Estimating the effect of the wMel release programme on the incidence of dengue and chikungunya in Rio de Janeiro, Brazil: a spatiotemporal modelling study

Gabriel Ribeiro dos Santos, Betina Durovni, Valeria Saraceni, Thais Irene Souza Riback, Sofia B Pinto, Katherine L Anders, Luciano A Moreira, Henrik Salje*

Summary
Background Introggression of genetic material from species of the insect bacteria Wolbachia into populations of Aedes aegypti mosquitoes has been shown in randomised and non-randomised trials to reduce the incidence of dengue; however, evidence for the real-world effectiveness of large-scale deployments of Wolbachia-infected mosquitoes for arboviral disease control in endemic settings is still scarce. A large Wolbachia (wMel strain) release programme was implemented in 2017 in Rio de Janeiro, Brazil. We aimed to assess the effect of this programme on the incidence of dengue and chikungunya.

Methods 67 million wMel-infected mosquitoes were released across 28 489 locations over an area of 86·8 km² in Rio de Janeiro between Aug 29, 2017 and Dec 27, 2019. Following releases, mosquitoes were trapped and the presence of wMel was recorded. In this spatiotemporal modelling study, we assessed the effect of the release programme on the incidence of dengue and chikungunya. We used spatiotemporally explicit mathematical models applied to geocoded dengue cases (N=283 270) from 2010 to 2019 and chikungunya cases (N=57 705) from 2016 to 2019.

Findings On average, 32% of mosquitoes collected from the release zones between 1 month and 29 months after the initial release tested positive for wMel. Reduced wMel introgression occurred in locations and seasonal periods in which cases of dengue and chikungunya were historically high, with a decrease to 25% of mosquitoes testing positive for wMel during months in which disease incidence was at its highest. Despite incomplete introgression, we found that the releases were associated with a 38% (95% CI 32–44) reduction in the incidence of dengue and a 10% (4–16) reduction in the incidence of chikungunya.

Interpretation Stable establishment of wMel in the geographically diverse, urban setting of Rio de Janeiro seems to be more complicated than has been observed elsewhere. However, even intermediate levels of wMel seem to reduce the incidence of disease caused by two arboviruses. These findings will help to guide future release programmes.

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Introduction Dengue virus continues to circulate endemically across tropical and subtropical regions worldwide, causing an estimated 50 million symptomatic infections per year.1 In addition, large-scale outbreaks of chikungunya virus, spread by the same Aedes mosquitoes that transmit dengue virus, have become increasingly common. In Brazil, more than 10·1 million cases of dengue and 1·2 million cases of chikungunya were reported across Brazil, more than 10·1 million cases of dengue and chikungunya in Rio de Janeiro, Brazil: a spatiotemporal modelling study
Because *Aedes* mosquitoes are a vector for many human pathogens, vector-oriented interventions have the potential to reduce the transmission of several pathogens at the same time. Typical interventions that target the vector, such as the spraying of pesticide, are often implemented in reaction to ongoing reported circulation of the virus and are not a long-term solution as they give rise to resistant strains of vectors. However, *w*Mel-infected mosquitoes can be released pre-emptively. Further, if implemented successfully, the population of these mosquitoes seems able to self-maintain, making the technology comparatively cost-efficient and sustainable. Because *Aedes* mosquitoes are a vector for many human pathogens, vector-oriented interventions have the potential to reduce the transmission of several pathogens at the same time. Typical interventions that target the vector, such as the spraying of pesticide, are often implemented in reaction to ongoing reported circulation of the virus and are not a long-term solution as they give rise to resistant strains of vectors. However, *w*Mel-infected mosquitoes can be released pre-emptively. Further, if implemented successfully, the population of these mosquitoes seems able to self-maintain, making the technology comparatively cost-efficient and sustainable.

