

# Exploring the urban arbovirus landscape in Rio de Janeiro, Brazil: transmission dynamics and patterns of disease spread



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## Summary

**Background** This study focuses on urban arboviruses, specifically dengue (DENV), chikungunya (CHIKV), and Zika (ZIKV), which pose a significant public health challenge in Rio de Janeiro state, Southeast Brazil. In our research, we highlight critical findings on the transmission dynamics of these arboviruses in Rio de Janeiro, identifying distinct patterns of disease spread.

**Methods** By combining genomic data with case reports from the Brazilian Ministry of Health, we have analysed the phylogenetics, prevalence and spatial distribution of these endemic viruses within the state.

**Findings** Our results revealed sustained DENV transmission primarily in the northern part of the state, a significant ZIKV epidemic in 2016 affecting all mesoregions, and two major CHIKV outbreaks in 2018 and 2019, predominantly impacting the northern and southern areas. Our analysis suggests an inverse relationship between arboviral case incidence and urban density, with less populous regions experiencing higher transmission rates, potentially attributed to a complex interplay of factors such as the efficacy of vector control measures, environmental conditions, local immunity levels, and human mobility. Furthermore, our investigation unveiled distinct age and gender trends among affected individuals. Notably, dengue cases were predominantly observed in young adults aged 32, while chikungunya cases were more prevalent among individuals over 41. In contrast, cases of ZIKV were concentrated around the 33-year age group. Intriguingly, females accounted for nearly 60% of the cases, suggesting a potential gender-based difference in infection rates.

**Interpretation** Our findings underscore the complexity of arbovirus transmission and the need for interventions tailored to different geographical mesoregions. Enhanced surveillance and genomic sequencing will be essential for a deeper, more nuanced understanding of regional arbovirus dynamics. Identifying potential blind spots within the state will be pivotal for developing and implementing more effective public health strategies, specifically designed to address the unique challenges posed by these viruses throughout the state.

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### Research in context

#### Evidence before this study

We conducted a comprehensive search of the PubMed database to identify previous evidence on the circulation of arboviruses across the various mesoregions within the state of Rio de Janeiro. Our search utilized the terms “Arboviruses”, “DENV”, “CHIKV\*”, “ZIKV”, “Rio de Janeiro”, and “Rio de Janeiro’s mesoregions” from inception until November 30th, 2023. Prior to this study, the urban arbovirus landscape in Rio de Janeiro, Brazil, was mostly unexplored, focusing only on the description of sporadic epidemics. Despite numerous studies on the prevalence and control of mosquito-borne diseases such as dengue, Zika, and chikungunya across Brazil, few have delved into the unique urban challenges presented by Rio de Janeiro. This city’s distinctive characteristics—high population density, complex environmental conditions, and varying levels of health infrastructure—create a unique scenario for arbovirus transmission. Such conditions exacerbate the challenges of disease management and control, setting Rio de Janeiro apart from other urban settings both within Brazil and globally.

#### Added value of this study

This study provides a comprehensive analysis of Rio de Janeiro’s urban arbovirus landscape, uncovering distinct patterns in disease transmission influenced by urbanization, ecological variations, and population density. We identified characteristic spatial and temporal trends, including sustained

DENV transmission, a significant ZIKV epidemic in 2016, and major CHIKV outbreaks occurred in 2018 and 2019. Interestingly, our analysis suggests an inverse relationship between arboviral case incidence urban density, with peri-urban areas experiencing higher transmission rates, potentially due to factors like vector control measures, environmental condition, local immunity levels, and human mobility. Demographic analysis revealed specific trends with DENV, CHIKV, and ZIKV affecting distinct age groups—predominantly young adults around 32, those over 41, and at 33 years, respectively, with a notable roughly 60% of cases being female. These findings underscore the complex dynamics of arbovirus spread and the need for tailored public health strategies.

#### Implications of all the available evidence

Our findings highlight the critical need for public health strategies that are specifically tailored to address the unique challenges posed by urbanization, ecological variations, and population density. These insights advocate for the development of targeted interventions and policies that consider the specific ecological and socio-demographic contexts of urban and peri-urban areas. Moreover, our research provides a valuable framework for the global health community, offering actionable for the effective management and control of arboviruses in similarly urbanized settings worldwide.

## Introduction

Urban arboviruses such as dengue (DENV), chikungunya (CHIKV), and Zika (ZIKV), continue to pose significant public health concerns in Brazil, expanding in conditions favoured by a confluence of human and ecological factors.<sup>1</sup> The latter include adequate levels of temperature, humidity, and rainfall, all exacerbated by the uneven distribution of water treatment services<sup>1,2</sup> creating ideal environments for the reproduction and dispersal of *Aedes spp.* mosquitoes, which are the primary vectors for transmission of these arboviruses.<sup>1</sup> Notably, the first reported epidemic compatible with a dengue viral infection occurred in 1845,<sup>3</sup> marking the beginning of its long history in Brazil. The reinfestation of Brazil by the *Aedes aegypti* mosquito in 1977 significantly contributed to the reintroduction of DENV in the 1980s.<sup>4</sup> Initially, serotypes 1 and 4 were identified in the state of Roraima located in the Northern part of the

country, and subsequently, in 1986, serotype 1 was also reported in Rio de Janeiro state.<sup>4</sup> Since then, dengue has become endemic in Brazil, with the country accounting for approximately 60% of reported cases in the Americas.<sup>5</sup> The state of Rio de Janeiro, with a population of over 16 million, has emerged as a focal point for urban arbovirus transmission, necessitating a deeper understanding of the epidemiological history and situation in the region.<sup>5,6</sup> The introduction of DENV-1 genotype V in the late 1980s marked the onset of sustained dengue activity in the state.<sup>5</sup> The emergence of DENV-2 in the early 1990s,<sup>4</sup> identified by its Asian/American genotype (genotype III) within the metropolitan area, led to significant epidemics and introduced the first severe forms of the disease.<sup>6</sup> The early 2000s saw the introduction of DENV-3 genotype III, causing to date the largest epidemic in the summer of 2001–2002,<sup>6</sup> which has also recently experienced a significant

resurgence.<sup>7,8</sup> DENV-4's identification in 2011 in Niterói and its subsequent spread highlights the ongoing evolution of dengue's epidemiological landscape in Rio de Janeiro.<sup>6,9</sup> This period was particularly marked by the co-circulation of two distinct genotypes of DENV-4, genotypes I and II, with genotype II becoming the predominant strain in the state.<sup>9</sup> This emergence further complicated the epidemiological scenario, leading to a notable increase in dengue cases, which showed a significant rise in the total number of reported cases compared to the year preceding the arrival of DENV-4.<sup>10</sup> Furthermore, between 2011 and 2013, DENV-4 was identified in 44.2% of dengue-positive cases, underscoring its substantial impact on the epidemic in Rio de Janeiro.<sup>6,9</sup>

