ABSTRACT

Dengue fever is re-emerging worldwide, however the reasons of this new emergence are not fully understood. Our goal was to report the incidence of dengue in one of the most populous States of Brazil, and to assess the high-risk areas using a spatial and spatio-temporal annual models including geoclimatic, demographic and socioeconomic characteristics. An ecological study with both, a spatial and a temporal component was carried out in Sao Paulo State, Southeastern Brazil, between January 1st, 2007 and December 31st, 2019. Crude and Bayesian empirical rates of dengue cases following by Standardized Incidence Ratios (SIR) were calculated considering the municipalities as the analytical units and using the Integrated Nested Laplace Approximation in a Bayesian context. A total of 2,027,142 cases of dengue were reported during the studied period. The spatial model allocated the municipalities in four groups according to the SIR values: (I) SIR<0.8; (II) SIR 0.8<1.2; (III) SIR 1.2<2.0 and SIR>2.0 identified the municipalities with higher risk for dengue outbreaks. “Hot spots” are shown in the thematic maps. Significant correlations between SIR and two climate variables, two demographic variables and one socioeconomical variable were found. No significant correlations were found in the spatio-temporal model.

The incidence of dengue exhibited an inconstant and unpredictable variation every year. The highest rates of dengue are concentrated in geographical clusters with lower surface pressure, rainfall and altitude, but also in municipalities with higher degree of urbanization and better socioeconomic conditions. Nevertheless, annual consolidated variations in climatic features do not influence in the epidemic yearly pattern of dengue in southeastern Brazil.

KEYWORDS: Dengue. Arbovirus. Infectious diseases outbreak. Epidemiologic study. Spatiotemporal analysis.

INTRODUCTION

Dengue fever is an acute infection caused by any of the four Dengue Virus (DENV) serotypes (DENV1-4). DENV belongs to the family Flaviviridae, genus Flavivirus, and is transmitted by female mosquitoes of the genus Aedes. Despite the growing economical investment in the prevention of dengue, it is still considered a neglected tropical disease and the most frequent vector-borne disease globally.

The annual incidence of dengue infections was estimated at around 400 million per year (284 to 528 million). The study on the Global Burden of Disease included 1,636 country-years of case reports of dengue from 76 countries. This study reported that dengue was increasing at a higher rate than any other communicable disease
until 2013, with a 400% increase over a period of just 13 years (2000 to 2013). The number of apparent cases more than doubled every decade, from 8.3 million in 1990 to 58.4 million in 2013\(^2\). Asia accounts for 75% of the dengue disease burden, followed by Latin America and Africa\(^3,5\). According to the Pan American Health Organization, Brazil and Colombia contributed with most of the cases in South America\(^6\). Brazil experienced a major outbreak in 2019, with over 2 million reported cases nationwide, including over 1,400 cases of severe dengue\(^5\).

The reasons for the global re-emergence of dengue are not fully understood, and several studies have been conducted to determine the factors associated with its increasing incidence\(^7\). Despite most cases following an epidemic pattern related to seasonal periods during a 12-month period, a widespread yearly occurrence of dengue has also been observed\(^6\).

Taking advantage of the mandatory notification of dengue in Brazil, we have explored the inconstant epidemiological pattern of dengue outbreaks in the last 12-year period in the most populous State of Brazil, using annual consolidated geoclimatic data in a spatiotemporal risk model analysis including demographic and socioeconomic factors.

MATERIALS AND METHODS

Design and location of study

This is an ecological study that was carried out in Sao Paulo State (SP). Each one of its 645 municipalities was used as the analytical units. SP is located in Southeastern Brazil (23°32’ S 46°38’ W) covering a total area of 248,219 km\(^2\). The State had an estimated population of 44,919,049 people in 2019, being one of the most populous areas in the Americas\(^7\). The State territory covers seven distinct climate types, taking into account the temperature and rainfall, varying from a subtropical climate in the mountainous area to a super-humid tropical type on the coast. SP is the richest Brazilian state with the second-highest Human Development Index (HDI 0.783) and gross domestic product per capita (GDP US$ 16,535) amongst the Brazilian States\(^2\).

