Assessing the epidemiological effect of wolbachia for dengue control

Louis Lambrechts, Neil M Ferguson, Eva Harris, Edward C Holmes, Elizabeth A McGraw, Scott L O'Neill, Eng E Ooi, Scott A Ritchie, Peter A Ryan, Thomas W Scott, Cameron P Simmons, Scott C Weaver

Dengue viruses cause more human morbidity and mortality than any other arthropod-borne virus. Dengue prevention relies mainly on vector control; however, the failure of traditional methods has promoted the development of novel entomological approaches. Although use of the intracellular bacterium wolbachia to control mosquito populations was proposed 50 years ago, only in the past decade has its use as a potential agent of dengue control gained substantial interest. Here, we review evidence that supports a practical approach for dengue reduction through field release of wolbachia-infected mosquitoes and discuss the additional studies that have to be done before the strategy can be validated and implemented. A crucial next step is to assess the efficacy of wolbachia in reducing dengue virus transmission. We argue that a cluster randomised trial is at this time premature because choice of wolbachia strain for release and deployment strategies are still being optimised. We therefore present a pragmatic approach to acquiring preliminary evidence of efficacy through various complementary methods including a prospective cohort study, a geographical cluster investigation, virus phylogenetic analysis, virus surveillance in mosquitoes, and vector competence assays. This multipronged approach could provide valuable intermediate evidence of efficacy to justify a future cluster randomised trial.

Introduction

Dengue is a major public health problem in tropical and subtropical regions, where almost 400 million infections are estimated to occur each year.[1] The cause is the flavivirus dengue virus, which has four serotypes (DENV-1–4) transmitted to human beings by mosquitoes. These viruses cause a systemic, debilitating, and mostly self-limiting illness, which without careful management can lead to hypovolaemic shock and death.[2] In the absence of a licensed vaccine or therapeutic drug, dengue prevention efforts are restricted to the control of its main mosquito vector, *Aedes aegypti*. With a few exceptions, the implementation of vector control methods has been largely unsuccessful because of the absence of a sustained commitment of resources[3] and the inability to scale up and successfully apply interventions over large geographical areas and modern megacities. Novel entomological approaches to dengue control have been developed[4] and some are now advancing to field testing.[5]

One of the most promising entomological strategies being developed for dengue control relies on the introduction of the intracellular bacterium wolbachia into *A aegypti*.[6] *Wolbachia pipientis* is a bacterial endosymbiont that was originally identified in ovaries of culex mosquitoes in the 1920s[7] and is thought to infect two-thirds of all living insect species.[8] The extraordinary evolutionary success of wolbachia is attributed to the ability to manipulate the biology of their hosts in diverse ways.[9] For example, wolbachia can induce reproductive abnormalities such as feminisation and cytoplasmic incompatibility between sperm and eggs. Since wolbachia is transmitted vertically via the egg, female-biased reproductive manipulations can drive wolbachia infections to high frequencies in wild populations. Cytoplasmic incompatibility, the most common manipulation in insects, occurs when wolbachia-infected male insects mate with wolbachia-free female insects and produce non-viable offspring. By contrast, wolbachia-infected female hosts produce successful offspring irrespective of the infection status of their mate.

The potential of wolbachia to control pest insect populations was realised half a century ago (figure 1). Wolbachia-induced cytoplasmic incompatibility was proposed to eliminate culex mosquitoes[10] or to introduce desirable genes into wild vector populations.[11] So far, however, wolbachia has never been operationally implemented as a vector control measure. Several major vectors of human pathogens are not naturally infected by wolbachia, including the main dengue virus vector *A aegypti*, and this was a substantial hurdle in using wolbachia to control vector populations. The mosquito vectors (*Anopheles* spp) of human malaria parasites were thought to be wolbachia free until a study in 2014 reported evidence for infection in field populations of *Anopheles gambiae*.[12]

