Q&A for Randomised Controlled Trial — Indonesia

August 2020

1. Why Yogyakarta?

Yogyakarta has regularly ranked among the top ten provinces in Indonesia for annual dengue incidence, over the past three decades. The World Mosquito Program and the University of Gadjah Mada, Yogyakarta, have since 2011 been working with local government, health authorities and communities to undertake pilot deployments of *Wolbachia* in Yogyakarta province. These pilot studies have demonstrated successful and durable *Wolbachia* establishment, with strong community support. These factors, together with a strong health system and human resource capacity for high quality clinical research, made it well-suited as the site for the first efficacy trial of WMP's *Wolbachia* method.

2. What is the key finding from the study?

The efficacy of *Wolbachia* (wMel strain) against virologically-confirmed dengue among persons aged 3-45 years was 77%. This means that dengue incidence was 77% lower among persons who lived in a *Wolbachia*-treated area compared to those who lived in an untreated area during the 27 months of the trial.

3. Have these results been peer reviewed?

These results have not yet been peer reviewed.

4. When will the full results of the trial be published?

Full results of the trial, including efficacy of *Wolbachia* against each of the four dengue virus serotypes, will be presented at an international scientific congress in November 2020 and submitted for publication in a peer reviewed journal. Publication is expected by late 2020 or early 2021.

5. What is a cluster randomised controlled trial?

A cluster randomised controlled trial is considered a gold-standard method for evaluating the efficacy of health interventions delivered at the community level. It involves the random allocation of an intervention to a subset (e.g. 50%) of predefined spatial units, and the comparison of disease outcomes amongst individuals living in areas with versus without the intervention. The random allocation of the intervention results in two study arms, one treated, one untreated, that are comparable at baseline in all factors except for the intervention under study. This allows for an unbiased estimate of the effect of the intervention on the disease of interest.

6. What is different about a test-negative cluster randomised controlled trial?

A test-negative design differs from a traditional cluster randomised trial in the way the disease outcomes are detected. Instead of recruiting a cohort of participants from treated and untreated communities and following them up over time to detect disease outcomes, a test-negative design recruits participants from among patients who present at healthcare facilities with a particular clinical syndrome, in our case a fever, and determines using a laboratory test who is positive or negative for the disease of interest (dengue). The efficacy of the intervention is then determined by comparing the likelihood of having received the intervention between persons who tested positive and those who tested negative. It can be more efficient, cheaper and logistically simpler to implement than a traditional cluster randomised controlled trial as it does not require prospective follow-up of thousands of participants over many years to attain a sufficient number of dengue cases for robust statistical analysis. Also, because all study participants are recruited from within the population of patients who present to a healthcare facility, it reduces the possibility of bias that may result from any differences in care-seeking behaviour between persons who did and did not receive the intervention.

7. How were the treatment areas for the study selected?

The study area was subdivided into 24 contiguous clusters, each with an area of approximately 1 km2 and an average population of 13,000 people. Among these 24 clusters, 12 were randomly allocated to receive *Wolbachia* deployments and 12 to remain untreated. This random assignment of the intervention was 'constrained' to ensure balance between *Wolbachia*-treated and untreated areas for some key factors including historical dengue incidence, incidence of non-dengue febrile illnesses, population demographics (proportion aged <15 years, socioeconomic status), population size, and area size. The final random allocation was drawn in a public randomisation event involving community leaders, to maximise transparency and acceptability of the process.

8. Where do the *Wolbachia*-carrying mosquitoes come from?

The *Wolbachia*-carrying mosquitoes are from a mosquito colony that has been maintained in Yogyakarta since 2013. *Wolbachia* was first introduced into mosquitoes from Yogyakarta by mating wild-type male Ae. aegypti mosquitoes from Yogyakarta with female *Wolbachia*-infected mosquitoes from Australia for 5 generations. This is called backcrossing and it serves to replace the Australian genetic background with the local Indonesian genetic background. Frequent outcrossing of the colony since 2013 with local wild-type mosquitoes ensures the genetic background of the mosquitoes is always well-matched to the mosquitoes flying around in Yogyakarta.

9. When and how were the *Wolbachia*-carrying mosquitoes deployed in Yogyakarta?

Wolbachia-carrying mosquitoes were released as eggs between March and December 2017. Households hosted these mosquito release containers, to which *Wolbachia*-carrying mosquito eggs, water and fish food were added once every two weeks, for 4-6 months. Adult *Wolbachia*-carrying mosquitoes emerged from the containers, then bred with wild-type Ae. aegypti mosquitoes until almost all Ae. aegypti in the intervention areas carried *Wolbachia*.

