Efficacy of *Wolbachia*-infected mosquito deploymentsfor the control of dengue in Indonesia

Supplementary appendix

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# Supplementary Methods

## 

## Constrained randomisation procedure

***Background***

Randomisation is a key step in the design of the AWEDcluster randomised trial. Importantly, it will provide a sense of “fairness” and transparency in allocation of the intervention. It is also an opportunity to ensure “balance” between intervention and non-intervention arms. Balance means that, overall, the intervention and non-intervention arms have similar dengue risk apart from the presence of *Wolbachia*. Balance aims to minimise the effect of confounding variables, so that any systematic difference in dengue incidence that occurs between *Wolbachia* intervention areas and untreatedareas during the trial can clearly be attributed to the effects of *Wolbachia*. Ensuring a well-balanced trial will reduce the need for statistical adjustment during the analysis phase, increasing the “face validity” of the trial. Balance also improves statistical power and efficiency, reducing the risk of Type II error (i.e. failure to detect an effect when the effect is, in fact, present).1

Simple randomisation can be relied upon to produce an overall balance between intervention and non-intervention arms when there is a large number of clusters available for randomisation. However simple randomisation can’t be relied on to produce balanced allocations where the sample size is small. For example, in our field efficacy trial involving just 24 clusters, it is possible simple random allocation may allocate most clusters with a high dengue risk to the same treatment arm through chance alone.

Covariate constrained (also referred to as “restricted”) randomisationis the best way to achieve balance when the number of clusters is small.1-3 It ensures balance and minimises loss of statistical power without the need for large numbers of strata. After deciding on appropriate balancing criteria, the approach identifies all potential allocations that will satisfy these criteria, and then randomly selects an allocation pattern from within this restricted list of possibilities. Covariate-constrained randomisation can be used because in general, when assessing the comparability of treatment and control arms, balance is assessed in terms of overall balance between treatment and control arms, rather than within each matched pair or within each stratum.2 This approach to randomisation does not generally affect the approach to analysis.2

Constrained randomisation for the AWED trial follows guidelines outlined by Hayes and Moulton, 2009.2 Balancing variables include those that may be potentially confounding covariates; may impact sample size; or may be useful for logistical considerations. Note that balance is necessary at both the cluster and individual levels.1 Sample size is an important balancing covariate as precision and power is maximised when sample sizes in treatment arms are similar. This includes both the number of clusters and the total number of sampled individuals within each treatment arm.1

In Yogyakarta, randomisation was conducted after community-level consent to participate in the RCT was obtained from local leaders (heads of urban villages). This minimises the likelihood that a cluster or part of a cluster declines to allow *Wolbachia* releases after randomisation has occurred and the intervention clusters are known, which would introduce a risk of the study arms becoming unbalanced not only in size but also in other important covariates. *Wolbachia* releases were successfully conducted in all clusters randomised to receive the intervention.

***Constraining variables***

|  |  |  |  |
| --- | --- | --- | --- |
| **Category** | **Constraining variable** | **Data source** | **Balancing criteria** |
| Potential confounding covariates | Incidence of notified DHF, per 1000 population (2013-15) | Yogyakarta District Health Office | Incidence in each arm deviates from overall incidence by +/- <5% |
| Incidence of presentations for other (non-dengue) febrile illness, per 1000 population (2014-15) | 18 primary care clinics in Yogyakarta (Puskesmas) | Incidence in each arm deviates from overall incidence by +/- <5% |
| % of population aged <15 years | Yogyakarta demographic bureau | Proportion aged <15y in each arm deviates from the overall proportion by +/- <5% |
| % of population >20 years that finished school | Yogyakarta demographic bureau | Proportion aged >20y that have finished school in each arm deviates from the overall proportion by +/- <5% |
| Population at risk | Population (2015) | Yogyakarta Statistics Office (BPS) | Population in each study arm is within 45-55% of the study area total |
| Number of clusters per treatment arm | N/A | Fixed at 12 clusters per arm |
| Logistical considerations | Cluster area (km2) | WMP Yogyakarta | Total area in each study arm is within 45-55% of the study area total |
| Cluster non-release area (km2)\* | WMP Yogyakarta | Non-release area in each study arm is within 45-55% of the study area total |
| Spatial strata | N/A | Each of 4 quadrants of the city will include 6 clusters, 3 intervention and 3 untreated |

\* to ensure the intervention arm cannot end up with an excess of “non-release” areas

***Constrained randomisation method:***

1. Calculated values for each balancing covariate for each of the 24 clusters and across the study area as a whole. Cluster values are summarised in Table S1.