Except in extreme scenarios in which case numbers decrease to almost zero, understanding the effect of spatially targeted interventions is complicated because cases of dengue and chikungunya vary substantially over space and time, driven by local variations in immunity, human behaviours, mosquito density, population density, building constructions, and climate, among other factors. Further, the movement of people outside a building constructions, and climate, among other factors. Further, the movement of people outside a release zone means that local residents can still become infected even if the intervention is 100% effective. Additionally, most infections are not detected, because they result in few symptoms or because health care is not sought.

As *w*Mel is deployed at increasing scale, a robust understanding of the effect of these releases on the incidence of arboviral disease in a range of epidemiological settings is needed. In this Article we studied the effect of *w*Mel-infected mosquito releases in Rio de Janeiro, Brazil, on dengue and chikungunya case occurrence in the city. Entomological results 1 year after the end of releases in the first two release areas in Rio de Janeiro showed successful *w*Mel introgression overall, but with heterogeneity in the prevalence of *w*Mel at the neighbourhood level. We made use of the public health systems of Rio de Janeiro, in which dengue and chikungunya cases from all hospitals and health clinics throughout the city have been systematically geocoded since 2010 for dengue and 2016 for chikungunya. We developed spatially explicit mathematical models to fit the timing and location of cases and estimated the effect of *w*Mel on the occurrences of dengue and chikungunya.

### Methods

#### Setting and *w*Mel field implementation

Our spatiotemporal modelling study was set in Rio de Janeiro, the second largest city in Brazil, with 6·7 million inhabitants over 1260 km². The city is a patchwork of highly dense, flat urban areas and uninhabited mountains covered with tropical forest. The *w*Mel release programme started in the northwest of the city in August, 2017. The release area was subdivided into five zones (RJ1, RJ2, RJ3.1, RJ3.2, and RJ3.3), covering a total area of 86·8 km² with around 890 000 inhabitants, and releases were phased through the different zones.

A pilot test in the city found that insecticide resistance was widespread in wild-type *Ae* *aegypti*, which can hinder the successful establishment of *w*Mel in an area, as the original *w*Mel mosquitoes would be more susceptible to...
insecticides used in the release zones. The release programme therefore crossed female wMel-infected mosquitoes with wild-type males, which allowed for a better matching of genetic profiles between wMel-infected and field mosquitoes.21 All releases used this newly developed, locally matched, insecticide-resistant mosquito strain. Releases started on Aug 29, 2017, and continued until Dec 27, 2019. Release points were distributed every 50 m in five release zones. Around 100 wMel-infected mosquitoes were released each time. A network of 1168 BG-Sentinel traps (Biogents, Regensburg, Germany) was used to monitor wMel introgression. The traps were regularly distributed throughout the release area with an average distance between two adjacent traps of 250 m. A trap was set 4 weeks after the first release in the area and mosquitoes were collected every two weeks until Dec 30, 2019. Up to ten A aegypti mosquitoes (male and female) per collection were tested individually for wMel using quantitative PCR. This testing regime gives an estimate of the overall proportion of mosquitoes that were infected in a given trap at a given time.

Case data
Every suspected case of dengue or chikungunya in Rio de Janeiro in a patient who presents to a health-care facility must be recorded in Brazil’s national surveillance system database, known as the Notifiable Diseases Information System. Suspected dengue cases are defined as patients who present with fever and at least two of the following manifestations: nausea or vomiting, rashes, myalgia or arthralgia, headache or retro-orbital pain, petechiae or a positive tourniquet test, and leukocytopenia. Suspected chikungunya cases are defined as patients with fever and arthralgia or arthritis.22 A differential diagnosis between chikungunya and dengue is based on the duration of fever (up to 7 days for dengue and up to 3 days for chikungunya) and the intensity of arthralgia, which is more intense in chikungunya. Lymphocytopenia is also frequent in chikungunya, whereas it is uncommon in dengue.23 A subset of cases were confirmed by laboratory testing. Cases of dengue were confirmed by PCR (38 870 [11·1%] of 350 102 cases), whereas cases of chikungunya were mainly confirmed through IgM serology (14 688 [23·5%] of all 62 415 suspected cases).

