Effectiveness of *Wolbachia*-infected mosquito deployments in reducing the incidence of dengue and other Aedes-borne diseases in Niterói, Brazil: A quasi-experimental study

Sofia B. Pinto, Thais I. S. Riback, Gabriel Sylvestre, Guilherme Costa, Julia Peixoto, Fernando B. S. Dias, Stephanie K. Tanamas, Cameron P. Simmons, Suzanne M. Dufault, Peter A. Ryan, Scott L. O’Neill, Frederico C. Muzzi, Simon Kutcher, Jacqui Montgomery, Benjamin R. Green, Ruth Smithyman, Ana Eppinghaus, Valeria Saraceni, Betina Durovni, Katherine L. Anders, Luciano A. Moreira

1 World Mosquito Program, Fiocruz, Rio de Janeiro, Brazil, 2 Gabinete da Presidência, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil, 3 World Mosquito Program, Institute of Vector Borne Disease, Monash University, Clayton, Australia, 4 Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam, 5 Division of Biostatistics, School of Public Health, University of California, Berkeley, California, United States of America, 6 City Health Secretariat, Niterói, Brazil, 7 City Health Secretariat, Rio de Janeiro, Brazil, 8 Centre for Strategic Studies, Fiocruz, Rio de Janeiro, Brazil, 9 Instituto Rene Rachou, Fiocruz, Belo Horizonte, Brazil

* These authors contributed equally to this work.

Citation: Pinto SB, Riback TIS, Sylvestre G, Costa G, Peixoto J, Dias FBS, et al. (2021) Effectiveness of *Wolbachia*-infected mosquito deployments in reducing the incidence of dengue and other Aedes-borne diseases in Niterói, Brazil: A quasi-experimental study. PLoS Negl Trop Disc 15(7): e0009556. https://doi.org/10.1371/journal.pntd.0009556

Editor: Claire Donald, University of Glasgow, UNITED KINGDOM

Received: January 31, 2021
Accepted: June 9, 2021
Published: July 12, 2021

Copyright: © 2021 Pinto et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: Zone-level entomological and arboviral disease notification data are available at https://doi.org/10.6084/m9.figshare.13662203.v3. Neighbourhood-level entomological and dengue case notification data are available at https://doi.org/10.6084/m9.figshare.13662230.v2.

Funding: This work was supported by the Brazilian Ministry of Health (DECIT/SVS, grant...
of releases, wMel introgression into local Ae. aegypti populations was heterogeneous throughout Niterói, reaching a high prevalence (>80%) in the earliest release zone, and more moderate levels (prevalence 40–70%) elsewhere. Despite this spatial heterogeneity in entomological outcomes, the wMel intervention was associated with a 69% reduction in dengue incidence (95% confidence interval 54%, 79%), a 56% reduction in chikungunya incidence (95%CI 16%, 77%) and a 37% reduction in Zika incidence (95%CI 1%, 60%), in the aggregate release area compared with the pre-defined control area. This significant intervention effect on dengue was replicated across all four release zones, and in three of four zones for chikungunya, though not in individual release zones for Zika.

Conclusions/Significance

We demonstrate that wMel Wolbachia can be successfully introgressed into Ae. aegypti populations in a large and complex urban setting, and that a significant public health benefit from reduced incidence of Aedes-borne disease accrues even where the prevalence of wMel in local mosquito populations is moderate and spatially heterogeneous. These findings are consistent with the results of randomised and non-randomised field trials in Indonesia and northern Australia, and are supportive of the Wolbachia biocontrol method as a multivalent intervention against dengue, chikungunya and Zika.

Author summary

The Aedes aegypti mosquito transmits dengue, chikungunya, Zika and other viral diseases between humans. Previous research has shown that when a symbiotic bacterium called Wolbachia—which exists naturally in many other insect species—is introduced into Ae. aegypti mosquitoes it makes them less able to transmit dengue and other viruses, and is passed from generation to generation via mosquito eggs. The authors report that after releasing Wolbachia-carrying Ae. aegypti in the Brazilian city of Niterói for periods during 2017 to 2019, between 33% and 90% of the Ae. aegypti mosquito population in four release zones were infected with Wolbachia by March 2020. The authors used controlled interrupted time series analysis to show that Wolbachia deployments were associated with a 69% reduction in dengue cases notified to the public health authorities, compared to a control area of Niterói that did not receive Wolbachia releases. Chikungunya and Zika case incidence was also significantly lower in the Wolbachia release areas. These results support previous findings from Indonesia and Australia, and show that Wolbachia mosquito releases are an effective and sustainable method for controlling dengue and other diseases spread by Ae. aegypti mosquitoes, even in large and complex urban environments.

Introduction

Dengue is a mosquito-borne disease transmitted primarily by the Aedes aegypti mosquito, which has increased globally in both case burden and geographic footprint over the past 50 years. Approximately 40% of the world’s population are at risk of dengue transmission, with an estimated 400 million infections per year resulting in 50–100 million clinical cases and 3.6 million hospitalisations [1, 2]. The economic cost to health systems and communities has been
estimated at $8.9 billion per annum [3]. In Brazil, more than 1.5 million dengue cases and 782 deaths were reported nationally in 2019, with in excess of 1300 cases per 100,000 population in the worst affected Central-West region. In the same year 132,000 cases of chikungunya—also transmitted by *Ae. aegypti* mosquitoes—were reported, including 92 deaths.

Current strategies for dengue control are limited to efforts to suppress immature and adult mosquito numbers, through spraying of insecticides and community campaigns to reduce breeding sites. Even where considerable resources are invested in these activities, sustained suppression of mosquito densities has been elusive, and seasonal outbreaks continue to occur [4, 5]. There is a well-recognised need for new, affordable and effective tools for control of dengue and other *Aedes*-borne arboviruses, including chikungunya and Zika [4, 6].

Stable introduction of the common insect bacterium *Wolbachia* (*wMel* strain) into *Ae. aegypti* has been shown in the laboratory to result in *Ae. aegypti* having reduced transmission potential for dengue and other *Aedes*-borne arboviruses including chikungunya, Zika, Yellow Fever and Mayaro virus [7–14]. Female *Ae. aegypti* mosquitoes infected with *wMel* transmit the bacterium with high fidelity to their offspring via infected eggs and *wMel* manipulates mosquito reproductive outcomes via a process called cytoplasmic incompatibility, which favours introgression of *wMel* into a wild-type population [13]. Accumulating evidence from field sites in Australia and Indonesia has demonstrated large reductions in dengue incidence in areas where short-term releases of *wMel*-infected mosquitoes have resulted in introgression and sustained high prevalence of *wMel* in local *Ae. aegypti* populations [15–17]. A recently completed cluster randomised trial of *wMel* Wolbachia deployments in Yogyakarta, Indonesia, conclusively demonstrated the efficacy of the method, with a 77% reduction in dengue incidence in *Wolbachia*-treated neighbourhoods compared to untreated areas [18]. The Yogyakarta CRT included chikungunya and Zika as secondary endpoints, but insufficient cases were detected to permit an evaluation of efficacy against these arboviruses. Acquiring field evidence for the effectiveness of *Wolbachia* in reducing transmission of these arboviruses is a priority, as is the accumulation of real-world evidence for public health impact from large-scale implementations of *wMel*-infected *Ae. aegypti* in the complex urban environments common throughout dengue-endemic areas.