A resurgence of interest in wolbachia-based strategies to control vector-borne diseases occurred about a decade ago with the advent of transinfection techniques (figure 1). Stable wolbachia infections in naive hosts can now be established by embryonic microinjections into the developing embryo germ line. Generally, wolbachia transinfection is more likely to be successful between closely related donor and recipient hosts, and the expression of wolbachia-induced phenotypes is conserved across hosts. In 2005, a stable infection by a wolbachia strain from the mosquito *Aedes albopictus* was established in *A aegypti*, which caused high rates of cytoplasmic incompatibility and rapidly spread to high frequencies in experimental populations.[13] Double transinfections of *A aegypti* with two different wolbachia strains from *A albopictus* quickly followed.[14]

Lancet Infect Dis 2015

Published Online
June 5, 2015
http://dx.doi.org/10.1016/S1473-3099(15)00091-2

Insect-Virus Interactions Group, Department of Genomes and Genetics, Institut Pasteur – CNRS URA 3012, Paris, France (L Lambrechts PhD); **MRC Centre for Outbreak Analysis and Modelling, School of Public Health, Imperial College London, London, UK** (Prof N M Ferguson PhD); **Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, CA, USA** (E Harris PhD); **Marie Bashir Institute for Infectious Diseases and Biosecurity, Charles Perkins Centre, School of Biological Sciences and Sydney Medical School, University of Sydney, Sydney, NSW, Australia** (Prof E C Holmes PhD); **School of Biological Sciences, Monash University, Melbourne, VIC, Australia** (E A McGraw PhD, Prof S L O'Neill PhD, P A Ryan PhD); **Program in Emerging Infectious Diseases, Duke-National University of Singapore Graduate Medical School, Singapore, Singapore** (E E Ooi PhD); **School of Public Health and Tropical Medicine and Rehabilitative Sciences, James Cook University, Cairns, QLD, Australia** (S A Ritchie PhD); **Department of Entomology and Nematology, University of California, Davis, CA, USA** (Prof T W Scott PhD); **Fogarty International Center, National Institutes of Health, Bethesda, MD, USA** (T W Scott); **Oxford University Clinical Research Unit, Centre for Tropical Medicine, Ho Chi Minh City, Vietnam** (Prof C P Simmons PhD); **Centre for Tropical Medicine, Nuffield Department of Medicine, University of Oxford, Oxford, UK** (C P Simmons); **Nossal Institute of Global Health, University of Melbourne, Carlton, VIC, Australia** (C P Simmons); **and Institute for Human Infections and**

Immunity and Department of Pathology, University of Texas Medical Branch, Galveston, TX, USA (Prof S C Weaver PhD)

Correspondence to:
Dr Louis Lambrechts, Insect-Virus Interactions Group, Department of Genomes and Genetics, Institut Pasteur – CNRS URA 3012, 75724 Paris Cedex 15, France
louis.lambrechts@pasteur.fr

A second wave of breakthroughs happened several years later with the discovery of wolbachia-induced phenotypes in mosquitoes that had a direct effect on pathogen transmission (figure 1). Until then, wolbachia was mainly regarded as a gene drive system. However, the possibility of transinfection of wolbachia strains from more distant hosts by cell culture adaptation before microinjection,[15] combined with the wide diversity of available wolbachia strains and properties, resulted in new associations between the bacteria and mosquitoes. Stable introduction of a life-shortening strain of wolbachia from drosophila into *A aegypti* halved the adult mosquito lifespan under laboratory conditions, thus mosquitoes were unlikely to live long enough to transmit dengue virus.[16] Furthermore, this life-shortening wolbachia strain directly inhibited the ability of a range of pathogens, including dengue virus, to infect and replicate in *A aegypti*.[17] Results from semifield and field trials in Australia have shown that wolbachia can be persistently established in wild *A aegypti* populations.[18,19] Together, these properties form the basis of a practical approach for suppression of dengue virus transmission through field release of wolbachia-infected mosquitoes.

Wolbachia deployment for dengue control

The next crucial step in the use of wolbachia for dengue control is to assess the efficacy of medium-scale wolbachia deployment in reducing human infection. The gold standard, a cluster randomised trial of wolbachia, has been discussed in detail.[20] A cluster randomised trial is an approach in which groups of people, rather than individuals, are randomly assigned to the alternative treatments under study. This design is particularly useful when the intervention cannot be directed toward selected individual participants, such as the release of wolbachia-infected mosquitoes. In the classic two-armed design, clusters without intervention provide contemporaneous controls. In a stepped-wedge design, the intervention is rolled out sequentially to all the clusters, therefore the clusters are their own controls over time.