All dengue and chikungunya cases are systematically geolocated by the Rio de Janeiro city health department using information on the home address of the patient where possible. A subset of notified cases could not be geolocated (66 832 [19·1%] of 350 102 cases of dengue and 4 710 [7·5%] of 62 415 cases of chikungunya), and were therefore not included in the analysis. This study was approved by the Brazilian National Institutional Review Board (CONEP, 59 756 16.2.0000.0008).

Spatial model
For our modelling approach, we divided the project area into 500×500 m cells (N=465 cells). We then counted the number of dengue and chikungunya cases that occurred in each 30-day period within each cell between Jan 1, 2010, and Sept 25, 2019, for dengue and between Jan 1, 2016, and Sept 25, 2019, for chikungunya (N=117 time periods, resulting in 54 405 total space–time units). We used WorldPop data to obtain detailed estimates of the distribution of the underlying population throughout the project area.24

We constructed Poisson regression models to separately fit the number of dengue cases and chikungunya cases for each space–time unit throughout the project area during the period 2010–19 for dengue and 2016–19 for chikungunya. To incorporate the spatial correlation in the location of dengue and chikungunya cases, we used integrated nested Laplace approximation, as implemented in R-INLA.25 This approach enabled us to introduce a spatial correlation term that explicitly incorporates the spatial dependence between locations. Additionally, to account for temporal correlation in the timing of cases, we used a temporally structured random effect by using an order one autoregressive model to the monthly time variable. We used the log of population size within the cell as an offset to the model.

To estimate the effect of the wMel release programme, we considered three separate measures. First, we used a binary variable for which each space–time unit was coded as 1 if wMel was detected in A aegypti mosquitoes across the traps within that location and within that month, and 0 if it was not. Second, we used the actual proportion of Aedes mosquitoes that were infected by wMel across these traps, using non-overlapping bins (0·0%, 0·1–10·0%, 10·1–20·0%, 20·1–30·0%, 30·1–40·0%, 40·1–50·0%, 50·1–60·0%, >60·0%). Finally, we considered wMel as a continuous variable and estimated the reduction in incidence of chikungunya and dengue for each unit increase in wMel. In all models, space–time units before the initiation of releases were given a value of 0. Space–time units with no traps and those in which no mosquitoes were caught were removed from the main analysis.

Sensitivity analysis
To assess the robustness of our approach, we conducted a range of sensitivity analyses. To test whether the released A aegypti mosquitoes were being recaptured in the traps (leading to falsely high estimates of wMel) and potentially driving our estimates, we conducted a sensitivity analysis in which we excluded space–time units that occurred within a short amount of time following releases. Two different exclusion criteria were designed. First, we excluded space–time units within which releases occurred, which resulted in the exclusion of 57% of space–time units with a non-zero wMel value. The second, more exclusive criterion was to exclude from the analysis space–time units both during the month of the release and the subsequent month. This approach resulted in 67% of space–time units with non-zero wMel values being excluded.

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We then repeated the analysis using an augmented dataset that inferred the percentage of *wMel*-infected *Aedes aegypti* (defined as %*wMel*) captured in places where data were missing. Missing %*wMel* estimates occurred either in places where no traps were set up despite releases having already occurred in the area or when traps did not capture any mosquitoes. We used a separate spatial regression model to estimate the number of *wMel*-positive mosquitoes within each space–time unit. The model to estimate %*wMel* can be summarised by $N_i(s, t) | \eta(s, t) \sim \text{Poisson}(N_t(s, t) \times \eta(s, t))$, with $\eta(s, t) = u(s) + v(t)$, where $N_i(s, t)$ is the number of infected *A. aegypti* captured in a given cell, $N(s, t)$ is the total number of *A. aegypti* captured in the same cell, $u(s)$ is a spatially structured random effect, $v(t)$ is a temporally structured random effect, $s$ is the spatial unit index, and $t$ is time. Both random effects were defined in the same way as for the case-count model (appendix p 2). We fit the model using the observed mosquito count and %*wMel* data. We then replaced the space–time locations that had missing %*wMel* estimates with values predicted by this *wMel* model, leading to an increase in the size of the dataset of 23%. We also ran a sensitivity analysis in which the *wMel* dataset was entirely replaced by values predicted by the *wMel* prediction model—not just in the space–time locations that had missing %*wMel* estimates.