2. Generated a large number of potential random allocations (n=100,000)

3. For each allocation, calculated the value of each balancing criterion in each study arm

4. Rejected any random allocations where any one or more of the balancing criteria described in Table 1 were not met.

5. Note that for the potential confounding covariates the comparisons between study arm values and overall values were calculated in two ways, and both were applied as constraints as described above in 4:

a. Individual-level: the aggregate rate or proportion calculated across the study arm was compared with the aggregate rate or proportion calculated across the whole population

b. Group-level: the mean of 12 cluster-level rates or proportions in the study arm was compared with the mean of all 24 cluster-level rates or proportions.

6. Assessed validity of the scheme:

a. Reviewed restricted number of potential allocations, ensuring the number was not too small relative to the overall number of possible allocations (as above). A minimum threshold of least 100 potential allocations was required.

b. Ensured that, within each stratum, no clusters are NEVER or ALWAYS allocated together, as this would result in an invalid randomisation scheme.

c. Examined dengue incidence correlation over time within clusters frequently randomised together, and compared against correlation within all pairwise combinations. Verified that dengue incidence in clusters frequently randomised together was not highly correlated

7. A total of 244 allocations met the balancing criteria (thus 488 possible distinct randomisations of intervention allocation). A random subset of 100 balanced allocations was selected, as the sampling frame for the final public randomisation event.

8. From this list a single allocation pattern was randomly selected, using numbered balls, at a public participatory event of community and government leaders in Yogyakarta in January 2017.

## 

## 

## Sample size calculations

There are no published formulae to estimate sample size for the proposed study design, ie. a cluster randomised trial with a test-negative design (TND), where the intervention effect is estimated from outcome-based sampling of test-positive and test-negative patients and ascertainment of their exposure status. Randomisation provides a basis of inference in comparing intervention clusters with control clusters as, under the null hypothesis, there should be no difference with regard to the relative appearance of test-positives and negatives in clusters, on average, across the two arms. Thus we proposed as the primary analytical approach a comparison of the exposure odds among test-positive cases versus test-negative controls (for data aggregated across all clusters), with the null hypothesis that the odds of residence in a *Wolbachia*-treated cluster is the same among test-positive cases as test-negative controls. The resulting odds ratio thus provides an estimation of the intervention effect and, as demonstrated previously, provides an unbiased estimate of the relative risk providing that the key assumptions underlying the TND are upheld.

A secondary approach employs as a summary measure for a group-level analysis the proportion of test-positive cases amongst all tested participants in each cluster, with a comparison of the average of these proportions in the intervention arm versus the untreated arm forming the basis of hypothesis testing for intervention effect. The null hypothesis is that the average proportion of total enrolled participants that are cases is the same in treated and untreated study arms. The alternative hypothesis is that the proportion of enrolled participants that are cases is lower in the *Wolbachia* treated arm than the untreated arm.