At this time, a cluster randomised trial for the wolbachia-based approach would be premature for several reasons. First, many strains of wolbachia are available for deployment, each with its own characteristic effects on dengue virus blocking and mosquito fitness. A process of selection through field testing is needed before one or more final strains can be chosen for a particular release area. Additionally, although deployment in north Queensland, Australia, has provided a basic template for release, this environment differs substantially from the large urban centres in southeast Asia and Latin America where a cluster randomised trial would probably be done. During deployment, the effectiveness of release strategies and community engagement has to be monitored to make potential adjustments. Examples of adaptive changes made during previous deployments include releasing larger numbers of mosquitoes, changing the intensity of trap grids to monitor wolbachia spread, supplementing releases with different mosquito developmental stages, and altering locations of deployment on the basis of community concerns.[18,21] However, a standard cluster randomised trial approach would lock in all aspects of the release, preventing extemporaneous improvements in design. Finally, a classic two-armed trial would have to be large, with more than 40 clusters each including about 100 study participants monitored for infection to detect a 50% reduction in dengue with 90% power.[20] Such a design has been roughly estimated to cost in excess of US$5–10 million.

Here, we argue that well designed observational studies could provide an array of valuable indirect evidence that supports wolbachia as a dengue intervention and, hence, justifies continued development, ultimately leading to a definitive efficacy trial. Ideally, several observational studies would be done in different settings and their outcomes combined in a meta-analytic framework to assess the effect on disease and infection incidence. Five possible approaches that could be used separately or in combination to acquire such evidence are a paediatric cohort study, a geographical cluster investigation, virus sequence analysis, virus detection in mosquitoes, and vector competence assays.

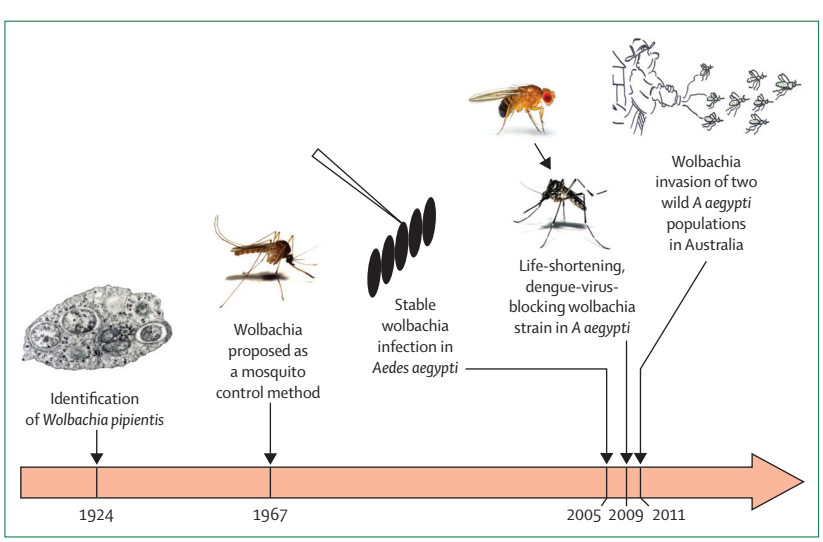

Figure 1: **Key developments in wolbachia-based dengue control strategies**

Paediatric cohort study

A prospective longitudinal cohort study that tracks seroconversion rates in children could measure both the true incidence of dengue virus infections and the relative risk of infection between wolbachia-treated and untreated areas.[20] Because the overall dengue virus seroconversion rate is generally 5–10% per year in endemic countries,[22] a cohort would need to include at least several thousand individuals to be compatible with the statistical requirements of a cluster randomised trial with sufficient power to detect a moderate intervention effect.[20] A smaller cohort

