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Assessment of the relationship between entomologic indicators of *Aedes aegypti* and the epidemic occurrence of dengue virus 3 in a susceptible population, São José do Rio Preto, São Paulo, Brazil

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ABSTRACT

The aims of this study were to describe the occurrence of dengue in space and time and to assess the relationships between dengue incidence and entomologic indicators. We selected the dengue autochthonous cases that occurred between September 2005 and August 2007 in São José do Rio Preto to calculate incidence rates by month, year and census tracts. The monthly incidence rates of the city were compared to the monthly Breteau indices (BI) of the São José do Rio Region. Between December 2006 and February 2007, an entomological survey was conducted to collect immature forms of *Aedes aegypti* in Jaguaré, a São José do Rio Preto neighborhood, and to obtain entomological indices. These indices were represented using statistical interpolation. To represent the occurrence of dengue in the Jaguaré neighborhood in 2006 and 2007, we used the Kernel ratio and to evaluate the relationship between dengue and the entomological indices, we used a generalized additive model in a spatial case–control design. Between September 2005 and August 2007, the occurrence of dengue in São José do Rio Preto was almost entirely caused by DENV3, and the monthly incidence rates presented high correlation coefficients with the monthly BI. In Jaguaré neighborhood, the entomological indices calculated by hectare were better predictors of the spatial distribution of dengue than the indices calculated by properties, but the pupae quantification did not show better prediction qualities than the indices based on the container positivity, in relation to the risk of dengue occurrence. The fact that the municipality's population had a high susceptibility to the serotype DENV3 before the development of this research, along with the almost total predominance of the occurrence of this serotype between 2005 and 2007, facilitated the analysis of the epidemiological situation of the disease and allowed us to connect it to the entomological indicators.

1. Introduction

Dengue infection is caused by four antigenically distinct serotypes of the dengue virus (DENV1, DENV2, DENV3, and DENV4), and its main vector is the *Aedes aegypti* mosquito. Each serotype infection provides specific long-term immunity but only transient immunity to the other serotypes (WHO, 2010). Dengue is considered the most significant arbovirus that affects humans. Dengue is estimated to annually cause 390 million infections, including

Abbreviations: BI, Breteau Index(cis); GIS, geographic information system; SINAN, notifiable diseases information system.

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96 million cases of classical dengue and 20,000 deaths caused by dengue (Bhatt et al., 2013).

In Brazil, the numbers of cases and deaths related to dengue have been increasing over time because of the introduction or reintroduction of virus serotypes and the simultaneous circulation of the four serotypes (Drumond et al., 2012, 2013; Figueiredo et al., 2008; Teixeira et al., 2013, 2005). In the state of São Paulo, the number of dengue cases have followed the same pattern, increasing from 2001 (51,668 cases) to 2007 (92,345 cases), 2010 (189,330 cases), and 2013 (201,498 cases) (CVE, 2014).

Tracking the viral serotypes that are circulating is an important tool for epidemiological research, particularly in relation to the spatial and temporal dynamics of the disease and infection incidence (Mondini et al., 2009). Molecular techniques based on PCR provide both advanced diagnostics of viral infections, mainly dengue and yellow fever, and quick and precise answers for disease-surveillance systems (Bronzoni et al., 2005; Teixeira et al., 2005).

Regarding entomological indicators, many authors have suggested the quantification of the pupae of *Ae. aegypti* as an adequate way to evaluate the risk of dengue transmission (Arredondo-Jimenez and Delgado-Valdez, 2006; Barrera et al., 2006; Focks, 2003; Focks and Chadee, 1997; Nathan and Focks, 2006; Romero-Vivas and Falconar, 2005). In contrast, Sanchéz et al. (2010, 2006) showed that in areas with low infestation levels, the Breteau Index (BI) was suitable to indicate areas that had a higher risk of dengue occurrence, and Morrison et al. (2008) stated that other studies are necessary to validate those results before their application to surveillance activities and vector control of the disease.

Authors, such as Focks (2003), Focks et al. (2000) and Nathan and Focks (2006) have suggested that indicators calculated by the area of properties and by inhabitants would have a better prediction capacity for the disease-occurrence risk than solely indicators calculated by property, recommending the development of studies that address these issues.