CHIKV and ZIKV, first identified in Rio de Janeiro in 2015,<sup>10–12</sup> have introduced new challenges at both local and national levels. CHIKV is notably linked to chronic conditions that significantly affect the quality of life, especially due to the persistent joint pain experienced by patients post-recovery.<sup>13</sup> While CHIKV's incidence in 2023 has seen a decline, the rising cases in neighbouring states signal a potential threat of re-emergence.<sup>14</sup> ZIKV, circulating since 2015,<sup>10</sup> drew widespread concern during Brazil's 2016 epidemic,<sup>12</sup> primarily due to its association with Guillain-Barré syndrome<sup>15</sup> and congenital Zika syndromes.<sup>12,16,17</sup> In recent years, the overlapping clinical symptoms among these arboviruses, the potential for cross-immunity between ZIKV and DENV,<sup>18</sup> and changes in surveillance during the COVID-19 pandemic have contributed to a drop in case reports. However, as the COVID-19 pandemic progressed and subsequent decline, a resurgence in arbovirus cases was observed.<sup>19,20</sup>

The evolving landscape of arboviral diseases in both the state and the country has been significantly highlighted by the introduction, re-introduction and circulation of different dengue virus serotypes and other arboviruses. The introduction of DENV-3 and DENV-4 alongside the already circulating DENV-1 and DENV-2, has notably complicated the epidemiological landscape. Since late 2014, the emergence of other arboviruses, such as CHIKV, ZIKV, has further diversified the arboviral challenges faced by public health systems. This scenario presents a complex interplay of multiple viruses, underscoring the critical need for integrated surveillance and control strategies to effectively manage the spread of these diseases.

Given Rio de Janeiro's role as a significant hub for business and tourism in Brazil's southeastern region, attracting an influx of 1.6 million airline passengers annually, understanding the urban arboviral epidemiology and spatial dynamics within this context is essential. The lack of detailed data on the genomic epidemiology of these co-circulating viruses highlights an urgent need for focused public health strategies and preparedness plans. Our study aims to explore the phylogenetic history and

spatial distribution of these viruses in the Rio de Janeiro state, seeking to enhance our understanding of their historical and contemporary transmission patterns. This comprehensive approach is vital for informing public health responses and mitigating the impact of these viruses on affected populations.

## Methods

### Sequence data collection

We retrieved all available viral genome sequences of DENV 1–4, CHIKV, and ZIKV from the state of Rio de Janeiro from public repositories such as NCBI<sup>21</sup> and GISAID (<https://www.gisaid.org/>) up to November 30th, 2023. Prior to any phylogenetic inference, we undertook a rigorous quality assessment of these sequences. This process involved excluding incomplete or problematic sequences, as well as those lacking comprehensive metadata (for example collection date; country of origin). Sequences underwent genotype assignment using the Genome Detective Tool.<sup>22</sup> Additionally, we removed sequences containing more than 10 ambiguous bases. To assess the spread of the virus within the state, we considered other existing and representative sequences collected globally. Acknowledging the impact of sampling bias on ancestral state reconstruction methods, we aimed to reflect the global diversity of genomes in our sampled datasets. In Brazil, given the high number of arbovirus cases, we adopted a downsampling approach to ensure balanced representation by selecting reference sequences based on the reported incidence of cases across Brazilian regions and the availability of genome sequences, thereby mitigating potential bias from uneven sequence data distribution. For countries in the Americas other than Brazil, we did not employ the same downsampling strategy due to the limited number of available genome sequences. For countries outside the Americas not directly linked to the epidemics in Rio de Janeiro and Brazil, our downsampling criteria were based on metadata availability and sequence quality, as previously described (Figure S1).

The Americas was categorised into four regions for simplification: i) North America, consisting solely of the United States; ii) the Caribbean (n = 19), including countries such as Anguilla, Antigua and Barbuda, Aruba; iii) Central America (n = 8), covering Belize, Costa Rica, El Salvador, Guatemala; and iv) South America (n = 10), encompassing Argentina, Bolivia, Chile, Colombia. The final datasets for each viral pathogen contained the following numbers of sequences: DENV-1 accounted for n = 2580 sequences (including 91 genomes from Rio de Janeiro); DENV-2 accounted for n = 2176 sequences (including 144 genomes from Rio de Janeiro); DENV-3 accounted for n = 2220 sequences (including 13 genomes from Rio de Janeiro); DENV-4 accounted for n = 1579 sequences (including 14 genomes from Rio de Janeiro); CHIKV accounted for

n = 1627 sequences (including 130 genomes from Rio de Janeiro); ZIKV accounted for n = 481 sequences (including 30 genomes from Rio de Janeiro).