Dengue fever cases and sources of information

Confirmed autochthonous dengue fever cases registered by the Sistema de Informacao de Agravos de Notificacao (SINAN – Information System on Notifiable Diseases) were used, in the period between January 2007 and December 2019, according to the date of onset of symptoms. Geoclimatological, demographic and socioeconomic characteristics of each city in SP were collected from the national and State database, including the Instituto Brasileiro de Geografia e Estatistica (IBGE – Brazilian Institute of Geography and Statistics), Fundacao Sistema Estadual de Analise de Dados (SEADE Research Foundation), and the global land-surface dataset ERA-5. All sources are available for public access.

Study variables

Dengue cases were extracted per epidemiological week, followed by the annual total for each municipality. The incidence was calculated annually according to the estimate population of each municipality every year\(^2\). Climatological data, including minimum, mean and maximum daily values for rainfall (millimeters), temperature (°C), dew point (°C), and surface pressure (Pa) were extracted daily, followed by the annual (mean) consolidation for the modeling, yearly. Further covariates were also included, as follow: geographical characteristics, including latitude (°), longitude (°) and altitude (meters). Biome data were available by cities as Atlantic forest, savanna or both (as available in databases). Demographic characteristics were also included by municipality, as follow: demographic density (inhabitants per km\(^2\)) based on the area of territorial unit each year, degree of urbanization (%), population living in urban and rural settings (%), and the annual geometric rate of growth (%). Finally, socioeconomic characteristics of each city were also tested and included in the model: average monthly wage of formal workers (number of minimum wage = US$211,11 in 2019 reais), GDP per capita, population in rural activities (%), HDI, infant mortality (deaths per 1,000 live births), number of public health-care units, number of public hospital beds (coefficient per 1,000 inhabitants), number of registered nurses and physicians (rate per 1,000 inhabitants), and the proportion of the population with access to potable water, garbage colletion and sewer.

Exploratory spatial analysis

Contiguity matrix

Each municipality of SP was considered a federative entity of a lower hierarchical level created within the federative unit; however, the municipalities are grouped in 11 intermediate geographical regions due to local common characteristics (Supplementary Figure S1A)\(^8\). These intermediate regions suggest a close relationship and a spatial dependence between them, and between the municipalities included in each of the groups. Thus, the Queen-type contiguity matrix was used, which considers
Geoclimatic, demographic and socioeconomic characteristics related to dengue outbreaks in Southeastern Brazil

The neighboring municipalities with at least one common border point, regardless of the direction of the points. The Supplementary Figure S1B represents the Queen-type contiguity matrix of SP, evidencing an average of 5.68 connections per municipality. The municipality of Ilhabela was excluded from the spatial analysis because it is an island.

**Calculation of crude and empirical Bayesian rates of the incidence of dengue**

The crude and empirical Bayesian rates of the incidence of dengue for each city were calculated and thematic maps of the respective rates were then created. The empirical Bayesian local estimator considers the adjustment of the rate of each city by the rates of its neighbors through the contiguity matrix. In order to assess the spatial autocorrelation, Moran’s Global Index and Geary’s Global Statistics were also calculated.

**Data analysis**

This ecological study presents both, a spatial and a temporal component.

