STUDY PROTOCOL
The impact of large-scale deployment of Wolbachia mosquitoes on arboviral disease incidence in Rio de Janeiro and Niterói, Brazil: study protocol for a controlled interrupted time series analysis using routine disease surveillance data [version 1; peer review: 1 approved, 1 approved with reservations]

Betina Durovni1,2, Valeria Saraceni3, Ana Eppinghaus4, Thais I.S. Riback2, Luciano A. Moreira2,5, Nicholas P. Jewell6,7, Suzanne M. Dufault3,6, Scott L. O’Neill8, Cameron P. Simmons8, Stephanie K. Tanamas8, Katherine L. Anders8

1Centre for Strategic Studies, Fiocruz, Rio de Janeiro, Brazil
2World Mosquito Program, Fiocruz, Rio de Janeiro, Brazil
3City Health Secretariat, Rio de Janeiro, Brazil
4City Health Secretariat, Niterói, Brazil
5Instituto Rene Rachou, Fiocruz, Belo Horizonte, Brazil
6Division of Epidemiology and Biostatistics, School of Public Health, University of California, Berkeley, Berkeley, CA, USA
7Centre for Statistical Methodology, London School of Hygiene & Tropical Medicine, London, UK
8World Mosquito Program, Institute of Vector Borne Disease, Monash University, Melbourne, VIC, Australia

Abstract
Background: Rio de Janeiro and Niterói municipalities in southeastern Brazil experience large dengue epidemics every 2 to 5 years, with >100,000 cases notified in epidemic years. Costs of vector control and direct and indirect costs due to the dengue, chikungunya and Zika were estimated to total $650 million USD in 2016, but traditional vector control strategies have not been effective in preventing arboviral disease outbreaks. The Wolbachia method is a novel and self-sustaining approach for the biological control of arboviral diseases, in which the transmission potential of Aedes aegypti mosquitoes is reduced by stably transfecting them with the Wolbachia bacterium. This paper describes a study protocol for evaluating the effect of large-scale non-randomised releases of Wolbachia mosquitoes on the incidence of dengue, Zika and chikungunya in the municipalities of Niterói and Rio de Janeiro. This follows a lead-in period since 2014 involving intensive community engagement, regulatory and public approval, entomological surveys, and small-scale pilot releases.

Method: The planned releases during 2017-2019 cover a combined area of 121 km² with a resident population of 1.1 million, across the two cities. Untreated areas with comparable historical dengue profiles and sociodemographic characteristics have been identified a priori as
comparative control areas in each municipality. The proposed pragmatic epidemiological approach combines a controlled interrupted time series analysis of routinely notified suspected and laboratory-confirmed arboviral cases, together with monitoring of arbovirus activity utilising outbreak signals routinely used in public health disease surveillance.

**Discussion:** If the current project is successful, this model for control of arboviral disease through Wolbachia releases can be expanded nationally and regionally.

**Keywords**
Wolbachia, dengue, chikungunya, Zika, vector-borne disease, disease surveillance, controlled interrupted time series, Brazil

This article is included in the Disease Outbreaks gateway.

**Corresponding author:** Katherine L. Anders (katie.anders@worldmosquito.org)

**Author roles:**
- **Durovni B:** Conceptualization, Investigation, Methodology, Project Administration, Writing – Original Draft Preparation
- **Saraceni V:** Conceptualization, Investigation, Writing – Original Draft Preparation
- **Eppinghaus A:** Investigation, Writing – Review & Editing
- **Riback TIS:** Investigation, Methodology, Writing – Review & Editing
- **Moreira LA:** Conceptualization, Funding Acquisition, Investigation, Resources, Writing – Review & Editing
- **Jewell NP:** Conceptualization, Formal Analysis, Methodology, Writing – Review & Editing
- **Dufault SM:** Formal Analysis, Methodology, Writing – Review & Editing
- **Simmons CP:** Conceptualization, Methodology, Writing – Review & Editing
- **Tanamas SK:** Conceptualization, Methodology, Writing – Original Draft Preparation
- **Anders KL:** Conceptualization, Methodology, Writing – Review & Editing

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Abbreviations
BG trap: BG-Sentinel trap; IBGE: Instituto Brasileiro de Geografia e Estatística (Brazilian Institute of Geography and Statistics); ITS: interrupted time series; MoH: Ministry of Health; PAHO: Pan American Health Organisation; PCR: polymerase chain reaction; qPCR: quantitative polymerase chain reaction; RCT: randomised controlled trial; SINAN: Sistema De Informação De Agravos De Notificação (Brazilian National Notifiable Diseases Information System); WHO: World Health Organisation

Background
The global incidence of dengue has increased dramatically in recent decades. Although cases are underreported, it is estimated that 390 million dengue virus infections occur every year, and of these 96 million have clinical manifestations of dengue or severe dengue. Globally, 3.9 billion people in 128 countries are at risk of infection. The primary vector of dengue is the Aedes aegypti mosquito, which is also capable of transmitting other arboviruses including chikungunya, Zika, yellow fever and Mayaro.

