STUDY PROTOCOL

REVISED The impact of large-scale deployment of *Wolbachia* mosquitoes on dengue and other *Aedes*-borne diseases in Rio de Janeiro and Niterói, Brazil: study protocol for a controlled interrupted time series analysis using routine disease surveillance data [version 2; peer review: 2 approved, 1 approved with reservations]

Previously titled: The impact of large-scale deployment of *Wolbachia* mosquitoes on disease incidence in Rio de Janeiro and Niterói, Brazil: study protocol for a controlled interrupted time series analysis using routine disease surveillance data

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v2 **First published:** 01 Aug 2019, **8**:1328
https://doi.org/10.12688/f1000research.19859.1
Latest published: 11 Jun 2020, **8**:1328
https://doi.org/10.12688/f1000research.19859.2

Abstract

Background: Rio de Janeiro and Niterói are neighbouring cities in southeastern Brazil which 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 *Aedes*-borne diseases dengue, chikungunya and Zika were estimated to total $650 million USD in 2016, but traditional vector control strategies have not been effective in preventing mosquito-borne disease outbreaks. The *Wolbachia* method is a novel and self-sustaining approach for the

Open Peer Review

Reviewer Status ? ✓ ✓

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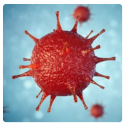
version 2 (revision) 11 Jun 2020			✓ report

biological control of *Aedes*-borne diseases, in which the transmission potential of *Aedes aegypti* mosquitoes is reduced by stably transfecting them with the *Wolbachia* bacterium (*w*Mel strain). This paper describes a study protocol for evaluating the effect of large-scale non-randomised releases of *Wolbachia*-infected mosquitoes on the incidence of dengue, Zika and chikungunya in the two cities 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 *Wolbachia* releases during 2017-2019 covered a combined area of 170 km^2 with a resident population of 1.2 million, across Niterói and Rio de Janeiro. Untreated areas with comparable historical dengue profiles and demographic characteristics have been identified *a priori* as comparative control areas in each city. The proposed pragmatic epidemiological approach combines a controlled interrupted time series analysis of routinely notified suspected and laboratory-confirmed dengue and chikungunya cases, together with monitoring of *Aedes*-borne disease activity utilising outbreak signals routinely used in public health disease surveillance.
Discussion: If the current project is successful, this model for control of mosquito-borne 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.

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version 1 01 Aug 2019	? report	✓ report	

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Competing interests: No competing interests were disclosed.

Grant information: This study is funded by the Bill and Melinda Gates Foundation (OPP1099252); the Brazilian Ministry of Health (DECIT; 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.

How to cite this article: Durovni B, Saraceni V, Eppinghaus A *et al.* **The impact of large-scale deployment of *Wolbachia* mosquitoes on dengue and other *Aedes*-borne diseases in Rio de Janeiro and Niterói, Brazil: study protocol for a controlled interrupted time series analysis using routine disease surveillance data [version 2; peer review: 2 approved, 1 approved with reservations]** F1000Research 2020, **8**:1328 https://doi.org/10.12688/f1000research.19859.2

First published: 01 Aug 2019, **8**:1328 https://doi.org/10.12688/f1000research.19859.1

REVISED **Amendments from Version 1**

The rationale for the non-randomised study design has been described in more detail. Language has been clarified throughout to refer to Aedes-borne disease instead of arboviral disease. The Niteroi intervention area has been updated to reflect additional releases within part of the original control area. Additional detail has been provided on the statistical methods for the ITS analysis. A graph of historical chikungunya incidence in the intervention areas and control areas is now included.

Any further responses from the reviewers can be found at the end of the article

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[1]. The primary vector of dengue is the *Aedes aegypti* mosquito, which is also capable of transmitting other arboviruses (i.e. mosquito-borne viruses) including chikungunya, Zika, yellow fever and Mayaro[2].

The first reported dengue outbreak in Brazil was in 1845, with subsequent outbreaks in 1880–1912 and 1916–1923[2]. 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[3]. In 1986, dengue virus serotype 1 (DENV1) was introduced to Rio de Janeiro and an estimated 1 million people were infected[3,4]. 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[2,3]. 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[2].

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[5,6].

Between 2016 and 2017, 762 deaths were attributed to severe dengue in Brazil[7]. 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[8]. 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[9]. In the absence of an effective vaccine for these arboviruses, disease prevention depends on vector control. Vector control guidelines in Brazil[10] 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[11].

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 transinfection 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 mosquitoes stably transfected with *Wolbachia pipientis* (*w*Mel strain) is reduced by 66–75%[12,13], 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[13]. 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[14–17].

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[18–22].