**Model fit**

We examined model fit by comparing predicted case counts within space–time units with observed case counts. We split the dataset into training and testing sets using two different methods. The first was to randomly split the case dataset into two equal parts, with each space–time unit having an equal probability of being within each set. The second was to split up large spatiotemporal regions, consisting of 20% of the global

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See Online for appendix
dataset, as the testing dataset. We then fit the model using the training set only and predicted the number of cases per space–time unit in the testing set. In estimating the case count in larger spatiotemporal regions, the model was asked to predict in places it had no information about over a whole year and across a wide area.

**Role of the funding source**

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

**Results**

Between Aug 29, 2017, and Dec 27, 2019, an estimated 67 million wMel-infected *A aegypti* mosquitoes were released in 658 179 individual release events at 28 489 different locations in the five release zones (figure 1A, B, appendix p 3). The mean average number of releases per month was 24 377. A total of 36 894 mosquito trap collection events were recorded, of which 23 071 contained at least one *A aegypti* mosquito. The overall proportion of trapped mosquitoes that were wMel-positive was 33·8% (95% CI 33·4–34·2; figure 1C), although differences were seen across the release region and over time, with a higher prevalence in release zone RJ1 (52·3% [51·2–53·3]) and a lower prevalence in release zone RJ3.1 (19·8% [19·4–20·3]; figure 1B).

Between 2010 and 2019, 283 270 cases of dengue were reported in Rio de Janeiro’s health-care centres, with an average of 28 327 cases per year. Chikungunya was designated a notifiable disease in 2016 and has been continuously reported since, with an average of 14 426 cases per year (57 705 total cases since 2016; figure 1D). Cases of both chikungunya and dengue have been reported throughout the city (appendix p 4). Before the start of the project, the temporal evolution of cases of both diseases in the project area followed the same trend as in the rest of the city. In 2019, we observed a decrease in the number of reported cases of both diseases in the project area compared with the rest of the city (appendix p 5).

We observed strong seasonality in the case data and the mosquito data. For both dengue and chikungunya, we found that transmission peaked during March and April (figure 2A). The number of *A aegypti* and *Aedes albopictus* mosquitoes trapped as part of the study also followed a similar seasonal trend, peaking in March (figure 2B, appendix p 6). By contrast, the proportion of mosquitoes that were infected with wMel was inversely correlated with the number of cases (Spearman’s $r$=-0.82), decreasing to around 25% in March and April and peaking at 49% in August, when the incidence of disease is lowest (figure 2C). The seasonal signal is still marked when removing subareas RJ3.2 and RJ3.3, where releases occurred later than in the other areas (appendix p 7). The correlation of wMel with the number of releases by month was less substantial (Spearman’s $r$=-0.34).

To explore the overall relationship between wMel introgression and case incidence both before and after the release programme, we initially compared the prevalence of wMel in each space–time unit with the number of cases reported in the same cell and time period across different years (figure 3). We looked at case incidence in the same time period as the wMel data and in the equivalent time period in previous years. These comparisons showed that wMel introgression was more successful in months and locations in which dengue and
chikungunya case incidence tended to be lower each year.

To estimate the effect of the intervention accounting for underlying heterogeneities in space and time in both the case data and the mosquito data, we fit a spatially explicit model to locations in which cases were found within the project area. 2209 space–time units (34% of the project area) had no trapped A aegypti mosquitoes and were therefore excluded from the main analysis as we could not identify the proportion of wMel in those locations. The presence of wMel in local A aegypti mosquitoes had a strong effect on the number of cases of dengue and chikungunya in that location at that time (figure 4). The mean average incidence of dengue within a space–time unit in which wMel was detected was 0·62 (95% CI 0·56–0·68) times the incidence in space–time units in which wMel was not detected; for chikungunya, this value was 0·90 (95% CI 0·84–0·96; figure 4A). The model estimated that spatial correlation between the location of dengue cases within any month extended to 1460 m (95% CI 1071–1815), and to 2081 m (95% CI 1594–2610) for chikungunya cases.