***Sample size estimations prior to trial commencement***

Simulations were used to estimate the power to detect a range of intervention effect sizes using the two methods above, assuming 12 clusters per arm, a fixed total of 1000 true dengue cases enrolled and 4000 non-dengue controls. Empirical data on population, historical dengue incidence and incidence of other febrile illness in the 24 study clusters were used to define the baseline characteristics for the simulated scenarios. Nine overlapping two-year windows of dengue data (2003-2014) were sourced from the Yogyakarta surveillance system. Data for other febrile illness during 2014-2015 were sourced from individual Puskesmas using ICD10 codes for non-localising fever (fever of unknown origin R50; Typhus A75.9; and acute infection due to bacteria at an unspecified site A49). We randomly allocated half the clusters to receive the intervention; this random allocation was repeated one million times, and only those allocations were kept in which the balancing criteria specified in the constrained randomization methods were met (n=244 balanced allocations, and thus 488 possible distinct randomizations of intervention allocation). Dengue case numbers per cluster were either kept at baseline values (for the simulation at the null; ie RR=1) or reduced proportionately (for simulations of intervention effects of RR=0.6, 0.5, 0.4, 0.3). For each of these five ‘true’ effect sizes, applied to each of the 244 balanced allocations, the ‘observed’ effect size was calculated from the simulated data by the two methods outlined above; i) aggregated odds ratio for residence in a treated cluster among cases versus controls, and ii) t-test for comparison of the average cluster summary proportions (cases/cases+controls) between study arms. Statistical inference, from the t-test directly, or, for the odds ratios using permutation distribution approximations with standard errors adjusted to account appropriately for the clustered nature of the data, respectively, was used to calculate the proportion of constrained random allocations that yielded a significant result. This provided an estimate of Type I error at the null, and power away from the null (Table). Both of these approaches thus are using approximations to the exact permutation distribution.4 In practice, the appropriate reference distribution for inference will be based on the set of 244 potential balanced allocations.

These simulations estimated that approximately 1000 cases plus four times as many controls will be sufficient to detect a 50% reduction in dengue incidence with 80% power.

**Percent of random allocations that yield significant results on simulated data**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *Risk Ratio* | *T-test* | | *Odds Ratio test* | |
| *Constrained* | *Random* | *Constrained* | *Random* |
| 1 | 0.13 | 5 | 1 | 7 |
| 0.6 | 48 | 49 | 61 | 57 |
| 0.5 | 81 | 75 | 89 | 82 |
| 0.4 | 97 | 93 | 99 | 96 |
| 0.3 | 100 | 95 | 100 | 100 |

The results show that constrained randomization is somewhat conservative at the null but generally increases power moderately. The odds ratio test is more powerful than the t-test approach, and will thus be used as the primary analysis with the additional attraction of being standard for the traditional test-negative design.

***Sample size re-estimation after one year of participant recruitment***

A re-estimation of sample size requirements was conducted in January 2019 after one year of recruitment. The initial power calculation used 1000 dengue cases and 4000 non-dengue controls allocated to each cluster based on historical proportions of dengue cases and other febrile illnesses, assuming no variation in the proportion of cases by cluster. This method was found to overestimate power for small samples by not taking into account randomness in the sampling. The sample size re-estimation included power estimates for 200, 400, 600, 800 and 1000 dengue cases with 4 times as many controls. Cases and controls were allocated among clusters by sampling from multinomial distributions, which incorporated added randomness by allowing the proportion allocated to each cluster to vary across simulations. The re-estimation found that 400 dengue cases plus four times as many controls would be sufficient to detect a 50% reduction in dengue incidence with 80% power.

***Power re-estimation for* Wolbachia *contamination scenarios.***

Additional simulations were conducted in September 2019 to assess the potential impact on power if a number of untreated clusters were ‘lost’ to *Wolbachia* contamination. For the target minimum observed effect size of 50% (RR=0.5) and 400 enrolled dengue cases, contamination of 3 untreated clusters (assuming that contaminated clusters experience the full intervention effect for 1 out of the 3 years of trial recruitment) is expected to result in a ~7% loss of power, and contamination of 6 clusters to result in a ~14% loss of power.

