average dose assumes same infectious dose for all serotypes (average across serotypes) for mosquito-to-human transmission. Same viral profile uses a model of human viral kinetics that is the same for all serotypes. Alternative model uses the alternative saliva infection model where *w*Mel infection affects only the EIP.

limited association between mosquito abdomen viral titers and blood meal viremia (Fig. 3) suggests that the primary influence of the level of viremia in blood is on the probability of establishing abdominal infection but not on later dissemination once abdominal infection has been established.

Our study has several limitations. Quantification of the level of infectiousness of mosquito saliva along a continuous gradient, rather than just a binary measure of infectious status as described here, would allow impacts of reduction in DENV saliva titer due to *w*Mel to be explored. However, we note that in vitro titration methods that work well for highly passaged reference DENV strains do not work well with clinical isolates. Further studies are also needed to understand the vector competence phenotype of *Wolbachia*-infected *A. aegypti* after challenge with DENV genotypes different from those currently circulating in Vietnam. We note that each serotype of DENV in circulation in southern Vietnam during the study period was composed essentially of a single virus genotype (*13*), and thus, our results are unlikely to be confounded by large fitness differences between viruses of the same serotype. Our mosquito studies were conducted with a single, consistent set of environmental conditions: 27°C and 70% relative humidity. Previous experimental studies have noted shortening of the EIP (suggesting more rapid viral replication) as temperature is increased in the range from 26° to 30°C. Thus, the impact of *w*Mel on DENV transmission efficiency might also show some temperature dependence, although the direction and magnitude of such effects are not possible to predict a priori. Although it would be challenging (in cost and time) to repeat the clinical studies presented here for a wide range of environmental conditions, some exploration of the effect of temperature on *w*Mel phenotype would be a worthwhile topic for future work.

Finally, there is an element of arbitrariness in the model structure. The relatively parsimonious, biologically motivated model structures adopted allowed biologically reasonable extrapolation to low and high viremia and gave quality of fit to the data comparable with logistic regression with the same degrees of freedom. Future modeling efforts could move toward using a truly dynamic model of DENV infection in the mosquito.

We have determined that *w*MelPop confers on *A. aegypti* profound resistance to DENV infection. Establishment of *w*MelPop-infected *A. aegypti* at high frequency in a dengue-endemic setting would result in complete abatement of DENV transmission; however, this might prove challenging given the fitness costs conferred by *w*MelPop infection. Establishment of *w*Mel-infected *A. aegypti*, as has occurred in some communities in northern Australia (*11*), is also predicted to have a substantial effect on transmission, but may be insufficient to entirely control dengue in settings where the basic reproduction number is high. Other complementary interventions may therefore be needed to offset the lower efficacy of *w*Mel in high transmission intensity settings, for example, traditional vector control methods and new approaches such as using adult male *Wolbachia-A. aegypti* releases for population suppression. Additionally, dengue vaccines (*14*) might work in concert with *Wolbachia* intervention to achieve long-term disease control. Finally, it will be desirable to evaluate other *Wolbachia-A. aegypti* strains; for example, the well-established *w*AlbB-*A. aegypti* strain deserves evaluation in this viremic blood challenge system and in the field (*15*). The prospect of a "menu" of *Wolbachia* options, alongside other dengue interventions, could enable a bespoke approach to dengue control in a range of epidemiological and socioeconomic contexts.