The first reported dengue outbreak in Brazil was in 1845, with subsequent outbreaks in 1880–1912 and 1916–1923. As a result of a coordinated effort from the Pan American Health Organization (PAHO) and the World Health Organization (WHO) to eradicate Ae. aegypti, Brazil was considered free of the mosquito in 1955, but the vector was reintroduced into the country two decades later. In 1986, dengue virus serotype 1 (DENV1) was introduced to Rio de Janeiro and an estimated 1 million people were infected. Since then, dengue has become a major public health problem. From 1986 to 1993, outbreaks occurred approximately every 2–5 years, and from 1993 dengue became endemic with seasonal peaks in cases during the rainy season (December to May), but with ongoing transmission throughout the year. Between 2000 and 2007, more than 3 million dengue cases were reported (caused by DENV serotypes 1, 2 and 3) and in 2010, DENV4 re-emerged after 28 years of absence.

The recent introduction of Zika and chikungunya in dengue hyperendemic areas of Brazil has aggravated the situation. The overlapping clinical features, absence of serological assays for the Zika virus that can reliably distinguish between acute disease and past exposure, and the association of pregnancy-associated Zika virus infection with microcephaly and other neurologic complications represents a great challenge for public health that will require new strategies and innovations.

Between 2016 and 2017, 762 deaths were attributed to severe dengue in Brazil. Additionally, in 2017, 127 deaths were confirmed to be caused by chikungunya. Yellow fever has spread from the North of Brazil to the Southeast over the last years, affecting humans and non-human primates. From July 2017 to April 2018, 1,266 cases of yellow fever including 415 deaths were confirmed in Brazil, with 223 cases and 73 deaths occurring in Rio de Janeiro State. No autochthonous cases were reported in Rio de Janeiro city or Niterói, although some residents from those cities acquired yellow fever while traveling to other places in Brazil. A mass vaccination campaign against yellow fever began in 2016.

The costs of vector control, direct medical costs, and indirect costs related to dengue, Zika and chikungunya in Brazil were estimated to be 2.3 billion Brazilian reais ($650 million USD) in 2016. In the absence of an effective vaccine for these arboviruses, disease prevention depends on vector control. Vector control guidelines in Brazil are focused on elimination or larvicide treatment of mosquito breeding sites and the control of adult mosquito populations with insecticides sprayed as ultra-low volume. The limited potential of these traditional vector control strategies to achieve large-scale and sustained reductions in dengue incidence is evidenced by the continuing public health burden of dengue throughout endemic areas where these measures are routinely employed, and the lack of robust efficacy data from well-designed trials to inform their optimal implementation.

The Wolbachia method (www.worldmosquito.org) is a novel, natural, and self-sustaining approach to reduce arboviral diseases transmitted by Ae. aegypti mosquitoes. The symbiotic Wolbachia bacterium is found naturally in over 60% of insect species, but not in Ae. aegypti, and is passed from one generation to the next through the insect’s eggs. Stable transfection of Wolbachia into a local Ae. aegypti colony in the laboratory produces a lineage of Wolbachia-carrying mosquitoes which, upon release over several weeks, can achieve dissemination of Wolbachia into the local Ae. aegypti population through the processes of maternal inheritance and cytoplasmic incompatibility that give Wolbachia-carrying mosquitoes a reproductive advantage. The DENV-transmitting potential of Wolbachia infected mosquitoes is reduced by 66–75%, a phenotype which has been shown to persist in field mosquito populations up to five years after the end of releases. Mathematical modelling of this reduced transmissibility predicts a substantial and sustained reduction in dengue incidence in human populations where Wolbachia is established. Laboratory data indicate a similar reduction in the competence of Wolbachia-carrying Ae. aegypti for transmitting other viruses including Zika, chikungunya, yellow fever and Mayaro.

With releases now conducted in eight countries over the past eight years, the World Mosquito Program has demonstrated that Wolbachia can be successfully established and maintained in both small-scale and large-scale urban settings in multiple ecological environments.

A core objective of these releases has been to ensure strong community acceptance and government support for the approach, achieved through embedding community and stakeholder engagement within the project activities in each site. Observational evidence of the impact of Wolbachia releases on arboviral disease in pilot sites has been encouraging, with no evidence of local dengue transmission where Wolbachia has established at high levels. Following city-wide deployment in Townsville, Australia, there has been no confirmed local dengue transmission in Wolbachia-treated areas for four seasons since completion of releases, despite local transmission every year for the prior 13 years and ongoing importation of DENV infection in travelers. A cluster randomized controlled trial (RCT) to generate a robust and quantiative estimate of the impact of
Wolbachia on dengue incidence commenced in Yogyakarta, Indonesia in 2017, with reporting of results expected in 2021[23].