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[21]. A cluster randomized controlled trial (RCT) to generate a robust and quantitative 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 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. The primary reason that randomised deployment of the *Wolbachia* intervention was not undertaken (either by random allocation of treated and untreated areas, or by randomising the sequence of staged deployments) was the intensive community engagement effort required in release areas in advance of deployments, as well as the gains in logistical efficiency and entomological outcomes from staging deployments across large contiguous areas. The potential benefits of randomised allocation of deployments in reducing bias from known and unmeasured confounders would require the intervention (and control) areas to be divided into a large number of units for randomisation. This fragmentation of the deployment areas would have made the community engagement activities and deployment logistics infeasible. Having small, fragmented release areas interspersed with untreated areas would also have greatly increased the potential for contamination between treatment arms from both mosquito movement and human movement, thereby diluting the observable intervention effect.

Here we describe a protocol for evaluating the effect of large-scale non-randomized *Wolbachia* releases on the incidence of dengue, chikungunya and Zika in the cities of Niterói and Rio de Janeiro. The proposed strategy employs a controlled interrupted time series analysis of routinely notified suspected and laboratory-confirmed cases of dengue, chikungunya and Zika, together with monitoring of disease 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 mosquito-borne 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 the burden of *Aedes*-borne disease.

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

1. Estimate the reduction in dengue, chikungunya and Zika 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 km^2 and a population of 484,918 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 km^2. 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 km^2 and with 886,551 inhabitants, 39% of whom live in slums. The total release area in Niterói is approximately 83 km^2 covering a population of 373,117 (Table 1). For the purposes of *Wolbachia* deployment, the Rio de Janeiro and Niterói intervention areas are each divided into 4 release zones, respectively, which are aligned with neighbourhood administrative boundaries.

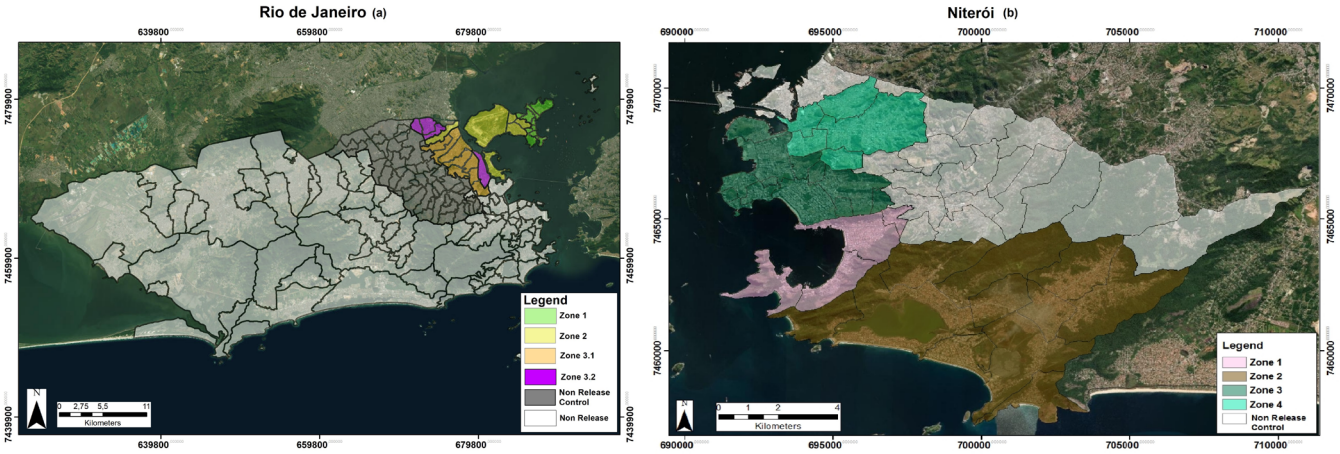

Figure 1. Map of (**a**) Rio de Janeiro and (**b**) Niterói *Wolbachia*-treated and untreated areas (produced in ArcMap version 10.5, ESRI, CA).

Table 1. Demographic characteristics of *Wolbachia* treated and untreated areas, Niterói and Rio de Janeiro (source: 2010 Brazil population census).

	Neighbourhoods	Population	Area (Km²)	Population density
Niterói				
Release Zone 1	4	23,747	9.2	2,581
Release Zone 2	11	68,695	50.6	1,357
Release Zone 3	13	178,891	12.6	14,197
Release Zone 4	5	101,784	10.8	9,424
Non-release control area	19	111,801	51.2	2,183
Total	52	484,918	134.4	3,608
Rio de Janeiro				
Release Zone 1	10	107,130	11.8	9,078
Release Zone 2	6	150,646	33.5	4,496
Release Zone 3.1	8	408,036	28.2	14,469
Release Zone 3.2	4	220,739	12.0	18,394
Non-release control area	51	1,512,608	117.3	12,895
Rest of city (no releases)	79	3,921,287	996.5	3,935
Total	158	6,320,446	1,199.3	5,270

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 synchronous historical dengue and chikungunya time series (Figure 2 and Figure 3). In Niterói, the remaining untreated area of the city has been designated as the comparative control zone (Figure 1).