**Spatial model**

The total number of cases of dengue, observed for each municipality \( i \) \((1 \leq i \leq 644)\) between 2007 and 2019 was modelled by a Poisson distribution. The expected number of events is calculated under the assumption that the dengue incidence rate is constant in all municipalities. Considering \( N_i \) as the yearly population in each municipality, the expected number of events \( E_i \) is calculated as:

\[ E_i = N_i \frac{\sum_{i=1}^{644} y_i}{\sum_{i=1}^{644} N_i} \]

Thus, the number of dengue cases in each municipality to be estimated by the model, defined as \( \eta_i \), is determined by a linear predictor on the logarithmic scale \( \eta_i = b_0 + u_i + v_i \), where \( b_0 \) represents the intercept or the overall general rate; \( u_i \) and \( v_i \) are two area specific effects, assuming Besag-York-Mollie (BYM) specification. The effect \( u_i \) is the spatially structured residual, modelled by an Intrinsic Conditional Autoregressive Structure (iCAR) denoted by: \( u_i \sim Normal (m_i , s_i^2) \), where \( m_i = \sum_{j \in N(i)} u_j \) and \( s_i^2 = \frac{\sigma^2}{\#V(i)} \). \( \#V(i) \) represents the neighboring municipalities with at least one common border point (contiguity matrix). The effect \( v_i \) is the unstructured residual, modelled by an exchangeable prior. An Integrated Nested Laplace Approximation (INLA) was used for estimating the parameters of the model. The selection of the statistical model was carried out using the Deviance Information Criterion, using the smallest value as the best-adjusted model, comparing the models without covariates, with climate variables only, and with all the covariates included. For each covariate, the estimate of the parameters and their respective credibility intervals were calculated as appropriated. Exponential values \( >1 \) were considered as positive correlations, and exponential values \( <1 \) as negative correlations. Standardized incidence ratios (SIR) were calculated and municipalities were grouped by risk.

**Spatio-temporal model**

Complementary to the spatial model described above, a spatio-temporal risk model was also created including variables with annual variations: climate characteristics. For this purpose, the time was defined as a structured (fixed) effect, known as a parametric spatio-temporal model, denoted by: \( y_{it} \sim Poisson (\lambda_{it}) \), where \( \eta_{it} = E_{it} \lambda_{it} \), \( t = 1, 2, \ldots, 13 \) (2007 to 2019).

**Statistics**

The statistical analysis, spatial and spatio-temporal models were performed using the R Statistic 4.0 (Windows, USA). The packages were used as follows: Rgdal to import digital maps, ClassInt to define the numerical ranges of variables in the thematic maps, Spdep to build the contiguity matrix, to obtain global and local empirical Bayesian rates, and to calculate the Global Moran Index and Global Geary Statistics, and R-INLA for the Bayesian modeling.

**RESULTS**

Dengue fever incidence in Sao Paulo State, from January 2007 to December 2019

A total of 2,081,142 cases of dengue were reported in SP during the studied period, revealing a gross incidence fluctuation with a peak of 1,481.88 cases per 100,000 inhabitants in 2015. Figure 1A. Figure 1B exhibits the particular epidemic pattern yearly per municipality, evidencing a remarkable peak of 32,183 cases per 100,000 inhabitants in the municipality of Bom Sucesso de Itarare, in 2012. Supplementary Tables S1 and S2 summarize the top 10 municipalities with the highest gross incidence and the highest absolute number of cases of dengue, respectively, during the studied period (2007 to 2019). Interestingly, nine out of 10 of the municipalities with the highest gross incidence were from intermediate regions located in the Northwest of SP. The municipality of Sao Jose do Rio Preto was present in both tables, with a...
The gross incidence of dengue fever cases per 100,000 inhabitants in a twelve-years period (2007 to 2019) involved almost the whole State, in contrast with the second highest peak of 838.79 cases per 100,000 inhabitants in 2019, which mainly involved the Northwestern region (Figure 1C). Exploratory maps of climate factors were also created (Supplementary Figure S2).

Bayesian rates and risk areas of dengue outbreaks

Figure 2 shows the maps created from the calculation of crude and empirical Bayesian local rates of dengue incidence for each municipality. Moran’s Global Index and Geary’s Global were 0.348 and 0.700, respectively. The municipalities with the highest incidence of dengue were located in the Northern region and on the coast, with lower incidence in the intermediate region of Sorocaba.