Pilot releases of *Wolbachia*-infected mosquitoes started in 2014 in Rio de Janeiro and in 2015 in Niterói, Brazil, and achieved successful establishment of *Wolbachia* throughout the two small pilot site communities, each with a population of 2500–2800 people [19, 20]. In 2017 Niterói became the first site in Brazil to move to scaled deployments across a large urban area. The intervention involved a phased approach including engagement with and acceptance by the community, communication strategies to ensure the communities were informed and supportive, releases of *Wolbachia*-infected *Ae. aegypti* mosquitoes, and monitoring of the levels of *Wolbachia* in *Ae. aegypti* in the field.

We report here the entomological and epidemiological outcomes of a large-scale non-randomised deployment of *Wolbachia*-infected *Ae. aegypti* mosquitoes in the Brazilian city of Niterói, for the control of dengue and other *Aedes*-borne diseases. The impact of *Wolbachia* deployment on dengue, chikungunya and Zika incidence was evaluated via a quasi-experimental study, using controlled interrupted time series analysis of routine notifiable disease surveillance data, in accordance with a pre-defined protocol [21].

**Methods**

**Ethics statement**

Approval to release *Wolbachia*-carrying *Ae. aegypti* mosquitoes into urban areas was obtained from three Brazilian governmental bodies: the National Agency of Sanitary Surveillance...
(ANVISA); the Ministry of Agriculture, Livestock and Supply (MAPA); and the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA), which issued a Temporary Special Registry (Registro Especial Temporário (RET), nr. 0551716178/2017). Ethical approval was also obtained from the National Commission for Research Ethics (CONEP—CAAE 59175616.2.0000.0008).

**Study setting**

Niterói, a municipality of the state of Rio de Janeiro is situated in the Guanabara Bay across from Rio de Janeiro city (22°52'58"S 43°06'14"W). According to the last national census in 2010 it had a population of 484,918 living in an area of 135 km². The city is divided into 7 health districts for administrative planning. For the evaluation of the impact of *Wolbachia* mosquito deployments, Niteroi was divided into four release zones and 1 control zone, which are aligned with neighbourhood administrative boundaries (Fig 1). Table 1 shows the baseline characteristics and release summary of each zone.

![Fig 1. Study site map showing the municipality of Niterói, comprising four zones in which releases of wMel-infected *Aedes aegypti* have been undertaken and one pre-defined parallel untreated control zone. Neighbourhood boundaries are shown in white. The inset shows the location of Niterói within the state of Rio de Janeiro, Brazil. Maps were generated in ArcGIS 10.7 (Esri, Redlands, CA, USA) using administrative boundaries freely available from the Brazilian Institute of Geography and Statistics (IBGE).](https://doi.org/10.1371/journal.pntd.0009556.g001)
Community engagement

WMP Brazil’s Communication and Engagement (C&E) strategy was developed prior to mosquito releases, following a thorough analysis of geographical, social, political, economic and cultural factors in the proposed release areas as previously described [22].

In Niterói the C&E plan was focused on three key areas: public schools, primary health care units and social leadership, due to their reach and influence within the release area, including into vulnerable communities. Community Reference Groups (CRGs) were also created, to serve as advisory committees populated by representatives of the planned release areas, to inform the activities of WMP Brazil. This group was also responsible for providing feedback on all communication materials and C&E strategies that were proposed throughout the WMP’s activities in their areas.

Prior to the release of wMel-infected mosquitoes in each area, a survey of awareness and acceptance of the method was conducted by an independent company. In order to reach a wide range of people living and working in the release areas, time-location sampling was used to survey passers-by in busy public locations in each neighbourhood. Respondents (n = 3485 in total) were 18 years and over, and lived or worked in the neighbourhood where the survey was conducted. The questionnaire was developed with the CRG, and included questions on awareness (“Have you heard about the Wolbachia method?”), understanding after explanation of the method (“Do you understand that this method replaces the population of Aedes aegypti mosquitoes with Aedes aegypti mosquitoes carrying Wolbachia, which have a reduced capacity to transmit dengue, Zika and chikungunya?”) and acceptance of the proposed wMel releases (“Do you agree with Fiocruz releasing these mosquitoes with Wolbachia here in your neighbourhood?”).

Mosquito production

The Rio wMel-infected Ae. aegypti line described in Garcia et al 2019 [23] was used for releases. The wMel-infected lines were maintained in controlled laboratory conditions, in 900
mesh-sided rearing cages. Each cage contained 2500–2750 adults, and was fed using donated non-transfusional usable human blood (agreement FIOCRUZ/ Hemominas OF. GPO/CCO-Nr224/16), once per week for two to three gonotrophic cycles. As a quality assurance procedure each blood bag was tested for dengue, Zika, chikungunya, Mayaro and yellow fever viruses, as described previously [9, 11, 24]. Two separate colonies were maintained, a broodstock (kept in Belo Horizonte) and a release-production colony (kept in Rio de Janeiro). Male *Ae. aegypti* adults (from F0–F1 field collected material) were introduced into the broodstock cages at a rate of 10–20% every 5 generations. This outcrossing frequency was sufficient to maintain kdr resistant genotypes within the broodstock colony throughout its maintenance (S1 Text and S1 Fig). Material from the broodstock colony was then transferred to the release-production colony where it was amplified through 2 amplifications without the addition of field collected males. A minimum sample of 168 mosquitoes from the release-production colony was screened for *wMel* infection on a weekly basis, using quantitative polymerase chain reaction (qPCR) as described below. *wMel* prevalence was 100% in all but three weekly screening events, and was never below 97%. Quantitative analysis of *wMel* in these samples detected a fairly constant wsp:rps17 copy number between 4 to 6 (S2 Fig).

From April 2017 until April 2018 immature stages for adult releases were reared at a density of approximately 1.0 larvae/ml and fed a diet of ground Tetramin Tropical Flakes (Tetra Holding [US] Inc. Germany, Product number 77101). From May 2018, immature stages for adult releases were reared at a density of approximately 2.75 larvae/ml and fed a diet of fish food: liver powder: yeast extract (4:3:1). We found no detrimental effects on outcomes, including development time, size, egg output or *wMel* density, with increases in larval density up to 2.75/ml. In both rearing regimes, when approximately 10–30% of larvae had pupated, the larvae/pupae were sieved and between 180–220 larvae/pupae were placed in a release device. The release device was a cylindrical PVC crystal tube approximately 28 mm in diameter and 250 mm in length, covered with a fixed mesh on one side and a removable mesh on the other side. Adults were allowed to emerge for 5–6 days and were maintained on a 10% sugar solution for 12–36 hours prior to releases. We estimated that the releases were slightly male biased with an average female: male ratio within the devices of 3:4. The release devices were then stacked, sugar-free into boxes for transport to the release site.

**Wolbachia deployments**

Mosquito deployments took place over a release area of 40 km² during a period of 35 months (February 2017—December 2019). Adult *wMel*-infected mosquitoes were released weekly from a moving vehicle. In zones 1–3 mosquito release points were initially determined using a 50 meter grid overlaid on the release areas, with one release point per grid square. In zone 4 the density of release points was adjusted for the residential population in each neighbourhood, with the aim of releasing a cumulative total of 100 mosquitoes per resident (average distance between release points on a regular grid was 41 meters). In all areas, the initial release points determined on the grids were then distributed to the nearest vehicle-accessible road for vehicle releases (S3 Fig). Releases were staged throughout the urban constructed areas in each release zone. Green non-constructed areas were excluded from releases as they provide less favourable habitats for *Ae. aegypti* and had few or no human residents. Initial release periods were 10–16 weeks duration, with subsequent re-releases conducted in local areas where *wMel* prevalence was <40% in 3 consecutive monitoring events as measured at least 4 weeks after the conclusion of releases. This 40% threshold was based on previous estimates of the unstable equilibrium point for *wMel*, above which invasion can occur [25]. This resulted in re-releases being conducted in approximately 30% of the initial release areas. Most areas of zones 1 and 2
had two periods of releases, zone 3 had three periods of releases and zone 4 only 1 release period.