Wolbachia release and monitoring

Staged *w*Mel *Wolbachia*-mosquito deployments will be implemented in Niterói and Rio de Janeiro, in order to achieve *Wolbachia* establishment across the two cities. 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

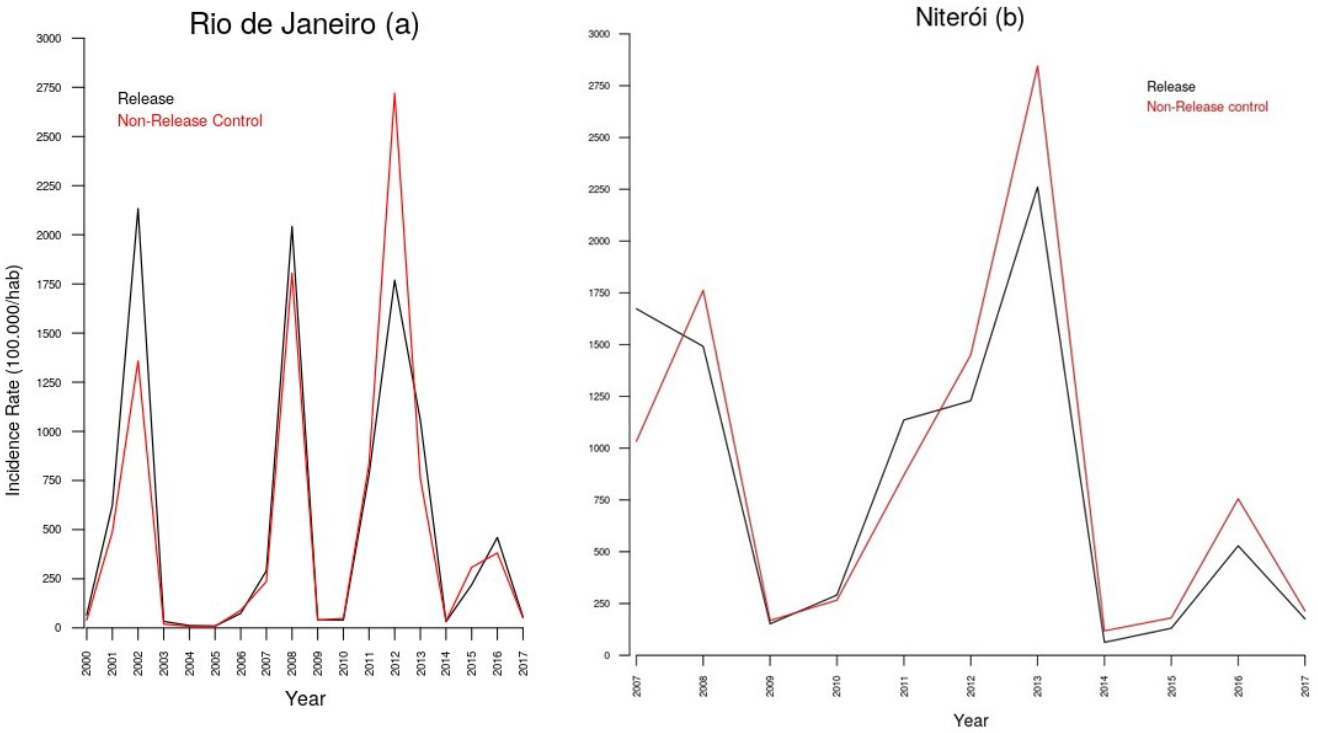

Figure 2. Dengue incidence in *Wolbachia*-treated vs untreated areas of (**a**) Rio de Janeiro (2000–2017) and (**b**) Niterói (2007–2017) (source: Brazilian National Disease Surveillance System (SINAN)).