## MATERIALS AND METHODS

### Study design

This was a prospective observational study that used viremic blood from acute dengue cases to blood-feed wild-type or *Wolbachia*-infected *A. aegypti* mosquitoes in the laboratory. The sample size was not prespecified and instead was based on pragmatic considerations around the duration of the study, which spanned two dengue seasons (from June 2012 to December 2013). We prespecified that data collection would stop in December 2013. We used biological replicates throughout the study; for example, multiple blood samples from independent patients but infected with the same DENV serotype. We also used biological replicates of the mosquitoes with a minimum of five blood-fed mosquitoes per cohort. Dengue patients were enrolled at the Hospital for Tropical Diseases (HTD) in Ho Chi Minh City, Vietnam. Patients were eligible for enrollment if (i) they were ≥1 year of age, (ii) with less than 72 hours of fever, and (iii) they were clinically suspected of having dengue and had a positive NS1 (nonstructural protein 1) rapid test. Exclusion criteria were (i) patients in intensive care unit and (ii) patients with intellectual disabilities. The baseline features of the dengue cases whose venous blood was used for vector competence studies are shown in table S1. On the day of enrollment, venous blood (EDTA anticoagulant) was collected and split for mosquito feeding and for quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) measurement of DENV RNA concentrations in plasma using a validated qRT-PCR assay that has been described previously (*16*). All patients provided written informed consent to provide blood samples. The study protocol was reviewed and approved by the Scientific and Ethical committee of the HTD (reference number CS/ND/09/24) and the Oxford Tropical Research Ethical Committee (reference number OxTREC 20-09).

The prespecified hypothesis was that *Wolbachia*-infected *A. aegypti* mosquitoes were more resistant to DENV infection. Hence, the primary entomological end point of interest was the proportion of mosquitoes with infected abdomens or saliva. This was addressed by scoring mosquito tissues for the presence or absence of DENV infection using a molecular test and thence modeling the results as a basis to predict the wider epidemiological impact on DENV transmission. All laboratory assays to test for DENV infection were performed by technicians blinded to the clinical and virological details of the patient blood sample and the *Wolbachia* status of the mosquitoes. All data were submitted to a Good Clinical Laboratory Practice database and cleaned before data lock.

**Viremic blood challenges of wild-type and Wolbachia-infected A. aegypti.** Vector competence studies were performed with wild-type *A. aegypti* from Cairns, Australia, and *A. aegypti* of the same origin but stably infected with *w*Mel or *w*MelPop. The wild-type versus *w*MelPop-*A. aegypti* studies were performed using eggs from outcrossed colonies maintained at Monash University, Australia. Colonies were maintained at population sizes of 400 with a 50:50 sex ratio. The wild-type versus *w*Mel-*A. aegypti* studies were performed with $F_2$ generation adults and obtained by hatching eggs collected from field sites in Cairns, Australia (*11*). For all studies, up to 100 three- to seven-day-old female *A. aegypti* mosquitoes were starved for 24 hours before being membrane fed on fresh acute blood from laboratory-confirmed dengue patients. All blood samples were placed into glass membrane feeders within 1 hour of the blood being collected, and mosquitoes were allowed access to the blood for 1 hour. Membrane feeders were water-jacketed and maintained at constant temperature during mosquito feeding (37°C). After cold knockdown, fully engorged mosquitoes were selected and then maintained in an environmental chamber with a 12:12-hour light/dark cycle, at 27°C and 70% relative humidity, with access to 10% sucrose solution.

**Detection of DENV in saliva and abdomen tissues.** Infectious virus in mosquito saliva was detected by placing the proboscis of a de-winged and de-legged mosquito into the end of a filtered micropipette tip containing 6 μl of sterile saliva medium (a 1:1 solution of 15% sucrose and inactivated fetal calf serum) for 30 min at room temperature. After 30 min, the 6-μl saliva medium was ejected and then drawn into a pointed glass capillary tube (tip diameter, <0.3 μm). The volume of saliva medium derived from one mosquito was then injected into the thorax of between four and six *A. aegypti* mosquitoes (4 to 7 days old; ~1 μl injected per mosquito), and the injected mosquitoes were maintained for 7 days in an environmental chamber with a 12:12 light/dark cycle, at 28°C and 80% relative humidity. After 7 days, the injected mosquitoes for each saliva sample were killed; the bodies were pooled, homogenized, and tested by qRT-PCR for DENV infection, with saliva samples scored as positive or negative depending on this result. Saliva samples were collected from all mosquitoes, but only saliva samples from mosquitoes with infected abdomens were evaluated for their infection status because pilot studies confirmed that abdomen infection was a prerequisite for the saliva to contain infectious virus. After collection of saliva samples, the abdomen was dissected from the mosquito body. Dissected abdomens were suspended in 0.5 ml of mosquito diluent (RPMI 1640 supplemented with 2% heat-inactivated fetal calf serum, antibiotics, and antimycotics). Individual mosquito abdomens were homogenized with 1-mm Zirconia/Silica beads for 15 min at 30 Hz using a TissueLyser II (Qiagen). Mosquito tissues were scored as being DENV-infected using a quantitative, internally controlled RT-PCR assay (*16*) on homogenized tissue, and the results were expressed as copies per tissue.