**Wolbachia monitoring**

Mosquitoes were collected weekly during and after releases using a network of BG Sentinel traps (Biogents AG, Regensburg, Germany, Product number NR10030) at an average density of 16 BG traps/km² throughout release areas (S4 Fig). Once wMel prevalence was detected at >60% in 3 consecutive monitoring events measured at least 4 weeks after the conclusion of releases, trap numbers were reduced to 50% within a neighbourhood (S4 Fig). Mosquitoes were sent to the laboratory for sorting, morphological identification and counting. The number of mosquitoes caught in each BG trap was recorded by species, sex, and in total. Mosquito samples were stored in 70% ethanol until screening for wMel-strain Wolbachia. Screening was performed weekly until week ending 8 April 2018 and fortnightly thereafter.

**Wolbachia molecular detection**

A maximum of 10 adult *Ae. aegypti* per BG trap per collection were screened for the presence of wMel using either quantitative polymerase chain reaction (qPCR), or a colorimetric loop-mediated isothermal amplification (LAMP) assay. Taqman qPCR was performed on a Roche LightCycler 480 as described previously [16, 26]. Briefly, the qPCR cycling program consisted of a denaturation at 95°C for 5 min followed by 40 cycles of PCR (denaturation at 95°C for 10 min, annealing at 60°C for 30 sec, and extension at 72°C for 1 sec with single acquisition) followed by a cooling down step at 40°C for 30 sec. LAMP reactions were performed in a Bio-Rad C1000 96-well PCR thermocycler with a 30min incubation at 65°C as previously described [16]. Individual reactions consisted of 2X WarmStartR Colorimetric LAMP Master Mix (New England BioLabs, Cat# M1800S), primers and 1 μL of target DNA from a 50μl single mosquito squash buffer extraction assay, in a total reaction volume of 17 μL. An individual mosquito was scored as positive for *Wolbachia* if the Cp (crossing point) value in qPCR was below 28, or if the well in the LAMP assay was yellow upon visual inspection. Equivocal results were counted as negative. Details of primer and probe nucleotide sequences are included in S1 Text.

**Epidemiological data**

Data on dengue and chikungunya cases notified to the Brazilian national disease surveillance system (SINAN) were used to evaluate the epidemiological impact of *Wolbachia* releases. Reporting of both diseases is mandatory in Brazil. Dengue notification data for Niterói is available from SINAN since 2007 and chikungunya since 2015. Notified dengue and chikungunya cases reported to SINAN are predominantly suspected cases based on a clinical case definition [27].

Between 2007–2014, approximately 15% of notified dengue cases had supportive laboratory test results, usually from IgM serology. Since the Zika epidemic in Brazil in 2015, laboratory confirmation of dengue has relied on PCR only due to cross-reactive serological responses, and only one dengue case notified in 2015–2020 included laboratory confirmation. For chikungunya, 24% of cases notified in 2015–2020 had supportive IgM serology results. For the purpose of this analysis, we include all notified dengue and chikungunya cases (suspected and laboratory confirmed).

Anonymized disaggregate data on notified suspected and laboratory-confirmed dengue, severe dengue, chikungunya and Zika cases were obtained from the SINAN system through the Health Secretariat of Niterói, for the period from January 2007 (January 2015 for
chikungunya and Zika) to June 2020. Population data by neighbourhood of residence from the Brazilian 2010 census (IBGE) was used to estimate the population in each Wolbachia release zone.

**Measurement of epidemiological impact**

The wMel intervention effect was estimated using controlled interrupted time series analysis performed separately for each release zone compared with the pre-defined control area, and for the aggregate release area compared with the control area, as described in a published study protocol [21]. The primary analysis included data from January 2007 (dengue) or January 2015 (chikungunya and Zika), until June 2020, encompassing 8–37 months of post-intervention observations. For zone-level analyses, negative binomial regression was used to model monthly dengue, chikungunya and Zika case counts in the intervention and control areas, with an offset for population size. Seasonal variability in disease incidence was controlled using flexible cubic splines with knots placed at 6-monthly intervals. For the primary analysis, a binary ‘group’ variable indicated the study arm (intervention or control). A binary ‘treatment’ variable distinguished the pre-intervention period and the post-intervention period. The zone-level post-intervention period was defined as four weeks after wMel releases had commenced throughout the whole zone; the corresponding post-intervention period was also applied to the control area for each zone-level analysis. The intervention effect was estimated from the interaction between the ‘group’ and ‘treatment’ variables, which allows explicitly for a level change in the outcome (dengue/chikungunya/Zika case incidence) in both intervention and control areas in the post-intervention period. Robust standard errors were used to account for autocorrelation and heteroskedasticity. A mixed-effects negative binomial regression was used to model monthly dengue, chikungunya or Zika case counts in the aggregate release area compared with the control area, with an offset for population size and controlling for seasonal variability in incidence using flexible cubic splines with knots placed at 6-monthly intervals. Clustering of dengue/chikungunya/Zika cases by release zone was modelled as a random effect by including a random intercept at the zone level and allowing for a random slope on the intervention. A binary ‘treatment’ variable distinguished the pre-intervention period and the post-intervention period, with the control area classified as ‘pre-intervention’ throughout. Robust standard errors were used to account for autocorrelation and heteroskedasticity. The zone-level and aggregate release area analyses included the pilot release area of Jurujuba within zone 1.

To account for within-zone heterogeneity in wMel establishment and dengue incidence, a secondary neighbourhood-level analysis was also performed in which Wolbachia exposure was determined by the measured wMel prevalence in Ae. aegypti collected from each neighbourhood, and a three-month moving average calculated to smooth the variability in monthly wMel prevalence, categorised into quintiles of exposure. In zone 1 we excluded the neighbourhood of Jurujuba where pilot wMel releases were staggered across seven sectors over a period of 16 months and wMel monitoring was initially done only in small pockets of the neighbourhood where releases had already occurred, because the wMel time-series during this staged release period was not representative of the whole of Jurujuba neighbourhood (whereas the dengue cases data was aggregate for the whole neighbourhood). This analysis included data to March 2020 only, as no Wolbachia monitoring was possible April–June 2020 due to restrictions on movement in response to the Covid-19 pandemic. Mixed-effects negative binomial regression was used to model monthly dengue case notifications by neighbourhood, in each of the four release zones individually and in all zones combined, compared with the pre-specified control zone. The model included population size as an offset and neighbourhood as a random effect.
effect. Given the large number of zero dengue case counts (zero-inflation) at the neighbour-
hood level, an alternative analysis using a zero-inflated negative-binomial model with robust
standard errors to account for clustering was considered. Model fit was not improved by
accounting for zero-inflation, as assessed using the Akaike Information Criterion (AIC), and
was thus not used in the analyses. This secondary analysis was not performed for chikungunya
or Zika due to the sparsity of case data at the neighbourhood level.

