

MAPPING THE DYNAMICS OF DENGUE SEROTYPES IN THE 2023 EPIDEMIC: INSIGHTS FROM SPATIAL- TEMPORAL ANALYSIS AND EMERGENCE OF THE COSMOPOLITAN DENV-2 GENOTYPE IN RIBEIRÃO PRETO, SÃO PAULO, BRAZIL

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Abstract: Ribeirão Preto, situated in the northeastern region of São Paulo, spans an area of 651km², housing nearly all of its 698,642 inhabitants within its urban perimeter. The city experiences a tropical savanna climate, characterized by hot and rainy summers and dry winters with significant thermal amplitude. With a history of successive dengue epidemics since 1990 and the circulation of all four dengue serotypes (DENV), the municipality is classified as endemic and possibly hyperendemic for dengue. Dengue, a neglected tropical disease, poses a significant public health threat, fueling epidemics worldwide and potentially leading to hemorrhagic fever. While infection with one serotype confers permanent immunity, subsequent infections, particularly with DENV-2 or DENV-3, can increase the risk of severe cases. This study aims to delineate the spatial and temporal distribution of each DENV serotype based on geographic coordinates of residence and symptom onset, identify hotspots and clusters of cases, and discern differences between dengue with warning signs and severe dengue by serotype. Collaboration between the Blood Center Research Group (GP) and the Municipal Laboratory (LM) facilitated the integration of DENV data into the Notifiable Diseases Information System (Sinan). Subsequently, the DENV-GIS Dashboard, employing Business Intelligence (BI) data modeling, was developed to conduct space-time analysis (STA) of circulating DENV in 2023. Using ArcGIS for Power BI by Fabric resources, the study unveiled a dengue incidence rate of 5% for cases with warning signs and severe symptoms attributed to DENV-1, while DENV-2 exhibited a significantly higher rate of 21%. Spatial distribution analysis identified a distinct cluster of DENV-2 cases, indicating a focal point of transmission. This discovery facilitated the demarcation of a specific area

warranting further investigation. Notably, within this delineated zone, the presence of the Cosmopolitan DENV-2 Genotype was confirmed. Thus, the DENV-GIS Dashboard emerges as a valuable analytical-operational tool for STA of DENV, facilitating the identification of geographic regions for targeted intervention in endemic control. This study underscores the importance of continuous surveillance and strategic intervention to mitigate future epidemics in regions with persistent dengue exposure.

Keywords: Serotypes of Dengue Virus (DENV); Business Intelligence (BI); Geographic Information System (GIS)

INTRODUCTION AND JUSTIFICATION

Dengue is a neglected tropical disease that has become a serious public health problem, causing successive epidemics worldwide over the years and potentially leading to hemorrhagic fever. [1, 11] There are five dengue serotypes (DENV) described in the literature [2], of which four have already been isolated in autochthonous circulation samples in Ribeirão Preto (RP), with the first cases confirmed in 1990 with the introduction of DENV-1, followed by DENV-2 in 1998, DENV-3 in 2001, and DENV-4 in 2012. Dengue infection provides lifelong immunity against the acquired serotype; however, it has been observed in Southeast Asia that previous infections increase the risk of severe forms in subsequent infections, particularly when ending with DENV-2 or DENV-3. [3, 7-8] In a 2022 study, with data from epidemic years in RP, a statistically significant difference was found in the hospitalization rate (HR) by serotype, with epidemic years featuring massive circulation of DENV-2 having the highest HR in RP's historical series. In 2023, the Municipal Health Department (SMS), through a partnership with a Research Group

from the Blood Center of Ribeirão Preto (GP), was able to identify serotypes from a significant portion of all samples sent to the Municipal Laboratory (LM). In view of this, the SMS, through data science, sought to correlate the results obtained by the GP, which identified the serotypes through reverse transcription polymerase chain reaction (RT-PCR) molecular tests, with samples for the enzyme-linked immunosorbent assay (ELISA) of non-structural protein 1 (NS1) from the LM, and from this relationship, complete the DENV in the Notifiable Diseases Information System (Sinan). This justified the construction of a tool to reveal DENV cases by geographic coordinates of residence and symptom onset. Thus, this dataset (DS) was imported into a Business Intelligence (BI) associated with a Geographic Information System (GIS) to design the DENV-GIS Dashboard.