## Calculation of *Wolbachia* Exposure Index for per-protocol analysis

Participants were asked about their mobility during the ten days prior to illness onset using a structured interview administered at enrolment. This records the duration of time spent at home, work or school, and other locations visited during daylight hours (5am – 9pm) in the ten-day period. The geographic coordinates of those locations were derived by geo-locating them on a digital map, with the assistance of the respondent. A weighted *‘Wolbachia* exposure index’ (WEI) was defined for each participant as WEI = ∑(tj\*wj), where *tj* is the participant’s time spent at location *j* as a proportion of their total observed time, and *wj* is the measured cluster-level *Wolbachia* frequency at location *j* in the calendar month of participant enrolment, resulting in a WEI value on a continuous scale from 0 to 1. Aggregate *Wolbachia* prevalence for each cluster was calculated each month from all *Ae. aegypti* trapped in that cluster. For any calendar month where mosquito collection was not done, the average of the cluster-level *Wolbachia* prevalence in the one previous and one subsequent month was used. For visited locations within the quasi-experimental study area, which comprises seven urban villages located adjacent to the northwestern border of the trial study site,5 the measured village-level *Wolbachia* prevalence from the screening event closest in time to the participant’s enrolment was used. Visited locations outside of both the AWED study area and the quasi-experimental study area were assumed to have a *Wolbachia* prevalence of zero. The process of calculating WEI was conducted blinded to participants’ case/control status, by partitioning the travel history data from the laboratory diagnostic data, to remove any possibility of observer bias.

An additional per-protocol analysis calculated WEI using only the cluster-level *Wolbachia* prevalence in the participant’s cluster of residence (in the calendar month of participant enrolment), ignoring the participant’s recent travel history. This recognises that dengue exposure risk may be higher at home versus other locations, rather than assuming an even distribution of exposure risk across daytime hours and locations visited.

Cases and controls were classified by strata of their WEI: 0-<0.2; 0.2-<0.4; 0.4-<0.6; 0.6-<0.8; and 0.8-1. This acknowledges that the WEI is not a highly precise measure, and serves to reduce error in exposure classification. The ITT methods described were extended to allow for this individual level covariate using a regression approach,6 adjusted for time. A mixed effects logistic regression model was fitted, incorporating a random effect for cluster membership. Such models yield an estimate, and associated confidence interval, for the relative risk. Efficacy was then calculated as 100\*(1-RR). The WEI strata was first included as an unordered covariate to calculate stratum-specific IRRs (relative to the baseline 0-<0.2 stratum). The WEI strata was additionally included as an ordinal covariate and the slope of the WEI variable was tested for a difference from zero.

## *w*Mel-infected *Aedes aegypti* mosquito production

An existing colony of local *Ae. aegypti* containing the *w*Mel *Wolbachia* strain, created for the 2016-2017 quasi-experimental study releases in Yogyakarta City,5 was used as the founder colony for the releases described here. It was backcrossed for three generations with wild-type males collected from the study intervention area to generate the release colony, which was then maintained as described previously.5 The insecticide resistance profile of the *w*Mel*-*infected *Ae. aegypti* release material was matched to the local wild-type population as described previously.5

## *w*Mel-infected *Aedes aegypti* deployments

*Wolbachia*-carrying mosquitoes were released as eggs using mosquito release containers (MRCs). These were 2-litre plastic buckets each containing one oviposition strip with 100–150 eggs, Tetra Pleco Wafers fish food (Tetra GmbH, Germany), and 1 litre of water. MRCs were covered and placed outside houses, protected from direct sun and rain. Holes drilled near the top of the bucket walls allowed adult mosquitoes to escape. Releases occurred between March and December 2017, with 9–14 rounds of releases in each intervention cluster. Releases stopped in each cluster when the prevalence of *Wolbachia* in field-caught mosquitoes was >60% for three consecutive weeks releases. MRCs were reset every two weeks. An MRC was placed in 1–2 randomly selected locations within each 50x50 m grid square across the intervention area. Permission was obtained from property owners to place MRCs on private property.

## Entomological monitoring

Prevalence of *Wolbachia* in the local *Ae. aegypti* population was monitored by weekly collection of adult mosquitoes via a network of 348 BG Sentinel traps (Biogents, Germany). Written consent was obtained from heads of households hosting BG traps The median (range) trap density was 16.0 (13.2–18.1) BG/km2 in the intervention clusters and 14.9 (10.3–16.8) BG/km2 in the untreated clusters (Table S1). Mosquitoes were demobilised at -20°C for ≥1 hour, then identified by morphological features. The number of mosquitoes caught in each BG trap was recorded by species, sex, and in total. *Ae. aegypti* were stored at -20°C in 80% ethanol until testing for *w*Mel infection.