Sensitivity analyses
As a sensitivity analysis, we excluded pre-intervention observations prior to 2012 to achieve
greater balance between pre-intervention and post-intervention period lengths while main-
taining sufficient data to inform on pre-intervention trends [28].

Power estimation
Power was estimated for the ITS analysis using 1000 simulated datasets drawn from a negative
binomial distribution fitted to a ten-year time series (2007–2016) prior to Wolbachia deploy-
ment, of monthly dengue case notifications from release and control zones in Niterói and Rio
de Janeiro. The simulated time series of dengue case numbers in the control zones as well as
the pre- Wolbachia release dengue case numbers in the treated zones were drawn directly
from this model-generated distribution. Post- Wolbachia release dengue case numbers in the
treated zones were drawn from the same model-generated distribution, modified by an addi-
tional parameter for an intervention effect of Relative Risks = 0.6, 0.5, 0.4, 0.3. For each of
these four ‘true’ effect sizes and a null effect (RR = 1), applied to each of the 1000 simulated
time series, the ‘observed’ effect size was calculated from a negative binomial regression model
of monthly case counts in the treated and untreated zones, as described above. Post-interven-
tion time periods of 1, 2 or 3 years were simulated, with the pre-intervention period fixed at 7
years. The estimated power to detect a given effect size was determined as the proportion of
the 1000 simulated scenarios in which a significant intervention effect (p < 0.05) was observed.
These simulations indicate 80% power to detect a reduction in dengue incidence of 50% or
greater after three years of post-intervention observations, and a reduction of 60% or greater
after two years.

Results
Wolbachia establishment in Niterói
Awareness (prior knowledge of the Wolbachia method) ranged from 36 to 50% and acceptance
(acceptance with the proposed wMel releases in the neighbourhood) ranged from 65 to 92%, in
the public survey conducted prior to releases in Niterói. No negative media nor negative com-
munity incidents were registered, and the Community Reference Group endorsed the start of
releases.

Heterogeneity in wMel Wolbachia establishment was observed in three of the four release
zones (Fig 2). In the initial release area of zone 1, Wolbachia prevalence was greater than 80%
in the first quarter of 2020 (up to 11 months post-release) and there was low variability across
the neighbourhoods. Local wMel introgression has been more variable in zones 2 and 3, with a
median wMel prevalence of 40–70% among neighbourhoods during the post-release period
(11 months and 9 months, respectively). In zone 4, a longer post-intervention observation
period is required to evaluate the trajectory of wMel establishment. Aedes albopictus is present
throughout the city and was detected at a similar abundance in our monitoring network dur-
ing and after releases (S5 Fig).
Arboviral disease trends pre- and post-Wolbachia intervention

During the ten years prior to the start of scaled *Wolbachia* mosquito releases in Niterói in early 2017, seasonal peaks in dengue case notifications occurred each year (Fig 3A), usually in March and April (Fig 3D). A median of 2,818 dengue cases were notified each year 2007–2016 (per capita incidence 581/100,000 population), with a minimum of 366 cases in 2014 (75/100,000) following a maximum of 11,618 in 2013 (2,396/100,000). In the three years following the start of phased *Wolbachia* releases, annual city-wide dengue case notifications were 895, 1,729 and 378 in 2017, 2018 and 2019 respectively, and the seasonal peaks in dengue incidence...
occurred predominantly in the areas of Niterói that had not yet received Wolbachia deployments (Fig 4).

Chikungunya surveillance commenced in January 2015. Between 44 and 533 chikungunya cases were notified annually in Niterói in 2015–2019, with the exception of 2018 when an explosive outbreak resulted in 3091 reported cases; 95% of those occurred in the six months January to June. The highest per capita incidence of chikungunya during the 2018 outbreak was in the untreated control zone (1,413 cases/100,000 population; Fig 5), followed by zone 4 where Wolbachia deployments had not yet commenced (958/100,000). In zones 1, 2, and 3 where deployments were underway and zone-level Wolbachia prevalence was between 20–55%, the incidence of chikungunya case notifications during the 2018 outbreak was 106/100,000, 244/100,000 and 201/100,000, respectively.

There were 8,247 Zika cases reported in Niterói between 2015 and June 2020, 91% (n = 7,532) of which were reported in 2015–2016 when Brazil experienced an unprecedented Zika outbreak (Fig 6). From 2017, when phased wMel deployments began in Niterói, until June 2020 a total of 715 Zika cases were notified in Niterói, of which of 95 were reported from areas where wMel deployments had already occurred: 12 in zone 1, 28 in zone 2, 48 in zone 3, and 7 in zone 4.
Fig 4. Dengue incidence and wMel infection prevalence in local *Aedes aegypti* mosquito populations, by release zone. Panels A,C,E,G: Lines show the monthly incidence of dengue case notifications per 100,000 population (left-hand Y axis) in Niterói release zones 1–4 (solid line in each panel) compared with
the untreated control zone (dashed line), January 2007—June 2020. Light blue shading indicates the beginning of the epidemiological monitoring period in each zone, one month after initial releases were completed in each respective zone. Darker blue shading indicates the aggregate wMel infection prevalence (right-hand Y axis) in each zone in each calendar month from the start of the epidemiological monitoring period until March 2020 (no wMel monitoring April—June 2020). Panels B,D,F,H show the same data but zoomed into the period from May 2017 –March 2020 and with the dengue incidence axis rescaled, to show more clearly the trends in release and control zones in the post-intervention period.

https://doi.org/10.1371/journal.pntd.0009556.g004

Reduction in dengue, chikungunya and Zika incidence post-Wolbachia intervention

Using interrupted time series (ITS) analysis to account for underlying temporal trends in case incidence and staggered implementation of the intervention, we found that wMel Wolbachia deployments were associated with a significant reduction in dengue incidence in each of the four release zones (Fig 6A). The magnitude of this reduction ranged from 46.0% (95%CI 21.0, 63.0) in zone 3 to 75.9% (95%CI 62.1, 84.7) in zone 2. Overall, Wolbachia deployments were associated with a 69.4% (95%CI 54.4, 79.4) reduction in dengue incidence in Niterói (Fig 7 and S1 Table).

https://doi.org/10.1371/journal.pntd.0009556.g005

Fig 5. Chikungunya incidence and wMel infection prevalence in local Aedes aegypti mosquito populations, by release zone. Lines show the monthly incidence of chikungunya case notifications per 100,000 population (left-hand Y axis) in Niterói release zones 1–4 (solid line in each panel) compared with the untreated control zone (dashed line), January 2015—June 2020. Light blue shading indicates the beginning of the epidemiological monitoring period in each zone, one month after initial releases were completed in each respective zone. Darker blue shading indicates the aggregate wMel infection prevalence (right-hand Y axis) in each zone in each calendar month from the start of the epidemiological monitoring period until March 2020 (no wMel monitoring April—June 2020).

https://doi.org/10.1371/journal.pntd.0009556.g005
This _Wolbachia_ intervention effect against dengue was also apparent overall, and in each zone, in the neighbourhood-level analysis that considered quintiles of _wMel_ prevalence in local _Aedes aegypti_ populations, although we found evidence of only marginal additional reductions in dengue incidence at higher levels of _Wolbachia_ beyond 20–40% _wMel_ prevalence (S6 Fig and S2 Table). There was substantial month-to-month variation in _wMel_ quintiles within neighbourhoods (S7 Fig), which was reduced but not removed by taking a three-month moving average of _wMel_ prevalence. The results were little changed in the sensitivity analysis, which excluded pre-intervention observations prior to 2012 (S8 Fig and S3 Table).