OBJECTIVES

This study sought to demonstrate singularities in the spatial distribution by geographic coordinates of residence and the temporal distribution by symptom onset of DENV cases in 2023 in RP. Among the specific objectives, it was necessary to verify fortuitous differences between the rates of dengue with warning signs and severe dengue for circulating DENV in 2023 and to assess the distribution of each serotype by residence coordinates to identify possible hotspots or clusters of cases and, finally, to compose a temporal evolution of cases to evaluate possible seasonal patterns or permanence.

METHOD

This study was conducted in 2023, and its casuistry - up to the publication deadline on September 20 - consisted of 21,530 suspected cases, of which 10,980 were confirmed as dengue, with 215 classified as dengue with warning signs, 14 as severe dengue, and,

among these, 8 dengue-related deaths and 1 death under investigation. Among the records with serotype identification, there were 3,473 NS1 samples from LM, with 3,464 processed by GP and others processed by other partner institutions, which, after relationship and completeness of information in Sinan, resulted, as of this date, in 919 DENV-1 and 75 DENV-2. Notification data were obtained by the Municipal Health Department Epidemiological Surveillance (VE-SMS) accessing Sinan, NS1 data were provided through LM flow, and PCR data were provided by partners with GP and other institutions, e.g., the Virology Research Center FMRP-USP and Adolfo Lutz Institute of Ribeirão Preto. There were two execution stages: in the first stage, a relationship was created between the received DS to generate reports of interest. Specifically, three DS were received, two from LM, one with the results of processed NS1 samples and another with the assignment of a sequential number for samples sent to the GP Laboratory, and one from the GP, the latter with PCR results from the GP and identification of the serotype for detected dengue cases linked to this sequential number assigned by LM. Periodically, VE-SMS already relates NS1 results to Sinan notifications to identify which Districts each result belongs to; similarly, in this stage, we related PCR results to the LM sample number and then created a key that relates this set to a Sinan export database. With this relationship created, three reports were elaborated: one with records not found in Sinan with all data for verification and proper notification, another with Sinan records to fill in DENV results, and a final report with internal control indicators and PCR vs. NS1 bio-statistics to determine if there is sensitivity variation of NS1 among serotypes. In the second stage, we used the properly completed Sinan with serotypes as DS to perform data analysis and obtain space-

time analysis (STA) [4-6, 9-10, 12, 14-15] by serotype. It is also important to carry out data treatment phases to select and create variables of interest and remove others, as well as to properly categorize these and other variables evoked by requirements gathering. In this regard, we highlight the use of an application for input data treatment (Power Query, M), for calculated measures and data modeling with business intelligence (Power BI, DAX), and for geocoding of locations in a geographic information system [16-17] (ArcGIS, Python), to construct an ArcGIS for Power BI by Fabric Dashboard. Finally, we developed two interface pages, both with a time bar for adjusting the period of interest, a serotype selector, a card with the notification value, and a set of graphic elements for segmentation of classification, sex, and age group; however, the first screen has a bubble map (DENV-1 and DENV-2) above a timeline in months, and the second screen has a heat map with a temporal automation bar.

RESULTS

Especially for this study, based on the dengue historical series available in the internal report of VE-SMS, it was possible to construct a timeline with the incidence coefficient of confirmed cases per year, identifying epidemic years and revealing the percentage proportion of each circulating serotype in the universe of samples processed for serotype surveillance each year. This result revealed that since 1998, when DENV-2 was introduced in RP, DENV-1 has alternated its predominance within a range of 2 to 4 consecutive years, and currently, in 2023 (3rd year >50%), it has yielded percentage participation to DENV-2, with 2024 possibly resembling 2001 or 2016 in this DENV-1 to DENV-2 transition cycle.