In Brazil, planned scale up of Wolbachia deployments from demonstration projects in Rio de Janeiro and Niterói municipalities to large-scale releases was accelerated by the declaration of Zika as a public health emergency by the WHO in early 2016, and the recommendation by WHO’s Vector Control Advisory Group in March 2016 that the Wolbachia method be evaluated in rigorously monitored pilot deployments under operational conditions, to build evidence of epidemiological effectiveness against Aedes-borne viruses[24]. Given the imperative from stakeholders and funders to scale up deployment within a relative short time frame, and to retain sufficient flexibility to optimize methods for large-scale deployment in the varied micro-environments within Niterói and Rio de Janeiro, an RCT or other carefully controlled deployment was not considered feasible. Instead releases under operational conditions, and with pragmatic evaluation of disease impact using data routinely collected for public health purposes, was favoured.

Here we describe a protocol for evaluating the effect of large-scale non-randomized Wolbachia releases on the incidence of dengue, Zika and chikungunya in the municipalities of Niterói and Rio de Janeiro. The proposed strategy employs a controlled interrupted time series analysis of routinely notified suspected and laboratory-confirmed arboviral cases, together with monitoring of arbovirus activity with outbreak signals routinely used in public health disease surveillance. This methodology allows measurement of the impact of the intervention at the population level over time, accounting for the seasonal trends and inter-annual fluctuations often observed in dengue and other arboviral disease incidence.

Methods
Study design
The aim of this epidemiological study is to test the hypothesis that the establishment of Wolbachia in local Ae. aegypti populations in Rio de Janeiro and Niterói leads to a reduction in arboviral disease burden.

The impact of Wolbachia deployment on arboviral disease incidence will be evaluated using routine notifiable disease surveillance data to describe associations between temporal and spatial trends in arbovirus disease and the deployment of Wolbachia across Niterói and Rio de Janeiro, with two objectives:

1. Estimate the reduction in dengue incidence in the aggregate treated areas of Niterói and Rio de Janeiro compared to an untreated control area, and in each treated zone compared to the untreated control area, each year for five years after Wolbachia establishment.

2. Quantify the occurrence of dengue outbreak signals in Wolbachia treated areas compared to untreated areas in Niterói and Rio de Janeiro, for five years after Wolbachia establishment, using outbreak indicators employed routinely for public health monitoring of dengue activity: i) control diagrams comparing the weekly dengue incidence (five-week moving average) against the five-year historical average in that same area; and ii) outbreak incidence threshold of 300/100,000 population in any month.

Study setting and population
Rio de Janeiro and Niterói cities are located in the State of Rio de Janeiro, Brazil. Niterói has an area of 134 km² and a population of 487,562 in 2010. Rio de Janeiro is the second largest city in Brazil with 6,320,446 inhabitants in 2010 and an area of 1,200 km². The two cities sit on opposite sides of the Guanabara Bay and are linked by a long bridge, which transports a large commuter population between Niterói and Rio de Janeiro.

The cities are divided into health districts, for the purpose of planning and delivering care - ten in Rio de Janeiro and seven in Niterói. In Rio de Janeiro, Wolbachia deployments will be conducted in one of the administrative areas of the city (Figure 1; produced in ArcMap version 10.5, ESRI, CA), in an area of approximately 90 km² and with 886,551 inhabitants, 39% of whom live in slums. The total release area in Niterói is approximately 31 km² covering a population of 268,536 (Table 1).

Figure 1. Map of (a) Rio de Janeiro and (b) Niterói Wolbachia-treated and untreated areas (produced in ArcMap version 10.5, ESRI, CA).
Table 1. Sociodemographic characteristics of *Wolbachia* treated and untreated areas, Niterói and Rio de Janeiro (source: 2010 Brazil population census).

<table>
<thead>
<tr>
<th>Neighbourhoods</th>
<th>Population</th>
<th>Area (km²)</th>
<th>Population density</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Niterói</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zone 1</td>
<td>4</td>
<td>23,747</td>
<td>9.2</td>
</tr>
<tr>
<td>Zone 2</td>
<td>11</td>
<td>68,695</td>
<td>50.6</td>
</tr>
<tr>
<td>Zone 3</td>
<td>13</td>
<td>178,891</td>
<td>12.6</td>
</tr>
<tr>
<td>Non-release control area</td>
<td>24</td>
<td>216,229</td>
<td>62.1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>52</td>
<td>487,562</td>
<td>134.5</td>
</tr>
<tr>
<td><strong>Rio de Janeiro</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zone 1</td>
<td>10</td>
<td>107,130</td>
<td>11.8</td>
</tr>
<tr>
<td>Zone 2</td>
<td>6</td>
<td>150,646</td>
<td>33.5</td>
</tr>
<tr>
<td>Zone 3.1</td>
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<td>32.6</td>
</tr>
<tr>
<td>Zone 3.2</td>
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<td>220,739</td>
<td>12.0</td>
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<tr>
<td>Non-release control area</td>
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<tr>
<td>Non-release area</td>
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<td>996.5</td>
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<tr>
<td><strong>Total</strong></td>
<td>160</td>
<td>6,320,446</td>
<td>1203.7</td>
</tr>
</tbody>
</table>

For the purposes of *Wolbachia* deployment, the Rio de Janeiro and Niterói intervention areas are divided into 4 and 3 release zones, respectively, which are aligned with neighbourhood administrative boundaries.