A total of 897 severe dengue cases were reported in Niterói between 2007 and early 2020, 691 of which were from one of the four intervention zones and 206 from the control zone. Only three of these cases occurred in the post-intervention period, two in zone 2 and one in zone 3. The control zone has not had any severe dengue cases reported since 2016. These numbers were too sparse to be analysed using our ITS model, even when allowing for zero-inflation.

We found in ITS analysis that chikungunya incidence was also significantly reduced following _Wolbachia_ deployments in Niterói as a whole (56.3% reduction in incidence; 95%CI 15.9, 77.3) and in three of the four individual release zones (Fig 8 and S1 Table). Zika incidence was
reduced by 37% (95% CI 1.5, 59.5) following Wolbachia deployments in Niterói as a whole, though not in individual release zones (Fig 9 and S1 Table).

**Discussion**

Large-scale phased deployments of wMel strain Wolbachia-infected *Aedes aegypti* mosquitoes in Niterói, Brazil during 2017–2019, resulted in wMel establishment in local *Ae. aegypti* populations at an infection frequency of 33–90% by March 2020, when field monitoring was paused due to the emergence of SARS-CoV-2 in Brazil. More than one-quarter of the total 373,000 residents of the intervention area were living in neighbourhoods where local wMel prevalence was 60% or greater by March 2020, predominantly in zones 1 and 2 where releases commenced earliest. In the remaining intervention areas, wMel prevalence was more heterogeneous and a resumption of entomological monitoring is planned in order to evaluate the long-term trajectory of wMel introgression into the local *Ae. aegypti* population.

Despite this heterogeneity in Wolbachia establishment, a significant reduction in the incidence of dengue, chikungunya and Zika case notifications was observed in Wolbachia-treated areas of Niterói, compared with a pre-defined untreated control area. This epidemiological impact on dengue was replicated across all four release zones, and in three of the four zones for chikungunya. Aggregate across the whole intervention area, the wMel deployments were associated with a 69% reduction in dengue incidence, a 56% reduction in chikungunya incidence and a 37% reduction in Zika incidence. Given the recognised lack of evidence for efficacy of routinely available approaches to arboviral disease control [4] based on elimination of
breeding sites and insecticide-based suppression of adult mosquito populations, and considering the magnitude of the historical burden of *Aedes*-borne disease in Niterói, an intervention effect of this magnitude represents a substantial public health benefit.

Results from a recent cluster randomised trial of *wMel*-infected *Ae aegypti* deployments in Yogyakarta Indonesia demonstrated 77% efficacy in preventing virologically confirmed dengue cases [18], with comparable efficacy against all four dengue virus serotypes. Previous non-randomised controlled field trials in Indonesia [15] and northern Australia [16, 17] demonstrated 76% and 96% effectiveness, respectively, in reducing the incidence of dengue cases notified to routine disease surveillance systems. In each of those sites the trajectory of *wMel* establishment was more rapid and more homogeneous across the release area than observed in Niterói. In the present study, the epidemiological impact in the area of Niterói where *wMel* introgression occurred most rapidly and homogeneously (zone 1) was highly comparable with the Indonesian studies: 77% (95%CI 64, 86). Another *Wolbachia* strain, *wAlbB*, has been successful introgressed into *Ae. aegypti* field populations in Kuala Lumpur, Malaysia [29], although with instability in *wAlbB* frequencies in some release areas after cessation of releases, which the authors attributed to immigration of wild-type mosquitoes into the small release sites (area 0.05–0.73 km\(^2\)) from surrounding untreated areas.

The reasons for slower and more heterogeneous *wMel* introgression here, compared to Indonesia and Australia, are not fully understood. A likely contributing factor is that these scaled deployments have largely used adult mosquitoes released from vehicles, which does not deliver as spatially homogeneous a deployment as occurred previously in Indonesia and

---

Fig 8. Estimated reduction in the incidence of chikungunya following *Wolbachia* deployments in Niterói, in each release zone individually and in the aggregate release area. Point estimates (circles) and 95% confidence intervals (horizontal bars) from controlled interrupted time series analysis of monthly chikungunya case notifications to the Brazilian national disease surveillance system (Jan 2015 –June 2020).

https://doi.org/10.1371/journal.pntd.0009556.g008
Australia. In contrast, the small-scale pilot releases in the Jurujuba neighbourhood of Niterói in 2015 achieved rapid and sustained introgression of wMel after 8–31 weeks of egg-based releases [20]. Additionally, Niterói release areas were complex urban environments with high rise areas and large informal settlements, where field activities were frequently interrupted by security issues and where physical barriers to spread [30], spatial heterogeneity in mosquito abundance [31], and limited mosquito dispersal [32] could have contributed to slower wMel introgression. The wild-type egg bank in such a setting is likely to be large and spatially heterogeneous, and would take time to be depleted, which is likely to have contributed to the heterogeneity in wMel introgression and intermediate frequencies of Wolbachia observed in our study. Regular monitoring of the wMel-Ae. aegypti broodstock has demonstrated insecticide susceptibility profiles comparable with wild-type material, so a concern of increased susceptibility to insecticide is not considered to be an issue here. Impaired maternal transmission by wMel-infected females [33, 34] and loss of induction of cytoplasmic incompatibility by wMel-infected males [35] has been observed by others at high, but field relevant, temperatures. Exposure of immature Ae. aegypti to very high temperatures in small water containers cannot be excluded as a contributing factor to the wMel introgression patterns observed in Niterói, especially in the more informal settlements where the urban landscape is more vulnerable to temperature variations. Entomological monitoring in future years will help clarify the long-term trajectory of wMel introgression in Niterói.

In large and complex urban environments, a homogeneous high level of introgression of wMel may prove operationally challenging and slow to achieve, even with optimised release
methods and longer post-release monitoring. This poses the question of what minimum threshold of wMel prevalence is needed to achieve interruption of local arbovirus transmission, and whether a dose-response relationship is observed between wMel prevalence and disease reduction. Predictions from mathematical models have suggested that even in conservative scenarios where scaled Wolbachia deployments only reduce the reproduction number (R0) of dengue by 50%, this could lead to reductions in global case incidence of 70% [1], although the impact is predicted to be highly spatially heterogeneous, with smaller relative reductions in areas with highest transmission intensity. Our findings support this prediction of epidemiological impact with imperfect wMel-mediated transmission blocking, by demonstrating that measurable reductions in dengue, chikungunya and Zika disease accrue even at a moderate prevalence of wMel in local Ae. aegypti populations. A secondary analysis based on measured wMel prevalence and dengue case notifications at the neighbourhood-level found only a marginal increase in the wMel intervention effect beyond 20–40% prevalence, which was unexpected. This analysis also indicated substantial variability in wMel prevalence over time (within neighbourhoods). This may be attributable in part to sampling variability due to small Ae. aegypti catch numbers in some areas but may also indicate true local instability in Wolbachia levels. When combined with people’s mobility and risk of acquiring dengue outside their neighbourhood of residence these factors may help explain the non-linear association between measured neighbourhood-level monthly wMel infection prevalence and dengue risk. The absolute abundance of wild-type Ae. aegypti, independent of wMel prevalence, is also relevant to understanding local dengue risk and could not be accounted for in our time series analyses because of a lack of baseline (pre-intervention) mosquito collection data. We cannot exclude that incompatibility within the population of wMel positive and negative Ae. aegypti could impact overall population size and contribute to the observed epidemiological outcomes. Overall, a contribution of indirect effects, confounding by mosquito population size, and imperfect wMel exposure measurement to the observation of an epidemiological impact even at moderate wMel prevalence cannot be excluded, and this observation needs replication in other settings.