Geographic information systems (GIS), in addition to being tools for spatial analysis, enable the spatial component to be included in studies of metaxenic diseases and allow the associated variables to be mapped and spatially analyzed. Some examples of GIS applications are the studies developed by Barrera et al. (2000), Mondini and Chiaravalloti-Neto (2008) and Tran et al. (2004).

The aims of this study were to describe the occurrence of dengue in space and time and to assess the potential use of entomologic indicators that were based on the quantification of pupae and on the quantification of containers with immature forms of *Ae. aegypti* calculated by property and by area to predict dengue occurrence.

2. Materials and methods

The municipality of São José do Rio Preto is located in the northwest region of the state of São Paulo (Fig. 1A), Brazil (20°49′11″ S and 49°22′46″ W). The altitude of this municipality is 475 m above sea level, and it is situated in a region of tropical climate with an average annual temperature of 25 °C and an average annual precipitation of 1410 mm. São José do Rio Preto is the main city in an area consisting of 102 municipalities. The city has more than 400,000 inhabitants, an almost universal water supply system, and development indices comparable to those of developed areas.

After the reintroduction of *Ae. aegypti* in the city in 1985, the first autochthonous cases of dengue occurred in 1990, when the presence of DENV-1 was detected. In 1998, DENV-2 was detected, in 2005 DENV-3 was detected (Mondini et al., 2009), and in 2011, DENV-4 was detected (Rocco et al., 2012). Since the 2000s, the city has been considered endemic for dengue (Mondini et al., 2005). All of the autochthonous dengue cases that occurred in the urban area of São José do Rio Preto between September 2005 and August 2007 were considered in this study. The cases were confirmed by serologic testing (ELISA-IgM), RT-PCR or clinical–epidemiological criterion and were registered in the Notifiable Diseases Information System (SINAN). During this period, serologic testing was used to confirm the dengue cases until the incidence had reached approximately 300 cases per 100,000 inhabitants. After that, according to the government case definition, the clinical–epidemiologic criterion was used to confirm the dengue cases.

Between September 2005 and August 2007, a viral diagnostic test was performed using techniques of molecular biology with blood samples from patients with clinical diagnoses of dengue fever (Bronzoni et al., 2005) who were selected in health units. Their sera were stored at −80 °C. Viral RNA was extracted from 140 μl of serum with the QIAamp Viral RNA Mini kit (QIAGEN, Inc.) as described by the manufacturer. The methodology described by Bronzoni et al. (2005) was used to detect the dengue virus serotypes present.

The annual and monthly incidence rates of dengue were calculated for the municipality during the period from September 2005 to August 2007. The monthly rates were compared to the BI (number of containers with *Ae. aegypti* per 100 premises) estimated monthly rates for the region of São José do Rio Preto, based on the data recorded in the System SISAWEB of the Endemic Control Superintendence. We calculated, for the annual periods from September 2005 to August 2006 and September 2006 to August 2007, the Pearson correlation coefficients between the monthly incidences and BI, lagged two and three months in relation to the rates, built scatter plots and fitted LOESS curves.

A geo-codification of dengue cases was performed based on the patients' residence addresses by means of metric interpolation using the cartographic base of the street axes of São José do Rio Preto. Thematic maps were then produced showing the dengue incidence rate in the municipality according to its urban census tracts, and the cases for which the serotypes were identified were mapped for the period from September 2005 to August 2007.

An evaluation of the relationship between dengue and entomological indicators was performed in the Jaguaré neighborhood, which is located on the northern zone of the city (Fig. 1B). This neighborhood has approximately 37,000 inhabitants, approximately 11,000 properties and an area of 397.5 ha. The criteria for selection were: the highest levels of infestation of *Ae. aegypti* in relation to the other neighborhoods, a high incidence of dengue, the presence of residents with different socioeconomic statuses, and residential and non-residential properties.

An entomological survey was conducted in the Jaguaré neighborhood between December 2006 and February 2007. This period was chosen because these months are the most favorable months for the development of the vector in this region and include an infestation peak (Barbosa et al., 2010). In this survey, a team of field researchers were contracted for this activity and accordingly trained; they visited all of the properties in the neighborhood and collected all of the observed larvae during the 3rd and 4th stages and all of the pupae in containers with the presence of culicids. The collected specimens were identified and quantified. To minimize the number of properties that could not be inspected, the visits were performed during the working days of the week until 7 pm and on Saturdays. Other visits were performed to target properties that were not inspected during the first visit. More details about this survey are provided by Favaro et al. (2013).