In Rio de Janeiro, two administrative areas adjacent to the release area have been designated *a priori* as a comparative control zone (Figure 1), based on comparable sociodemographic characteristics and synchronous historical dengue time series (Figure 2 and Table 1). In Niterói, the remaining untreated area of the city has been designated as the comparative control zone (Figure 1).

**Wolbachia release and monitoring**

Staged *Wolbachia*-mosquito deployments will be implemented in Niterói and Rio de Janeiro, in order to achieve *Wolbachia* establishment across the two municipalities. Pilot releases commenced in late 2015, and city-wide deployments are ongoing through to the end of 2019. *Wolbachia*-containing adult mosquitoes will be released at one location per 50 x 50 meter grid square for a minimum of 16 consecutive weeks in each release zone. In areas where *Wolbachia* frequency remains low after 16 weeks of releases, or where particularly high wild type *Ae. aegypti* populations are observed, *Wolbachia*-containing mosquito eggs will also be released to complement adult releases with the aim of accelerating *Wolbachia* establishment. Monitoring of *Wolbachia* frequency will be done using BG-Sentinel mosquito traps (BioGents, Germany), distributed throughout the release area at a density of 16 traps per km². Traps will be serviced weekly and all collected mosquitoes identified morphologically by microscopy. From eight weeks after the start of releases, a maximum of 10 *Ae. aegypti* (male and female) per trap will be tested individually for *Wolbachia* using quantitative polymerase chain reaction (qPCR). *Wolbachia* screening will be performed biweekly during releases and until establishment, then every 1–3 months thereafter. All surplus *Ae. aegypti* will be biobanked. Mosquito collection and screening results will be stored in a custom designed web-based data repository. The *Wolbachia* prevalence in screened *Ae. aegypti* will be reported aggregated to each release zone, calculated as the total number of *Ae. aegypti* mosquitoes that tested positive for *Wolbachia* aggregated across all BG traps in the zone, divided by the total number of *Ae. aegypti* that were screened in that zone.

**Epidemiological data sources**

The two proposed strategies for the evaluation of the impact of large-scale *Wolbachia* deployments on arboviral disease incidence in Niterói and Rio de Janeiro municipalities make use of existing data on dengue, chikungunya and Zika case notifications to the Brazilian national disease surveillance system (SINAN). Dengue surveillance has been in place since its re-emergence in 1986 and data is available from the SINAN system since 2000. Zika and chikungunya became notifiable diseases in 2015.

Suspected cases of dengue and other arboviral diseases are required to be reported to the city health department, according to a case definition of fever plus two other symptoms including malaise, headache, myalgia, nausea, vomiting, cutaneous rash, and arthralgia. Dengue case notifications include an indication of disease severity (dengue, dengue with alarm signals, severe dengue, fatal dengue). A variable proportion of notified suspected cases are tested by IgM serology or
PCR, following the Brazilian guidelines and the timing between onset of symptoms and blood collection. The number of cases in a given period of time may limit the availability of tests, and PCR testing is routinely performed only for severe and fatal cases, pregnant women and young children. In 2016, 4.8% of notified dengue cases in Rio de Janeiro and 11.5% in Niterói were supported by a positive IgM serology result, and only 0.2% of notified cases in Rio and 0.03% in Niterói had a positive PCR result. The ability to confirm dengue cases by serology is impaired since the Zika outbreak due to serological cross-reactivity between the dengue and Zika viruses. As PCR testing is performed only in certain patient populations, the proportion positive is unlikely to be generalisable to all notified cases. Therefore, for the purpose of our analyses we will use all notified dengue cases (suspected and laboratory-confirmed) as the primary endpoint.

In the absence of a reliable serological test that does not cross-react with dengue, Zika lab diagnosis is done solely on the basis of molecular detection (real-time PCR) up to the first 5 days in serum and 15 days in urine. This has severely limited the ability to confirm Zika virus infection among notified cases (3.5% in 2016, 19.3% in 2017).

Chikungunya diagnoses can be confirmed either through PCR or serology, as it does not cross-react with Zika or dengue. The proportion of notified cases with supportive laboratory findings is higher than for dengue: 31.9% and 18.2% in Rio and Niterói, respectively, in 2016.

Anonymized disaggregate (line-listed) data on notified suspected and laboratory-confirmed dengue, chikungunya and Zika cases will be extracted from the SINAN system for the historical pre-intervention period (2000–2016 for dengue, and 2015–2016 for chikungunya and Zika) and the prospective post-intervention period (2017–2023). The dataset will include age, sex, neighbourhood of primary residence, date of illness onset, date of notification, reporting health clinic, disease severity, hospitalisation, death, and where available, geocoordinates of primary residence, type of diagnostic test performed, diagnostic test result, and final diagnostic classification. Population data from the Brazilian census (IBGE) and population by neighbourhood of residence obtained from the municipalities of Rio and Niterói will be used to estimate the population in each release zone. The incidence rate (number of new dengue, Zika or chikungunya cases divided by the population at risk) will be expressed per 100,000 inhabitants.