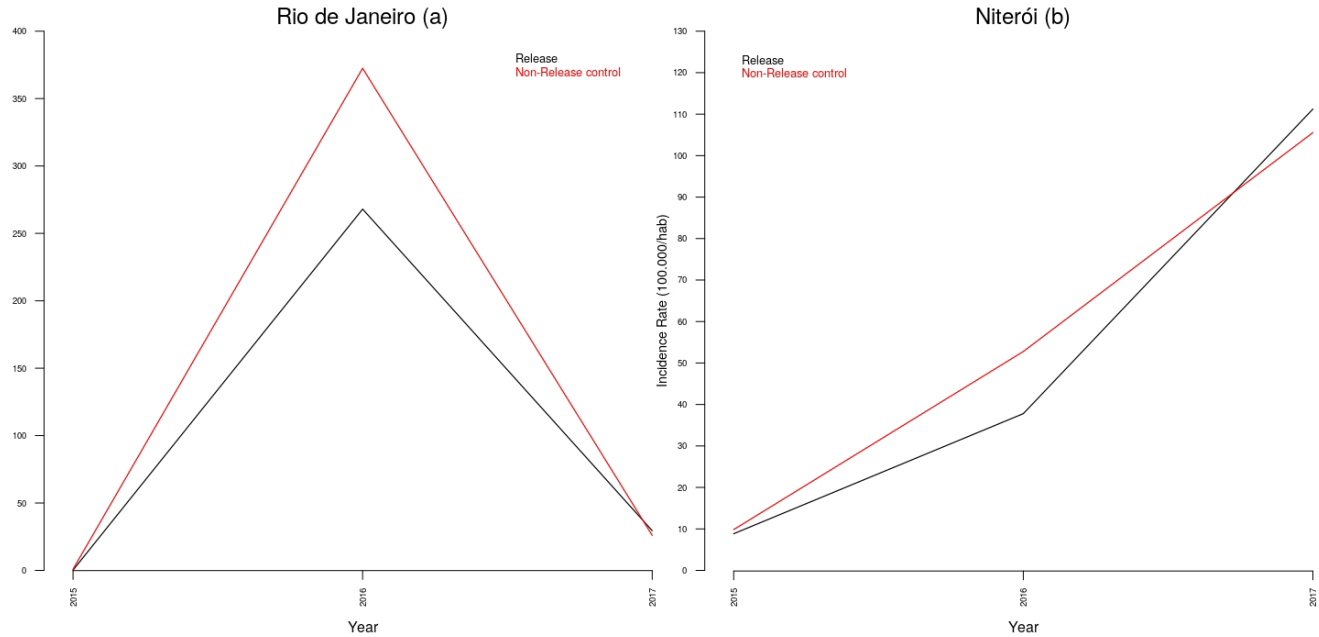

Figure 3. Chikungunya incidence in *Wolbachia*-treated vs untreated areas of (**a**) Rio de Janeiro (2015–2017) and (**b**) Niterói (2015–2017) (source: Brazilian National Disease Surveillance System (SINAN)).

a density of 16 traps per km^2. 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 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[25], 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[26] 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[26]. 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 cities 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.

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[27]. It uses a set of historical observations of an outcome of interest (in this context monthly dengue case notifications) to establish an underlying trend, which is assumed to be 'interrupted' by the introduction of an intervention (in this case *Wolbachia* releases). 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 the untreated control area), provides an estimate of the intervention effect. Segmented regression will be used to estimate the effect of *Wolbachia* releases on monthly case counts of dengue and chikungunya, using an appropriately defined model: e.g. negative binomial regression for autocorrelated count data with population offset, adjusted for temporal effects using indicator variables or flexible cubic splines, and assuming a step change post-intervention. The *Wolbachia* intervention effect will be estimated from the interaction between a binary 'area' variable (intervention vs control area) and a binary 'period' variable (post-intervention vs pre-intervention). The post-intervention period will be defined for the control area beginning at the same time as the post-intervention period in the comparative release zone. This allows explicitly for a level change in the outcome (dengue case incidence) in both intervention and control areas in the post-intervention period, for example in a scenario where other secular events coincident with the *Wolbachia* deployments may have influenced dengue incidence in both intervention and control areas independent of *Wolbachia*. An additional analysis will consider *Wolbachia* frequency as a continuous covariate or categorised into quintiles of exposure reflecting the measured *Wolbachia* prevalence in the local mosquito population. The outcome distribution is assumed to be negative binomial to allow for overdispersion. Robust standard errors will be used to account for autocorrelation and heteroskedasticity. If an excess of zero counts is apparent, the proposed negative

binomial model will be nested within a zero-inflated negative binomial model of the same structure. A likelihood ratio test can be performed to test model fit.

Separate analyses will be performed for each release zone compared with its pre-defined control area, and for the aggregate release areas in each city compared with the control area for each city. The availability of comparative control areas – well-matched to the release area in demographic characteristics and historical arboviral disease incidence (Figure 2 and Figure 3) – permits a robust, controlled analysis in which the confounding effects of seasonality and inter-annual variability can be adjusted for. The intervention effect will be estimated after 12 months of post-release observations have accumulated, and each 12 months thereafter for five years using cumulative post-release observations, for each release zone individually and in aggregate for each city.

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) chikungunya and Zika cases notified to the disease surveillance system.

Although the historical time series for chikungunya and Zika 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[26], 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[20,22,23] 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 two cities 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. 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[28]. 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[12,14,16,17]. The World Mosquito Program[29] is deploying *w*Mel *Wolbachia*-infected *Ae. aegypti* mosquitoes in Brazil with the purpose of achieving a large-scale and sustained reduction in arboviral disease burden in two cities 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[24].