The entomological indicators considered for *Ae. aegypti* included two indices calculated per property (the BI and the number of pupae per property) and two indices calculated by area (the number of containers with immature forms per hectare and the number of pupae per hectare). The property areas in hectares were obtained from the geo-codified lot map that was provided by the city hall.

Fig. 1. (A) Municipality of São José do Rio Preto, state of São Paulo, Brazil. (B) Jaguaré neighborhood and urban perimeter of São José do Rio Preto.

These indicators were calculated for the whole neighborhood and by blocks (blocks with less than five properties were grouped with adjacent blocks). In addition, the number of pupae per inhabitant was estimated for the entire neighborhood.

Based on the entomological indicators calculated per block, thematic maps were built to visualize their spatial distributions in the Jaguaré neighborhood. We assumed that the centroids of the geometric representations of the blocks could be considered as representatives of polygon objects and, therefore, as samples of a continuous geographic phenomenon in physical space that could be used to model a stochastic Gaussian punctual process (Bailey and Gatrell, 1995).

The ordinary kriging method was used to obtain a mathematical model and to construct thematic maps based on statistical interpolation using the method of generalized weighted least squares estimation, with the weights defined by the semi-variance function (semivariogram). The isotropic semivariogram was defined with a spherical function with the parameters nugget, contribution and amplitude. We used the package geoR from the program R version 3.0.2 to perform the statistical interpolation (Ribeiro and Diggle, 2001).

Regarding dengue cases in the Jaguaré neighborhood, we considered just the ones that occurred between January and May 2006 and 2007. These both time periods represented more than 90% of the total number of cases recorded during these two years and they include the peaks of incidence and vector infestation. In justifying the inclusion of the 2006 period in the analysis of the relationship between entomologic indicator and dengue occurrence, we considered that the infestation patterns during the summers of 2005/6 and 2006/7 did not have expressive variations; there were no atypical climatic variations, no alterations of the control activities developed and no structural alterations in the neighborhood. In addition, this comparison was opportunistic because the epidemic that occurred in 2006 exposed the completely susceptible population to the recently introduced serotype DENV3 (Mondini et al., 2009).

To represent the autochthonous cases of dengue that occurred in the Jaguaré neighborhood in 2006 and 2007, we used the Kernel ratio obtained from the division of the Kernel function applied to the sites of dengue occurrence by the result of the application of this function to the centroids of the lots corresponding to the residences in the neighborhood, weighted by the number of inhabitants per residence (Câmara and Carvalho, 2004). This process generated measurements that corresponded to the incidence rates. These rates were presented in thematic maps according to quintiles.

We used a generalized additive model (GAM), in a spatial case–control designed, to evaluate the relationship between dengue and entomological measures (Hastie and Tibshirani, 1986). The autochthonous dengue cases were considered the cases themselves and the controls to represent the inhabitants of the neighborhood (the source population of the dengue cases) were generated randomly, in a proportion of 1:1 with the cases. We calculated the amount of controls for each census tract, weighting by its population. ArcGIS10.2 software was used to generate the random spatial distribution of the controls. Values of the entomological measures for each case and each control were obtained using the maps of the entomological indicators generated by kriging, considering the geographical coordinates of the cases and controls (Barbosa et al., 2014).

The GAM were adjusted considering the occurrence of dengue (case or control) as a dependent variable for each study period

(January to May of 2006 and of 2007) and each entomological index as a possible explanatory variable (e.g., we built a model for each study period and each entomological index). The geographic coordinates of the cases and controls were used as the spatial component of the models. It was considered as smoothing functions of the coordinates with a band width of 100 m representing, in this situation, the mean flight radious of *Ae. aegypti* (Barbosa et al., 2014; Hastie and Tibshirani, 1986). The entomological measures were scaled (using their means and standard deviations) allowing to compare with the results of the models. We used the software R version 3.0.2 with the epigam library to adjust these models (Barbosa et al., 2014).

This study was approved by the Ethical Review Board of the Faculdade de Medicina de São José do Rio Preto.

