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Efficacy of Wolbachia-Infected Mosquito Deployments for the Control of Dengue

A. Utarini, C. Indriani, R.A. Ahmad, W. Tantowijoyo, E. Arguni, M.R. Ansari, E. Supriyati, D.S. Wardana, Y. Meitika, I. Ernesia, I. Nurhayati, E. Prabowo, B. Andari, B.R. Green, L. Hodgson, Z. Cutcher, E. Rancès, P.A. Ryan, S.L. O'Neill, S.M. Dufault, S.K. Tanamas, N.P. Jewell, K.L. Anders, and C.P. Simmons, for the AWED Study Group*

ABSTRACT

BACKGROUND

Aedes aegypti mosquitoes infected with the *w*Mel strain of *Wolbachia pipientis* are less susceptible than wild-type *A. aegypti* to dengue virus infection.

METHODS

We conducted a cluster-randomized trial involving releases of *w*Mel-infected *A. aegypti* mosquitoes for the control of dengue in Yogyakarta, Indonesia. We randomly assigned 12 geographic clusters to receive deployments of *w*Mel-infected *A. aegypti* (intervention clusters) and 12 clusters to receive no deployments (control clusters). All clusters practiced local mosquito-control measures as usual. A test-negative design was used to assess the efficacy of the intervention. Patients with acute undifferentiated fever who presented to local primary care clinics and were 3 to 45 years of age were recruited. Laboratory testing was used to identify participants who had virologically confirmed dengue (VCD) and those who were test-negative controls. The primary end point was symptomatic VCD of any severity caused by any dengue virus serotype.

RESULTS

After successful introgression of *w*Mel into the intervention clusters, 8144 participants were enrolled; 3721 lived in intervention clusters, and 4423 lived in control clusters. In the intention-to-treat analysis, VCD occurred in 67 of 2905 participants (2.3%) in the intervention clusters and in 318 of 3401 (9.4%) in the control clusters (aggregate odds ratio for VCD, 0.23; 95% confidence interval [CI], 0.15 to 0.35; P=0.004). The protective efficacy of the intervention was 77.1% (95% CI, 65.3 to 84.9) and was similar against the four dengue virus serotypes. The incidence of hospitalization for VCD was lower among participants who lived in intervention clusters (13 of 2905 participants [0.4%]) than among those who lived in control clusters (102 of 3401 [3.0%]) (protective efficacy, 86.2%; 95% CI, 66.2 to 94.3).

CONCLUSIONS

Introgression of *w*Mel into *A. aegypti* populations was effective in reducing the incidence of symptomatic dengue and resulted in fewer hospitalizations for dengue among the participants. (Funded by the Tahija Foundation and others; AWED ClinicalTrials.gov number, NCT03055585; Indonesia Registry number, INA-A7OB6TW.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Address reprint requests to Dr. Simmons at Monash University, 12 Innovation Walk, Clayton, VIC 3800, Australia, or at cameron.simmons@worldmosquito.org.

*A list of investigators in the AWED Study Group is available in the Supplementary Appendix, available at NEJM.org.

Drs. Utarini and Indriani and Drs. Anders and Simmons contributed equally to this article.

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Dengue is a mosquito-borne, acute viral syndrome caused by any of the four serotypes of dengue virus (DENV).[1] In 2019, the World Health Organization designated dengue as one of the top 10 global health threats.[2] An estimated 50 million to 100 million symptomatic cases occur globally each year.[3,4] Dengue epidemics occur annually or at multiyear intervals, and the surge in case numbers places considerable pressure on health services.[5]

Aedes aegypti mosquitoes are the primary vectors of dengue. Efforts to control *A. aegypti* populations with the use of insecticides or environmental management methods have not been effective in controlling dengue as a public health problem in most countries.[6] Few randomized trials of *A. aegypti*–control methods have been conducted, and none have used the end point of virologically confirmed dengue (VCD).[7] A trial of community mobilization to reduce the *A. aegypti* population in Nicaragua and Mexico showed modest efficacy (29.5%) against dengue seroconversion in the saliva of residents.[8]

Wolbachia pipientis — a common, maternally inherited, obligate intracellular type of bacteria — infects many species of insects but does not occur naturally in *A. aegypti*.[9] Stable transinfection of *A. aegypti* with some strains of wolbachia confers resistance to disseminated infection by DENV and other arboviruses.[10-13] Thus, the introgression of "virus-blocking" strains of wolbachia into field populations of *A. aegypti* is an emerging dengue-control method.[14-17] The approach involves regular releases of wolbachia-infected mosquitoes into a wild mosquito population over a period of several months. Wolbachia facilitates its own population introgression by manipulating reproductive outcomes between wild-type and wolbachia-infected mosquitoes: the only viable mating outcomes are those in which the progeny are infected with wolbachia.[13]

Here, we report the results of a cluster-randomized trial that assessed the efficacy of deployments of *A. aegypti* mosquitoes infected with the *w*Mel strain of wolbachia in reducing the incidence of VCD in Yogyakarta, Indonesia. The trial builds on earlier entomologic and epidemiologic pilot studies in this geographic setting.[14,18,19]

METHODS

TRIAL DESIGN AND OVERSIGHT

The Applying Wolbachia to Eliminate Dengue (AWED) trial was supported by the Tahija Foundation and was hosted by Universitas Gadjah Mada, Indonesia. The protocol was published previously[20,21] and is available with the full text of this article at NEJM.org.