**Detection of Wolbachia status in mosquito tissues by real-time PCR.** For quality control purposes, *Wolbachia* infection status was scored using a multiplex PCR assay on nucleic acid extracts from mosquito abdomens. *A. aegypti* ribosomal protein S17 (Ae-RpS17) was used as an internal control. *Wolbachia* strain *w*Mel was detected with primers/probes specific to the WD0513 gene, and *w*MelPop was detected with primers/probes specific to the polymorphic insertion sites of the IS5 at loci IS5-WD1310. Sequences of primers/probes for *Wolbachia* and DENV detection are shown in table S2. PCR was performed on a LightCycler 480 II machine using LightCycler 480 Probes Master according to the manufacturer's instructions.

**Data release.** See Supplementary Materials for the *w*Mel and *w*MelPop data analyzed in this paper.

## Statistical analysis

**Nonparametric assessment of the differences in tissue infection rates in wMel versus wild-type mosquitoes.** We applied a standard sign test for paired data, treating the experimental data as pairs of binomial observations corresponding to the proportions infected of the *w*Mel and wild-type mosquito groups fed on a particular blood sample, which were sampled on a particular day. Rows in Table 1 present the number of observation pairs for which the proportion of *w*Mel mosquitoes infected was less than, equal to, or greater than the proportion of wild-type mosquitoes infected for different data subsets, designated as $n_{pairs}(p_{wMel} < p_{WT})$, $n_{pairs}(p_{wMel} = p_{WT})$, and $n_{pairs}(p_{wMel} > p_{WT})$, respectively. If there was no difference between the infection rates of *w*Mel and wild-type mosquitoes, $n_{pairs}(p_{wMel} < p_{WT})$ would be expected to be drawn from a binomial distribution with $P = 0.5$ and $N = n_{pairs}(p_{wMel} < p_{WT}) + n_{pairs}(p_{wMel} > p_{WT})$. The two-sided $P$ value in the final column of Table 1 is the probability of a sample from that distribution being equal to or more extreme than the observed value of $n_{pairs}(p_{wMel} < p_{WT})$.

**Transmission model.** Because the probability of a mosquito becoming infected with DENV from a blood meal depends strongly on the viral titer in that blood meal, quantitative assessment of the impact of *Wolbachia* on transmission requires a mathematical model that couples the dynamics of infection within the human host with those in the vector. We found no previously published mathematical models of DENV transmission that included such coupling, so the framework presented below needed to be developed specifically for this study.

We define $\rho_h(v|\tau)$ to be the probability density that the plasma viral titer of a human host is $v$ at time $\tau$ after infection; we model viral dynamics in humans probabilistically to represent the variation seen between individuals. We assume that the probability that a mosquito taking a blood meal on that individual becomes infected depends on the viral titer in the blood at the time of feeding: let $p_i(v)$ be the probability that a vector becomes infected when feeding on a human with a plasma viral titer of $v$. If a mosquito becomes infected, then we assume that its infectiousness to humans depends on the time elapsed from the infecting blood meal and the plasma viral titer of the blood meal. We define $p_m(v|t)$ to be the probability that a mosquito infected by taking a blood meal with viral titer $v$ will infect another human host it bites time $t$ later; this distribution captures the EIP.