This study has some limitations. Deployments of wMel-infected Ae. aegypti were not randomised, so there is the potential for measurement of the intervention effect to be confounded by other factors that differ between the release areas and the pre-defined control area. Routine disease surveillance data is imperfect both in specificity (not all notified cases are true dengue/chikungunya/Zika cases) and in sensitivity (not all dengue/chikungunya/Zika cases are notified). However, the risk of these factors influencing the measurement of the epidemiological endpoint here is reduced by the inclusion of a parallel control with a historical dengue time series that is highly synchronous with each of the release areas for ten years pre-intervention. The replication of the dengue intervention effect in each of the four release zones, and for chikungunya in three zones, also mitigates the possibility that any parallel change in vector control practices or healthcare seeking behaviour in intervention areas could have confounded the observed result. For chikungunya and Zika, there is substantial uncertainty around the point estimate of the intervention effect because case notifications for these two diseases were very sparse in both release and control areas outside of a single large outbreak in 2018 and 2015–16.

We have demonstrated that wMel introgression can be achieved across a large and complex urban environment over a period of three years to a prevalence in local Ae. aegypti which, while still heterogeneous, is sufficient to result in a measurable reduction in dengue, chikungunya and Zika case incidence. Ongoing entomological and epidemiological monitoring will provide additional information on the trajectory of wMel establishment in areas where releases have occurred more recently, or introgression has been slower, and on the full magnitude and durability of the public health benefit.
Supporting information

S1 Text. KDR genotyping methods.

S1 Fig. KDR genotype analysis of consecutive wMelRio brood stock generations from F25 to F43 showing 4 outcrossing events. Outcrossing events were performed on brood females in generation F26, F30, F36 and F41. ‘_RT’ represents the offspring of those outcrossing events and are marked with an orange box. Field collected samples in Rio and Niteroi always show highly resistant genotypes with a roughly 50:50 frequency distribution of R1 and R2 mutations. The wMelRio brood stock line tends to increase its R1 frequency with standard inbred rearing and some small % of susceptible genotypes start to appear around the 3rd Generation. The resulting outcross event normally restores the near 50:50 R1:R2 frequency distribution and reduces susceptible genotype frequencies as well. Methods for kdr genotyping, primers and probes are presented in supplementary methods.

S2 Fig. Analysis of wMel quantification in the release generation. Quantification of wMel was performed weekly on up to 4 day old mosquitoes from the release generation, emerged within the release device, prior to releases. wsp::rps17 copy numbers were fairly constant between 4 to 6, from June 2018 to December 2019. Error bars represent standard deviation of the mean. Total numbers of mosquitoes tested are represented by black dots.

S3 Fig. Spatial distribution of mosquito release locations in Niterói release zone 1 (A), zone 2 (B), zone 3 (C) and zone 4 (D). Approximate locations of adult mosquito releases are shown by blue markers. The Jurujuba pilot release area in zone 1 is indicated with hatched shading. Maps were generated in ArcGIS 10.7 (Esri, Redlands, CA, USA) using base map and data from OpenStreetMap under open database license (CC BY-SA).

S4 Fig. Spatial distribution of mosquito monitoring locations in Niterói release zone 1 (A), zone 2 (B), zone 3 (C) and zone 4 (D). Approximate locations of BG adult mosquito traps are shown for each zone. Pink markers indicate BG traps that were removed throughout the monitoring period. Black markers indicate BG traps that were removed in three of four neighbourhoods in zone 1 and six of 11 neighbourhoods in zone 2 once releases were completed and wMel prevalence was >60% in 3 consecutive monitoring events measured at least 4 weeks after the conclusion of releases, in order to reduce monitoring costs. The Jurujuba pilot release area in zone 1 is indicated with hatched shading. Maps were generated in ArcGIS 10.7 (Esri, Redlands, CA, USA) using base map and data from OpenStreetMap under open database license (CC BY-SA).

S5 Fig. Analysis of the abundance of Aedes albopictus and Aedes aegypti in BG trap collections in Niterói release zones. Panels A–E show the mean number of mosquitoes caught per trap per day for each species, each month, in release zones 1–4 and in the Jurujuba pilot release area. Panels F–J show the relative frequency of each species, each month, in release zones 1–4 and in the Jurujuba pilot release area. Shaded areas represent release periods. Dotted shaded areas indicate that only part of the zone was receiving releases.

S6 Fig. Estimated reduction in dengue incidence with increasing wMel prevalence in Aedes aegypti populations in Niterói neighbourhoods. This analysis uses a three-month moving
average of wMel% and excludes the Zone 1 pilot release area of Jurujuba. Point estimates (markers) and 95% confidence intervals (horizontal bars) are from controlled interrupted time series analysis of monthly dengue case notifications to the Brazilian national disease surveillance system (Jan 2007 –March 2020), by neighbourhood, in each release zone and in the aggregate release area. wMel prevalence was calculated as the percentage of trapped Ae. aegypti positive for wMel, in each neighbourhood each month, grouped by quintile. The lowest quintile (wMel 0–20%) served as the reference category for calculation of the incidence rate ratio (IRR) and included the monthly observations within that quintile from the respective release zone, as well as all observations from the untreated control zone (n = 3,021 neighbourhood-months observed and n = 11,278 notified dengue cases).

S7 Fig. Notified dengue cases and wMel% quintile monthly time series by neighbourhood, in Niterói release zones 1–4. wMel% quintile was based on the wMel prevalence in a single month (current wMel%) or a three-month moving average (Averaged wMel%).

S8 Fig. Estimated reduction in dengue incidence with increasing wMel prevalence in Aeles aegypti populations in Niterói neighbourhoods–sensitivity analysis. This sensitivity analysis excludes all observations prior to 2012, five years prior to the start of releases. Point estimates (markers) and 95% confidence intervals (horizontal bars) are from controlled interrupted time series analysis of monthly dengue case notifications to the Brazilian national disease surveillance system (Jan 2012 –March 2020), by neighbourhood, in each release zone and in the aggregate release area. wMel prevalence was calculated as the percentage of trapped Ae. aegypti positive for wMel, in each neighbourhood in a moving three-month window, grouped by quintile. The lowest quintile (wMel 0–20%) served as the reference category for calculation of the incidence rate ratio (IRR) and included the observations within that quintile from the respective release zone, as well as all observations from the untreated control zone (n = 1,881 neighbourhood-months observed and n = 6,996 notified dengue cases).

S1 Table. Dengue, chikungunya and Zika incidence rate ratios in Wolbachia-release zones compared to the control zone. IRRs are from negative binomial regression models of monthly case counts (Jan 2007 –June 2020 for dengue; Jan 2015 –June 2020 for chikungunya and Zika), with an offset for population size and 6-monthly flexible cubic splines to account for seasonal effects. The mixed effects model for the aggregate Niteroi release area included a random effect for release zone.

S2 Table. Dengue incidence rate ratios with increasing wMel prevalence in Aeles aegypti populations in Niterói neighbourhoods. IRRs are from mixed effects negative binomial regression models of monthly dengue case counts (Jan 2007 –March 2020) by neighbourhood, with an offset for population size, 6-monthly flexible cubic splines to account for seasonal effects, and a random effect for neighbourhood.