Spatial risk model

Consolidated spatial models from 2007 to 2019 are presented in Figure 3. Model 1 represents the SIR without covariates (Figure 3A) [DIC value = 6464.63], and Model 2 includes the SIR with climate variables only (Figure 3B) [DIC value = 6464.43]. Finally, the Model 3 includes all the studied variables [DIC value = 6464.69], allocating the municipalities in four risk groups according to their SIR values: (I) SIR<0.8; (II) SIR 0.8<1.2; (III) SIR 1.2<2.0; and SIR>2.0 that identified the municipalities with higher risk for dengue outbreaks. Description of data by grouped risk is presented in the Supplementary Table S3. “Hot spots” of dengue outbreak are mainly located in the Northern of SP (Figure 4). The Model 3 evidenced a significant correlation between SIR and two climate variables (surface pressure and rainfall), two demographic variables (altitude and
degree of urbanization), and one socioeconomic variable (number of public hospital beds). The estimate of these significant covariates are shown in Table 1.

Spatio-temporal risk model

No significant correlations were found in the spatio-temporal model (Supplementary Table S4 and Supplementary Figure S3).

DISCUSSION

In recent years, at least five human epidemic arboviruses have emerged or re-emerged mainly in tropical and subtropical settings: DENV, Zika virus, West Nile virus, Yellow Fever virus and Chikungunya virus. The expansion of mosquito-borne viral infections has been related to a combination of urbanization, globalization and weather changes, causing a significant and growing burden worldwide\textsuperscript{20,21}. Brazil is not an exception, showing an increase of cases of dengue mainly in the Atlantic Coast and in the interior region of SP\textsuperscript{22}.

Here, we revealed an irregular epidemiological behavior of dengue in one of the most affected Brazilian States\textsuperscript{22}. As presented (Figure 1A), the graphical annual incidence in SP does not show any clear pattern in the last 12-year period, exhibiting a disorganized “up and down” curve through the years. In the same way, Rodrigues et al.\textsuperscript{22} also revealed a disorganized cyclic curve in Brazil, where the incidence of dengue (per 100,000 inhabitants) oscillated from 212 (2001-2002 period), to 74 (2003-2004 period), 206 (2005-2006 period), 224 (2007-2008 period), 354 (2009-2010 period), and finally to 211 (2011-2012 period). Immunological issues may explain these annual variations regarding the predominance of different DENV serotypes, leading to a greater exposure to reinfection and/or immune amplification. In fact, the replacement of serotype clades has been associated with new outbreaks and disease severity\textsuperscript{23}. Thus, the predominance of DENV-1 and DENV-2 in 2000 followed by the introduction of DENV-3 in 2003, may explain the outbreaks at the begging of the century\textsuperscript{24,25}, and the re-emergence of DENV-1, associated with the importation of DENV-4 in 2010, justifying the peaks of dengue in the last decade\textsuperscript{26}. Moreover, the expansion of DENV-4 can also be accounted for the peaks observed in SP during 2013 to 2015, as well as for the source for further expansion of dengue across other Brazilian States\textsuperscript{27}.

Dengue has been largely related to demographic and climate changes; indeed, the behavior of the incidence of dengue is commonly correlated to weather variables as temperature, rainfall, surface pressure and/or dew point\textsuperscript{28-31}. However, the nature of climate and its relationship with dengue is not that simple and does not always follow a logical correlation\textsuperscript{30,32,33}. Firstly, our spatial risk model evidenced a preferential location of the cases of dengue across the studied area with well-defined clusters mainly distributed in Northern SP. Additionally, some climate variables were significantly correlated with its incidence: a positive correlation with surface pressure, and a negative correlation with altitude and rainfall. Nevertheless, the spatio-temporal model used in this study was not able to reveal any significant correlation with any of the geoclimatic, demographic and socioeconomic variables analyzed, probably due to a loss of sensitivity to detect fluctuations of these variables by the use of consolidated annual data.