Field-caught *Ae. aegypti* were screened for *w*Mel *Wolbachia* by qualitative PCR Taqman assay on a Roche LightCycler 480. The qPCR conditions consisted of a denaturation step at 95°C for 5 minutes followed by 45 cycles of PCR (denaturation at 95 °C for 10 seconds, annealing at 60 °C for 15 seconds, and extension at 72 °C for 1 second with the single acquisition) followed by a cooling down step at 40°C for 10 seconds. Specific primers targeting the gene encoding *Ae aegypti Rps17* and *wMel WD0513* were used as previously described,7 but with replacement of the Cy5-BHQ3 fluorophore-quencher pair in the *w*Mel probe with the fluorophore-quencher LC640-IowaBlack (Integrated DNA technologies).8 Testing was at weekly intervals when *Wolbachia* prevalence was <80% and 4-weekly intervals when establishment was achieved (≥80% cluster level prevalence for two consecutive testing weeks).

## Arbovirus diagnostic investigations

For nucleic acid testing, viral RNA was extracted from participant’s plasma samples using a High Pure Viral Nucleic Acid Kit (Roche) according to the manufacturer’s instructions. An internally controlled, multiplex real-time RT-PCR assay was used to detect viral RNA from Zika virus (ZIKV), DENV or Chikungunya virus (CHIKV). The internal control was equine arteritis virus (EAV), a standard amount of which was spiked into plasma samples prior to RNA extraction. The primers and probes used for detection of ZIKV,9 CHIKV,10 and DENV11 were as described previously. The primers and probes for DENV targeted the 3’ UTR region of the four DENV serotypes. Primers and probes for detection of EAV were as described previously.12 PCR was performed on a Roche Lightcycler II thermocycler. PCR conditions available from the authors upon request. PCR was performed in batches of test samples and every PCR plate carried external positive controls (supernatants from cultures of DENV-1-4, CHIK and ZIKV) and negative controls. The DENV serotype specific PCR was performed using a commercial assay (Dengue Simplexa kit (DiaSorin Molecular) on the Liaison MDX thermocycler according to the manufacturer’s instructions. The Bio-rad Platelia Dengue NS1 Ag test (BioRad Cat. No. 72830), a one step sandwich format microplate enzyme immunoassay for the detection of DENV NS1 antigen, was performed according to the manufacturer’s instructions. The Panbio Dengue IgM Capture Elisa (Abbot Cat. No. 01PE20) and the Panbio Dengue IgG Capture Elisa (Abbot Cat. No. 01PE10) were used to detect IgM and IgG antibodies to dengue antigen in plasma and were used according to the manufacturer’s instructions .