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 intervention[27,30,31] and is a pragmatic alternative design where an RCT is considered infeasible, particularly in the presence of a well-matched untreated control area[32,33]. While the ITS approach could also be combined with randomization of the intervention, 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 relative short time frame, randomized allocation of the *Wolbachia* deployments was not considered feasible as it would have necessitated a fragmented approach to community engagement and release logistics which would have considerably complicated and delayed the implementation. The Brazil deployments were also an opportunity to optimise methods for large-scale deployment, and to evaluate the public health impact of a large contiguous release where the intervention area is likely to encompass a greater proportion of people's daily movements, compared to a cluster randomised trial design. 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. The non-randomized and unblinded nature of the *Wolbachia* intervention means it is plausible that community awareness could alter health-care seeking behaviour, diagnostic or reporting practices in areas where *Wolbachia* releases have been conducted. However we believe it is unlikely this would be of sufficient magnitude to result in a biased estimation of the intervention effect, since the intervention is deployed throughout such a large, diverse and complex setting (>120km^2, with population >1 million). We also cannot exclude the possibility of a concurrent change in socio-demographic or ecological factors related to dengue risk, or a change in dengue control practices, between the intervention and control areas independent of the *Wolbachia* intervention, but given the strong synchrony in dengue incidence between intervention and control zones for over a decade prior to releases this is considered unlikely.

Human mobility also presents a potential challenge to this study, especially during the period in which staged releases are occurring and *Wolbachia* levels are heterogeneous, 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[8], 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[34].

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[21]. 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.

Acknowledgements

The authors acknowledge the contributions of Peter Ryan, Simon Kutcher, Jacqui Montgomery, and Frederico Muzzi (World Mosquito Program) to the planning and implementation of *Wolbachia* deployments in Rio de Janeiro and Niterói.

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Open Peer Review

Current Peer Review Status: ? ✓ ✓

Version 2

Reviewer Report 22 December 2020

https://doi.org/10.5256/f1000research.27081.r74998

✓ **Stephen Waterman**
Dengue Branch, Division of Vector-Borne Diseases (DVBD), National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Centers for Disease Control and Prevention (CDC), San Juan, Puerto Rico

This manuscript describing the protocol for an intervention trial of Wolbachia replacement in *Aedes aegypti* populations with the wMel Wolbachia strain to prevent dengue, Zika and chikungunya in the complicated densely populated urban environments of Rio de Janeiro and Niteroi, Brazil, has been revised somewhat in response to previous open reviewers. In response to both reviewers, the authors explain the pragmatic reasoning behind not conducting the study as a cluster randomized trial. In addition to community pressure to deploy the intervention quickly and in wide areas as a result of the Zika outbreak, the authors indicate that the necessary community engagement to set up clusters was not feasible given the resources available and that the smaller geographic units are more susceptible to contamination. In response to reviewer 1, the authors clarify the Wolbachia strain used and state that the wMel strain has persisted at high levels for over two years in Indonesia and as many as 8 years in northern Australia.

Each city has four intervention release zones (ranging from 9 to 51 square kms and populations densities from 1,400-18,000 persons/square km) and a large non-release control area. Adult mosquito releases took place between late 2015 and the end of 2019 and lasted for at least 16 weeks in each release zone. Wolbachia introgression frequency monitoring is done with BG traps and PCR testing of a sample of the mosquitoes collected. The study design main analysis methodology is controlled interrupted time series which makes use of routinely collected public health disease surveillance data and requires that consistent historical data is available supporting the comparability of the intervention and control areas. The analysis compares the incidence of suspect and confirmed dengue cases before and after the intervention in the treatment and control zones. Additional details on the regression analysis have been provided. Power estimates from simulations based on binomial distribution of ten year historical data suggest that the study has 80% to detect a 50% reduction after three years and a 60% reduction after two years.

This study is important to assess the impact of Wolbachia replacement in a highly Aedes aegypti

transmitted disease endemic urban area in Latin America, as the other studies conducted by the World Mosquito Program have been done in Asia or in non-endemic Australia. Since the study is not randomized, including as much detail as possible in the description of the methods and the results in eventual publications will be important to assess the strengths and weaknesses of the study.

Comments

- Additional details regarding the Wolbachia mosquito releases and trapping results would be of interest. Why were releases done as adult mosquito releases and not eggs as in the Yogyakarta Indonesia RCT trial?[1] Were releases in all release zones begun simultaneously or staggered? Were the BG trap adult female Aedes aegypti mosquito counts/densities comparable in the different zones and control area? Will the study design enable the assessment of the impact of Wolbachia releases on *Aedes* species abundance?

- Large dengue outbreaks had not occurred prior to the intervention in the study cities since 2013. Is there available seroprevalence data for dengue and Zika in the intervention zones and the control area prior to the wMel intervention? Do we know if pre-intervention reported Zika incidence in 2016 was comparable in the intervention and control areas?

- The analysis plan is to report results two years after the completion of releases. If the releases were staggered will the initial publication include data from all the intervention zones?