The rainfall is usually positively correlated with the incidence of dengue\textsuperscript{31}. Actually, it seems logical to hypothesize that more rain would favor the grow of vectors\textsuperscript{29}. Interestingly, we found a negative correlation with the annual rainfall, leading to a greater risk of outbreaks of dengue in municipalities with dryer conditions. Our results corroborated previous data on cases of dengue in the Brazilian territory, where most of the cases of dengue were significantly accumulated in areas with semi-arid climate...
conditions, being 1.2 to 11 times higher than in tropical and subtropical settings\textsuperscript{22}. These contradictory findings could be explained by weekly and monthly variations in rainfall, especially in the first four months of the year when precipitations reach their highest levels in Brazil, leading to an increment of the larvae density\textsuperscript{34,35}. Additionally, the proliferation of the vector may not be caused directly by climate change as well, but rather, by the human response to changes in the patterns of rainfall\textsuperscript{36}, increased or decreased use of water storage containers\textsuperscript{36}. The interaction between surface pressure and dengue seems to be dynamic, involving weekly variations and a complex interaction with other climate characteristics\textsuperscript{37}. In the same way, the negative correlation with the altitude may also be explained by more favorable conditions to the development of larvae in lower altitudes. In any case, high altitudes (over 2,000 meters) do not seem to be an impediment to the growth of the vector\textsuperscript{38}.

SP covers an extensive territory with a diversity of geoclimatic conditions, but also a variety of demographic and socioeconomic settings among their municipalities; the neighboring between them disclose a nested relationship. Interestingly, demographic and socioeconomic characteristics were positively and significantly correlated with the incidence of dengue. A greater number of cases was observed in municipalities with higher degree of urbanization and in those with more a higher availability of public hospital beds, given by the index per 1,000 inhabitants. These two variables tend to be side by

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3}
\caption{A) Thematic map of spatial model (Model 01) without covariates; B) Thematic map of spatial model (Model 02) with climatic covariates; C) Thematic map of spatial model (Model 03) with all the studied covariates.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4}
\caption{Thematic map showing the cities with a standardized incidence ratios >2, reveling the “hot spots” of dengue cases in Sao Paulo State.}
\end{figure}
The adaptation of *A. aegypti* to urban context is well known. It is expected that, in rural conditions, the inhabitants are more exposed to local fauna, including a higher exposure to insect bites; however, the urbanization of the cities contributes to create an ideal scenario for the growing of larvae across multiple focuses of standing water (old tires, tanks, flower pots, inadequate garbage collection etc.), boosting vector infestation and making human beings the preferred target for the hematophagous needs of female mosquitoes. Our results reinforced that dengue is in fact an “urban disease”.

On the other hand, the positive correlation with the index of available public hospital beds may be interpreted in two different ways: (I) a greater urbanization translates into a greater need for health facilities, and consequently for the construction and availability of more health facilities for the general population; and (II) the health-care network, especially due to the neighboring of the municipalities, may lead to human displacements, translocating the cases of dengue from smaller municipalities with lower urbanization degree and less health-care facilities, to bigger metropolis in which referral hospitals are usually located. Here we did not find any significant correlation with GDP per capita or HDI; however, an inverse association between the incidence of dengue and GDP per capita (RR=0.98) and a direct association with HDI (RR=3.64) have been described in Brazil. Despite the SINAN records, the probable site of infection, including the travel records of the patients and their date of the onset of symptoms, it is not possible to assure the exact location of the transmission of dengue, especially due to the disease incubation period, and the frequent human displacements across the State.

### Limitations of the study

Despite the novel insights into the epidemiology of dengue, our study has some limitations. The aggregating data on the cases of dengue and climate characteristics by year led to a loss of sensitivity in critical disparities in the number of new cases and climate changes. Thus, a spatio-temporal model using consolidated annual data as we assessed here, appears to be unsatisfactory to predict risk factors related to the climate. Interactive effects of different meteorological factors, fluctuations in regional weather conditions, seasonality and daily variations can provide more information to the prediction models of outbreaks of dengue.