Supplementary Tables

## **Table S1: Baseline characteristics of clusters (sociodemographic, historical disease incidence, area and mosquito trapping)**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Population (2015), N | Population aged <15 years, % | Completed high school, % | Notified DHF incidence per 10,000 (2013-15) | Other febrile illness incidence per 10,000 (2014-15) | Total cluster area, km2 | Residential (release) area,  km2 | BG trap density per km2 of residential (release) area |
| *Intervention* | *153,403* | *22* | *74* | *19.7* | *199.3* | *12.24* | *11.04* | *15.7* |
| Cluster 1 | 12,747 | 21 | 56 | 19.3 | 210.0 | 0.94 | 0.88 | 15.9 |
| Cluster 2 | 10,179 | 21 | 78 | 19.3 | 91.2 | 0.69 | 0.69 | 14.5 |
| Cluster 6 | 22,085 | 20 | 78 | 15.3 | 175.3 | 0.94 | 0.93 | 15.1 |
| Cluster 7 | 19,587 | 21 | 79 | 11.9 | 185.8 | 1.11 | 0.98 | 16.3 |
| Cluster 9 | 13,132 | 21 | 83 | 17.2 | 205.1 | 1.14 | 1.06 | 13.2 |
| Cluster 10 | 8,127 | 22 | 60 | 31.4 | 268.2 | 1.17 | 1.00 | 16.0 |
| Cluster 12 | 18,947 | 20 | 85 | 15.8 | 113.4 | 1.13 | 1.08 | 16.7 |
| Cluster 14 | 15,101 | 21 | 83 | 18.4 | 196.4 | 1.27 | 1.14 | 14.9 |
| Cluster 16 | 10,474 | 22 | 79 | 22.6 | 333.3 | 0.89 | 0.86 | 16.3 |
| Cluster 19 | 9,726 | 22 | 84 | 20.9 | 75.5 | 0.83 | 0.83 | 15.7 |
| Cluster 21 | 8,348 | 23 | 55 | 27.5 | 385.0 | 1.04 | 0.87 | 16.1 |
| Cluster 24 | 4,950 | 24 | 61 | 17.1 | 153.0 | 1.09 | 0.72 | 18.1 |
| *Untreated* | *158,279* | *22* | *77* | *19.0* | *215.6* | *13.38* | *12.13* | *14.3* |
| Cluster 3 | 17,702 | 21 | 78 | 16.3 | 372.8 | 1.02 | 0.92 | 16.3 |
| Cluster 4 | 6,471 | 20 | 61 | 13.2 | 228.5 | 0.93 | 0.73 | 15.1 |
| Cluster 5 | 12,936 | 21 | 81 | 22.7 | 112.5 | 1.1 | 1.06 | 12.3 |
| Cluster 8 | 16,026 | 21 | 76 | 16.9 | 241.7 | 1.12 | 1.08 | 14.8 |
| Cluster 11 | 14,983 | 21 | 79 | 19.6 | 120.9 | 0.86 | 0.8 | 12.5 |
| Cluster 13 | 28,541 | 21 | 80 | 13.5 | 158.0 | 1.64 | 1.54 | 15.6 |
| Cluster 15 | 8,976 | 22 | 82 | 17.9 | 182.9 | 0.95 | 0.87 | 10.3 |
| Cluster 17 | 4,031 | 23 | 90 | 19.5 | 390.9 | 0.73 | 0.63 | 15.9 |
| Cluster 18 | 21,185 | 21 | 76 | 17.3 | 113.3 | 1.6 | 1.43 | 16.8 |
| Cluster 20 | 10,780 | 22 | 91 | 28.0 | 100.7 | 1.03 | 1.01 | 15.8 |
| Cluster 22 | 9,971 | 23 | 69 | 23.9 | 416.3 | 1.33 | 1.16 | 12.9 |
| Cluster 23 | 6,677 | 24 | 63 | 19.0 | 148.5 | 1.07 | 0.9 | 13.3 |

## **Table S2: Baseline characteristics of trial participants**

|  |  |  |
| --- | --- | --- |
|  | Age, median (IQR) | Female sex, n (%) |
| Total enrolled participants | 11.99 (6.99, 21.11) | 3074 (48.72) |
| **By treatment allocation** |  |  |
| Intervention arm | 12.02 (6.90, 21.08) | 1431 (49.24) |
| Untreated arm | 11.97 (7.02, 21.16) | 1643 (48.27) |
| **By diagnostic outcome** |  |  |
| VCD cases | 11.03 (7.16, 18.14) | 189 (49.09) |
| VCC cases | 28.52 (16.92, 40.71) | 1 (25.00) |
| Test-negative controls | 12.08 (6.97, 21.37) | 2884 (48.71) |
| Unclassifiable results | 12.44 (10.76, 16.94) | 3 (27.27) |
| **By inclusion in analysis dataset** |  |  |
| Included in analysis | 11.99 (6.99, 21.11) | 3074 (48.72) |
| Excluded from analysis | 10.46 (6.08, 19.42) | 899 (49.02) |
| **By follow-up status** |  |  |
| Not hospitalized | 12.10 (7.06, 21.16) | 2707 (48.60) |
| Hospitalised | 11.87 (6.92, 20.46) | 138 (46.78) |
| Died | - | - |
| Lost to follow-up | 10.80 (6.40, 20.80) | 229 (51.46) |