- Are the secondary endpoint analyses of severe dengue cases and fatalities intended to strengthen the efficacy evidence with data on confirmed diagnoses, to help address the cost effectiveness of the intervention, or to address a question of whether Wolbachia reduces the severity of the disease when transmission does occur?
- The protocol states that the study will continue through 2023 and analyses will be done each year for 5 years. Since the releases finished at the end of 2019, will an analysis of the sustained Wolbachia introgression and cumulative epidemiologic impact after 5 years be possible?

Minor comments

- The statement in the last paragraph of page 9 that we have no evidence of dengue, Zika or chikungunya transmission where high levels of Wolbachia have been established is somewhat misleading in that the authors are referring to northern Australia where Aedes aegypti transmitted diseases are not endemic and the force of infection is considerably lower than Asia and Latin America.

- The comment on page 9, next to last paragraph, with regard to the licensed CYD-TDV vaccine being rendered less feasible in high burden areas because of the need for pre-vaccination screening actually better applies to low to moderate burden areas. WHO included in its 2018 vaccine recommendations[2] that areas with dengue seroprevalence of 80% or greater in 9 years might consider forgoing pre-vaccination screening. In low burden regions the risk of vaccinating false serologic positives increases and the cost effectiveness decreases. The authors may want to clarify whether they think Wolbachia replacement could make dengue vaccines unnecessary.

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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: Epidemiology, dengue and arbovirology, tropical medicine

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.

Version 1

Reviewer Report 09 December 2019

https://doi.org/10.5256/f1000research.21787.r57000

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, Australia[1] 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* status[2]. 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 uninfected[3].

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* invasion[4] 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 “km^2”.

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.

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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.

Author Response 01 Jun 2020

Katherine ANDERS, Monash University, Melbourne, Australia

Major comments

1. 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, Australia 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 status. 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 uninfected.
Thank you for drawing our attention to this oversight. We have now indicated the strain used (wMel) in the abstract, the sixth paragraph of the introduction, the 'Wolbachia release and monitoring' subheading of the Methods, and the first paragraph of the discussion. While it is true that other Wolbachia strains have been shown to have higher thermostability than wMel in the mosquito larval life stages, the effect of heatwaves on wMel Wolbachia prevalence in Wolbachia-treated areas of northern Australia has been shown to be transient (Ross et al, PLoS NTD 2020). Long-term monitoring of WMP's release sites in northern Australia and Yogyakarta, Indonesia, has demonstrated sustained high levels of Wolbachia 2 – 8 years post-release. Entomological monitoring of Wolbachia prevalence in local Ae. aegypti populations in the Rio and Niteroi release areas is currently ongoing, and so will provide empirical evidence of the level to which wMel introgression is sustained over time.

2. **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.**
It is true that Wolbachia could theoretically spread from the deployment areas into the untreated control zones, however the rate of spread is known to be relatively slow especially in dengue urban environments like these (100-200m per year; Schmidt et al PLoS Biol 2017). Given the size of the control zone (117km2 in Rio de Janeiro, and 51km2 in Niteroi), it is highly unlikely that Wolbachia establishment will occur in any meaningful way beyond the border area of the control zones within the 5 year timeframe of this study.

3. **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.**
The randomization itself would not necessarily take a lot of time, although there would be additional work required to define an adequate number of spatial units for randomization (ideally based on a power calculation), and define those spatial units with 'hard' borders which minimise contamination between treated and untreated areas. The time delays come from the added complexity of conducting the intensive community and stakeholder engagement that is needed prior to releases, and conducting the deployments themselves, across a fragmented set of release areas interspersed with untreated control areas, compared to conducting these engagement and deployment activities in a contiguous release area. Please see our response to Reviewer 1's comments #1, #7 and #11 for further discussion of the reasons for non-randomisation, and for the references to where we have

improved discussion of this point in the manuscript text.

4. 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
The descriptive analysis will simply describe the seasonal and interannual trends, as stated. I.e. the calendar months in which dengue incidence has historically been highest and lowest in the two cities, and the range in annual case counts and per capita incidence. We don't see that this level of detail is of value to the reader here. We don't understand the reviewer's suggestion about a schematic of expected trend in notifications. We show in figure 2 the historical trends in dengue incidence in the intervention area and untreated control area for each city; the lines for intervention and control area can be expected to diverge if Wolbachia successfully interrupts dengue transmission.
Minor comments

5. 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.
We have changed the wording in the title to 'Aedes-borne disease' as suggested.

6. I think the clause in the fourth line [of the abstract] will flow better if "Aedes-borne diseases dengue, chikungunya and Zika" is changed to "Aedes-borne diseases (dengue, chikungunya and Zika).
We have kept the original wording here, because strictly these three are not the only Aedes¬-borne diseases, but they are the Aedes-borne diseases were are considering in this protocol.