Together, these three distributions represent the complete transmission cycle; all that is additionally required to calculate the basic reproduction number (the average number of human infections gen-

erated by a typical infected human in the absence of immunity), $R_0$, for a serotype is the average number of female mosquitoes per human host, $m$, the mortality hazard for adult female mosquitoes, μ, and the biting rate of female mosquitoes, κ. Then

$$R_0 = m\kappa^2 \int_0^\infty \left( \int_0^D \rho_h(v|\tau) d\tau \right) p_i(v) \left( \int_0^\infty p_m(v|t) e^{-\mu t} dt \right) dv \quad (1)$$

Here, $D$ is the maximum time for clearance of virus in humans. This equation is the standard definition of the reproduction number for a vector-borne disease, generalized to account for viral dose dependence in the mosquito.

We wish to estimate the distributions $\rho_h(v|\tau)$, $p_i(v)$, and $p_m(v|t)$ for each of the four DENV serotypes and for *Wolbachia*-infected and wild-type mosquitoes. However, the available data did not allow every parameter to be estimated independently for each combination of serotype and *Wolbachia* infection status, so it was necessary to assume that only a subset of parameters varied between serotypes or were affected by *Wolbachia*.

Our primary interest is the extent to which *Wolbachia* reduces transmission, as characterized by the ratio of $R_0$ of a DENV serotype in a wild-type *A. aegypti* population to that in a *Wolbachia*-infected *A. aegypti* population; values of $m$ and κ in Eq. 1 are not needed for calculating this ratio. However, the assumed value of μ, the mortality hazard of adult mosquitoes, can affect estimates. *A. aegypti* mortality varies seasonally and by setting, with release-recapture studies typically giving daily survival probabilities below 85% (*17*–*19*). Because one possible phenotype of *w*Mel on dengue replication in mosquitoes that we explore below is a lengthening of the EIP, we conservatively assume that daily survival is constant at its seasonal maximum of 90% (μ = 0.1/day) (*19*). This results in a larger proportion of transmission being from older mosquitoes than assuming a lower value for daily survival, and hence reduces the potential impact of EIP lengthening on dengue transmission.

We estimate $\rho_h(v|\tau)$ from serial plasma viremia levels measured in 262 consecutively enrolled dengue cases in the IDAMS (International Research Consortium on Dengue Risk Assessment, Management, and Surveillance) study (clinicaltrials.gov identifier, NCT01550016) in Ho Chi Minh City, Vietnam. Of these 262 cases, 73 cases were hospitalized and 189 were managed entirely as ambulatory patients for the duration of their illness. The serial viremia measurements in these 189 ambulatory cases have been described previously (*13*), and the data are shown in fig. S1. Here, we use the following data fields for each measurement: study participant identifier, DENV serotype, day of illness when sample was collected, and $\log_{10}$ viral titer per milliliter of plasma (measured with qRT-PCR) in sample. We modeled viral kinetics in a human host with a simple biphasic exponential growth/decay function, where average (across all patients) viral titer at time $t$ after infection is given by:

$$v(t) = \frac{e^{at}}{1 + e^{(a+b)t-c}} \quad (2)$$

We assume that individual patient $\log_{10}$ viral titers are drawn from a normal distribution with mean $\log[v(t)]$ and standard deviation σ, thus defining the distribution $\rho_h(v|\tau)$. Because the dates of infection are unknown, we estimate time of infection from the day of illness onset by assuming a fixed 7-day incubation period for dengue. Parameters $a$, $b$, and $c$ were fitted independently for each serotype, whereas σ was fitted assuming it to be the same for all serotypes.

**Mosquito infection model.** The probability that a mosquito feeding on blood with viral titer $v$ will become infected, $p_i(v)$, is estimated from data on abdominal infection status in mosquitoes infected as part of this study. We used a simple dose-response model:

$$p_i(v) = 1 - \exp\left[ -\left( \frac{\log v + \delta_W}{\theta_S} \right)^\gamma \right], \text{ if } \log v + \delta_W > 0$$

$$p_i(v) = 0 \text{ otherwise} \quad (3)$$

The single parameter $\delta_W$ was found to be sufficient to capture the phenotypic impact of *Wolbachia*. This parameter was assumed to be 0 for wild-type and was estimated for *w*Mel-infected mosquitoes. Its effect is to modify the infecting dose of virus by a fixed factor. The parameter $\theta_S$ determines the infectious dose and is estimated independently for each serotype $S$, whereas γ determines the slope of the dose-response curve and is assumed not to vary by serotype. We did not model an effect of day of measurement (after mosquito feeding) for abdominal infection data because no significant differences were seen between the 7-, 10-, 14-, and 18-day time points examined here.