of 1000–1500 children, although underpowered in the context of a trial, could be greatly enhanced by the use of concurrent approaches. Fine-scale entomological surveillance (eg, a grid of traps) would allow the spatio-temporal dynamics of wolbachia prevalence to be monitored and distinguish wolbachia-free areas from areas where wolbachia had established in real time. Raw entomological data could be interpolated over time and space by use of standard methods and serve as a covariate for dengue virus seroconversion. As with other epi-demiological investigations, participants residing in the study area, but acquiring infections outside of the intervention area, represent a complication to this approach.[23,24] However, geographical cluster studies of dengue cases and fine-scale spatiotemporal phylogenetic analyses of genomic virus strain sequences would help to address this concern.

Figure 2: **Geographical cluster methods**
The central dot represents the home of an individual with confirmed dengue (red: area with wolbachia; green: area without wolbachia). People living within a 100 m radius (black dots) are screened for concomitant or secondary dengue virus infection (crosses denote homes of additional dengue virus-infected individuals).

Geographical cluster investigation

Dengue virus infections are acute, often clinically inapparent, or have non-specific signs and symptoms, and thus are difficult to detect across populations in real time. Active surveillance of human infections can be efficiently achieved with geographical cluster sampling around dengue index cases.[25,26] Here, index case refers to the laboratory-diagnosed clinical dengue case that initiates a cluster investigation within a geographically restricted area around the home of a person with a documented infection. Geographical cluster investigations could be used to compare the fine-scale spatial signature of dengue virus transmission in areas with and without wolbachia (figure 2). This method would test the hypothesis that concurrent or subsequent infections, or both, around an index case are reduced in areas where wolbachia-infected mosquitoes are established. Additionally, inward migration of dengue infections acquired outside the treatment area could be a confounding factor.[23,24] However, detailed phylogenetic analysis of virus sequences or monitoring the movement patterns of study participants could potentially resolve this issue. Nonetheless, if a wolbachia intervention reduces local transmission at a microscale, cluster investigation methods should detect this reduction. An efficacious intervention would result in fewer index cases in the wolbachia-treated areas or a reduction in concurrent infections as measured by a lower frequency of cases that are spatiotemporally linked to the index case, or both.

Figure 3: **Schematic representation of how wolbachia intervention might change patterns of virus genetic diversity**
Assuming that many lineages of various dengue virus serotypes (coloured dots) cocirculate before the intervention, a reduction in local dengue virus transmission is expected to result in a reduction in viral genetic diversity in the intervention area and a relative increase in average dispersion distances.

Virus sequence analysis

Increasing access to viral genome sequence data has promoted the development of new methods to infer dengue epidemiological dynamics on the basis of analyses of changing patterns in viral genetic diversity in time and space.[27,28] Assuming that many lineages of various serotypes cocirculate before an intervention, a reduction in local transmission should decrease the viral genetic diversity across serotypes in the intervention area because of a major viral demographic bottleneck, and increase the average dispersion distances travelled by dengue virus into the intervention area (figure 3). Phylogenetic analysis is a straightforward way to identify the introduction of foreign viral lineages into the study area, provided that genetic diversity accumulates at a sufficiently high rate. Previous work on dengue virus microevolution in southeast Asia suggested that spatial patterns of genetic diversity are shaped by frequent virus immigration and highly focal transmission.[28–30] Although the level of phylogenetic resolution to be obtained is unknown, deep sequencing methods have improved substantially, increasing the power of this approach. Although we expect local dengue virus transmission to be reduced in wolbachia-treated areas, some viruses will continue to be imported by human-mediated dispersal. However, these viruses will not persist locally, reducing the strong spatial clustering that is typically reported in dengue virus phylogenies.