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**Figure 2.** Dengue incidence in Wolbachia-treated vs untreated areas of (a) Rio de Janeiro (2000–2017) and (b) Niterói (2007–2016) (source: Brazilian National Disease Surveillance System (SINAN)).
Controlled interrupted time series analysis

Interrupted time series (ITS) analysis is a valuable study design for evaluating the effectiveness of a population-level health intervention that is implemented at a clearly defined point in time. It uses a set of historical observations of an outcome of interest (in this context monthly arboviral case notifications) to establish an underlying trend, which is assumed to be ‘interrupted’ by the introduction of an intervention (in this case *Wolbachia* releases). Descriptive analyses of seasonal and inter-annual trends in arboviral disease notifications, by release zone and municipality, will first be made. Comparison of the trend in monthly case notifications in the post-intervention period with the hypothetical scenario of no intervention (the ‘counterfactual’, inferred from the historical time series and untreated control area), provides an estimate of the intervention effect. Segmented regression using an appropriately defined impact model (e.g. negative binomial regression for autocorrelated count data with population offset, assuming a step change post-intervention) will be used to estimate the effect of *Wolbachia* releases on the arboviral disease endpoints in each release zone, and for the aggregate release areas in each municipality. In Rio and Niterói, the availability of comparative control areas – well-matched to the release area in socioeconomic characteristics and historical dengue incidence (Figure 2) – permits a robust, controlled analysis in which the confounding effects of seasonality and inter-annual variability can be adjusted for. Zone-level ITS analyses will be performed 12 months after completion of releases in each zone, and each 12 months thereafter, with release zones considered ‘treated’ for the purpose of this analysis based on completion of releases, regardless of the long-term *Wolbachia* monitoring results. Aggregate release-area level analyses will be performed for each municipality 12 months after completion of releases in the last zone and each 12 months thereafter.

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* deployment, 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 additional 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-intervention 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.

The primary endpoint for the ITS analysis will be dengue cases notified to the disease surveillance system. The secondary endpoints will be: a) the count of severe dengue cases reported to the surveillance system, b) the count of fatal dengue cases reported to the surveillance system, and c) Zika and chikungunya cases notified to the disease surveillance system.

Although the historical time series for Zika and chikungunya incidence is short, we will nonetheless describe the incidence during and after *Wolbachia* deployments relative to the *a priori* defined non-release control areas for both Niterói and Rio de Janeiro.

Dengue outbreak signals

As a complementary approach for evaluating the public health impact of large-scale *Wolbachia* releases, we will also use the following dengue outbreak alert tools routinely used in public health practice. We hypothesise that these dengue outbreak signals will not be triggered in areas where *Wolbachia* has been established.

1. Control diagram (endemic channel)

The definition of a dengue outbreak or epidemic has changed over time in Brazil. In Rio de Janeiro and Niterói, a control diagram is currently used to monitor dengue incidence. Briefly, the control diagram is constructed from a five-week centered moving average of weekly notified dengue incidence for the past five years excluding epidemic years. An early signal of a dengue outbreak/epidemic is triggered when the weekly incidence of dengue crosses the upper limit of the control diagram, with the upper limit defined as [mean+(standard deviation*1.96)]. Incidence that remains above the upper limit of the control diagram for two or more consecutive weeks constitutes a dengue outbreak. For the purpose of monitoring the impact of *Wolbachia* releases on dengue, we will construct annual control diagrams with weekly dengue incidence, by city and for each release and non-release zone, to monitor the occurrence of dengue outbreaks. The number of dengue outbreak signals triggered per year will be reported.

2. Classical incidence threshold

Another outbreak definition that has been used by the Ministry of Health (MoH) in previous years is a dengue incidence threshold of ≥300 cases/100,000 population in a given month. Although not included in current MoH guidelines, this provides an alternative endpoint for evaluating dengue activity at a population level in the post-intervention period, compared with pre-intervention, and we will report the number of months in a given year where dengue incidence crosses this threshold.
Current study status
This study is ongoing. Wolbachia releases are expected to be completed by the end of 2019, and the collation and analysis of disease surveillance data will continue until 2023.

Dissemination of study results
Based on the results of the power estimation above, the study outcome will be evaluated and reported two years after the completion of releases. The findings will be submitted for peer review and publication in an appropriate open access journal, together with aggregate supporting data.

Discussion
The municipalities of Rio de Janeiro and Niterói in southeastern Brazil have been affected by dengue for more than 30 years, with epidemics occurring every 2 to 5 years. In recent years, outbreaks of the other Aedes-borne diseases chikungunya and Zika have presented further public health challenges, and since July 2017 at least 221 human cases of yellow fever have been confirmed in Rio de Janeiro State, including in urban areas. Vector control strategies, based on elimination of mosquito breeding sites and use of insecticides to reduce adult populations, have not been effective in preventing dengue outbreaks. The Wolbachia method is a novel and self-sustaining approach for the biological control of arboviral diseases. The signature feature of Wolbachia is to reduce the arbovirus-transmission potential of Wolbachia-infected mosquitoes. The World Mosquito Program is deploying Wolbachia-infected Aedes aegypti mosquitoes in Brazil in the purpose of achieving a large-scale and sustained reduction in arboviral disease burden in urban areas where these diseases are public health priorities.