It is worth mentioning that in 2022, this VE-SMS, through a study, revealed that

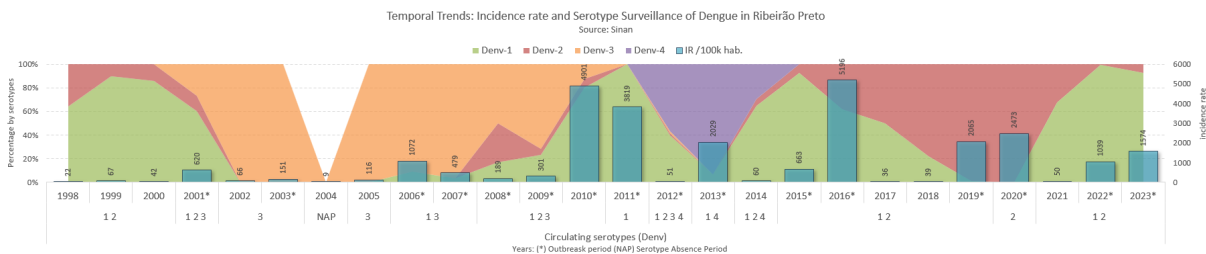


Figure 1: Temporal movements of serotypes dengue virus in Ribeirão Preto

The distribution of dengue serotypes in Ribeirão Preto

Ano	Tipo 1	Tipo 2	Tipo 3	Tipo 4	Total	Selected	(>90%)	Serotype
2007	1	0	31	0	32	2007	97%	3
2008	1	2	3	0	6		50%	
2009	45	10	139	0	194		72%	
2010	57	5	9	0	71		80%	
2011	217	0	0	0	217	2011	100%	1
2012	21	1	1	30	53		57%	
2013	15	0	0	201	216	2013	93%	4
2014	11	1	0	5	17		65%	
2015	128	10	0	0	138		93%	
2016	13	8	0	0	21		62%	
2017	1	1	0	0	2		50%	
2018	4	14	0	0	18		78%	
2019	1	121	0	0	122	2019	99%	2
2020	0	30	0	0	30	2020	100%	2
2021	38	18	0	0	56		68%	
2022*	110	0	0	0	110	2022*	100%	1
Total	677	230	208	236	1351			

Source: Sinan - Net & Online - (*) ref. 20/set/2022.

Hospitalizations - observed data table

Group	Yes	No	Total
DENV1	649	29877	30526
DENV2	1470	30655	32125
DENV3	16	2707	2723
DENV4	147	13032	13179
Total	2282	76271	78553

vs.

Hospitalizations - multinomial data table

Group	Yes	No	Total
DENV1	887	29639	30526
DENV2	933	31192	32125
DENV3	79	2644	2723
DENV4	383	12796	13179
Total	2282	76271	78553

H = hospitalization
C = confirmed cases
HR = hospitalization rate

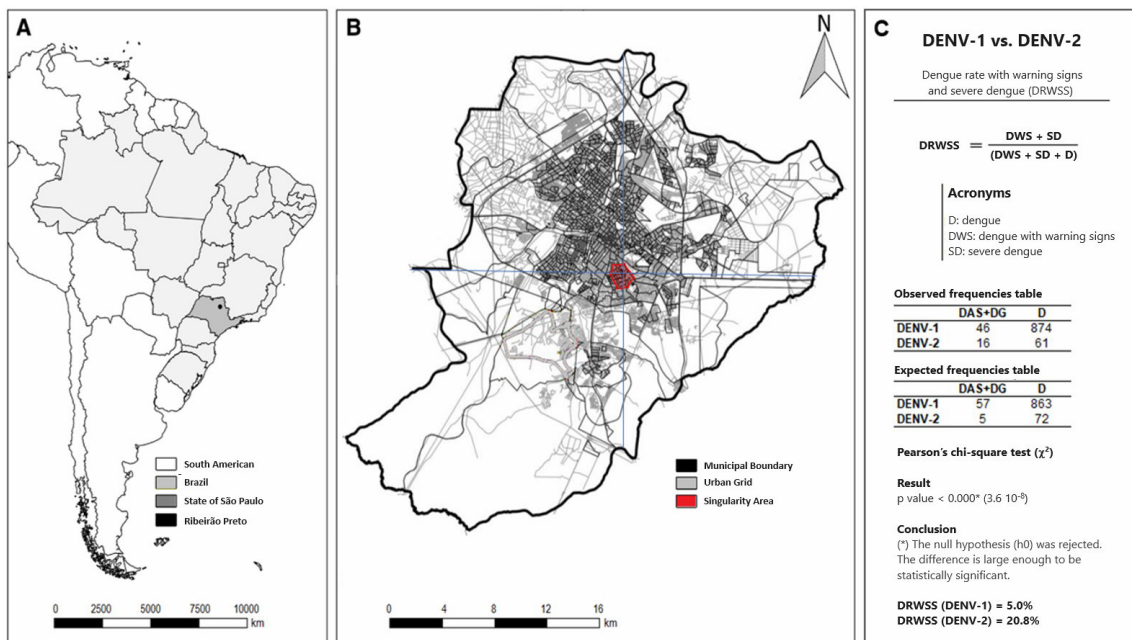
Group	Year	H	C	HR
DENV1	11 e 22	649	30526	2%
DENV2	19 e 20	1470	32125	5%
DENV3	2007	16	2723	1%
DENV4	2013	147	13179	1%