Community approval for *w*Mel releases was obtained from the leaders of 37 urban villages after a campaign of community engagement and mass communication. Written informed consent for participation in the clinical component of the trial was obtained from all the participants or from a guardian if the participant was a minor. In addition, participants 13 to 17 years of age gave written informed assent. The trial was conducted in accordance with the International Council for Harmonisation guidelines for Good Clinical Practice and was approved by the human research ethics committees at Universitas Gadjah Mada and Monash University. The trial data were analyzed by the independent trial statisticians. The funders had no role in the analysis of the data, in the preparation or approval of the manuscript, or in the decision to submit the manuscript for publication.

RANDOMIZATION

The baseline characteristics of the trial clusters are described in Table S1 in the Supplementary Appendix, available at NEJM.org. In brief, the trial site was a contiguous urban area of 26 km^2 with a population of approximately 311,700. The trial site was subdivided into 24 clusters, each approximately 1 km^2 in size, and where possible, having geographic borders that would slow the dispersal of mosquitoes between clusters. Of the 24 clusters, 12 were randomly assigned to receive deployments of open-label wolbachia-infected mosquitoes (intervention clusters), and 12 clusters were assigned to receive no deployments (control clusters, termed "untreated clusters" in the protocol) (Fig. 1 and Fig. S1). In intervention clusters, most community members were unaware of the cluster assignment because release containers were placed discretely in a minority of residential properties for a limited time. No placebo was used in the control clusters. Constrained randomization was used to prevent a chance im-

balance in the baseline characteristics or in the spatial distribution of the intervention and control clusters (see the Supplementary Appendix).

WOLBACHIA DEPLOYMENT AND ENTOMOLOGIC MONITORING

A. aegypti infected with the *w*Mel strain of wolbachia were sourced from an outcrossed colony, as described previously.[14] In 2013, we found that this *w*Mel-infected Indonesian mosquito line was less likely than wild-type *A. aegypti* to transmit DENV (Figs. S2 and S3). Mosquito eggs were placed in intervention clusters from March through December 2017. Each cluster received between 9 and 14 rounds of deployments (Table S2). Details regarding mosquito releases and monitoring of *w*Mel in the mosquito populations are provided in the Supplementary Appendix. Monitoring was performed with the use of a network of 348 adult mosquito traps (BG-Sentinel, BioGents).

PARTICIPANT ENROLLMENT

Participants were recruited from a network of 18 government-run primary care clinics in Yogyakarta and the adjacent Bantul District. Eligible participants were 3 to 45 years of age, had fever (either reported by the participant or measured in the clinic and defined as a forehead or axillary temperature of >37.5°C) with onset 1 to 4 days before presentation, and had resided in the trial area every night for the 10 days preceding the onset of illness. Participants were not eligible if they had localizing symptoms suggestive of a specific diagnosis other than an arboviral infection (e.g., severe diarrhea, otitis, and pneumonia) or were enrolled in the trial within the previous 4 weeks.

PROCEDURES

Participants provided demographic information, a geolocated residential address, and a detailed travel history for the 3 to 10 days before the onset of illness. A 3-ml venous blood sample was obtained for arbovirus diagnostic testing. No other diagnostic investigations were performed. Participants were contacted 14 to 21 days after enrollment to obtain vital status and to determine whether they had been hospitalized since enrollment. No information on the clinical severity of VCD cases was collected, and no information on clinical diagnoses or severity of non-VCD cases was acquired.