### Phylogenetic reconstruction

Sequences were aligned using MAFFT employing default parameters.<sup>23</sup> The alignment was manually curated to remove artefacts at the terminal regions and within the alignment using Aliview.<sup>24</sup> Phylogenetic analysis of these sequences was performed using the maximum likelihood method implemented in IQ-TREE v.2.<sup>25</sup> The tree was inferred with the general time reversible (GTR) model of nucleotide substitution and a proportion of invariable sites (+I) as selected by the ModelFinder application. Branch support was assessed by the approximate likelihood-ratio test based on the bootstrap and the Shimodaira–Hasegawa-like procedure (SH-aLRT) with 1000 replicates. The resulting tree topologies were inspected for temporal molecular clock signals using the clock functionality of TreeTime.<sup>26</sup> ML-trees were then transformed into time scaled phylogenies in TreeTime. Outlier sequences that deviated from the strict molecular clock assumption as flagged by TreeTime were removed with the Ape package in R<sup>27</sup> until a good time scaled phylogeny was obtained. The percentage of outliers ranged from 0.8% for DENV-1 to 1.5% for DENV-2, to 1.8% for DENV-3, to 1.3% for DENV-4, to 1.2% for CHIKV, and 1.7% for ZIKV. For each viral population, we produced time scaled tree topologies and performed discrete ancestral state reconstruction (of locations) to infer the global dissemination of each virus using the *migration* package extension of TreeTime under a GTR model. The *migration* package systematically explores the entire tree, including internal nodes and tips, to evaluate the potential and putative events. Finally, using a custom python script, we counted the number of state changes from the resulting annotated tree topologies by iterating over each phylogeny from the root to the external tips. We count state changes when an internal node transitions from one location to a different location in the resulting inferred internal node or tip(s). The timing of putative transition events was recorded, which served as the estimated import or export event. All results were visualized using ggplot libraries.<sup>28</sup>

### Epidemiological data

Data regarding weekly reported cases of DENV, ZIKV, and CHIKV in the state of Rio de Janeiro were sourced from the Sistema de Informação de Agravos de Notificação (SINAN) (<https://portalsinan.saude.gov.br/dados-epidemiologicos-sinan>), as provided by the Brazilian Ministry of Health.<sup>19</sup> While SINAN has been collecting epidemiological data on arboviral infections since its inception, it is important to note that the reliability and comprehensiveness of this data have significantly improved post-2021. Accordingly, our analysis specifically

utilized the curated dataset from 2015 onwards to ensure robustness and accuracy. According to the Ministry's definition, a case is considered “confirmed” either by laboratory criteria (molecular and serological) or clinical-epidemiological criteria, considering the clinical symptoms and epidemiological history of that patient. It is also noted by the Ministry that during epidemic seasons, most cases are confirmed based solely on clinical criteria. The dataset included metadata such as the date of sample collection, the identified virus, patient gender, age, and the municipality and state of residence. We processed this data to categorise each municipality into one of the six mesoregions of the state. The northern part of the state encompassed *Noroeste Fluminense* (NWF) and *Norte Fluminense* (NOF). The central region included *Baixadas* (CL) and *Centro Fluminense* (FC), while the southern region comprised *Metropolitana do Rio de Janeiro* (MR) and *Sul Fluminense* (SF). Monthly climate data for Rio de Janeiro was obtained from Copernicus.eu satellite climate data (<https://www.copernicus.eu/en>). We derived the temperature data summary by computing the yearly lowest, mean, and maximum values. In addition, to evaluate cross-correlations of the spatial distributions of the three different viruses we utilised the *spatialEco* package V2.0-0 in R (<https://github.com/jeffrejevans/spatialEco>), which follows the recent advancements of Chen YG in spatial cross-correlation analyses.<sup>29</sup> For each pair of viruses, after aggregating incidence across the observation period, we used the cross-Correlation function to quantify Local Indicators of Spatial Association (LISA) clusters (high–high, high–low, low–high, low–low) and the spatial autocorrelation global Moran's I.

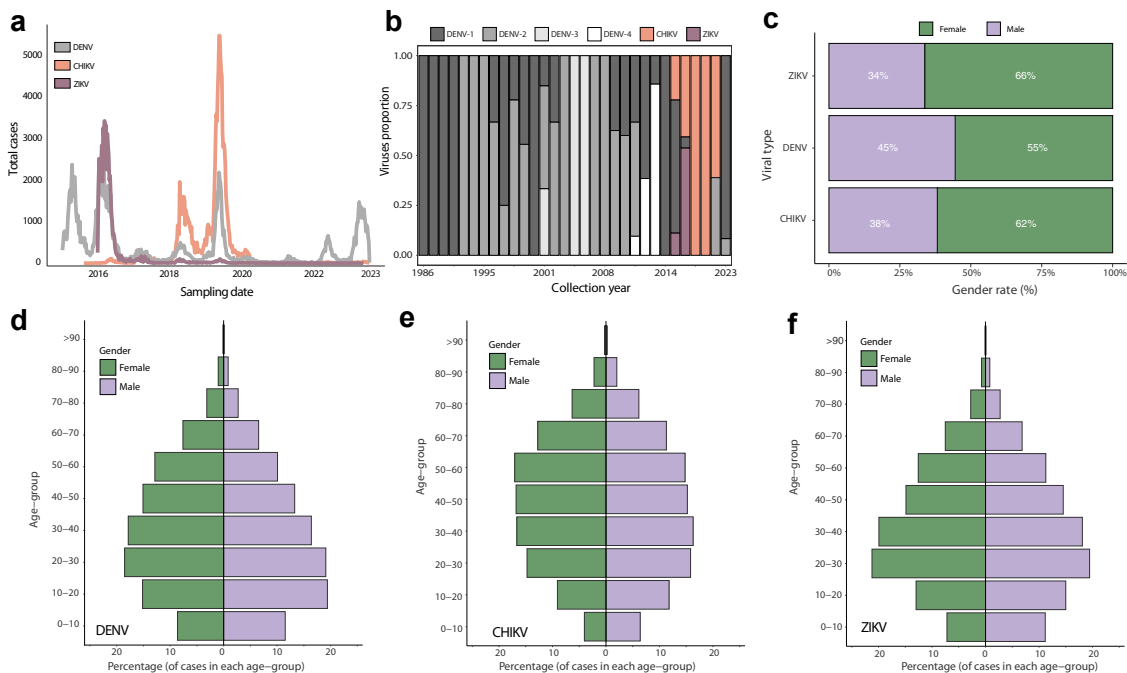
### Role of funding source

The funding source supported the design and execution of this study, including data collection, analysis, and interpretation. It had no role in the writing of the manuscript or the decision to submit it for publication.