Pearson's χ^2 test

Valor de p: 1.7 E-126 $p < 0.01$

The null hypothesis (h0) that the count numbers are sampled from a multinomial distribution is rejected

Figure 2: The hospitalization rate per serotypes dengue virus in Ribeirão Preto



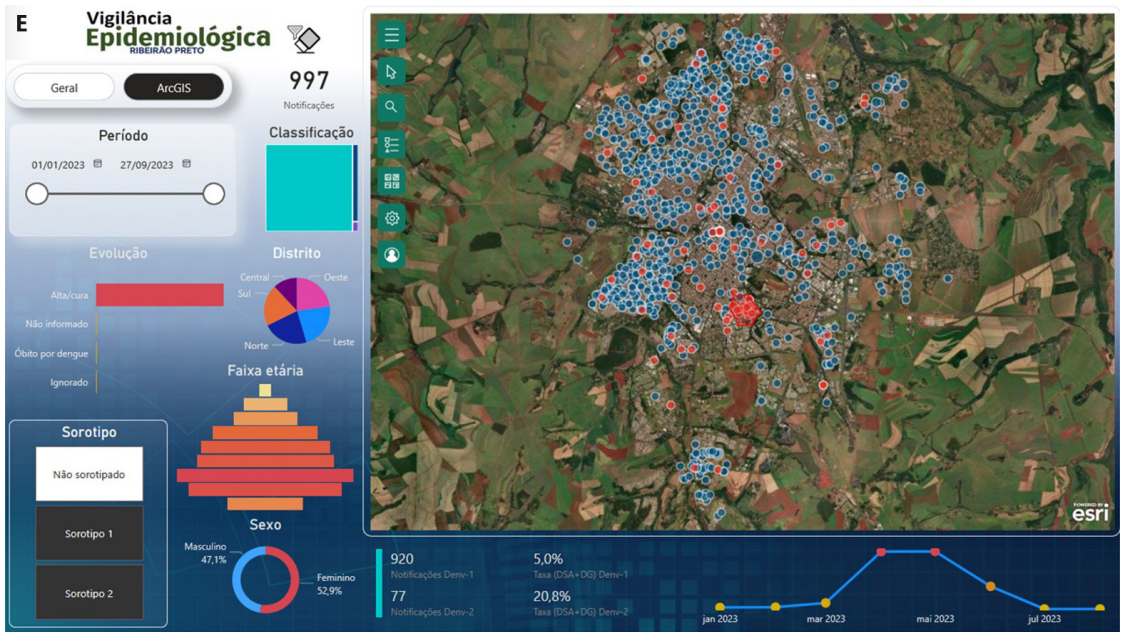
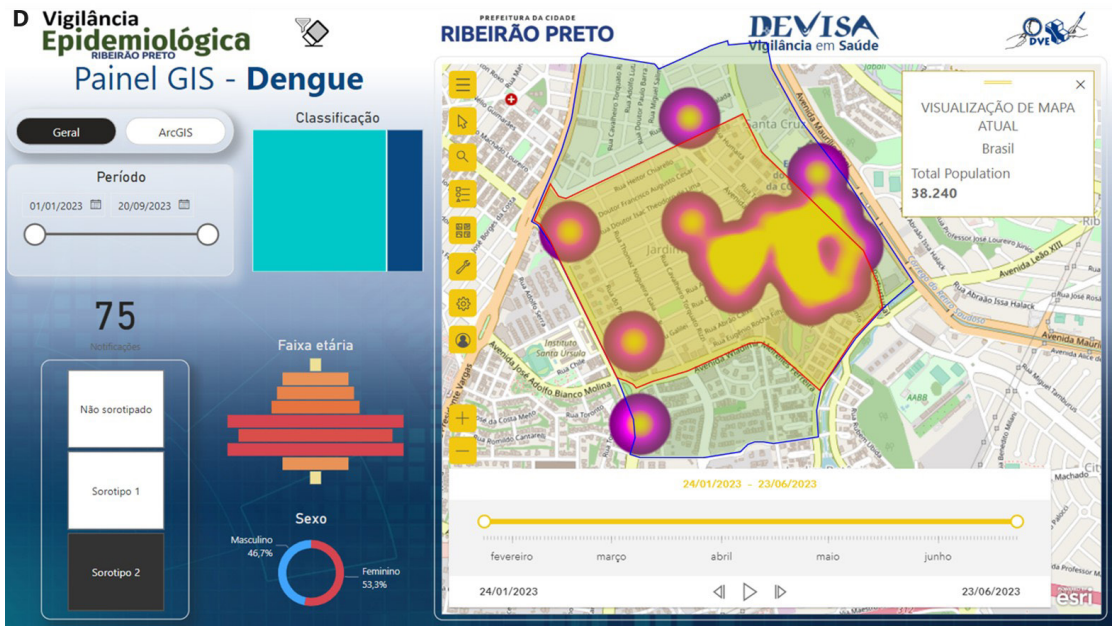