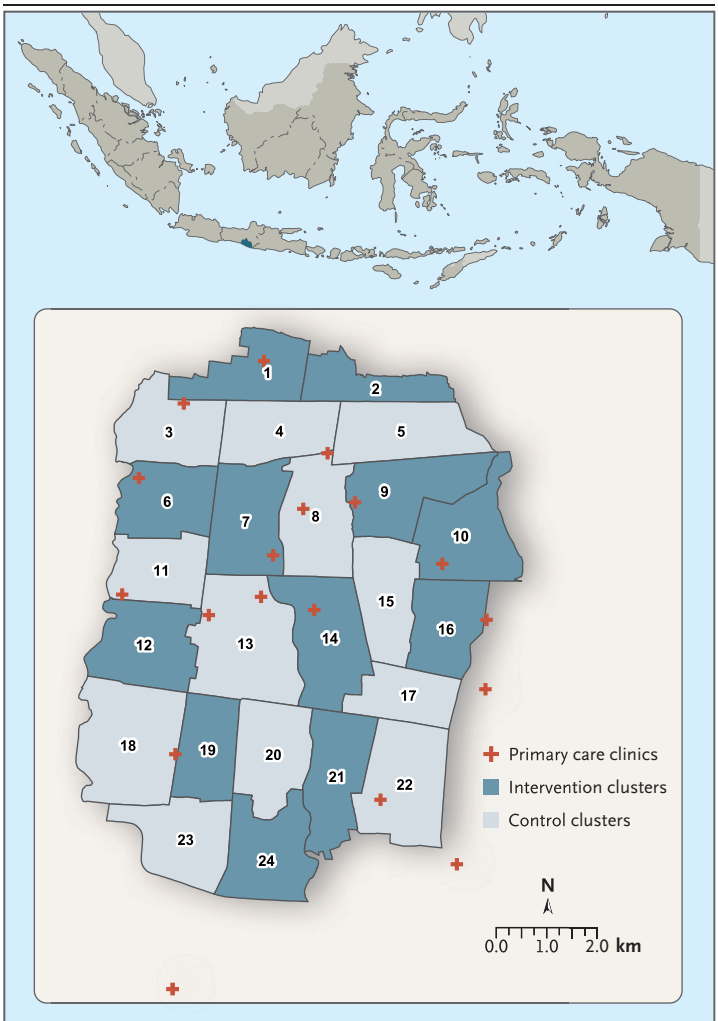

Figure 1. Map of the Trial Location and Clusters.

A map of Indonesia is shown at the top, with the location of Yogyakarta Province shaded in dark blue. The enlarged area at the bottom shows the trial area in Yogyakarta City, which includes a small area of neighboring Bantul District (clusters 23 and 24). Intervention clusters (which received deployments of *Aedes aegypti* mosquitoes infected with the *w*Mel strain of *Wolbachia pipientis*) are shaded in dark blue, and control clusters (which received no deployments) are shaded in light blue. Red crosses indicate the locations of the primary care clinics where enrollment was conducted.

DIAGNOSTIC INVESTIGATIONS AND CLASSIFICATIONS

Trial participants were classified as having VCD if the plasma sample obtained at enrollment was positive for DENV in a multiplex (DENV, chikungunya virus, and Zika virus) reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay

or in an enzyme-linked immunosorbent assay (ELISA) for DENV nonstructural protein 1 (NS1) antigen (Platelia dengue NS1 [Bio-Rad]). Participants were classified as test-negative controls if the plasma sample obtained at enrollment was negative by RT-PCR for DENV, chikungunya virus, and Zika virus and also negative by ELISA capture assay for DENV NS1 antigen and dengue IgM and IgG. The diagnostic algorithm is provided in Figure S4. The DENV serotype was determined with the use of a separate RT-PCR assay (Simplexa) by an independent laboratory at the Eijkman Institute, Jakarta. Details of the diagnostic methods are provided in the Supplementary Appendix.

PRIMARY, SECONDARY, AND SAFETY END POINTS

The primary end point was symptomatic VCD of any severity caused by any DENV serotype. The secondary end points reported here are symptomatic VCD caused by each of the four DENV serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) and symptomatic, virologically confirmed chikungunya and Zika virus infections. Safety end points included hospitalization or death within 21 days after enrollment.

STATISTICAL ANALYSIS

The sample size that was needed to show a 50% lower incidence of dengue in the intervention clusters than in the control clusters, which was considered the minimum effect size for public health value, evolved over time. The full description of the sample-size calculations is provided in the Supplementary Appendix. In brief, we determined that 400 cases of VCD and 1600 test-negative controls would be needed to give the trial 80% power to detect a 50% lower incidence of VCD among participants in intervention clusters than among those in control clusters. The emergence of severe acute respiratory syndrome coronavirus 2 in Yogyakarta in March 2020 prevented the continued recruitment of participants in clinics, and enrollment ended on March 18, 2020. On May 5, 2020, the trial steering committee endorsed the recommendation of the trial investigators to terminate the trial, at which time 385 participants with VCD had been enrolled.

The statistical analysis plan was published previously[22] and is available with the protocol. The trial population used in the efficacy analysis included all enrolled participants with VCD and all test-negative controls, excluding participants who had been enrolled before the establishment of wolbachia throughout the intervention clusters (i.e., 1 month after the last release in the last cluster) and excluding test-negative controls who had been enrolled in a calendar month in which no dengue cases were observed among participants. The primary intention-to-treat analysis considered wolbachia exposure as a binary classification on the basis of residence in an intervention cluster or a control cluster. Residence was defined as the primary place of residence during the 10 days before illness onset. The intervention effect was estimated from an aggregate odds ratio comparing the exposure odds (residence in an intervention cluster) among participants with VCD with that among test-negative controls, with the use of the constrained permutation distribution as the foundation for inference. The null hypothesis was that the odds of residence in an intervention cluster would be the same among participants with VCD as that among test-negative controls. The efficacy of the intervention was calculated as $100 \times (1 - \text{aggregate odds ratio})$. A prespecified exploratory analysis evaluated the efficacy of the intervention in preventing hospitalization with VCD.