7. Change "km2" to "km2"
This change has been made.

8. 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
See response to comment #13, Reviewer 1.
Historical chikungunya data for the intervention areas and control areas is now included as Figure 3.

Competing Interests: None

Reviewer Report 07 November 2019

https://doi.org/10.5256/f1000research.21787.r51960

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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 arbobviruses 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 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 Chikungunga? 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.

Author Response 01 Jun 2020

Katherine ANDERS, Monash University, Melbourne, Australia

Main comments

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

We agree with the reviewer that randomisation could be combined with ITS, and in principle this could be a stronger design. However randomisation of the deployment (either by random allocation of parallel treated and untreated areas, or by randomising the sequence of staged deployments) would have necessitated the study site being divided up into a large number of spatial units for randomisation. Logistically, this would have made the community engagement and deployment activities infeasible within the funding and time constraints of the project, and in the context of the large and complex urban setting. Intensive stakeholder and community engagement, and deployment planning, could only have commenced after the randomisation process had occurred and the intervention areas were determined. Field activities would then have had to be conducted in a spatially fragmented way, complicating the stakeholder and community engagement and the deployment logistics and inevitably lengthening time frames. Smaller non-contiguous release areas interspersed with untreated areas would also have greatly increased the potential for contamination between treatment arms from both mosquito movement and human movement, thereby diluting the observable intervention effect. Please see our responses to comments #7 and #11 below, and Reviewer 2 comment #3.

2. 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?

It is plausible that community awareness could alter health-care seeking behaviour, or diagnostic / reporting practices, in areas where Wolbachia releases have been conducted.

However we find it unlikely that this could lead to a large and sustained difference in dengue case notifications across two cities as large and complex as these, where the total intervention area exceeds 120km2 and 1 million inhabitants. Dengue (and chikungunya and Zika) is a notifiable disease, so there remains a requirement for health care providers to report any suspected cases meeting the standard case definition. We have included a discussion of this as potential limitation, in the fourth paragraph of the discussion. From the beginning of the project's engagement with communities, all messaging has reinforced the continued importance of routine activities related to arbovirus surveillance and care.

Minor comments

3. 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.
Since the three arboviruses of interest are specifically those vectored by Aedes mosquitoes, for greater clarity we have replaced 'arboviruses' with 'Aedes-borne viruses' in most instances throughout the abstract and article text, including in the manuscript title. Elsewhere we have replaced 'arboviral disease' with the more intuitive term 'mosquito-borne disease'. In the last sentence of paragraph one, it is made clear that the term 'arbovirus' encompasses dengue, chikungunya, Zika, yellow fever and Mayaro viruses, and that these are specifically Aedes-borne arboviruses.

4. Based on the title referring to arboviruses, as a reader I was expecting the first sentence or so to at least mention 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".
As above, the manuscript title has been adjusted to replace 'arboviral disease' with 'Aedes-borne disease' to improve clarity for the reader. The first sentence introduces dengue (the disease) as a global public health challenge, and the last sentence of that paragraph defines dengue, chikungunya and Zika (and yellow fever and Mayaro) as Aedes-borne diseases (and arboviruses). A definition of arbovirus as meaning a mosquito-borne virus has also been added to this sentence.

5. In the fourth paragraph referring to yellow fever, please clarify if it is an arbovirus.
Yellow fever has been defined as an Aedes-borne arboviruses in the last sentence of the first paragraph of the introduction.

6. The last sentence of the sixth paragraph: "Laboratory data indicate a similar reduction in the competence of Wolbachia-carrying Ae. aegypti for transmitting other viruses include ZIka, chikungunya, yellow fever and Mayaro". If all listed are arboviruses, please edit 'viruses' to 'arboviruses'.
The text has been corrected as suggested.

7. In the ninth 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.
Please see our response to Major comment #1 above. We have added text to the end of the ninth paragraph of the introduction, expanding on this justification for not randomising, as suggested.

8. Related to my previous comments on arboviruses, in the tenth paragraph…again it could really help the reader to edit this sentence to something like "…three arboviruses, namely dengue, Zika and chikungunya…"
Consistent with our response to comment # above, we have adjusted the wording in the paragraph to refer to dengue, Zika and chikungunya as 'Aedes-borne diseases' or 'mosquito-borne diseases'.

9. 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.
Thank you for pointing out this inconsistency, we have corrected the wording of the aims and first objective to be inclusive of dengue, chikungunya and Zika (as the Aedes-borne diseases of interest). The second objective still relates only to dengue, as the outbreak indicators used routinely for public health purposes in Rio and Niteroi apply only to dengue outbreak monitoring. As explained under 'Controlled interrupted time series analysis' (3rd and 4th paragraphs, page 11), the primary endpoint for the ITS analysis will be dengue for which there is the longest historical case time series, with chikungunya and Zika analysed as secondary endpoints in the ITS.