10. How do you prevent the *Wolbachia*-carrying mosquitoes from flying into untreated areas?

Natural borders (roads, rivers, non-residential areas) were used to define cluster boundaries as much as possible, to limit the spatial spread of *Wolbachia* into untreated areas. Some contamination of untreated clusters did occur, and this is accounted for in a secondary (per-protocol) analysis that will be published as part of the detailed trial results in a peer-reviewed journal.

11. Did the *Wolbachia* contamination in untreated areas affect the results of the trial?

Some contamination of *Wolbachia* into 'untreated' clusters did occur, and this is accounted for in a secondary (per-protocol) analysis that will be published as part of the detailed trial results in a peer-reviewed journal. This contamination means that some people classified as living in an untreated area may have had a partial protective effect from *Wolbachia*. This would result in a dilution of the true intervention effect, which means our result is, if anything, an underestimate of *Wolbachia*'s true effect on dengue incidence.

12. People move around the city, they don't just stay in their area of residence. How can you know whether they got their dengue infection in a *Wolbachia*-treated or untreated area?

The analysis that produced this primary efficacy result classifies participants as simply *Wolbachia*-exposed or not, based on whether their residence was in an area that received *Wolbachia* deployments or not. We collected information from participants at enrolment about the locations where they had spent time during the 10 days prior to illness onset, from which we can determine the proportion of their time spent in *Wolbachia*-treated and untreated areas. This will be accounted for in a secondary (per-protocol) analysis that will be published as part of the detailed trial results in a peer-reviewed journal.

13. Was the efficacy of *Wolbachia* equivalent for all four dengue virus serotypes?

Serotype-specific efficacy is a secondary endpoint which will be reported when the full trial results are published in a peer-reviewed journal.

14. Did the trial require that other routine vector control activities stop in intervention areas and/or untreated areas?

No, *Wolbachia* deployments were in addition to routine vector control activities. No changes were introduced to existing activities in either the intervention or untreated areas.

15. Besides *Wolbachia* were there differences in mosquito-control activities between the treated and untreated areas?

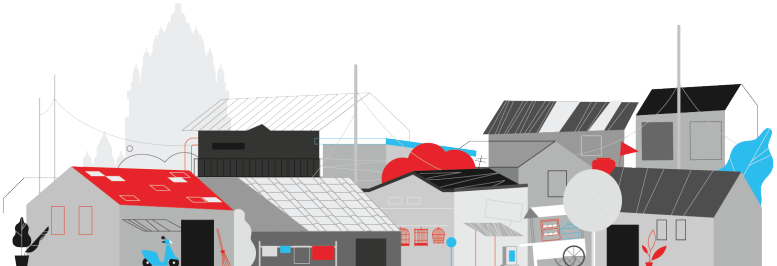
Routine vector control measures are focused on community-based activities to reduce mosquito breeding sites, application of larvicide and focal insecticide fogging in areas around notified cases. These activities are managed at the village level and their implementation varies somewhat between communities, but the community engagement messaging both before and during the trial emphasised that usual vector control and bite prevention practices should be continued throughout both intervention and untreated areas.

16. Were the communities blinded to whether they were in a treated or untreated cluster? If not, how do you know that this didn't affect the results of the trial?

The nature of the *Wolbachia* intervention means that blinding was considered infeasible, as it would have doubled the resources and time required to conduct field releases of mosquitoes; for example, with inactive eggs as the placebo. The risks to study validity from a non-blinded deployment (for example, if a belief that *Wolbachia* is protective against dengue cases leads residents of treated areas to be less likely to seek care for a febrile illness) were also seen to be minimised by the fact that dengue cases and test-negative controls are drawn from the same patient population, who are clinically indistinguishable at the time of presentation and enrolment at clinics. The test-negative cluster randomised controlled trial design is tolerant to the possibility that healthcare seeking behaviour is modified by a person's knowledge of their *Wolbachia* status, as long as this modified behaviour applies equally to test-positive dengue cases and test-negative controls.

17. Did you have consent from all residents of the treated areas to release *Wolbachia*-carrying mosquitoes in their community?