Figure 3: A) Map of Ribeirão Preto in Brazil, South American; B) The singularity area of DENV-2 in Ribeirão Preto; C) DRWSS 5% for DENV-1 versus 21% for DENV-2; D) First screen of the DENV-GIS; E) Second Screen of the DENV-GIS

the group of epidemic years with DENV-2 predominance had the highest hospitalization rate (HR), being 4.58%, followed by 2.13% for DENV-1, 1.12% for DENV-4, and 0.59% for DENV-3. Similarly, for the present study, a chi-square (X^2) test was performed, by which ($p < 0.001$) we identified that the dengue rate with warning signs and severe dengue (DRWSS) can be four times higher in DENV-2 compared to DENV-1 cases in 2023. We also conducted a bio-statistical evaluation between PCR and NS1 results and identified that, despite maintaining specificity over 97%, NS1 sensitivity decreases from 65% in DENV-1 to 27% in DENV-2.

This further reinforces the interest in the space-time analysis (STA) result regarding DENV-2 cases this year, in order to identify if there were any spatial singularities on the RP map and how these cases occurred over time by symptom onset. As a result, the DENV-GIS Dashboard, through STA, revealed that there is a homogeneous spatial distribution for DENV-1, whereas for DENV-2, a singularity was identified, for which it was even possible to construct an area of interest. Through the heat map, under temporal variation, this cluster was almost perennial, meaning that from the onset to the end of cases, the hotspot was present on the map, with greater intensity during the epidemic peak period. In the DENV-GIS Dashboard, when selecting only DENV-1 and DENV-2 in the “serotype filter” and combining the segmentation of categories “dengue with warning signs” and “severe dengue” in the “final classification” selector, it is possible to observe a proportional increase in DENV-2 cases compared to DENV-1. As a result, through a calculated measure in this DS, it was found that the DRWSS was 5% for DENV-1 and almost 21% for DENV-2. Moreover, when selecting only DENV-2, an atypical cluster of points can be observed in the spatial distribution of residence

geographic coordinates forming an almost perennial cluster in the temporal interval, and through STA, it was possible to construct an area of interest to be evaluated for operational feasibility and the best way to work with it, expecting to anticipate the next year.

As the last result produced until then, this DENV-GIS Dashboard was presented at the September 2023 arboviruses technical meeting, where, after plenary discussion, it was agreed that this DENV-2 singularity may represent the geographic region of entry of the Cosmopolitan genotype in RP (identified by the GP genomic study) and should be considered for preventive activities as early as 2023, anticipating the 2024 scenario.

CONCLUSION

Based on the results displayed by the DENV-GIS Dashboard, it is possible to conclude that there is a singularity in the spatial distribution by geographic coordinates of residence for DENV-2 in 2023 on the RP map. It is also concluded that, through the temporal distribution by symptom onset of DENV-2 cases in the heat map, the identified spatial singularity remains almost perennial over time, possibly being the geographic region of entry of the Cosmopolitan genotype of DENV-2 in 2023. It is worth noting that, based on the results presented in this study, it was also possible to verify that there is a statistically significant difference between the DRWSS of co-circulating DENV-1 and DENV-2 in 2023, with DENV-2 presenting the highest rate, namely, 21% of confirmed cases were converted into dengue with warning signs. Therefore, it is concluded, furthermore, that the DENV-GIS Dashboard could be understood as an operational support tool with STA of dengue cases by DENV in order to define polygons or geographic regions of greater interest to work on vector control. It is, therefore, a GIS instrument aimed at

evaluating results in the elapsed time set and risk stratification, intending to substantiate elements for the next year, highlighting the interest in mitigating subsequent infections, especially ending in DENV-2 or DENV-3, in a continuously exposed population region, thereby reducing the frequency of cases with severe complications.

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INSTITUTIONAL REVIEW BOARD STATEMENT AND INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

The sequences generated in this study were deposited in GenBank under the following accession by GP.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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