In March 2016, the WHO convened a Vector Control Advisory Group to review new and existing vector control tools for use in the response to the Zika virus outbreak. Based on the available evidence that Wolbachia reduces the Zika, dengue and chikungunya transmission potential of Ae. aegypti mosquitoes and field data showing long-term establishment of Wolbachia in mosquito populations in a range of environmental settings, the WHO recommended carefully monitored pilot implementation of the Wolbachia method in affected countries.

While RCTs are still considered the gold standard, they are not always feasible or agreeable to the community and government. The controlled ITS analysis is a quasi-experimental design that is commonly used to evaluate population-level public health interventions and is a pragmatic alternative design where an RCT is considered infeasible, particularly in the presence of a well-matched untreated control area. Given the public health emergency posed by the Zika epidemic at the time of this study’s inception and the need to scale up Wolbachia deployment in Rio de Janeiro and Niterói within a relatively short timeframe, an RCT or other carefully controlled deployment was not considered feasible. The controlled ITS is appropriate for the pragmatic evaluation of large-scale Wolbachia deployments given the long and reliable time series of dengue mandatory reporting data from both Rio de Janeiro and Niterói that allows for a longitudinal assessment of dengue trends before and after the Wolbachia intervention. Assessment of an impact of the intervention on chikungunya and Zika may be more difficult given their shorter time series.

Notifiable disease surveillance data can be limited by a lack of specificity in case definitions and inconsistent reporting practices, which may influence our ability to detect a true intervention effect on arboviral disease incidence. A subset of notified dengue cases are supported by laboratory diagnostic results, but these have several limitations: i) laboratory testing occurs infrequently (<15% of notified cases), particularly during outbreaks, ii) the cross-reactivity of IgM serology between dengue and Zika limits the utility of serological data since 2015, and iii) the restricted use of PCR in only certain patient populations limits the generalisability of PCR-positivity rates to all notified cases. We therefore base our analyses on all notified cases (suspected and confirmed). Benefits of using these routinely collected data include the availability of a long time series, reduced costs for data collection and timely acquisition of data.

Human mobility also presents a challenge to this study as individuals are likely to spend time in both Wolbachia-treated and untreated areas, making it difficult to determine the place of illness acquisition among notified cases of arboviral disease. Travel between areas means that the true Wolbachia exposure status of individuals resident in Wolbachia-treated and untreated areas becomes more similar, thereby diluting the observed intervention effect towards the null.

The introduction of Zika and chikungunya viruses brought new challenges to health surveillance and a greater willingness for better vector control in affected regions. With the re-emergence of yellow fever in Brazil, there is even more potential public health benefit if the Wolbachia intervention successfully reduces the vector competence of Ae. aegypti mosquitoes in the field and reduces arboviral disease incidence. No specific treatment for dengue, chikungunya or Zika currently exists. Although a vaccine against dengue (Sanofi Dengvaxia) was licensed in 2015, it was recently found to enhance the severity of subsequent dengue infection in individuals who were seronegative at the time of vaccination. As a result, a serology test prior to the administration of the vaccine is required to confirm previous dengue infection, increasing costs and decreasing feasibility in high-burden areas.

Releases of Wolbachia are completed or underway in eight countries, with no evidence of local transmission of dengue, Zika or chikungunya in places where Wolbachia is established at high levels. The implementation of the Wolbachia intervention is complex and has not been done on a large scale in very densely populated urban areas in the Americas before. The implementation of the project was preceded by careful work to engage the community and gain public acceptance for the intervention, even in the most difficult contexts of poverty and urban violence present in both Rio de Janeiro and Niterói. Engagement, entomological monitoring, and public health impact assessment...
activities were developed in close partnership with local governments. If the current project is successful, this model can be expanded to the rest of the country and the Americas.

Ethics approval and consent to participate
The study was approved by the Brazilian Institutional Review Board (Conep) (CAAE: 59175616.2.0000.0008). The study uses pre-existing non-identifiable disease surveillance data, which does not require individuals’ consent.

Data availability
No data is associated with this article.

Grant information
This study is funded by the Bill and Melinda Gates Foundation (OPP1099252); the Brazilian Ministry of Health (DECI; TED 09/2016); and the US Centers for Disease Control (CDC).