3. Results

The incidence rates of dengue fever in São José do Rio Preto for the annual periods from September 2005 to August 2006 (2005/6) and September 2006 to August 2007 (2006/7) were 3014.6 and 2370.1 cases per 100,000 inhabitant-years, respectively (12,526 autochthonous cases in 2005/6 and 10,051 in 2006/7). Fig. 2A and B shows these rates according to the urban census sectors and in quintiles, in which we noted that the presence of heterogeneity varied between 0 and 13,184.3 cases per 100,000 inhabitant-years during the first year and between 0 and 8206.3 during the second year.

Regarding the characterization of the circulating serotypes, in 2005/6, the viral serotypes present were identified by PCR in 336 of 718 serum samples, of which 331 contained DENV3, four contained DENV2, and one contained both of these serotypes. In 2006/7, 94 samples were processed, of which 46 were positively identified: 45 as DENV3 and one as DENV2. The dengue cases for which the viral serotype was identified for the two annual periods analyzed are shown in Fig. 2C and D, respectively.

Fig. 3 shows the partial monthly incidence rate (including only the dengue cases confirmed by either serological testing or RT-PCR) and the total monthly incidence rate (including the dengue cases confirmed by serological testing, RT-PCR and by the clinical–epidemiologic criterion). During 2005/6, the total incidence rate was almost equal to the partial incidence rates until March, and, in the next year period, between January and March of 2007, approximately half of the confirmed dengue cases were confirmed by either serological testing or RT-PCR. The incidence rates were greater in 2005/6 than in 2006/7, although showed similar behavior during the two annual periods studied. Moreover, both demonstrated incidence peaks in April and concentrations of more than 90% of the cases between January and May.

Fig. 3 also presents the monthly BI between September 2005 and August 2007 for the region of São José do Rio Preto; we noted similar infestation levels and seasonal patterns in the two years, with higher values between December and March. A comparison of the two curves showed that the incidence followed the infestation curve, with the infestation peaks preceding the incidence peaks by two to three months. The highest correlation coefficients, for both annual periods, were obtained when we lagged the BI for two month and their values were 0.94 ($p = 0.000$) and 0.84 ($p = 0.001$), respectively, for 2005/6 and 2006/7. The scatter plots and the LOESS curves were better fitted for the first annual period compared to the second (Fig. 4).

In the Jaguaré neighborhood, 1528 and 563 autochthonous cases of dengue fever were recorded for the periods 2005/6 and 2006/7, respectively, of which 96.2% and 90.9% of cases occurred between January and May. Fig. 5A and B shows the incidence rates resulting from the Kernel ratio in that period for the two years studied, and we note the dissemination of the disease throughout the entire neighborhood, with higher values in 2006.

Fig. 6A and B shows the spatial risk maps with the odds ratio (OR) isolines for dengue and Fig. 6C and D shows the respective *p* value maps for 2006 and 2007, as a result of applying GAM considering only the spatial distribution of the cases and controls. The areas with OR over 1 and significant are broader in 2006 than in 2007, with is in agreement with the incidence rate maps presented in Fig. 5A and B.

In the entomological survey performed in the Jaguaré neighborhood, 9875 properties were inspected (89.8% of the neighborhood's properties). Totals of 36,119 larvae and 4178 pupae of *Ae. aegypti* were found in 1051 properties and 1867 containers. The larvae and pupae were found, respectively, in 1015 and 442 properties and in 1788 and 647 containers. The following entomological indicators of *Ae. aegypti* were obtained in the Jaguaré neighborhood: 18.9 containers with immature forms per 100 properties (BI) and 0.42 pupae per property, 5.2 containers with immature forms and 11.6 pupae per hectare, and 0.07 containers with immature forms and 0.15 pupae per inhabitant.

Fig. 7 shows the maps of the entomological indicators obtained by kriging based on the information aggregated by blocks. The BI and the number of pupae of *Ae. aegypti* per property are shown in Fig. 7A and B, and the number of containers harboring *Ae. aegypti* per hectare and the number of *Ae. aegypti* pupae per hectare are shown in Fig. 7C and D. Both indicators calculated per property showed the same pattern, which also occurred for the indicators calculated per hectare. However, the comparison between the indicators calculated per property and those calculated per hectare revealed differences, especially in the southern and western regions of the Jaguaré neighborhood: a low infestation based on the indicators calculated per hectare and a high infestation based on the indicators calculated per property (Fig. 7).