Virus detection in mosquitoes

Local *A aegypti* populations will need to be monitored after the release of wolbachia-infected mosquitoes for changes in wolbachia prevalence and possibly in mosquito density. Several sampling methods that effectively capture female *A aegypti* have been developed.[31–34] Virus detection could be combined with routine molecular tests to detect the presence of wolbachia. Detecting *A aegypti* mosquitoes infected with dengue virus is challenging because of the low infection rates (typically about 0·1%) in adult females across the population, although infection rates can be higher in locations of geographical cluster investigations.[25] Because mosquitoes that test positive for the virus are not necessarily infectious, the proportion of infected mosquitoes does not directly translate into an estimate of virus transmission unless virus disseminated from the mosquito midgut or in saliva is assayed too. However, this approach is limited by the sensitivity of assays and variation of in-vitro saliva collections. Nonetheless, a successful intervention is expected to reduce the incidence of viraemic and infectious individuals and, therefore, reduce the incidence of dengue virus infection in mosquitoes in areas where wolbachia infection predominates.

Vector competence assays

After the release of wolbachia-infected mosquitoes, it will be necessary to verify that the phenotype of reduced vector competence is maintained over time in field-collected mosquitoes.[35] Vector competence assays consist of experimentally exposing laboratory-reared mosquitoes to either an artificial infectious blood meal or the blood of a viraemic person.[36] The proportion of infectious mosquitoes (ie, with virus detected in saliva) is then measured over time. The ability of wolbachia-infected mosquitoes to deliver dengue virus in their saliva is strongly reduced compared with wolbachia-free mosquitoes.[19] Ideally, vector competence experiments would be extended to human-to-mosquito-to-human transmission experiments in a human challenge model.[37] Vector competence assays will provide additional indirect evidence about the effect of the intervention, especially if the virus interference effect is strong.

Conclusions and perspectives

The present challenge is to convert a promising strategy into a validated public health intervention through rigorous assessment of its epidemiological effect. The various approaches described in this Personal View are not a substitute for a cluster randomised trial. Nonetheless, this strategy has at least two major strengths that can lay the foundations for a future trial. First, the proposed investigations are not dependent on the uniform application of the intervention, which by nature will vary through time and space. Instead, an association between the presence of wolbachia and proxies of dengue virus transmission (eg, seroconversion or occurrence of secondary cases around index cases) can be inferred dynamically from the spatiotemporal correlation between these factors. Second, comprehensive findings and detection of correlations between environmental and biological factors will likely improve fundamental understanding of dengue epidemiology that will inform and underpin future trial designs. A multipronged approach would help to assess the potential effects on other *A aegypti*-borne arboviruses (eg, chikungunya virus) and the likelihood of unexpected outcomes, such as viral evolution to escape the inhibitory effects of wolbachia, or other unforeseen adverse events.

Measuring the epidemiological effect of wolbachia deployment to reduce dengue virus transmission is challenging. The intervention is not based on individuals, as a vaccine trial would be, but on populations defined by spatial areas. The fundamental test of the effect of the intervention is a comparison between areas where wolbachia-infected mosquitoes are present versus areas where they are not (figures 2, 3). Although restricted dispersal of *A aegypti*[38] and, therefore, spread of wolbachia is expected to maintain spatial delineation of the intervention, a buffer zone will be necessary to avoid unexpected overlap between treatment and control areas. The intervention needs to be deployed over a geographical area large enough to ensure that a sufficient number of dengue cases (or absence of cases if the intervention is effective) is captured. Previous knowledge of the study area will help to assign intervention and control areas with similar baseline transmission trends. Virus importation into the intervention area (through human-mediated dispersal[23,24]), which is likely to occur and might reduce the signal-to-noise ratio, can be explored with geographical cluster studies and by accounting for movement of study participants.

One advantage of our proposed approach is that interpretation of seroconversion data from a small-scale paediatric cohort can be enhanced by data from geographical cluster investigations, viral sequencing, and virus detection in mosquitoes, collectively resulting in a body of evidence that could support continued development of wolbachia as a public health method. A true placebo treatment (ie, release of wolbachia-free mosquitoes) is not ethically possible. However, the human and mosquito samples can be blinded before laboratory testing.

We have described a pragmatic approach for the assessment of novel entomological interventions for dengue control through a coordinated, cross-disciplinary, ecological study that combines several proxies of efficacy at the epidemiological, entomological, and virological levels. The approach relies on a combination of methods that have been successfully used to monitor dengue epidemiological dynamics, in addition to novel methods. Although this approach has no precedent for dengue, it has the potential to provide valuable intermediate evidence of efficacy that

supports the wolbachia method and justifies funding for a cluster randomised trial or deployment.