An additional prespecified cluster-level intention-to-treat analysis was performed by calculating the proportion of participants with VCD in each cluster. The difference in the average proportions of participants with VCD between the intervention clusters and the control clusters was used to test the null hypothesis of no intervention effect (a t-test statistic) and to derive an estimate of the cluster-specific relative risk, with inference based on the constrained permutation distribution.[23,24] The same methods used in the intention-to-treat analyses described above were used in the analyses for the secondary end point of serotype-specific efficacy. The analyses included participants with VCD caused by one of the four DENV serotypes and used the same control population as that used in the primary analysis. There was no prespecified plan to control for multiple testing in the analysis of secondary end points.

Per-protocol analyses considered exposure contamination by assigning a wolbachia exposure index to each participant on the basis of the *w*Mel prevalence in their cluster of residence only, or by combining this frequency with the partici-

pant's recent travel history. A generalized linear model was fitted, with balanced bootstrap resampling based on cluster residence, to estimate the relative risk of VCD and associated confidence interval in each quintile of wolbachia exposure, relative to the risk of VCD in participants in the bottom quintile of wolbachia exposure. Details are provided in the Supplementary Appendix.

RESULTS

ESTABLISHMENT OF *w*MEL IN *A. AEGYPTI* POPULATIONS

A map of Indonesia showing the trial clusters is provided in Figure 1. *w*Mel was durably established in the *A. aegypti* populations in each of the 12 intervention clusters (Fig. 2). The monthly median cluster-level *w*Mel prevalence was 95.8% (interquartile range, 91.5 to 97.8) during the 27 months of clinical surveillance.

TRIAL PARTICIPANTS

A total of 53,924 patients at 18 primary care clinics were screened for trial eligibility from January 8, 2018, to March 18, 2020, and 8144 patients were enrolled. Of these, 6306 participants met the requirements for inclusion in the analyses: 2905 participants who resided in *w*Mel intervention clusters and 3401 who resided in control clusters (Fig. 3). Four participants with virologically confirmed chikungunya (one in an intervention cluster and three in control clusters) were excluded from the analyses. No cases of Zika virus infection were detected. The median age of the participants was 11.6 years (interquartile range, 6.7 to 20.9), and 48.8% of participants were female (Table S3). A total of 295 of the 6306 participants (4.7%) who were included in the analyses were hospitalized during the time between enrollment and follow-up (14 to 21 days later). The incidence of hospitalization for any cause was lower among participants who resided in intervention clusters (81 of 2905 [2.8%]) than among those who resided in control clusters (214 of 3401 [6.3%]) (odds ratio, 0.43; 95% confidence interval [CI], 0.32 to 0.58) (Table S4). This lower incidence was evident across all clinics (Fig. S5). No participants died between enrollment and follow-up. Of the 6306 participants, 385 (6.1%) had VCD, and 5921 (93.9%) were classified as test-negative controls. Age and sex were well matched in these two populations (Table S3).

INTENTION-TO-TREAT ANALYSES

The incidence of VCD was significantly lower among participants who lived in the intervention clusters (67 cases among 2905 participants [2.3%]) than among participants who lived in the control clusters (318 cases among 3401 participants [9.4%]) (odds ratio, 0.23; 95% CI, 0.15 to 0.35; P=0.004). This represented a protective efficacy of 77.1% (95% CI, 65.3 to 84.9) (Fig. 4). The intervention effect was evident by 12 months after *w*Mel establishment (Fig. S6). The protective efficacy was similar against all serotypes but was highest against DENV-2 (83.8%; 95% CI, 72.1 to 90.6) and lowest against DENV-1 (71.0%; 95% CI, 18.2 to 89.7) (Fig. 4). The lower boundary of the 95% confidence interval for protective efficacy against all four serotypes was greater than 0. There were 13 hospitalizations for VCD among 2905 participants (0.4%) in intervention clusters and 102 hospitalizations among 3401 participants (3.0%) in control clusters (protective efficacy, 86.2%; 95% CI, 66.2 to 94.3) (Fig. 4 and Table S5).

An additional prespecified intention-to-treat analysis assessed the number of participants with VCD as a proportion of all participants in each cluster. In all but one of the intervention clusters, the proportion of VCD cases was lower than that in control clusters, yielding a relative risk of 0.23 (95% CI, 0.06 to 0.47) (Fig. 5). Figure S7 shows the proportion of participants with VCD and the *w*Mel prevalence over time in individual clusters. When stratified according to serotype, the relative risk of VCD caused by the two most prevalent serotypes (DENV-2 and DENV-4) was significantly lower in the intervention clusters than in the control clusters (Fig. S8).