In the absence of human challenge studies, we lack direct measurements of mosquito infectiousness, $p_m(v|t)$; here, we examine the closest proxy available, namely, detection of infectious DENV in mosquito saliva. We defined $q_m(v|t)$ to be the proportion of mosquitoes infected by taking a blood meal with viral titer $v$ that will have detectable infection in saliva time $t$ later. We assume the following functional form for $q_m(v|t)$:

$$q_m(v|t) = \left( 1 - \exp\left[ -\frac{1}{\varepsilon_W \phi_s} \left( \frac{t^\kappa}{\beta_W^\kappa + t^\kappa} \right) \right] \right) \quad (4)$$

This semi-mechanistic form gives power-law $[\sim(t/\beta_W)^\kappa]$ temporal growth of saliva infection for small $t$. This growth saturates at a time governed by parameter $\beta_W$; thus, this parameter governs the EIP. Because we needed to use this model outside the observed range of $7 \le t \le 18$ days, it was important to choose a functional form for the time dependence of saliva infection status that was well behaved and biologically plausible for both small and large $t$. The model above gives close to zero probability of detectable infection for small $t$ (<7 days) and a probability that plateaus at large $t$ (>18 days). Similar to the abdominal infection model, the serotype-specific parameters $\phi_S$ govern the infectious dose. A dose-response shape parameter (akin to γ in Eq. 3) was also examined but found to result in overfitting, with estimates having 95% CIs overlapping 1.

Two parameters, $\beta_W$ and $\varepsilon_W$, specify the phenotypic impact of *Wolbachia* for the saliva infection model. Hence, *Wolbachia* can affect either the proportion of mosquitoes ever developing infectiousness in saliva or the rate at which saliva infectiousness increases (and thus, the EIP), or both. The former is estimated separately for wild-type and *w*Mel-infected mosquitoes, whereas the latter scales the infectious dose parameters for *w*Mel versus wild-type, and hence has a value of 1 for wild-type and is estimated for *w*Mel-infected mosquitoes.

When both $\beta_W$ and $\varepsilon_W$ were fitted (our baseline model), estimates for $\beta_{WT}$ and $\beta_{wMel}$ were nearly identical, with a substantial overlap of the 95% CIs. Thus, nearly the entire phenotypic effect was attributed to $\varepsilon_W$—representing a net reduction in the probability of infection in saliva in *w*Mel-infected versus wild-type mosquitoes, irrespective of the time elapsed since the infecting blood meal (Fig. 5B). However, because the lower CI of $\varepsilon_W$ was just below 1 for the baseline model, we

fitted three simpler models, assuming (A) $\beta_{WT} = \beta_{wMel}$ and $\varepsilon_W = 1$ (that is, no phenotypic effect of *w*Mel); (B) $\beta_{WT} = \beta_{wMel}$ (that is, the phenotypic effect of *w*Mel is acting solely through $\varepsilon_W$); (C) $\varepsilon_W = 1$ (that is, the phenotypic effect of *w*Mel is acting solely through a difference between $\beta_{WT}$ and $\beta_{wMel}$—effectively a lengthening of the EIP due to *w*Mel infection). Model B had the highest DIC, with the baseline model (with both $\beta_W$ and $\varepsilon_W$ fitted) next (DIC difference from B of 1.1), followed by model C (DIC difference from B of 2.4) and then model A with much worse (DIC difference from B of 47). Because the phenotypic effect of *w*Mel infection substantively affects the overall estimates of the impact of *Wolbachia* on transmission, we choose to present the estimates for model C (where $\varepsilon_{wMel} = 1$) as an alternative to the baseline model. This alternative model (Fig. 5C) fitted the data qualitatively well (Fig. 4), albeit worse than the baseline model (difference in DIC, 1.4). Although model B had the highest DIC, the small numerical difference compared with the model fitting both $\beta_W$ and $\varepsilon_W$ meant that we opt to retain the latter as our baseline, as it best represents the uncertainty in the phenotypic effect of *w*Mel infection and is slightly more pessimistic than model B in the estimates of the impact of *w*Mel on the $R_0$ of dengue.