Contributors
LL wrote the first draft of the manuscript. All authors contributed equally to editing of the manuscript.

Declaration of interests
We declare no competing interests.

Acknowledgments
We thank Stephanie James and Fil M Randazzo for their insights, and two anonymous reviewers for helpful comments. This work was supported by a grant from the Foundation for the National Institutes of Health through the Grand Challenges in Global Health Initiative of the Bill & Melinda Gates Foundation.

References

1 Bhatt S, Gething PW, Brady OJ, et al. The global distribution and burden of dengue. *Nature* 2013; **496:** 504–07.
2 Simmons CP, Farrar JJ, Nguyen V, Wills B. Dengue. *N Engl J Med* 2012; **366:** 1423–32.
3 Morrison AC, Zielinski-Gutierrez E, Scott TW, Rosenberg R. Defining challenges and proposing solutions for control of the virus vector *Aedes aegypti*. *PLoS Med* 2008; **5:** e68.
4 McGraw EA, O'Neill SL. Beyond insecticides: new thinking on an ancient problem. *Nat Rev Microbiol* 2013; **11:** 181–93.
5 James S, Simmons CP, James AA. Ecology. Mosquito trials. *Science* 2011; **334:** 771–72.
6 Iturbe-Ormaetxe I, Walker T, O' Neill SL. *Wolbachia* and the biological control of mosquito-borne disease. *EMBO Rep* 2011; **12:** 508–18.
7 Hertig M, Wolbach SB. Studies on *Rickettsia*-like micro-organisms in insects. *J Med Res* 1924; **44:** 329–74, 7.
8 Hilgenboecker K, Hammerstein P, Schlattmann P, Telschow A, Werren JH. How many species are infected with *Wolbachia*?—A statistical analysis of current data. *FEMS Microbiol Lett* 2008; **281:** 215–20.
9 Werren JH, Baldo L, Clark ME. *Wolbachia*: master manipulators of invertebrate biology. *Nat Rev Microbiol* 2008; **6:** 741–51.
10 Laven H. Eradication of *Culex pipiens fatigans* through cytoplasmic incompatibility. *Nature* 1967; **216:** 383–84.
11 Curtis CF. Population replacement in *Culex fatigans* by means of cytoplasmic incompatibility. 2. Field cage experiments with overlapping generations. *Bull World Health Organ* 1976; **53:** 107–19.
12 Baldini F, Segata N, Pompon J, et al. Evidence of natural *Wolbachia* infections in field populations of *Anopheles gambiae*. *Nat Commun* 2014; **5:** 3985.
13 Xi Z, Khoo CC, Dobson SL. *Wolbachia* establishment and invasion in an *Aedes aegypti* laboratory population. *Science* 2005; **310:** 326–28.
14 Ruang-Areerate T, Kittayapong P. *Wolbachia* transinfection in *Aedes aegypti*: a potential gene driver of dengue vectors. *Proc Natl Acad Sci USA* 2006; **103:** 12534–39.
15 McMeniman CJ, Lane AM, Fong AW, et al. Host adaptation of a *Wolbachia* strain after long-term serial passage in mosquito cell lines. *Appl Environ Microbiol* 2008; **74:** 6963–69.
16 McMeniman CJ, Lane RV, Cass BN, et al. Stable introduction of a life-shortening *Wolbachia* infection into the mosquito *Aedes aegypti*. *Science* 2009; **323:** 141–44.
17 Moreira LA, Iturbe-Ormaetxe I, Jeffery JA, et al. A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, Chikungunya, and *Plasmodium*. *Cell* 2009; **139:** 1268–78.
18 Hoffmann AA, Montgomery BL, Popovici J, et al. Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature* 2011; **476:** 454–57.
19 Walker T, Johnson PH, Moreira LA, et al. The *w*Mel *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. *Nature* 2011; **476:** 450–53.