Table 1 presents the models showing the relationship between occurrence of dengue, for both study periods, and for each one of the entomological indicators evaluated, adjusted for the spatial distribution of the cases and controls. In 2006, all entomological indicators were considered risk factors to the occurrence of dengue (OR > 1 and significant), however those calculated per hectare presented higher values of OR than the indices per property. In 2007, only the indices calculated per hectare were considered risk factors for the occurrence of dengue, nevertheless with lower values than in 2006. Indicators calculated per hectare showed, in both study periods, to be better predictors of the occurrence of the disease than those calculated per property, furthermore, they demonstrated to have a similar prediction power.

The results presented above are in consonance with the entomological index maps (Fig. 7A–D), the incidence rate maps (Fig. 5A and

Table 1
Dengue risk estimated models* for each one of the scaled** entomological indices evaluated, adjusted for the spatial distribution of the cases and controls, Jaguaré neighborhood, municipality of São José do Rio Preto, state of São Paulo, Brazil.

	Odds ratio	95% Confidence interval	*p* value***
January to May of 2006			
Breteau Index	1.06	1.03–1.09	0.000
Pupae per property	1.09	1.06–1.13	0.000
Container per hectare	1.35	1.31–1.39	0.000
Pupae per hectare	1.31	1.27–1.35	0.000
January to May of 2007			
Breteau index	0.98	0.92–1.04	0.434
Pupae per property	1.01	0.95–1.07	0.735
Container per hectare	1.17	1.11–1.23	0.000
Pupae per hectare	1.17	1.11–1.24	0.000

* All models were significant, with $p < 0.000$.
** Scaled using the mean and the standard deviation.
*** *p* value of the variable in the model.