10. 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. Perhaps add a sub-heading of "release areas" above the rows where the names of the release zones are provided.
Thank you for this suggestion, we have improved the labelling of the areas in Table 1 to make clearer which are the release zones and non-release areas. Please note that we have also updated this table and Figure 2 to reflect an additional release zone in Niteroi (and the consequent reduced size of the control area).

11. 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 randomisation could not be used. An interrupted time series design could still be adopted, but one with randomisation could offer even stronger evidence.
Please see our responses to comments #1 and #7 above. Additional discussion of the justification for not randomising, and the potential benefits from the pragmatic evaluation of a large-scale contiguous release, has been added to the third paragraph of the discussion

section on page 12.

12. "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.
Unfortunately socio-economic data are not available aggregate to the release zones. We have now corrected this text to clarify that the comparability of the untreated control areas is demonstrated by the synchronous historical dengue time series.

13. Incidentally, is no historical data available on Zika and Chikungunga? 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?
Historical chikungunya data for the intervention areas and control areas is now included as Figure 3.

14. 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.
Yes, this is about the timing of running the analyses, ie. the intervention effect will be estimate for each zone when 12 months of post-release observations have accumulated in that zone, and repeated each 12 months thereafter (for five years) on cumulative post-release observations. And the same for the city-level analyses. The text at the end of the first paragraph under the heading 'Controlled interrupted time series analysis' has been edited to clarify this. There is no separate analysis for control areas, these are included in the regression model as the comparator for each individual release zone, and for the city-level analysis. This has been clarified in the text.

15. "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?
We recognise this inconsistency may have been confusing, and have changed throughout to

use 'city/cities' in place of 'municipality/municipalities'.

16. 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?
Of the common count models, negative binomial models have the most flexible approach for handling overdispersion in count data by assuming that the variance is a quadratic function of the mean. Common causes of overdispersion can range from an excess of zero counts, unexplained heterogeneity, and/or temporal autocorrelation. The models as proposed will be using flexible cubic splines to explain secular and temporal trends, hopefully leaving very little remaining unexplained heterogeneity. To account for any lingering heteroskedasticity or temporal autocorrelation, heteroskedasticity-robust estimation will be used for estimating variance. This has now been made explicit in the description of the Controlled interrupted time series methods. If an excess of zero counts is apparent, the proposed negative binomial model will be nested within a zero-inflated negative binomial model of the same structure. A likelihood ratio test can be performed to test model fit. This is also now described in the methods.

17. 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
The regression model will use monthly dengue case time series data for the treated area (either an individual release zone or aggregate city-level release area, as specified in the text) and the control area (one control area per city). The treatment effect will be estimated from the interaction between a binary 'area' variable (treated vs control) and a binary 'period' variable (post-Wolbachia vs pre-Wolbachia). This allow explicitly for a level change in the outcome (dengue case incidence) in both intervention and control arms in the post-intervention period, for example in a scenario where other secular events coincident with the Wolbachia deployments may have influenced dengue incidence in both intervention and control areas independent of Wolbachia. The text under 'Controlled interrupted time series' has been revised to clarify this.

18. 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?
Quasi-experimental designs, such as the proposed ITS analysis, aim to address the validity concerns associated with non-randomization in several ways. By using a region's historic data to inform the counterfactual outcome, the ITS analysis aims to minimize threats to internal validity. Second, by adding control regions and performing a controlled ITS, we better account for seasonal and secular influences during both the pre- and post-intervention periods. While it is true that there may be other factors associated with dengue risk that differ between treated and control zones in a non-randomised design, we make the implicit assumption in the controlled ITS that these differences are consistent throughout both the pre- and post-intervention period. Any divergence in the post-intervention period from the synchrony in dengue time series observed between intervention and control zones in the pre-intervention period is therefore assumed to represent an intervention effect. However we cannot exclude the possibility of a concurrent

change in socio-demographic or ecological factors related to dengue risk, or a concurrent change in dengue control practices, between the intervention and control areas independe of the Wolbachia intervention. We have expanded on this limitation in the discussion.

19. 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.
The inclusion of the control area time series in the regression model and estimation of the intervention effect from an interaction between 'area' and 'period' effectively does allow for a 'fake' interruption in the series for the control area, as it allows for a step change in the control area as well as the treated area, post-intervention. The interaction parameter from which the intervention effect is estimated indicates whether there was any change in dengue incidence following the Wolbachia intervention in areas where Wolbachia releases occurred, over and above any change in dengue incidence that was observed in the untreated control area.

20. 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?
Yellow fever is not included as an endpoint for the study because there is not a history of urban yellow fever in the cities of Rio and Niteroi. The recent outbreak in Rio de Janeiro state that is mentioned early in the discussion included some urban cases, but in other parts of the state. We have removed that sentence from the first paragraph of the discussion, to avoid confusion.

Competing Interests: None