20 Wolbers M, Kleinschmidt I, Simmons CP, Donnelly CA. Considerations in the design of clinical trials to test novel entomological approaches to dengue control. *PLoS Negl Trop Dis* 2012; **6:** e1937.
21 Harris AF, McKemey AR, Nimmo D, et al. Successful suppression of a field mosquito population by sustained release of engineered male mosquitoes. *Nat Biotechnol* 2012; **30:** 828–30.
22 Endy TP, Yoon IK, Mammen MP. Prospective cohort studies of dengue viral transmission and severity of disease. *Curr Top Microbiol Immunol* 2010; **338:** 1–13.
23 Stoddard ST, Forshey BM, Morrison AC, et al. House-to-house human movement drives dengue virus transmission. *Proc Natl Acad Sci USA* 2013; **110:** 994–99.
24 Stoddard ST, Morrison AC, Vazquez-Prokopec GM, et al. The role of human movement in the transmission of vector-borne pathogens. *PLoS Negl Trop Dis* 2009; **3:** e481.
25 Yoon IK, Getis A, Aldstadt J, et al. Fine scale spatiotemporal clustering of dengue virus transmission in children and *Aedes aegypti* in rural Thai villages. *PLoS Negl Trop Dis* 2012; **6:** e1730.
26 Reyes M, Mercado JC, Standish K, et al. Index cluster study of dengue virus infection in Nicaragua. *Am J Trop Med Hyg* 2010; **83:** 683–89.
27 Rabaa MA, Ty Hang VT, Wills B, Farrar J, Simmons CP, Holmes EC. Phylogeography of recently emerged DENV-2 in southern Viet Nam. *PLoS Negl Trop Dis* 2010; **4:** e766.
28 Raghwani J, Rambaut A, Holmes EC, et al. Endemic dengue associated with the co-circulation of multiple viral lineages and localized density-dependent transmission. *PLoS Pathog* 2011; **7:** e1002064.
29 Jarman RG, Holmes EC, Rodpradit P, et al. Microevolution of dengue viruses circulating among primary school children in Kamphaeng Phet, Thailand. *J Virol* 2008; **82:** 5494–500.
30 Rabaa MA, Klungthong C, Yoon IK, et al. Frequent in-migration and highly focal transmission of dengue viruses among children in Kamphaeng Phet, Thailand. *PLoS Negl Trop Dis* 2013; **7:** e1990.
31 Barrera R, Mackay AJ, Amador M. An improved trap to capture adult container-inhabiting mosquitoes. *J Am Mosq Control Assoc* 2013; **29:** 358–68.
32 Krockel U, Rose A, Eiras AE, Geier M. New tools for surveillance of adult yellow fever mosquitoes: comparison of trap catches with human landing rates in an urban environment. *J Am Mosq Control Assoc* 2006; **22:** 229–38.
33 Ritchie SA, Buhagiar TS, Townsend M, et al. Field validation of the gravid Aedes trap (GAT) for collection of *Aedes aegypti* (Diptera: Culicidae). *J Med Entomol* 2014; **51:** 210–19.
34 Vazquez-Prokopec GM, Galvin WA, Kelly R, Kitron U. A new, cost-effective, battery-powered aspirator for adult mosquito collections. *J Med Entomol* 2009; **46:** 1256–59.
35 Frentiu FD, Zakir T, Walker T, et al. Limited dengue virus replication in field-collected *Aedes aegypti* mosquitoes infected with *Wolbachia*. *PLoS Negl Trop Dis* 2014; **8:** e2688.
36 Nguyet MN, Duong TH, Trung VT, et al. Host and viral features of human dengue cases shape the population of infected and infectious *Aedes aegypti* mosquitoes. *Proc Natl Acad Sci USA* 2013; **110:** 9072–77.
37 Thomas SJ. Dengue human infection model: re-establishing a tool for understanding dengue immunology and advancing vaccine development. *Hum Vaccin Immunother* 2013; **9:** 1587–90.
38 Harrington LC, Scott TW, Lerdthusnee K, et al. Dispersal of the dengue vector *Aedes aegypti* within and between rural communities. *Am J Trop Med Hyg* 2005; **72:** 209–20.