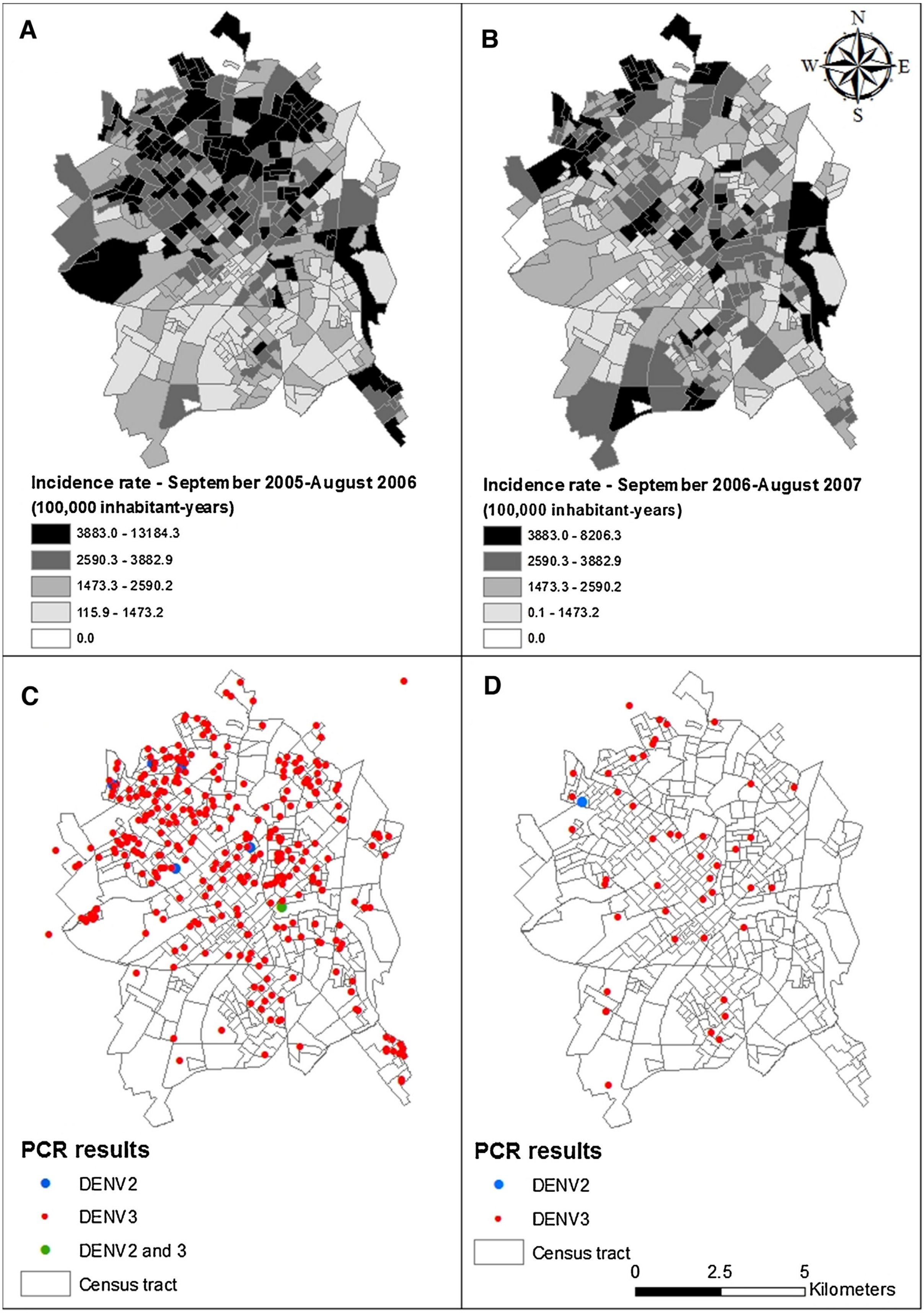

Fig. 2. Incidence rates of dengue by urban census tracts, September 2005 to August 2006 (A) and September 2006 to August 2007 (B). Cases of dengue with serotype identification, September 2005 to August 2006 (C) and September 2006 to August 2007 (D). Municipality of São José do Rio Preto, state of São Paulo, Brazil.

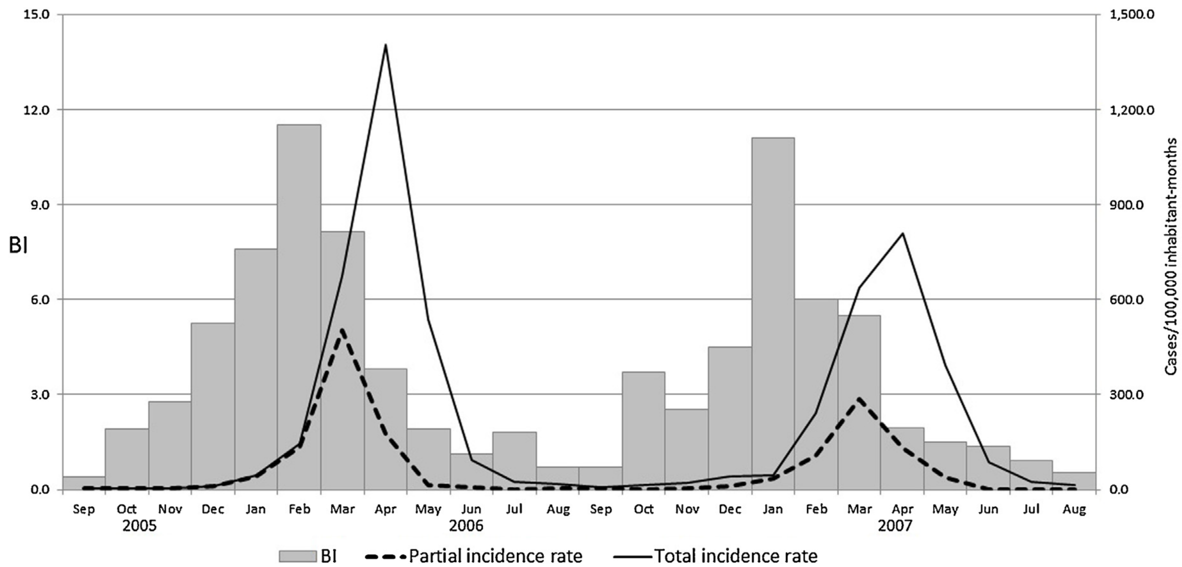

Fig. 3. Partial and total monthly incidence rates of dengue, municipality of São José do Rio Preto, and monthly Breteau indices (BI), region of São José do Rio Preto, September 2005 to August 2007, state of São Paulo, Brazil.