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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References


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Adeshina I. Adekunle
Australian Institute of Tropical Health and Medicine, James Cook University, Townsville, Australia

Review:
The authors present the study protocol for an ongoing large deployment of *Wolbachia*-infected *Aedes-aegypti* mosquitoes in Rio de Janeiro and Niteroi municipalities in southeastern Brazil where *Aedes*-borne diseases, especially dengue, are common. The study design is a quasi-experimental study (QES) that allows the application of the controlled interrupted time series analysis to evaluate whether *Wolbachia* strategy is effective in this region. The manuscript is well written and the study, when completed, has great potential for evaluating the promising *Wolbachia* strategy for controlling *Aedes*-borne diseases. However, I have some concerns about this study and the ability of the interrupted time series method to infer reduction in disease burden if present:

**Main comments:**
- *Aedes-aegypti* mosquitoes movement.
First of all, the authors failed to indicate the strain of *Wolbachia* used in this study. Different strains of *Wolbachia* have different characteristics that enable them to invade the wild type population and establish themselves. The *wMel* strain was used in Townsville, Australia¹ and was claimed to be successful. However, with climate change and variability in temperature, some *Wolbachia*-infected mosquitoes with different strains, include *wMel*, cannot maintain their *Wolbachia* status². Hence, even if the intervention works, an introduction of infected patients into such a population will result in an outbreak and all the gains from *Wolbachia* strategy would have been lost as the majority of the mosquitoes are now uninfected³.

Figure 1 in the manuscript shows the map of the treated and untreated zones. These are adjacent zones, especially for the treated and untreated zones. Mosquitoes movement from untreated zone to treated zone could prevent *Wolbachia* invasion⁴ and vice versa. Hence, and since this is a study that takes a few years, mosquitoes migration cannot be ruled out. Thus, the control zones (untreated) is not suitable anymore.
- The controlled interrupted time-series method.
The authors stated that the reason for favoring quasi-experimental design (QED) over randomized controlled trial (RCT) was due to time constraints as stakeholders and funders wanted to scale-up the
deployment of Wolbachia to accelerate the control of the emergent Zika virus in this region. However, in this set-up, to carefully randomize zones as treated or not should not take much time. In fact, since the incidence of Aedes-borne virus infections is similar between treated and untreated zones (Figure 2), that should be a motivation for randomizing the zones. I am thinking there are other reasons for ditching RCT. The authors should elaborate on this.

For the controlled interrupted analysis, the authors plan to perform first, the descriptive analysis of the seasonal and inter-annual trends in the Aedes-borne infection notifications. It will be great if the authors are more specific about the types of descriptive analysis they intend to carry out. Also, a schematic of the expected trend in the notifications accounting for confounders will greatly help the protocol.

Minor comments:

Title
1. The title indicated controlling “arboviral disease incidence”. The authors can be more specific if they change that to either “Aedes-borne disease incidence” or something more specific.

Abstract
1. I think the clause in the fourth line will flow better if “Aedes-borne diseases dengue, chikungunya and Zika” is changed to “Aedes-borne diseases (dengue, chikungunya and Zika).
2. Change “km2” to “km²”.

Methods
1. Figure 2 shows the comparative incidence of dengue for the treated and untreated regions. What about Zika and Chikungunya that the study is trying to control too? I think this is a dengue control study that has the potential to control Zika and Chikungunya in these regions. It will be great if the authors make it appear so. As it is, it seems to be a study deliberately targeting all the three Aedes-borne diseases.

References

Is the rationale for, and objectives of, the study clearly described?
Yes

Is the study design appropriate for the research question?
Yes

Are sufficient details of the methods provided to allow replication by others?
Yes
Are the datasets clearly presented in a useable and accessible format?
Not applicable

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Infectious disease modelling and bio-statistics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 07 November 2019

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Liz Turner
Department of Biostatistics and Bioinformatics, Duke University, Durham, NC, USA

This very well written manuscript describes the protocol for a quasi-experimental study using controlled interrupted time series. The study is currently being conducted in Brazil. The selected design is an innovative use of the controlled interrupted time series which can leverage routinely collected surveillance data to seek to address causal questions of interest. Moreover, the intervention being evaluated, namely the deployment of *Wolbachia* mosquitoes with the goal to reduce arboviral disease incidence, is innovative and shows great promise.

I have two main comments:

- A desire to see more justification for why randomization was not possible. The difficulties of randomization in this setting are mostly described but, as you'll see from my more detailed comments below, it’s not absolutely convincing that randomization would not have been possible here, even within the interrupted time series design.

- Because the measurement of incidence of the arboviruses of interest is mostly through surveillance data and it may be difficult to blind communities to the use of *Wolbachia*, how can it be determined that in the treatment zones post-*Wolbachia* release any smaller incidence is not just related to the fact that there is lower reporting as community members expect the incidence of those viruses to go down? I have a big concern about the possibility of differential assessment of outcomes in treated and control zones. Can the authors elaborate on this in relevant places in the text?

Here I offer some more minor points that may help with clarity for the reader:

**Abstract**

- It could help the reader if when “dengue, chikungunya and Zika” are first mentioned in the abstract, they are also named as arboviruses i.e. to help readers not necessarily familiar with them to be certain that all three fall in to this class of viruses.

**Background**
Based on the title referring to arboviruses, as a reader, I was expecting the first sentence or so to at least mention of arbovirus. Would it be possible to do so? This could be achieved very easily by saying something like “The global incidence of the arbovirus dengue has increased dramatically in recent decades.”

In the 4th paragraph referring to yellow fever, please clarify if it is an arbovirus.