### Results

The analysis of weekly notified Dengue cases from 2015 to 2023 highlights distinct epidemic peaks, with significant outbreaks occurring in early 2015, from late 2015 to early 2016, in early 2019, and again in early 2023 (Fig. 1a). This pattern aligns with trends observed both within the country and internationally,<sup>30,31</sup> where a notable decrease in Dengue notifications was seen following 2016. This reduction coincides with the emergence of the ZIKV (Fig. 1a). Concurrently, the state witnessed the introduction of CHIKV in mid-2015. However, CHIKV's most significant outbreak did not occur until the 2018–2019 period, during which the weekly case count surpassed 5000 in 2019.

Analysis of genomic data over the years (Fig. 1b), identified that Dengue serotypes presented temporal dynamics with an oscillatory behaviour characterised by



**Fig. 1: Dynamics of circulating urban arboviruses in Rio de Janeiro, Southeast Brazil.** a) A time series from 2015 to 2023 showing weekly reported cases in the state, categorized by virus type (DENV in grey, CHIKV in dark green, ZIKV in purple). b) Proportion of available viral genomes from samples collected in the state of Rio de Janeiro, highlighted by year and virus type, with colors representing each virus (DENV in scale grey, CHIKV in dark green, ZIKV in purple); c) Gender rate of the reported cases for DENV, CHIKV and ZIKV; d–f) Breakdown of reported cases by gender and age for DENV, CHIKV and ZIKV respectively.

recurrent peak genome prevalence. DENV-2 was the most sequenced viral strain in the state, followed by DENV-1. DENV-3 was only present in 2001 and 2004–2005, while DENV-4 was only detected in 2011–2013. Notably, CHIKV and ZIKV genomes were documented in years following their emergence, but seemingly disappearing from public databases thereafter.

When examining the age profile of infected individuals, we found that the median age varied by virus: 32 years for DENV, 41 years for CHIKV, and 33 years for ZIKV. Notably, there was a slight female predominance, with females accounting for more than 55% of the cases, reaching up to 66% (Fig. 1c). Cases were most frequently reported in two age groups for both females and males 30–40 years across all viral infections and 20–30 years (all but CHIKV), with those aged 40–60 years being particularly affected by CHIKV (Fig. 1d–f). However, the 0–10-year age group was the least affected, and the distribution of cases was slightly higher among females compared to males (Fig. 1d–f).

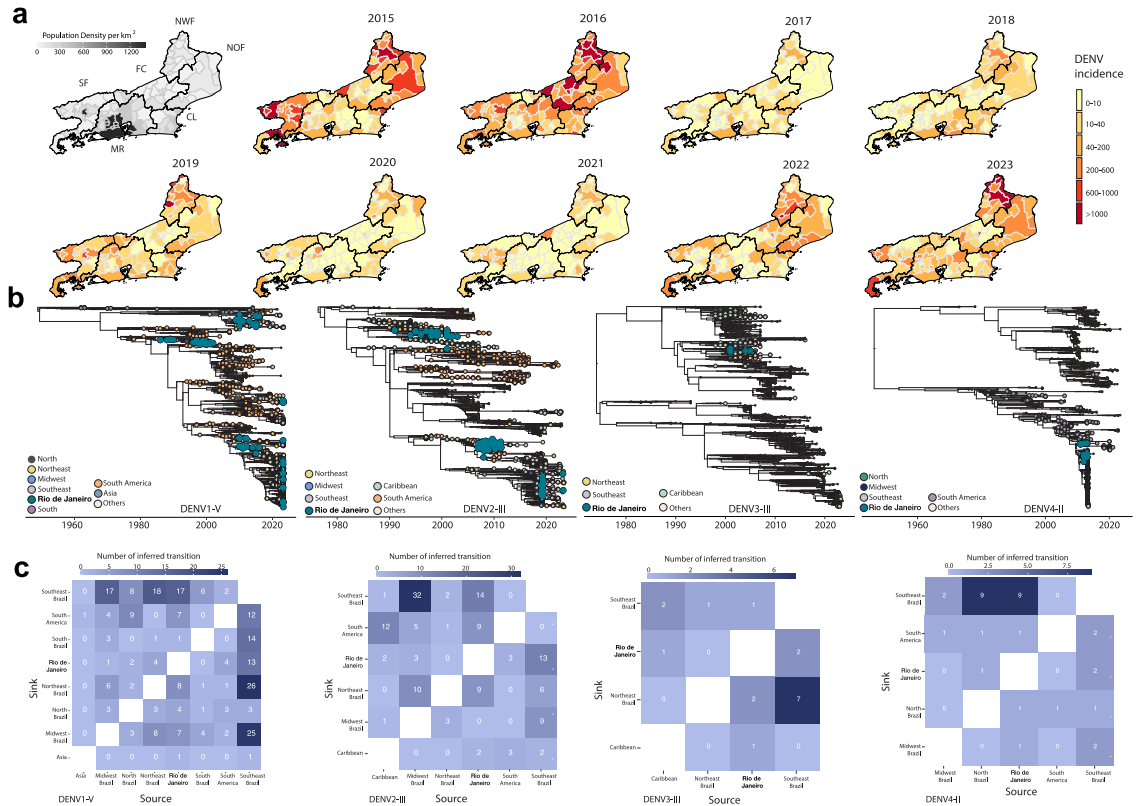
### Dengue virus transmission dynamics in the state of Rio de Janeiro

The analysis of spatial distribution patterns of dengue cases across the mesoregions of Rio de Janeiro, depicted in Figs. 1 and 2a, revealed distinct epidemic waves in 2015, 2016, and again in 2023. In 2015, the highest

incidence was observed in the North mesoregions (NWF, NOF) and the South (SF). Intriguingly, the Central mesoregions (CL and FC) also experienced an increase in case numbers during this period (Fig. 2a). However, this increase did not align with human density, as evidenced by a Pearson correlation coefficient of  $-0.16$  and a P value of  $1.15 \times 10^{-11}$ . This discrepancy prompted further investigation into the role of climatic conditions, particularly temperature. Interestingly, the correlation between dengue incidence and temperature was found to be low. Despite this, our results highlight that peri-urban regions, among others, have reported the highest mean temperatures over the past nine years (Figure S2).