Although biome is treated as a categorical variable, two details should be carefully analyzed: the extension across to the territory of each municipality, and the constant deforestation due to the expansion of urban areas and to agricultural activities (mainly sugar cane cultivation in Western SP). Strict monitoring and mapping of constant changes in the local biomass could provide better information on the influence of this factor in the incidence of dengue.

The infestation rates of the vector were not included due to the potential data bias considering the non-systematic data collection (no-randomly selected point of collections), low coverage throughout the territory of each municipality and incomplete data for each unit in the surveillance system. Demographic and socioeconomic characteristics may also vary over time, but no dramatic changes have been observed in the last decade according to the IBGE and SEADE Research Foundation databases. In this study, we used annual estimate values for the demographic variables (incidence calculation per year), and the recently updated SEADE database (2019).

## Perspectives

Our findings led to a better understanding of the epidemiological behavior of dengue to improve the public health strategies, facilitating the identification of the target and focusing the governmental interventions for the control of the disease in “hot” areas as the proper target of economic resources, inhabitants education (regional social media, local institutions etc.), the vector control and

### Table 1 - Parameters estimation (exponential) *a posteriori* of the variables with significant correlation in the fixed spatial model, in Sao Paulo State, between January 1st, 2007 and December 31st, 2019.

| Parameter                                | Mean     | Credibility interval       |
|------------------------------------------|----------|---------------------------|
| Intercept                                | 3.212    | -1.36E+21 -2.29E+16       |
| Surface pressure (Pascal)*               | 1.000    | 1.000                     |
| Rainfall (millimeters)*                  | 0.999    | 0.998 1.000               |
| Altitude (meters)                        | 0.997    | 0.996 0.998               |
| Degree of urbanization (%)†              | 1.020    | 1.014 1.026               |
| Public hospital beds (rate per 1,000 inhabitants)† | 1.105    | 1.061 1.149               |

*Annual mean; †SEADE Research foundation, 2019.
the surveillance of the cases of dengue. These results may serve as a base for further studies to analyze local data (by municipality or intermediate regions) in order to assess possible predictive regional variables. We have ruled out that annual climate variations may be involved in variations of dengue, so that further studies should focus in daily, weekly, monthly or sessional data to better explain the observed inconsistent fluctuations.

CONCLUSION

The incidence of dengue exhibited inconstant and unpredictable variations every year in Southeastern Brazil. The higher rates of dengue are concentrated in geographic clusters with lower surface pressure, rainfall and altitude, but also in municipalities with higher degree of urbanization and better socioeconomic conditions. Annual variations in climate characteristics seem not to influence the epidemic yearly pattern of dengue in Southeastern Brazil.

ACKNOWLEDGMENTS

We thanks the Department of Infectious Diseases, Hospital das Clínicas, University of Sao Paulo, Brazil.

AUTHORS’ CONTRIBUTIONS

SV contributed with the study design, data collection, data analysis, data interpretation, literature search and writing; AKN contributed with the statistical analysis and data interpretation; FCN contributed with the study design, data interpretation, and writing; CAPJ and AC contributed with data collection, data interpretation and writing; ES and EJAL contributed with the study design, data collection, data interpretation, literature search and writing.

CONFLICT OF INTERESTS

All authors declare none.

FUNDING

This study was supported by Center for Arbovirus Discovery, Diagnostics, Genomics & Epidemiology (CADDE) project MR/S0195/1, and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) grant Nº 18/14389-0. SV and AC received a medical residency scholarship from the Ministry of Health, Brazil. CAPJ is supported by FAPESP (grant Nº 2019/21858-0), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), finance code 001.

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Supplementary Material available from: https://doi.org/10.48331/scielodata.URRIFK