We next fit separate models that explored the relationship between the observed proportion of A aegypti mosquitoes within each space–time unit that were infected with wMel and the incidence of dengue and chikungunya in that space–time unit. We found that space–time units in which 0·1–10·0% of the mosquitoes were infected with wMel had 0·93 (95% CI 0·83–1·04) times the incidence of dengue and 0·98 (95% CI 0·93–1·04) times the incidence of chikungunya compared with locations in which no wMel was detected (figure 4B). In areas with a wMel prevalence of greater than 60%, these values decreased to 0·29 (95% CI 0·24–0·36) for dengue and 0·77 (95% CI 0·65–0·89) for chikungunya. In separate models that considered wMel as a continuous variable, we estimated that each 10% increase in the prevalence of wMel was associated with 0·85 (95% CI 0·83–0·87) times the incidence of chikungunya (appendix p 8).

To ensure that our results were not affected by the recapture of recently released mosquitoes, we repeated our analysis on a dataset in which data for all locations where a release event had occurred within a previous month had been removed. Additionally, because traps were not placed in all locations at all times, leading to missing estimates of %wMel in some space–time units, we also conducted a separate sensitivity analysis in which we initially predicted the %wMel in all space–time locations of the study region (appendix p 2). In both of these sensitivity analyses, the effect of wMel on the incidence of both dengue and chikungunya was consistent with our previous results (appendix p 9).

To assess the performance of our model, we repeatedly removed randomly selected space–time locations from the model (termed held-out locations) and predicted the number of cases in those locations using model fit on the remaining data. We then compared our estimates with the observed number of cases in that location. Good correlation was observed between the predicted and observed number of cases (appendix p 10), including when 50% of randomly selected space–time units were held out (appendix p 10), and when randomly selected spatially clustered cells (5 km² in area) were held out for one year at a time (appendix p 10).

Discussion

In this study, we have critically assessed the effect of a wMel release programme on the incidence of dengue and chikungunya in a diverse, urban setting. By December, 2019, 29 months after the phased releases began, the prevalence of wMel in local A aegypti mosquitoes in the five release areas in Rio de Janeiro was between 27% and 60%. Using a spatially and temporally explicit modelling framework we have shown that,
Despite varying prevalences of uMel across the study area, uMel releases still resulted in lower incidence of both dengue and chikungunya viruses during the first 2 years after the intervention, highlighting the potential of this technology.

Quantifying the protective effect of low-to-moderate uMel prevalence has not previously been possible, because rapid establishment has generally been observed following uMel deployments in other settings, and arboviral case notification data are not commonly available at a high spatial resolution. Here we report a dose–response relationship between uMel prevalence and relative reductions in dengue and chikungunya case incidence. A small but important protective effect against dengue was seen even at very low local uMel prevalence (<10%), and for locations in which the prevalence of uMel was greater than 60%, the protective effect was 76% (95% CI 64–71), which is comparable to previous results (using different methods) from the neighbouring municipality of Niterói and from Indonesia. Why uMel was unable to become quickly established in Rio de Janeiro despite large numbers of releases is unclear. The underlying incidence of dengue and chikungunya in the city is highly heterogeneous. We found that uMel introgression was lower in areas that have a high annual incidence of disease. Seasonal fluctuations in uMel introgression were also observed, with lower levels between February and May, the hottest period of the year. High temperatures have been linked to lower uMel acquisition in laboratory studies. The release programme also made fewer releases during the summer months, which could contribute to this observed seasonal effect. The areas of the city with persistently high dengue and chikungunya incidence could have other factors that complicate the uMel release programme, including large, heterogeneously distributed baseline mosquito populations, or be in areas that are hard to access, such as favela communities. A. albopictus also circulates in Rio de Janeiro but has not been implicated as being involved in dengue and chikungunya incidence in this city. A role for A. albopictus in affecting uMel introgression in A. aegypti remains unclear.