PER-PROTOCOL ANALYSES

In per-protocol analyses, a wolbachia exposure index was assigned to each participant on the basis of *w*Mel frequency in their cluster of residence only or by accounting also for *w*Mel frequencies and time spent in other locations. Protective efficacy against VCD increased with incremental increases in participants' wolbachia exposure index when taking into consideration the cluster of residence and recent travel history (Fig. S9A). When only the *w*Mel frequency in the cluster of residence was considered, a threshold effect was observed in that only cluster-level *w*Mel frequencies higher than 80% were protective (Fig. S9B).

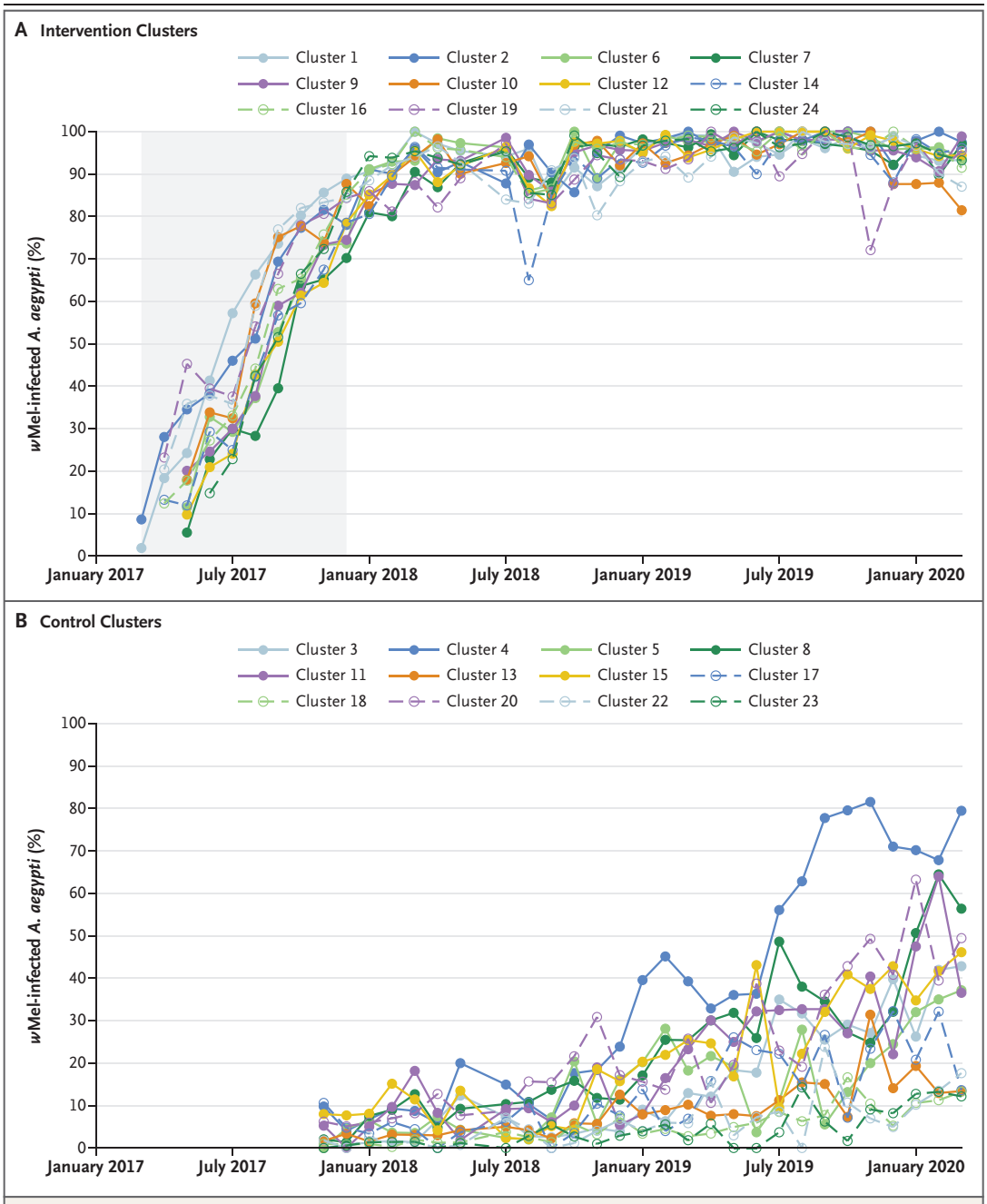

Figure 2. Introgression of *w*Mel into Local *A. aegypti* Mosquito Populations.