From 2020 to 2021, there was a notable decrease in case counts across all mesoregions. However, an upsurge was detected in 2023, with the North (NWF) region reporting the highest case numbers as of September 2023, as shown in Fig. 2a. It is important to note that during the COVID-19 pandemic, arbovirus notifications decreased, likely influenced by the implemented sanitary measures and the presence of overlapping symptoms between SARS-CoV-2 and arboviral infections.

Fig. 2b presents time-stamped maximum likelihood phylogenetic trees for all DENV serotypes/genotypes detected in the region since 1986, including DENV1



**Fig. 2: Dengue transmission dynamics in Rio de Janeiro.** a) The first map indicates the different mesoregion of Rio de Janeiro state: Baixadas (CL), Centro Fluminense (FC), Metropolitana do Rio de Janeiro (MR), Noroeste Fluminense (NWF), Norte Fluminense (NOF) e Sul Fluminense (SF). Weekly notified dengue cases normalized per 100 K individuals by mesoregion in 2015–2023. b) Time-stamped Maximum Likelihood trees for dengue serotypes (1–4) progress from left (serotype 1) to right (serotype 4). Tips on the trees are annotated with circles and colored based on their locations. Colors denote distinct sampling locations in accordance with the legend located to the left of each tree. Only significant sampling locations involved in DENV transmission with Rio de Janeiro have been highlighted, while others are grouped as ‘Others.’ Rio de Janeiro state genomes are highlighted in teal blue. c) Heatmaps display the number of inferred virus transitions, across the entire study timeframe, ranging from lower numbers in light blue to higher numbers in dark blue. Inside each square there is the number of the inferred transitions. The y-axis axis represents sinks/destinations, and the x-axis represents sources/origins, with one heatmap per serotype.

genotype V (DENV1-V), DENV2 genotype III (DENV2-III), DENV3 genotype III (DENV3-III), and DENV4 genotype II (DENV4-II).

Analysis indicated six probable introduction events of DENV1-V into Rio de Janeiro, with the initial introduction suggested to have a South American origin in the late 1980s. The Rio de Janeiro isolates exhibited significant clustering with those from different Brazilian regions and other South American countries, indicative of both internal and cross-border transmission dynamics. Notably, a substantial number of transition events were traced back to Rio de Janeiro, predominantly originating from Southeast Brazil (13 events), with Northeast Brazil and other South American countries each accounting for four events. Fewer transitions were observed from North (2 events) and Midwest Brazil (1 event). In contrast, transitions originating from Rio de Janeiro were primarily directed towards Southeast Brazil (17 events)

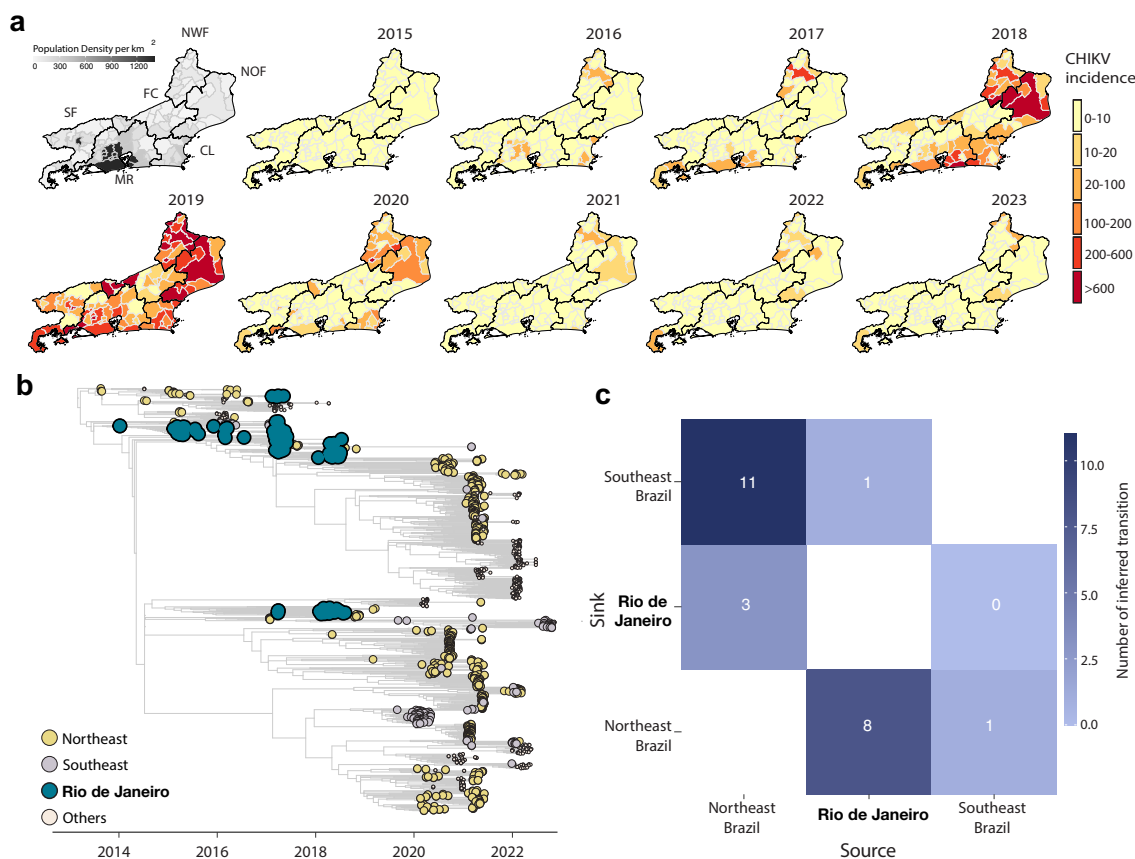
and Northeast Brazil (8 introductions), as shown in Fig. 2c.

Phylogenetic reconstruction also pointed to the likely introduction of DENV2-III into Rio de Janeiro from dispersal events in the Caribbean and South America during the 1990s, as depicted in Fig. 2b. The Rio de Janeiro isolates are grouped into three distinct clades. The earliest identified DENV2-III genome in Rio de Janeiro is linked to its introduction from the American region, followed by its spread to Northeast Brazil and subsequent reintroductions in the state and other South American countries. Similar to DENV1-V, the most frequent introduction events for DENV2-III originated from Southeast Brazil, totalling 13 events. The dissemination of DENV2-III from Rio de Janeiro notably affected other Brazilian regions, particularly Southeast Brazil (14 events) and Northeast Brazil (9 events), and also extended to other South American regions (9 events), as shown in Fig. 2c.