Extensive effort was invested in local community engagement leading up to this trial, extending from community leaders and key stakeholders to the media and the general public, with an aim of informing the community about the planned *Wolbachia* releases and addressing any concerns. Approval for releases was given by community leaders after extensive community consultation, with individual residents' consent obtained for hosting a mosquito release container at their property.



18. Will the intervention eventually eliminate dengue from Yogyakarta?

On the basis of these positive results, the District Health Office of Yogyakarta has begun working with the World Mosquito Program in Indonesia to plan for *Wolbachia* deployments into the untreated areas of Yogyakarta city, commencing later in 2020. Subsequent releases are planned in the neighbouring Sleman and Bantul districts of Yogyakarta province in 2021. Once *Wolbachia* is durably established throughout Yogyakarta city, it is conceivable that this could lead to local elimination of dengue transmission for years to come, although enhanced case finding and diagnosis would be needed to track progress towards and achievement of any elimination goal.

19. What do these results mean for other dengue endemic settings?

These results are consistent with published findings from non-randomised deployments in northern Queensland, and Indonesia, and with preliminary results from city-wide deployments in Brazil. This gives us confidence that the reduction in dengue incidence reported here is likely to be replicated across different epidemiological settings. It also supports evaluation of the impact of large-scale *Wolbachia* deployments using routinely available disease surveillance data, since it is neither feasible nor necessary to conduct a formal efficacy trial in each of the many settings in which *Wolbachia* deployments are likely to be of benefit.

It is possible that differences in the predominance of circulating DENV serotypes in different locations may influence the generalisability of these results to other settings, but in general we expect that in other locations where *Wolbachia* is established at a high level, we will see similarly significant reductions in local arboviral disease incidence. Indeed, where *Wolbachia* deployments are being conducted across a larger contiguous area, we expect the reduction in disease burden to be even greater because the vast majority of people's movements (and mosquito exposure) will be within the *Wolbachia*-treated area.

The main caveat in replicating these results elsewhere is that differences in ecology, climate, altitude and the complexity of the urban environment are likely to affect the trajectory of *Wolbachia* establishment, and consequently the timing of the impact on disease.

20. If my city or my country wants to deploy *Wolbachia*, how can we get involved?

If you would like to deploy *Wolbachia* in your city, please email us at contact@worldmosquito.org with information about you, your organization and how you would like to get involved.

Technical information

21. You report an odds ratio as the measure of intervention effect. How should this be interpreted, in terms of the relative reduction in dengue associated with the *Wolbachia* intervention?

A common measure of the intervention effect is the risk ratio that directly compares the risk of disease in the intervention and untreated arms. For the cluster-randomized test-negative design, the estimated odds ratio uses the test-negative counts from each arm to approximate the relative sizes of the susceptible care-seeking populations in each arm. In this way, the odds ratio estimates the risk ratio. In our trial, this means the odds ratio quantifies how much smaller the risk of dengue is in the *Wolbachia* intervention arm as compared to the untreated arm.

22. How can you interpret the results in terms of a reduction in dengue incidence when you don't have a denominator, as you would in a cohort study design?

In principle, one could estimate dengue incidence for both the intervention and untreated arms by dividing the number of cases in both arms by the sizes of the treated and untreated susceptible populations, respectively. However, these population sizes can be extremely difficult to determine accurately. The test-negative design uses the relative frequency of the test-negative controls in the two intervention arms as a proxy for the ratio of these population sizes based on the assumption that the *Wolbachia* intervention does not impact febrile illnesses other than dengue.

23. What does the 95% confidence interval tell us?

Because the true intervention effect is unknown, the 95% confidence interval provides a range of possible values. In this study, our best estimate of the intervention effect is the reported point estimate of 77%. If this study could be repeated many times, we would likely see slight variation in each repeated study's estimate of the true intervention effect due to sampling variability. The 95% confidence interval is constructed such that if this study were repeated many times, 95% of intervals estimated this way would contain the true intervention effect.

24. How is clustering accounted for in the analysis?

The non-independence of individuals within each cluster in CRTs causes statistical correlation that can affect inference. When clustering is not taken properly into account, conventional estimation approaches tend to underestimate the true variance, resulting in confidence intervals that are artificially narrow and p-values that are too small. In addition, cluster characteristics may induce test-positives and test-negatives to vary together (e.g. cluster population size/density).
Our estimate of the confidence intervals around the aggregate odds ratio account for the non-independence of test-positives and test-negatives within clusters, by using an estimator of the variance that incorporates an adjustment for the observed correlation of outcomes within clusters and allows for the covariance of test-positives