The saliva infection model shows no dependence on the plasma viral titer of the infecting blood meal; including such dependence did not significantly improve model fit, reflecting the lack of obvious viral titer dependence seen in the raw saliva infection data shown in Fig. 1. For example, substituting a term $(\omega_W + \log \nu)/\phi_S$ for $1/\varepsilon_W\phi_S$ in Eq. 4, fitting $\omega_{wMel}$ and assuming $\omega_{wMel} = 0$ (akin to the abdominal model) increased the DIC by 2.3 relative to the baseline model. Furthermore, the central estimate for $\omega_{wMel}$ was unreasonably large in magnitude (−6.1) given that $\log_{10}$ donor viral titers only varied in the range of 5.3 to 9.9, meaning that this model variant was approximating the behavior of the functionally simpler baseline model with no improvement in fit.

Our default (and simplest) approach to relating $p_m(\nu|t)$ to $q_m(\nu|t)$ is to assume proportionality, namely, $p_m(\nu|t) \propto q_m(\nu|t)$. However, other assumptions are plausible and can substantially affect the resulting estimates of the overall impact of *w*Mel on dengue transmission. We undertake some sensitivity analysis therefore by assuming that $p_m(\nu|t)$ is determined by a similar functional form to Eq. 4, but with modified parameters. We examine the impact of varying the infectious dose parameters by a fixed multiplier to mimic the effect of the infectious dose from mosquitoes to humans being either larger or smaller than that seen with the assay we used to assess infectious virus in saliva.

**Inferential framework.** Model fitting was undertaken in a Bayesian framework using Markov Chain Monte Carlo (MCMC) methods (*20*). To account for the overdispersion of the data (Figs. 1 and 2), a Beta-binomial likelihood function was used rather than a simple binomial likelihood. The Beta-binomial was parameterized in terms of the mean binomial proportion, Θ, and its overdispersion, ρ, defined such that the mean and variance of a sample of $n$ draws is given by $n\Theta$ and $n\Theta(1 - \Theta)[1 + (n - 1)\rho]$, respectively. The overdispersion parameter ρ was fitted separately for the abdominal and saliva data. Uninformative uniform priors were assumed for all parameters, with an upper bound of 200 for all parameters and a lower bound of 0 for all parameters other than δ, for which a lower bound of −200 was used. Sensitivity to changing the upper and lower bounds (where appropriate) on priors was tested, and none was found so long as the upper and lower bounds lay outside the 99.9th percentile of the posterior distribution. Parameters were updated individually, with a single update sweep defined as a sequence of proposed updates to each parameter in turn. For computational efficiency, a uniform proposal distribution was used for each parameter, centered around the current parameter value and with width manually tuned to give 20 to 40% acceptance rates (proposal acceptance rates were monitored separately for each parameter). MCMC chains were equilibriated with 100,000 update sweeps, and posterior distributions were estimated from the following 500,000 update sweeps, sampling once every 500 sweeps. Convergence was checked visually and by running multiple chains from different starting points. Analyses were undertaken in Microsoft Excel and the statistical language R.

In exploratory but nonexhaustive analyses, a variety of functional forms were explored for both $p_i(\nu)$ and $q_m(\nu|t)$; in particular, we examined how model fit could be significantly improved by making a parameter vary by serotype or by *Wolbachia* infection status while retaining parameter identifiability. We found little evidence for any serotype dependence beyond the overall scaling of the dose-response relationships expressed in the functional forms used above. Similarly, significant differences (assessed by nonoverlapping 95% CIs and the DIC) between estimates for wild-type and *w*Mel-infected mosquitoes were only seen for the parameters δ, ε, and, to a lesser extent (see discussion above), β.