Concerning DENV3-III, our findings showed a predominant association and clustering with regions in Southeast Brazil (two events) and the Caribbean (one event) (Fig. 2b). Following its first introduction, our data indicates that DENV3-III likely spread throughout the Northeast and Southeast mesoregions of Brazil. Finally, for DENV4-II, our analysis revealed that the isolates from Rio de Janeiro were predominantly associated with those from Southeast and North Brazil. This observation, despite being based on a limited dataset, provides evidence that DENV4-II was introduced into Rio de Janeiro from these regions. Specifically, we identified two independent introduction events from Southeast Brazil and one from North Brazil, as illustrated in Fig. 2c.

### Chikungunya virus transmission dynamics in Brazil, 2015–2023

Fig. 3a illustrates the spatial distribution of CHIKV cases across the mesoregions of Rio de Janeiro. From 2016 to the end of 2017, the prevalence of CHIKV remained relatively stable but with a significant surge in cases in the North (NWF and NOF) mesoregions in 2018, followed by notable increases in the Central (FC, CL), and South (MR) mesoregions in 2019. In 2020, reported CHIKV cases were relatively low, likely influenced by the COVID-19 pandemic. Additionally, it is interesting to note that, similar to previous observations, the incidence of CHIKV does not appear to be related to human density, as indicated in Fig. 3a (Pearson coefficient correlation =  $-0.15$ ,  $P$  value =  $1.70 \times 10^{-7}$ ).



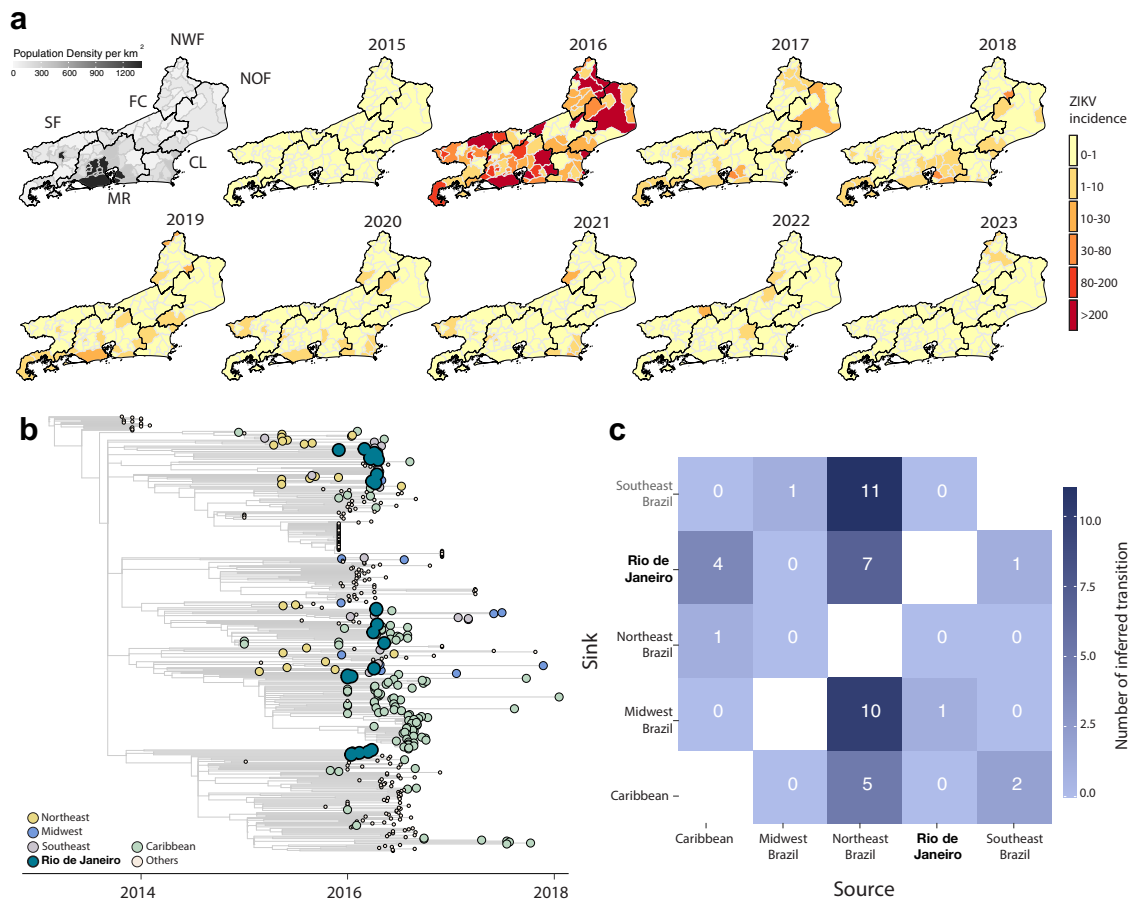
**Fig. 3: Chikungunya transmission dynamics in Rio de Janeiro.** a) The first map indicates the different mesoregion of Rio de Janeiro state: Baixadas (CL), Centro Fluminense (FC), Metropolitana do Rio de Janeiro (MR), Noroeste Fluminense (NWF), Norte Fluminense (NOF) e Sul Fluminense (SF). Weekly notified chikungunya cases normalized per 100 K individuals by mesoregion in 2015–2023. b) Time-stamped Maximum Likelihood tree for chikungunya. Tips on the tree are annotated with circles and colored based on their locations. Colors denote distinct sampling locations in accordance with the legend located to the left of each tree. Only significant sampling locations involved in CHIKV transmission with Rio de Janeiro transmission history are listed, while others are grouped as ‘Others.’ Rio de Janeiro state genomes are highlighted in teal blue. c) Heatmaps display the number of inferred virus transitions, across the entire study timeframe, ranging from lower numbers in light blue to higher numbers in dark blue. Inside each square there is the number of the inferred transitions. The y-axis axis represents sinks/destinations, and the x-axis represents sources/origins.