Age is in years. IQR: interquartile range, VCD: virologically-confirmed dengue, VCC: virologically-confirmed chikungunya

## **Table S3: Hospitalisation outcome by treatment arm for participants in the analysis dataset**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Intervention arm** | | **Untreated arm** | | OR (95% CI) |
|  | N | % | N | % |
| **All participants** | | | | |  |
| Not hospitalized | 2602 | 89.5 | 2968 | 87.2 | Ref |
| Hospitalised | 81 | 2.8 | 214 | 6.3 | 0.43 (0.32, 0.58) |
| Lost to follow-up | 223 | 7.7 | 222 | 6.5 | - |
|  | | | | |  |

## **Table S4: Hospitalisation outcome by treatment arm for VCD cases**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Intervention arm** | | **Untreated arm** | | OR (95% CI) |
|  | N | % | N | % |
| **VCD** | | | | |  |
| Not hospitalised | 52 | 77.6 | 197 | 61.9 | Ref |
| Hospitalised | 13 | 19.4 | 102 | 32.1 | 0.50 (0.22, 1.14) |
| Lost to follow-up | 2 | 3.0 | 19 | 6.0 | - |

# Supplementary Figures

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## **Figure S1: Diagnostic algorithm and classification of participants**

Among 8,144 total participants enrolled, 7,539 were enrolled after *Wolbachia* establishment (8 Jan 2018) and with a blood sample successfully collected, excluding 186 test-negative controls (95 in the intervention arm and 91 in the untreated arm) who were enrolled in a month with no test-positive dengue cases (September 2018). Four virologically-confirmed chikungunya (VCC) and 318 virologically-confirmed dengue (VCD) cases were identified by multiplex RT-PCR. Another 67 VCD cases were negative in RT-PCR but positive in dengue NS1 antigen enzyme-linked ELISA. 1,229 participants were classified as neither test-positive cases nor test-negative controls due to a positive or equivocal result for dengue IgM and/or IgG antibody, or due to insufficient sample volume for complete diagnostics or an inconclusive diagnostic result. 5921 participants with negative results in all diagnostic tests were classified as test-negative controls.

DENV: dengue virus; CHIK: chikungunya virus; Zika: Zika virus; PCR: polymerase chain reaction; VCC: virologically-confirmed chikungunya; VCD: virologically-confirmed dengue; NS1: dengue non-structural protein 1; ELISA: enzyme-linked immunosorbent assay; IgM: immunoglobulin M; IgG: immunoglobulin G.

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## **Figure S2: *w*Mel introgression into local *Aedes aegypti* mosquito populations.**

Lines show the percentage of *Ae. aegypti* collected from intervention clusters (A) and untreated clusters (B) that were *w*Melinfected, each month from the start of deployments (March 2017) to the end of participant enrolment (March 2020). Shaded area indicates the period from the first release in the first cluster (March 2017) to the last release in the last cluster (December 2017). There were between 9 and 14 fortnightly release rounds per cluster.

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## **Figure S3: Participant enrolment and hospitalisations by primary care clinic and treatment arm.**

Bars show the number of participants in the analysis dataset by clinic of enrolment (clinics numbered 1 - 18). Each clinic enrolled participants from both untreated (dark blue; yellow) and *Wolbachia*-treated (light blue; orange) clusters. The proportion of participants who were hospitalised varied between clinics, and was higher among participants from untreated (yellow) than *Wolbachia*-treated (orange) clusters.

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## **Figure S4: Intervention efficacy over time.**

Efficacy is calculated as 100\*(1-aggregate odds ratio) among participants enrolled within the first 12 months after *w*Mel establishment, within the first 24 months, and within the full 27 month trial period.

## 

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