The lines in each panel indicate the percentage of *A. aegypti* infected with *w*Mel collected from traps in intervention clusters (Panel A) and control clusters (Panel B) each month from the start of deployments (March 2017) to the end of participant enrollment (March 2020). The shaded area in Panel A indicates the period from the first release in the first intervention cluster (March 2017) to the last release in the last intervention cluster (December 2017). There were 9 to 14 release rounds, with each round lasting 2 weeks, in each intervention cluster.

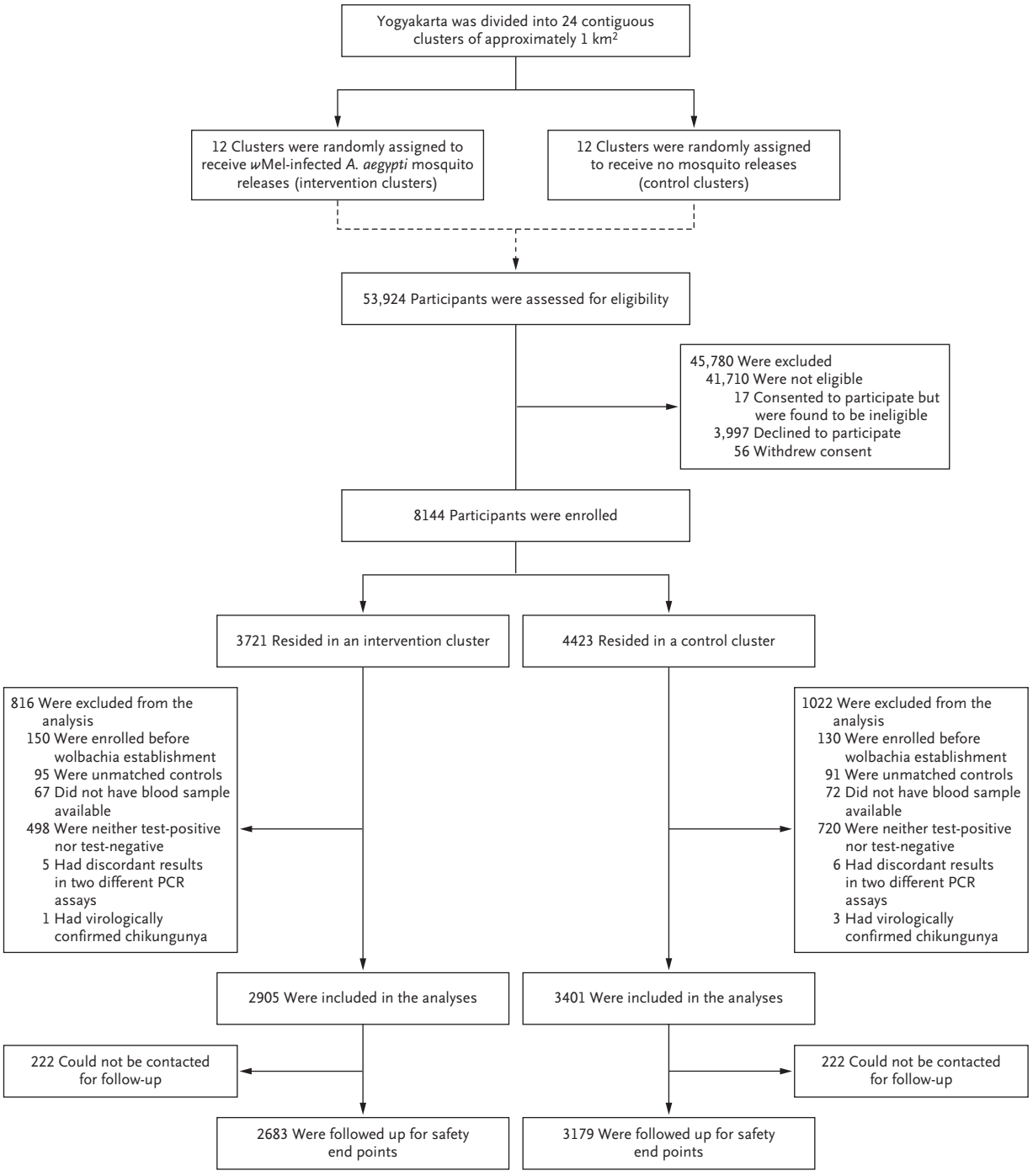

Figure 3. Cluster Randomization, Enrollment of Participants, and Inclusion in Analysis Data Set.

The most common reasons for exclusion from the analysis data set were enrollment before the prespecified time point of wolbachia establishment (i.e., January 8, 2018), enrollment in a calendar month in which no cases of virologically confirmed dengue were observed (i.e., unmatched controls in September 2018), or positive or equivocal dengue IgM or IgG serologic results at enrollment that precluded classification as a test-negative control. Participants were contacted 14 to 21 days after enrollment to determine vital status and whether they had been hospitalized for dengue since enrollment (safety end points). PCR denotes polymerase chain reaction.