The last sentence of the 6th paragraph: “Laboratory data indicate a similar reduction in the competence of Wolbachia-carrying Ae. aegypti for transmitting other viruses including Zika, chikungunya, yellow fever and Mayaro”. If all listed are arboviruses, please edit “viruses” to “arboviruses”.

In the 9th paragraph, I very much appreciate the rationale that there was demand for scale-up of implementation of the Wolbachia releases. I would appreciate a little more clarity on why randomization was not possible since, given that not all areas of the study will receive the treatment during the study, the scale-up argument doesn't seem sufficient to justify no randomization. I can very well imagine that there are many compelling reasons to not randomize, some of which are alluded to, and so it would help to be even clearer about this e.g. was it because of the existing partnerships and infrastructure making certain areas better for release than others? What else? Some of this is sort of implied in the text and it would be valuable to have just a few more explicit sentences regarding this.

Related to my previous comments on arboviruses, in the 10th paragraph where it is stated: “Here we describe a protocol for evaluating the effect of large scale non-randomized Wolbachia releases on the incidence of dengue, Zika and chikungunya in the municipalities of Niterói and Rio de Janeiro.”, again, it could really help the reader to edit this sentence to something like “…on the incidence of three arboviruses, namely dengue, Zika and chikungunya, in the……”.

Also, see a later comment based on the discussion as to why yellow fever is not also included in the study.

Methods – Study design

Why do the two stated objectives only relate to dengue? It will be important to make clear in the manuscript as it is strange that the overall goals are related to three arboviruses but the objectives only related to one of them.

Table 1: Are zones 1-3 in Niteroi and zones 1-3.2 in Rio the release areas? If so, please more clearly label this in the table as it is not immediately obvious to me when I look at the table even though I infer that it is the case. Perhaps add a sub-heading of “release areas” above the rows where the names of the release zones are provided.

Methods – Study setting and population

Overall, the rationale appears reasonable for the use of controlled interrupted time series but, in reference to my comments above, please look for some opportunities to be even clearer about why randomization could not be used. An interrupted time series design could still be adopted, but one with randomization could offer even stronger evidence.

“In Rio de Janeiro, two administrative areas adjacent to the release area have been designated a priori as a comparative control zone (Figure 1), based on comparable sociodemographic characteristics and synchronous historical dengue time series (Figure 2 and Table 1).” I do not see any information on these SES characteristics except for population whereas I see the
“synchronous historical dengue time series” data in Figure 2, which is really helpful. Can more info on SES be added to the appendix or is the data presented the extent of the available data? As a reader, I would want that information when judging the validity of the comparisons between treatment and control areas. Incidentally, is no historical data available on Zika and Chikungunya? If it is available, it would be really valuable to see that compared between treated and control. I see in the last paragraph of pg 6 that some such data is available so why not include it in the current manuscript?

Controlled interrupted time series

• It is stated: “Zone-level ITS analyses will be performed 12 months after completion of releases in each zone, and each 12 months thereafter, with release zones considered ‘treated’ for the purpose of this analysis based on completion of releases, regardless of the long-term Wolbachia monitoring results.” What does “performed 12 months after completion”? Is it literally about the timing of running the analyses, even though data from each month will be used? The reason I ask for clarification is that on first read I thought that this meant there was a switch to only using data at 12 months after completion of the releases. Also, what is the time frame for control areas where there is no release? Please be more specific.

• “Aggregate release area level analyses will be performed for each municipality 12 months after completion of releases in the last zone and each 12 months thereafter.” What are municipalities? Please define this term when it is first introduced as I don’t see this term defined elsewhere. Is it city i.e. Rio and Niteroi? If so, why not just use the same term throughout i.e. municipality or city, and only choose one of those terms?

• Regarding analyses, I recognize that there may not be space but it would be beneficial to provide some more details including:

  • Model diagnostics to be used e.g. what happens if there is even more dispersion than can be accommodated than the proposed negative binomial models?

  • How will contrasts be made between the treated and control communities? As far as I can tell, this is not specified, though perhaps I’m missing it? Please specific which parameters will be used to estimate the “treatment” effect.

  • How will the fact that the control zones are not randomized be accounted for in modelling i.e. although the set of control zones were selected to be comparable on average to those treated based on historical dengue incidence and SES, what about other factors that may be different between the treated and control zones?

  • Will there be a “fake” interruption in the series for the control areas? It doesn’t seem necessary and may be problematic but would be valuable to describe explicitly what will be done in the time series modelling for the control zones.

Discussion

• The discussion starts with a sentence on dengue, zika and yellow fever. Why is yellow fever not included in the outcome measures for the proposed study?

Overall, this is a fascinating study and a great use of an interrupted time series.

Is the rationale for, and objectives of, the study clearly described?

Yes
Is the study design appropriate for the research question?
Yes

Are sufficient details of the methods provided to allow replication by others?
Partly

Are the datasets clearly presented in a useable and accessible format?
Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Statistical methods in global health research including elimination science and impact evaluation with a focus on malaria and febrile illnesses

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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