S3 Table. Dengue incidence rate ratios with increasing wMel prevalence in Aeles aegypti populations in Niterói neighbourhoods–sensitivity analysis excluding pre-intervention observations prior to 2012 to achieve greater balance in the length of pre-intervention and post-intervention observation periods. IRRs are from mixed effects negative binomial regression models of monthly dengue case counts (Jan 2012 –March 2020) by neighbourhood, with
an offset for population size, 6-monthly flexible cubic splines to account for seasonal effects, and a random effect for neighbourhood.

**Acknowledgments**

The authors acknowledge the municipality of Niterói for their partnership and logistical support for this study.

**Author Contributions**

**Conceptualization:** Sofia B. Pinto, Gabriel Sylvestre, Cameron P. Simmons, Peter A. Ryan, Scott L. O’Neill, Betina Durovni, Luciano A. Moreira.

**Data curation:** Thais I. S. Riback, Julia Peixoto, Stephanie K. Tanamas, Benjamin R. Green, Katherine L. Anders.

**Formal analysis:** Stephanie K. Tanamas, Suzanne M. Dufault, Katherine L. Anders.

**Funding acquisition:** Scott L. O’Neill, Luciano A. Moreira.

**Investigation:** Sofia B. Pinto, Thais I. S. Riback, Gabriel Sylvestre, Guilherme Costa, Julia Peixoto, Fernando B. S. Dias, Cameron P. Simmons, Peter A. Ryan, Scott L. O’Neill, Frederico C. Muzzi, Simon Kutcher, Jacqui Montgomery, Benjamin R. Green, Ruth Smithyman, Betina Durovni, Luciano A. Moreira.

**Methodology:** Sofia B. Pinto, Gabriel Sylvestre, Stephanie K. Tanamas, Suzanne M. Dufault, Peter A. Ryan, Scott L. O’Neill, Simon Kutcher, Jacqui Montgomery, Benjamin R. Green, Ruth Smithyman, Ana Eppinghaus, Valeria Saraceni, Betina Durovni, Katherine L. Anders, Luciano A. Moreira.

**Project administration:** Julia Peixoto, Frederico C. Muzzi, Simon Kutcher, Jacqui Montgomery, Ana Eppinghaus, Valeria Saraceni, Luciano A. Moreira.

**Resources:** Ana Eppinghaus, Valeria Saraceni.

**Software:** Benjamin R. Green.

**Supervision:** Sofia B. Pinto, Guilherme Costa, Luciano A. Moreira.

**Visualization:** Thais I. S. Riback, Julia Peixoto, Katherine L. Anders.

**Writing – original draft:** Sofia B. Pinto, Thais I. S. Riback, Guilherme Costa, Stephanie K. Tanamas, Betina Durovni, Katherine L. Anders, Luciano A. Moreira.

**Writing – review & editing:** Stephanie K. Tanamas, Cameron P. Simmons, Suzanne M. Dufault, Peter A. Ryan, Scott L. O’Neill, Katherine L. Anders.

**References**

1. Cattarino L, Rodriguez-Barraquer I, Imai N, Cummings DAT, Ferguson NM. Mapping global variation in dengue transmission intensity. Sci Transl Med. 2020; 12(528). https://doi.org/10.1126/scitransmed. aax4144 PMID: 31996463

2. Stanaway JD, Shepard DS, Undurraga EA, Halasa YA, Coffeng LE, Brady OJ, et al. The global burden of dengue: an analysis from the Global Burden of Disease Study 2013. Lancet Infect Dis. 2016; 16 (6):712–23. https://doi.org/10.1016/S1473-3099(16)00026-8 PMID: 26674619

3. Shepard DS, Undurraga EA, Halasa YA, Stanaway JD. The global economic burden of dengue: a systematic analysis. Lancet Infect Dis. 2016; 16(8):935–41. https://doi.org/10.1016/S1473-3099(16) 00146-8 PMID: 27091092
4. Bowman LR, Donegan S, McCall PJ. Is dengue vector control deficient in effectiveness or evidence?: systematic review and meta-analysis. PLoS Negl Trop Dis. 2016; 10(3):e0004551. https://doi.org/10.1371/journal.pntd.0004551 PMID: 26986468

5. Sim S, Ng LC, Lindsay SW, Wilson AL. A greener vision for vector control: The example of the Singapore dengue control programme. PLoS Negl Trop Dis. 2020; 14(8):e0008428. https://doi.org/10.1371/journal.pntd.0008428 PMID: 32853197

6. Wilson AL, Boelaert M, Kleinschmidt I, Pinder M, Scott TW, Tusting LS, et al. Evidence-based vector control? Improving the quality of vector control trials. Trends Parasitol. 2015; 31(8):380–90. https://doi.org/10.1016/j.pt.2015.04.015 PMID: 25999026

7. Aliota MT, Peinado SA, Velez ID, Osorio JE. The wMel strain of Wolbachia reduces transmission of Zika virus by Aedes aegypti. Sci Rep. 2016; 6:28792. https://doi.org/10.1038/srep28792 PMID: 27364935

8. Aliota MT, Walker EC, Uribe Yepes A, Velez ID, Christensen BM, Osorio JE. The wMel strain of Wolbachia reduces transmission of chikungunya virus in Aedes aegypti. PLoS Negl Trop Dis. 2016; 10(4):e0004677. https://doi.org/10.1371/journal.pntd.0004677 PMID: 27124663

9. Dutra HL, Rocha MN, Dias FB, Mansur SB, Caragata EP, Moreira LA. Wolbachia blocks currently circulating Zika virus isolates in Brazilian Aedes aegypti mosquitoes. Cell Host Microbe. 2016; 19(6):771–4. https://doi.org/10.1016/j.chom.2016.04.021 PMID: 27156023

10. Moreira LA, Iturbe-Ormaetxe I, Jeffery JA, Lu G, Pyke AT, Hedges LM, et al. A Wolbachia symbiont in Aedes aegypti limits infection with dengue, chikungunya, and Plasmodium. Cell. 2009; 139(7):1268–78. https://doi.org/10.1016/j.cell.2009.11.042 PMID: 20064373

11. Pereira TN, Rocha MN, Sucupira PHF, Carvalho FD, Moreira LA. Wolbachia significantly impacts the vector competence of Aedes aegypti for Mayaro virus. Sci Rep. 2018; 8(1):6889. https://doi.org/10.1038/s41598-018-25236-8 PMID: 29720714

12. van den Hurk AF, Hall-Mendelin S, Pyke AT, Frentiu FD, McElroy K, Day A, et al. Impact of Wolbachia on infection with chikungunya and yellow fever viruses in the mosquito vector Aedes aegypti. PLoS Negl Trop Dis. 2012; 6(11):e1892. https://doi.org/10.1371/journal.pntd.0001892 PMID: 23133693

13. Walker T, Johnson PH, Moreira LA, Iturbe-Ormaetxe I, Frentiu FD, McNemiman CJ, et al. The wMel Wolbachia strain blocks dengue and invades caged Aedes aegypti populations. Nature. 2011; 476(7361):450–3. https://doi.org/10.1038/nature10355 PMID: 21866159