Subgroup	Intervention Clusters	Control Clusters	Protective Efficacy (95% CI)
	no. of participants/total no. (%)		
All cases of VCD	67/2905 (2.3)	318/3401 (9.4)	77.1 (65.3–84.9)
VCD cases according to DENV serotype			
DENV-1	12/2905 (0.4)	45/3401 (1.3)	71.0 (18.2–89.7)
DENV-2	20/2905 (0.7)	134/3401 (3.9)	83.8 (72.1–90.6)
DENV-3	5/2905 (0.2)	22/3401 (0.6)	75.3 (4.9–93.6)
DENV-4	17/2905 (0.6)	70/3401 (2.1)	73.6 (57.9–83.5)
Unknown	14/2905 (0.5)	53/3401 (1.6)	71.3 (50.8–83.3)
Participants hospitalized with VCD	13/2905 (0.4)	102/3401 (3.0)	86.2 (66.2–94.3)

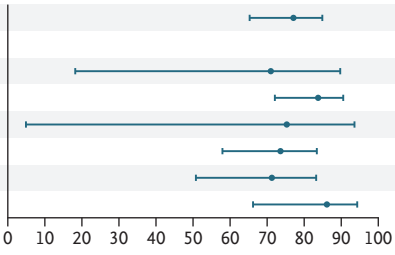

Figure 4. Efficacy in the Intention-to-Treat Analysis.

The protective efficacy is expressed as 100×(1−aggregate odds ratio). Cases of virologically confirmed dengue (VCD) for which the serotype was unknown were those that were negative by reverse-transcriptase PCR assay for dengue virus (DENV) and positive for DENV nonstructural protein 1 antigen. Seven participants (one in an intervention cluster and six in control clusters) had two DENV serotypes detected during the same febrile episode (four participants with DENV-1 and DENV-2, two participants with DENV-1 and DENV-4, and one participant with DENV-2 and DENV-4).

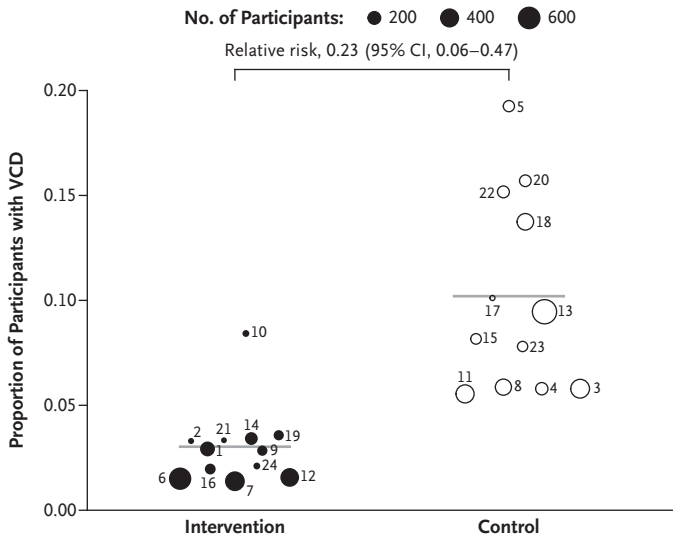

Figure 5. Cluster-Level Proportions of Participants with VCD.

Shown are the number of participants with VCD as a proportion of all participants in each intervention cluster (solid circles) and in the control clusters (open circles). The circle size is proportional to the total number of participants in the cluster (see the key at the top of the graph). Circles are labeled with the respective cluster number. Horizontal bars show the mean proportion of VCD cases in the intervention clusters and the control clusters. The relative risk was derived from a comparison of these mean proportions.

DISCUSSION

Establishment of *w*Mel in *A. aegypti* mosquitoes in Yogyakarta reduced the incidence of symptomatic VCD cases by 77% among residents 3 to 45 years of age. It is reassuring that protective efficacy was observed against all four DENV serotypes and with the greatest confidence observed against DENV-2 and DENV-4, since these were the most prevalent serotypes. The protective efficacy in preventing hospitalization with VCD, a pragmatic proxy of clinical severity, was 86%. In 11 of the 12 intervention clusters, the proportion of participants with VCD in each cluster was lower than that in control clusters, which shows consistent biologic replication of the intervention effect.

The conceptual underpinnings of the test-negative design used in this trial, and the statistical framework for population inference, have been described previously.[23] Acute undifferentiated fever for a duration of 1 to 4 days was set as the clinical basis for participant eligibility to avoid selection bias at the point of recruitment and to enable virologic detection of dengue cases. Trial procedures, such as the concealment from research staff of the wolbachia exposure status of the participants, were designed to prevent bias in follow-up, laboratory testing, and outcome classification. The mosquito releases in the intervention clusters were delivered openly (not placebo controlled) for several months in each cluster during 2017. There was no evidence that this changed the health care–seeking behavior of community members in subsequent years, because similar numbers of participants who

met the eligibility criteria were enrolled from the intervention and the control clusters.