Phylogenetic analysis suggests that CHIKV was initially introduced to Rio de Janeiro through a dispersal event from Brazil's Northeast mesoregion in early 2015 (Fig. 3b). This corresponds to the same region where it was first detected in mid-2014.<sup>32</sup> Subsequent dispersion from Rio de Janeiro to other regions of Brazil occurred in 2018 and 2019 (Fig. 3b), highlighting the state's pivotal role in the continued expansion and resurgence of CHIKV, especially in the Northeast and Southeast mesoregions of Brazil. However, the paucity of genome sequences from Rio de Janeiro in 2019 may have led to an underestimation of the state's impact on the broader Brazilian CHIKV context. The majority of inferred transmission events, involving Rio de Janeiro, were from Rio de Janeiro to Northeast Brazil (eight events,

Fig. 3c), with a bidirectional exchange pattern evident, as three introduction events from the Northeast back into Rio de Janeiro were also identified.

**Zika virus transmission dynamics in Brazil, 2015–2023**

Fig. 4a presents the geographical distribution of ZIKV cases across the mesoregions of Rio de Janeiro. In contrast to the patterns observed with DENV and CHIKV, ZIKV exhibited a single peak in cases in 2016 that affected nearly all macroregions across the state.<sup>32</sup> Following that peak, from 2017 to 2020, there were minor increases in the number of ZIKV cases, primarily observed in the North (NOF), Central (MR), and South (SF) regions. Interestingly, as with DENV and CHIKV,



**Fig. 4: Zika transmission dynamics in Rio de Janeiro.** a) The first map indicates the different mesoregion of Rio de Janeiro state: Baixadas (CL), Centro Fluminense (FC), Metropolitana do Rio de Janeiro (MR), Noroeste Fluminense (NWF), Norte Fluminense (NOF) e Sul Fluminense (SF). Weekly notified Zika cases normalized per 100 K individuals by mesoregion in 2015–2023. b) Time-stamped Maximum Likelihood tree of Zika isolates. Tips on the tree are annotated with circles and colored based on their locations. Colors denote distinct sampling locations in accordance with the legend located to the left of each tree. Only significant sampling locations involved in ZIKV transmission with Rio de Janeiro transmission history are listed, while others are grouped as 'Others.' Rio de Janeiro state genomes are highlighted in teal blue. c) Heatmaps display the number of inferred virus transitions, across the entire study timeframe, ranging from lower numbers in light blue to higher numbers in dark blue. Inside each square there is the number of the inferred transitions. The y-axis axis represents sinks/destinations, and the x-axis represents sources/origins.



we also noticed that the incidence of ZIKV does not appear to be related to human density (Pearson coefficient correlation =  $-0.15$ ,  $P$  value =  $8.16 \times 10^{-5}$ ) (Fig. 4a). This consistent observation across different viruses suggests that factors other than human population density might play a significant role in the spread of these diseases in the region. Starting in 2020 and continuing through 2023, the reported incidence of Zika cases continuously decreased. Our phylogenetic tree of ZIKV (Fig. 4b) revealed multiple clades, underscoring the occurrence of several introduction events in the country over time.

There were three probable introduction events into the state of Rio de Janeiro. ZIKV isolates originating from Rio de Janeiro are clustered with sequences from the Midwest, Northeast, and Southeast regions of Brazil, as well as with those from Caribbean regions. This complex pattern of virus dissemination highlights the intricate dynamics within Brazil and across the continent. The majority of inferred transmission events linked to Rio de Janeiro involved the influx of individuals moving into Rio de Janeiro, primarily from Northeast Brazil (seven events, as depicted in Fig. 4c), and from the Caribbean (four events).

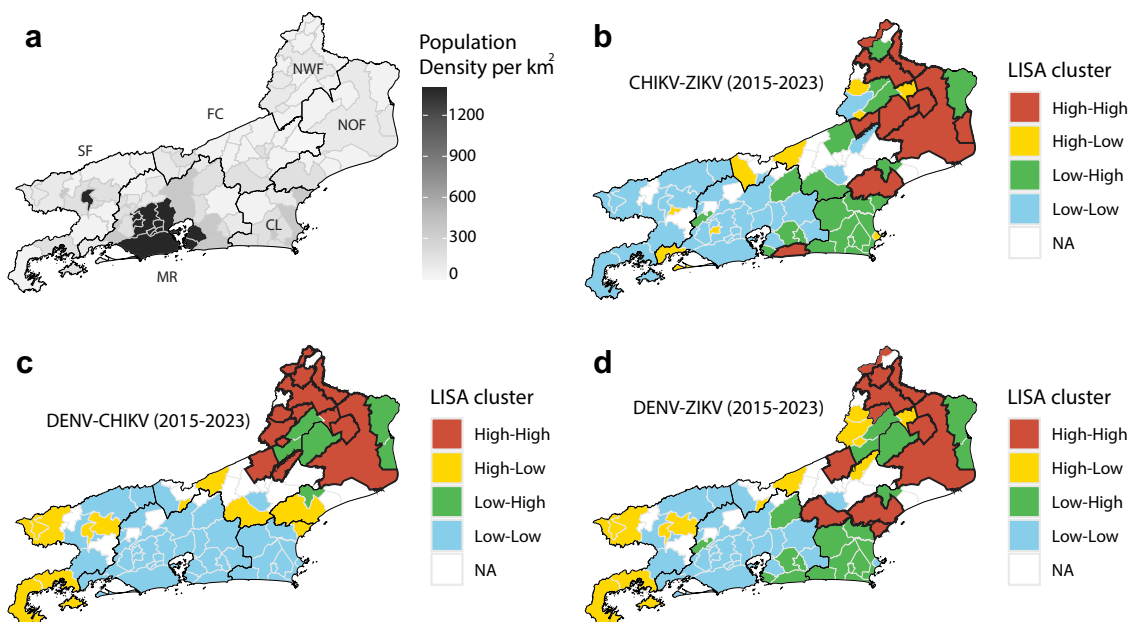
Finally, we assessed the spatial cross-correlation of incidence between the different viruses. Fig. 5 shows LISA clusters for each pair of viruses. Across all pairs of viruses, clusters of mutual high incidence (LISA clusters

high–high) were identified in the northeastern region, while clusters of mutual low incidence were generally identified in the south and southwestern regions (Fig. 5a–c). This result was in accordance with the observation that arboviral incidence was typically associated with lower human density (Fig. 5d). In fact, all of the municipalities with high human density were identified as belonging to the LISA clusters corresponding to mutual low incidence independently of the pair of viruses analysed (Fig. 5a–c).