## SUPPLEMENTARY MATERIALS

www.sciencetranslationalmedicine.org/cgi/content/full/7/279/279ra37/DC1
Table S1. Study population characteristics.
Table S2. List of primers and probes used.
Fig. S1. Human DENV viremia kinetics.
Supplementary data: Mosquito biting study data.

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**Funding:** Supported by the Wellcome Trust; the Bill and Melinda Gates Foundation (BMGF); the Foundation for the NIH, as part of the Grand Challenges in Global Health Initiative of BMGF; the National Health and Medical Research Council, Australia; the U.K. Medical Research Council; the National Institute of General Medical Sciences Models of Infectious Disease Agent Study initiative; and the European Union Seventh Framework Programme European Management Platform for Emerging and Re-emerging Infectious Disease Entities consortium. **Author contributions:** C.P.S., N.M.F., and S.L.O. designed the study; B.W., D.T.H.K., V.T.T., T.N.B.C., V.T.L., L.T.D., H.L.N., and N.V.V.C. performed the mosquito biting experiments; J.P., P.A.R., S.L.O., and E.A.M. developed the *Wolbachia*-infected *A. aegypti*; N.M.F., H.C., and R.A. performed the analysis; and N.M.F. and C.P.S. drafted the manuscript. **Competing interests**: N.F. is an informal and unpaid advisor on dengue control measures (including *Wolbachia* and vaccines) and dengue modeling for BMGF and Sanofi Pasteur Inc. C.S. has a paid consulting position with Sanofi Pasteur Inc., which has a business interest in developing dengue vaccines. S.L.O., P.A.R., and E.A.M. are named as coinventors on a patent for *Wolbachia* mosquito strains: Modified Arthropod and Method of Use; Filing # 14/304,919; Filing date: 06/14/2014. The other authors declare that they have no competing interests. **Data and materials availability:** The data collected in this study are provided in Supplementary Materials.

Submitted 15 August 2014
Accepted 26 February 2015
Published 18 March 2015
10.1126/scitranslmed.3010370

**Citation:** N. M. Ferguson, D. T. H. Kien, H. Clapham, R. Aguas, V. T. Trung, T. N. B. Chau, J. Popovici, P. A. Ryan, S. L. O'Neill, E. A. McGraw, V. T. Long, L. T. Dui, H. L. Nguyen, N. V. V. Chau, B. Wills, C. P. Simmons, Modeling the impact on virus transmission of *Wolbachia*-mediated blocking of dengue virus infection of *Aedes aegypti*. *Sci. Transl. Med.* **7**, 279ra37 (2015).

Science Translational Medicine

# Modeling the impact on virus transmission of *Wolbachia*-mediated blocking of dengue virus infection of *Aedes aegypti*

Neil M. Ferguson, Duong Thi Hue Kien, Hannah Clapham, Ricardo Aguas, Vu Tuan Trung, Tran Nguyen Bich Chau, Jean Popovici, Peter A. Ryan, Scott L. O'Neill, Elizabeth A. McGraw, Vo Thi Long, Le Thi Dui, Hoa L. Nguyen, Nguyen Van Vinh Chau, Bridget Wills and Cameron P. Simmons

*Sci Transl Med* **7**, 279ra37279ra37.
DOI: 10.1126/scitranslmed.3010370

**Use a bug to fight a bug**

Dengue is the most common mosquito-borne viral infection in humans. In this new work, Ferguson *et al.* have assessed the extent to which infecting mosquitoes with a bacterium called *Wolbachia* was able to prevent those mosquitoes from being infected with dengue virus after they were fed with blood collected from dengue patients. One *Wolbachia* strain (*w*MelPop) almost completely prevented dengue infection. A second strain (*w*Mel) partially blocked dengue infection. A mathematical model fitted to the data collected on the *w*Mel strain suggested that *w*Mel could reduce the transmissibility of dengue by 66 to 75%, enough to eliminate dengue in low or moderate transmission settings.

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