14. Ye YH, Carrasco AM, Frentiu FD, Chenoweth SF, Beebe NW, van den Hurk AF, et al. Wolbachia reduces the transmission potential of dengue-infected Aedes aegypti. PLoS Negl Trop Dis. 2015; 9(6):e0003894. https://doi.org/10.1371/journal.pntd.0003894 PMID: 26151040

15. Indriani C, Tantowiyo W, Rances E, Andari B, Prabowo E, Yusdi D, et al. Reduced dengue incidence following deployments of Wolbachia-infected Aedes aegypti in Yogyakarta, Indonesia: a quasi-experimental trial using controlled interrupted time series analysis. Gates Open Res. 2020; 4:50. https://doi.org/10.12688/gatesopenres.13122.1 PMID: 32803130

16. O’Neill SL, Ryan PA, Turley AP, Wilson G, Retzki K, Iturbe-Ormaetxe I, et al. Scaled deployment of Wolbachia to protect the community from dengue and other Aedes transmitted arboviruses. Gates Open Res. 2018; 2:36. https://doi.org/10.12688/gatesopenres.12844.3 PMID: 30596205

17. Ryan PA, Turley AP, Wilson G, Hurst TP, Retzki K, Brown-Kenny J, et al. Establishment of wMel Wolbachia in Aedes aegypti mosquitoes and reduction of local dengue transmission in Cairns and surrounding locations in northern Queensland, Australia. Gates Open Res. 2019; 3:1547. https://doi.org/10.12688/gatesopenres.13061.2 PMID: 31667465

18. Utarini A, Indriani C, Ahmad RA, Tantowiyo W, Arguni E, Ansari MR, et al. Efficacy of Wolbachia-Infected Mosquito Deployments for the Control of Dengue. N Engl J Med. 2021; 384(23):2177–86. https://doi.org/10.1056/NEJMoa2009243 PMID: 34107180

19. Garcia GA, Sylvestre G, Aguiar R, da Costa GB, Martins AJ, Lima JBP, et al. Matching the genetics of released and local Aedes aegypti populations is critical to assure Wolbachia invasion. PLoS Negl Trop Dis. 2019; 13(1):e0007023. https://doi.org/10.1371/journal.pntd.0007023 PMID: 30620733

20. Gesto JSM, Ribeiro GS, Rocha MN, Dias FB, Peikoto J, Carvalho FD, et al. Reduced competence to arboviruses following the sustainable invasion of Wolbachia into native Aedes aegypti from Southeastern Brazil. Sci Rep. 2021; 11:10039. https://doi.org/10.1038/s41598-021-89409-8 PMID: 33976301

21. Durovni B, Saraceni V, Eppinghaus A, Riback TIS, Moreira LA, Jewell NP, et al. The impact of large-scale deployment of Wolbachia mosquitoes on dengue and other Aedes-borne diseases in Rio de Janeiro and Niteroi, Brazil: study protocol for a controlled interrupted time series analysis using routine disease surveillance data. F1000Res. 2019; 8:1328. https://doi.org/10.12688/f1000research.19859.2 PMID: 33447371

22. Costa GB, Smithyman R, O’Neill SL, Moreira LA. How to engage communities on a large scale? Lessons from World Mosquito Program in Rio de Janeiro, Brazil [version 1; peer review: 1 approved, 2
approved with reservations]. Gates Open Res. 2020; 4(109). https://doi.org/10.12688/gatesopenres.13153.2 PMID: 33103066

23. Garcia GA, Hoffmann AA, Maciel-de-Freitas R, Villela DAM. *Aedes aegypti* insecticide resistance underlies the success (and failure) of *Wolbachia* population replacement. Sci Rep. 2020; 10(1):63. https://doi.org/10.1038/s41598-019-56766-4 PMID: 31919396

24. Rocha MN, Duarte MM, Mansur SB, Silva B, Pereira TN, Adelino TER, et al. Pluripotency of *Wolbachia* against Arboviruses: the case of yellow fever. Gates Open Res. 2019; 3:161. https://doi.org/10.12688/gatesopenres.12903.2 PMID: 31259313

25. Hoffmann AA, Montgomery BL, Popovic J, Iturbe-Ormaetxe I, Johnson PH, Muzzi F, et al. Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. Nature. 2011; 476(7361):454–7. https://doi.org/10.1038/nature10356 PMID: 21866160

26. Dar M, Giesler T, Richardson R, Cai C, Cooper M, Lavasani S, et al. Development of a novel ozone-and photo-stable HyPer5 red fluorescent dye for array CGH and microarray gene expression analysis with consistent performance irrespective of environmental conditions. BMC Biotechnol. 2008; 8:86. https://doi.org/10.1186/1472-6750-8-86 PMID: 19014508

27. Ministry of Health Brazil (Health Surveillance Secretariat). Guia de Vigilância em Saúde: volume único. Available from: http://bvms.saude.gov.br/bvs/publicacoes/guia Vigilancia_saude_4ed.pdf.

28. Bernal JL, Cummins S, Gasparrini A. Interrupted time series regression for the evaluation of public health interventions: a tutorial. Int J Epidemiol. 2017; 46(1):348–55. https://doi.org/10.1093/ije/dyw098 PMID: 27283160

29. Nazni WA, Hoffmann AA, NoorAfizah A, Cheong YL, Mancini MV, Golding N, et al. Establishment of *Wolbachia* strain wAlbB in Malaysian populations of *Aedes aegypti* for dengue control. Curr Biol. 2019; 29(24):4241–8 e5. https://doi.org/10.1016/j.cub.2019.11.007 PMID: 31761702

30. Schmidt TL, Filipovic I, Hoffmann AA, Rasic G. Fine-scale landscape genomics helps explain the slow spatial spread of *Wolbachia* through the *Aedes aegypti* population in Cairns, Australia. Heredity (Edinb). 2018; 120(5):386–95. https://doi.org/10.1038/s41437-017-0039-9 PMID: 29358725

31. Hancock PA, Ritchie SA, Koenraadt CJM, Scott TW, Hoffmann AA, Godfray HCJ. Predicting the spatial dynamics of *Wolbachia* infections in *Aedes aegypti* arbovirus vector populations in heterogeneous landscapes. J Appl Ecol. 2019; 56(7):1674–86. https://doi.org/10.1111/1365-2664.13423

32. Jasper M, Schmidt TL, Ahmad NW, Sinkins SP, Hoffmann AA. A genomic approach to inferring kinship reveals limited intergenerational dispersal in the yellow fever mosquito. Mol Ecol Resour. 2019; 19(5):1254–64. https://doi.org/10.1111/1755-0998.13043 PMID: 31125998

33. Mancini MV, Ant TH, Herd CS, Gingell DD, Murdochy SM, Mararo E, et al. High temperature cycles result in maternal transmission and dengue infection differences between *Wolbachia* strains in *Aedes aegypti*. bioRxiv. 2020.

34. Ross PA, Wiwatantaratanabutr I, Axford JK, White VL, Endersby-Harshman NM, Hoffmann AA. Wolbachia infections in *Aedes aegypti* differ markedly in their response to cyclical heat stress. PLoS Pathog. 2017; 13(1):e1006006. https://doi.org/10.1371/journal.ppat.1006006 PMID: 28056065

35. Ross PA, Ritchie SA, Axford JK, Hoffmann AA. Loss of cytoplasmic incompatibility in *Wolbachia*-infected *Aedes aegypti* under field conditions. PLoS Negl Trop Dis. 2019; 13(4):e0007357. https://doi.org/10.1371/journal.pntd.0007357 PMID: 31002720