## Discussion

In this study, we delved into the complex landscape of urban arboviruses in the state of Rio de Janeiro, Southeast Brazil, focusing on the public health implications of DENV, CHIKV, and ZIKV. Our analysis leverages genomic and incidence data sourced from the Brazilian Ministry of Health to reveal spatial–temporal patterns of these viruses within the region.

Our findings indicate that DENV has transitioned to an endemic status in Southeast Brazil, with significant outbreaks occurring in early 2015, late 2015–early 2016, early 2019, and again in 2023. Contrary to expectations, our analysis revealed no direct correlation between dengue incidence and population density. Further investigation into climatic conditions, particularly temperature, also revealed a surprisingly low correlation.



**Fig. 5: Spatial cross-correlation between virus incidence and human density for DENV, CHIKV, and ZIKV (2015–2023).** a) The first map displays the population density per km<sup>2</sup> calculated for the different mesoregions of Rio de Janeiro state: Baixadas (CL), Centro Fluminense (FC), Metropolitana do Rio de Janeiro (MR), Noroeste Fluminense (NWF), Norte Fluminense (NOF), and Sul Fluminense (SF). b–d) Panels b–d illustrate the spatial cross-correlation calculated for each pair of viruses (CHIKV-ZIKV; DENV-CHIKV; DENV-ZIKV) after aggregating incidence across the observation period (2015–2023), utilizing the cross-correlation function to quantify Local Indicators of Spatial Association (LISA) clusters (high–high, high–low, low–high, low–low), and the spatial autocorrelation global Moran’s I.

Despite this, our data highlight that peri-urban regions, among others, have reported the highest mean temperatures over the last nine years. Given the low correlation, our findings suggest that a complex interplay of factors influences the dynamics of dengue transmission. These factors likely include vector control initiatives, local immunity levels, and human mobility patterns. The interaction between these factors and climatic conditions may further complicate the transmission dynamics of such arboviruses at the national and regional levels. This revelation has critical implications for public health strategies and resource allocation. It indicates that interventions cannot solely focus on high-density urban areas but must also address the complex interplay of factors contributing to DENV transmission in less densely populated areas. Tailoring control measures to account for these nuanced dynamics, including the enhancement of vector surveillance and control programs, public health education, and the development of healthcare infrastructure to manage outbreaks, becomes pivotal.

Conversely, ZIKV presented a different trend, characterised by a single, widespread surge of infection in 2016 that affected all mesoregions in the state, leading to a substantial outbreak.<sup>33,34</sup> The national decrease in ZIKV cases thereafter is attributed to the onset of herd immunity, aligning with modelling studies' forecasts that herd immunity would significantly curb transmission within the virus's first decade of emergence.<sup>33,35</sup>

The pattern of inverse correlation between arbovirus incidence and urban density, initially noted for DENV, extends to CHIKV. This suggests that transmission is more pronounced in northern areas, despite the southern mesoregions' larger population. Factors such as socioeconomic conditions, and human mobility, particularly proximity to state borders, appear to facilitate the sustained transmission of these viruses.<sup>36</sup>

Our study further documents significant outbreaks of CHIKV in 2018 and 2019, followed by a notable decline in cases. The 2019 epidemic, particularly severe in the North (NWF and NOF) mesoregions, represents the most significant CHIKV outbreak in Rio de Janeiro to date.<sup>37,38</sup> The concurrent COVID-19 pandemic is likely to have led to a decrease in arbovirus reporting, a result of both a shift in surveillance priorities and the symptom overlap between SARS-CoV-2 and arboviral infections.<sup>39,40</sup> This situation underscores the importance of maintaining robust surveillance systems, which are crucial for developing effective regional intervention strategies.

Our demographic descriptive analysis revealed distinct patterns in the age and gender of individuals affected by these arboviruses in Rio de Janeiro. DENV predominantly affected young adults around 32 years old, consistent with the age group's susceptibility in urban settings. CHIKV cases were more common among individuals over 41, likely due to the more severe symptoms experienced by older populations. ZIKV showed a similar age distribution

to DENV, suggesting overlapping transmission dynamics. Interestingly, females represented 60% of the cases across all viruses, indicating a difference in infection rates based on gender.

### Limitations

The limited number of complete genome sequences available for these viruses, despite their high incidence, points to a significant gap in our understanding of the arbovirus landscape in Rio de Janeiro. This gap is further underscored by the recent approval of the live-attenuated QDENGUA vaccine by Takeda, highlighting the importance of prevention and effective treatment strategies tailored to the mesoregion level.<sup>41</sup> While our study provides insight into the spatial-temporal dynamics of DENV, CHIKV, and ZIKV in Rio de Janeiro, it is constrained by the limited availability of whole genome sequences and incomplete metadata, which may hamper a full understanding of transmission dynamics and restrict accurate estimates related to divergence time and ancestral location reconstructions. Future sequencing efforts are essential for addressing these limitations.

### Conclusions

Considering Rio de Janeiro's significance as a hub for human mobility, our findings underscore the intricate dynamics of arbovirus transmission in the region and the urgent need for targeted public health interventions to mitigate the substantial challenges posed by these diseases.

### Contributors

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### Data sharing statement

Input files (e.g. alignments and metadata), used in the study are shared publicly on GitHub (<https://github.com/genomicsurveillance/Arbovirus-genomic-surveillance/tree/main/Arbo-RJ>).

### Editor note

The Lancet Group takes a neutral position with respect to territorial claims in published maps and institutional affiliations.

### Declaration of interests

We declare no competing interests.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.lana.2024.100786>.

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