B) and the spatial risk maps (Fig. 6A and B). It can be seen that the indices per hectare, in both periods, had a higher degree of concordance with dengue than those calculated per property. In addition, it is worth noting that the degree of concordance for indices per hectare with dengue was higher in 2006 than in 2007, which is in accordance with the lower values of OR obtained in 2007.

4. Discussion

According to Teixeira et al. (2005), the dengue fever epidemics in Brazil have occurred after the introduction or reintroduction of certain virus serotypes when a high proportion of susceptible individuals exists in the population. This pattern could be verified in São José do Rio Preto, where the results obtained through PCR suggested that almost all of the cases could be attributed to DENV3, a virus whose introduction occurred in 2005 (Mondini et al., 2009). In addition to the high degree of susceptibility of the population, this scenario generated high incidences of dengue fever in 2005/6 and 2006/7, with cases throughout the urban area of the municipality.

To compare the entomological indicators and the incidence of dengue fever in a given neighborhood, it would be ideal to perform entomologic measures in a period when the dengue fever transmission occurred in a completely susceptible population (Mondini et al., 2009). In this study, the comparison performed between the indicators measured in December 2006 to February 2007 and the incidences of dengue fever in 2007 had the disadvantage of part of the population in the neighborhood having been immunized during the previous period by the serotype DENV3. This immunization altered the potential of the indicators of infestation to estimate the transmission risk of dengue fever because further transmission now also depended, among other factors, on the proportion of

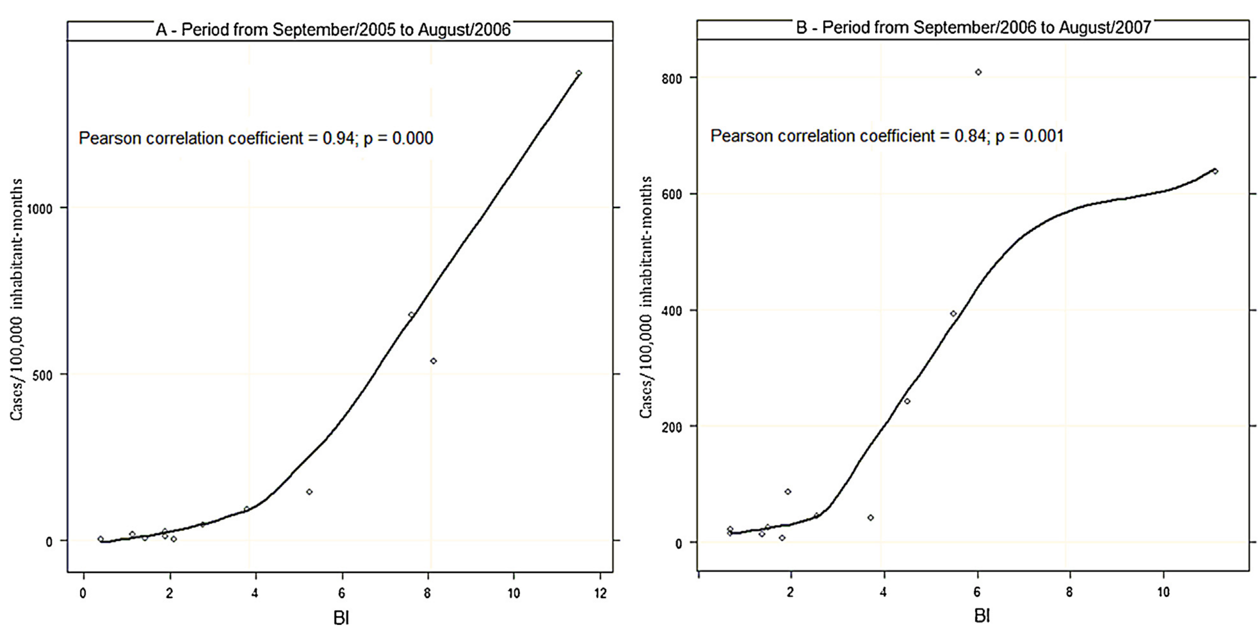

Fig. 4. Scatter plots with monthly incidence rates and BI, lagged two months in relation to the rates, fitted LOESS curves, and Pearson correlation coefficients for the annual periods 2005/6 (A) and 2006/7 (B).