We observed less of an effect of uMel on chikungunya than on dengue. The reasons for this difference are unclear, although A. albopictus could have a role. Although A. albopictus has not directly been implicated in the transmission of chikungunya virus in Rio de Janeiro, this species has been an important vector for the virus elsewhere, and is more rarely involved in the transmission of dengue virus. Even ongoing low levels of chikungunya virus transmission by A. albopictus would result in a seemingly reduced effect of the release programme on the transmission of this virus. Alternatively, there could be underlying biological differences in the relative transmissibility of the dengue and chikungunya viruses in uMel-positive A. aegypti mosquitoes.

Cluster randomised trials—such as that in Yogyakarta, Indonesia, and the trial currently being conducted in Belo Horizonte, Brazil (NCT04514107)—provide a gold-standard measure of the effectiveness of uMel on disease incidence. However, these large trials might not always be feasible, especially when resources are scarce. Our study highlights how the systematic geocoding of cases provides a valuable resource to understand where incidence is concentrated that can also act as a reference point to evaluate the effect of spatially targeted interventions. However, we have shown that, alongside detailed data, we need to use structured models to appropriately measure these datasets. For example, our finding that uMel introgression in a location was correlated with the incidence of dengue and chikungunya in that same location in the years before the intervention highlights the complexity of using observational case data to understand the effect of an intervention of which penetration is itself spatially and temporally uneven. Only through the use of spatiotemporally structured models can we disentangle these different correlation structures to identify the underlying effect of the intervention.

Our study considers data to the end of 2019. Extending the analysis period by including additional years would provide important insight into the durability of the intervention. Unfortunately, the COVID-19 pandemic has had substantial effects on the mosquito release programme and the city health department. In particular, the systematic geocoding of dengue and chikungunya cases by the city health department largely stopped in 2020. By the end of 2021, additional ovitrapping data...
showed that the average introgression level across the project area was more than 50%, suggesting that complete introgression might be possible. The study also has other limitations. In particular, arboreal viral cases are notified on the basis of the patient's place of residence, but people move around and beyond the city and patients might have acquired their infection elsewhere. Further, the majority of the cases are diagnosed on the basis of clinical presentation alone, meaning that some cases could have been misdiagnosed. If we include only confirmed cases in our model (128 dengue cases, 1078 chikungunya cases), the results are consistent with those that included all reported cases; however, the number of confirmed dengue cases is too small to obtain precise estimates (appendix p 11).

We also note the good correlation between the spatiotemporal distribution of unconfirmed cases and that of confirmed cases, suggesting that there is no substantial bias caused by the lack of case confirmation (appendix p 12). In the event that many of the unconfirmed cases were caused by other pathogens, such as influenza, that would not be affected by the mosquito release programme, our estimates of the effect of wMel would be biased towards the null and the true effect would be greater.

Our results provide further evidence that wMel can considerably reduce the public health burden from different arboviruses within the same community. The establishment of wMel in complex urban communities such as Rio de Janeiro is a major challenge, and understanding why the introgression of wMel into A. aegypti populations is faster and more homogeneous in some locations than in others will help to underpin its future success.

We developed a flexible analytical framework that successfully measured, at a fine scale, the effect of a spatially and temporally targeted intervention on the transmission of pathogens with complex dynamics. Our model identified the relationship between the level of introgression and the effect on disease transmission. This framework could be used to assess the efficiency of other spatiotemporally targeted interventions.

Contributors
GRdS visualised the project and did the formal analysis. GRdS and HS did the investigation and developed the method. VS, TISR, and SBP curated the data. BD and KLA validated the data and the method. GRdS wrote the initial draft, and SBP, KLA, LAM, and HS reviewed and edited the manuscript. BD, SBP, KLA, and LAM were involved in project administration. HS acquired funding, and LAM and KLS supervised the project.

Declaration of interests
We declare no competing interests.

Data sharing
All code and data used in these analyses are available at https://github.com/pdgcam/wMel_Rio_dengue_chik.

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