Populations of *w*Mel-infected mosquitoes were not static, and spatially heterogenous *w*Mel contamination was measured at the edges of control clusters in year 2 of the trial. Nonetheless, the efficacy estimates from per-protocol analyses, which accounted for individual participants' recent exposure to *w*Mel through changes in cluster-level *w*Mel prevalence or human movement, did not exceed those in the intention-to-treat analysis. We plan more nuanced exploratory analyses outside the scope of the current protocol to explore the fine spatial and temporal connections between *w*Mel prevalence and the risk of VCD.

The efficacy results reported here are consistent with a body of laboratory and field observations. Predictions from an ensemble of mathematical models have suggested that the reduced infectiousness observed in *w*Mel-infected *A. aegypti* could be sufficient to reduce the basic reproductive number to below 1 in many settings in which dengue is endemic, which could result in local elimination of disease.[3,25,26] Previous nonrandomized field studies in Australia[16,17] and Indonesia[14] provided evidence of large epidemiologic effects after *w*Mel was introgressed. A quasi-experimental study of *w*Mel deployments showed that the incidence of hospitalization with dengue hemorrhagic fever was 76% lower in seven urban villages on the northwestern border of Yogyakarta than in three control villages on the southeastern border of the city during the 30 months after mosquito deployment.[14] Together with the results of the trial reported here, these data suggest that when *w*Mel is established at high prevalence in local *A. aegypti* populations, reductions in the incidence of dengue follow. Another wolbachia strain, *w*AlbB, also has pathogen-blocking properties and can be introgressed into *A. aegypti* field populations.[15] This suggests the possibility of a portfolio of wolbachia strains, each with different strengths and weaknesses, for application as public health interventions in areas in which dengue is endemic.

Stable *w*Mel transinfection imparts a viral-resistant state in *A. aegypti* mosquitoes that attenuates superinfection by several medically important flaviviruses and alphaviruses. Multiple mechanisms have been proposed to explain this phenotype, including wolbachia-induced triggering of innate immune effectors[27,28] and changes in intracellular cholesterol transport.[29] DENV could plausibly evolve resistance to *w*Mel; however, the requirement for alternating infection of human and mosquito hosts, together with what appears to be a complex mode of action, could be a constraint to the adaptive emergence of resistant virus populations. Future research should survey arbovirus populations for signals of wolbachia-associated selective pressure.

The approach of *w*Mel introgression represents a novel product class for the control of dengue.[30] An attractive aspect of this strategy is that it is maintained in the mosquito population and does not need reapplication.[31] Future trials should explore the multivalency of the intervention, since laboratory studies[12,32-35] suggest *w*Mel could also attenuate transmission of Zika, chikungunya, yellow fever, and Mayaro viruses by *A. aegypti*.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

APPENDIX

The authors' full names and academic degrees are as follows: Adi Utarini, M.D., Ph.D., Citra Indriani, M.D., M.P.H., Riris A. Ahmad, M.D., Ph.D., Warsito Tantowijoyo, Ph.D., Eggi Arguni, M.D., Ph.D., M. Ridwan Ansari, M.Sc., Endah Supriyati, M.Sc., D. Satria Wardana, B.Sc., Yeti Meitika, B.Comp.Sci., Inggrid Ernesia, B.Sc., Indah Nurhayati, B.Sc., Equatori Prabowo, B.E., Bekti Andari, M.A., Benjamin R. Green, B.A., Lauren Hodgson, B.Sc., Zoe Cutcher, M.Phil. App.Epi., Edwige Rancès, Ph.D., Peter A. Ryan, Ph.D., Scott L. O'Neill, Ph.D., Suzanne M. Dufault, Ph.D., Stephanie K. Tanamas, Ph.D., Nicholas P. Jewell, Ph.D., Katherine L. Anders, Ph.D., and Cameron P. Simmons, Ph.D.

The authors' affiliations are as follows: the World Mosquito Program Yogyakarta, Center for Tropical Medicine (A.U., C.I., R.A.A., W.T., E.A., M.R.A., E.S., D.S.W., Y.M., I.E., I.N., E.P.), the Department of Health Policy and Management (A.U.), the Department of Biostatistics, Epidemiology, and Public Health (C.I., R.A.A.), and the Department of Child Health (E.A.), Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia; the Department of Biostatistics, School of Public Health, University of California, Berkeley, Berkeley (S.M.D., N.P.J.); the London School of Hygiene and Tropical Medicine, London (N.P.J.); Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam (C.P.S.); and the World Mosquito Program, Institute of Vector-Borne Disease, Monash University, Clayton, VIC, Australia (B.A., B.R.G., L.H., Z.C., E.R., P.A.R., S.L.O., S.M.D., S.K.T., K.L.A., C.P.S.).

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