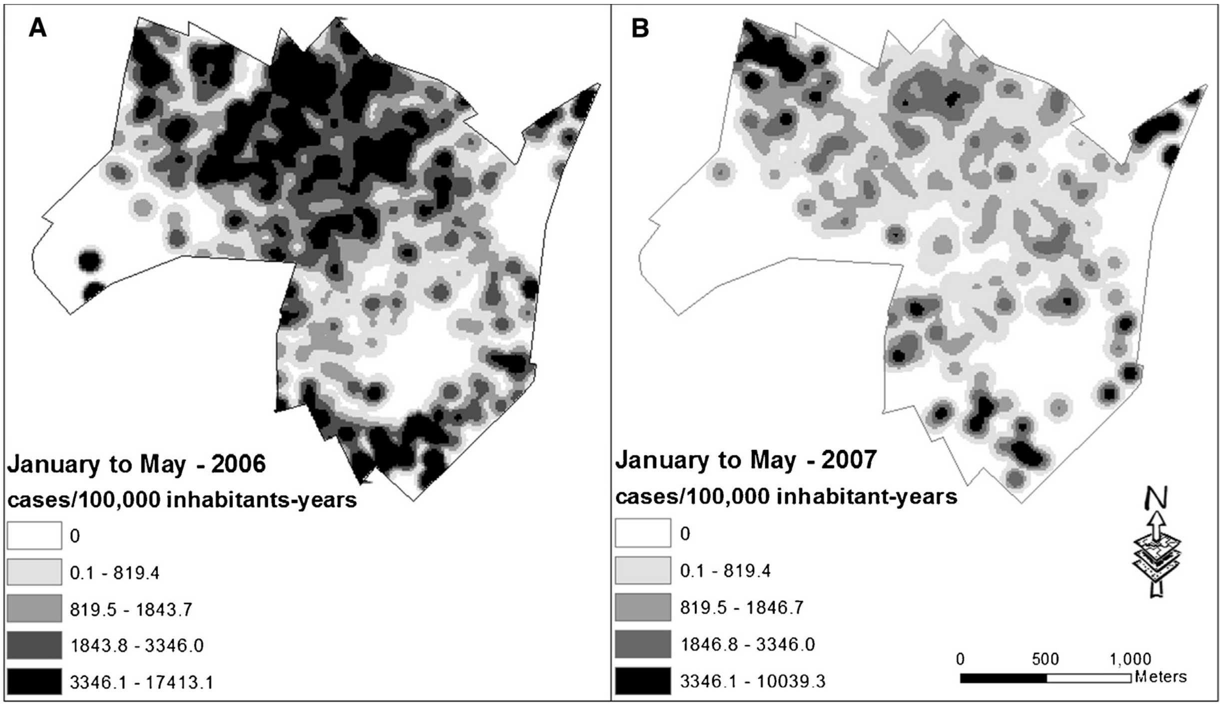

Fig. 5. Incidence rates of dengue from January to May of 2006 (A), and from January to May of 2007 (B), Jaguaré neighborhood, municipality of São José do Rio Preto, state of São Paulo, Brazil.

the population susceptible to DENV3. Many authors note that the entomological threshold for the transmission of dengue depends, among other factors, on the immunologic status of the population (Focks, 2003; Focks et al., 2000; Ghouth et al., 2012; Halstead, 2000; Morrison et al., 2008).

The decision to use entomologic measures obtained in the summer 2006/7 to compare with the incidence rates of dengue fever in both 2006 and 2007 in the Jaguaré neighborhood was based on the pattern of infestation observed between 2005 and 2007 for the region of São José do Rio Preto, which showed the same seasonal behavior and similar levels of infestation, as was noted in the methodology.

Regarding the capacity of the entomological indicators to indicate the spatial risk of dengue occurrence in 2006 and 2007, both indicators calculated per hectare showed better results than those calculated per property, throughout those periods. Moreover, the entomological indicators based on the number of containers with *Ae. aegypti* and on the number of pupae identified similar patterns of infestation, and the latter did not provide additional information in relation to the findings provided by the classic indicators.

For authors such as Focks (2003), Focks and Chadee (1997), and Nathan and Focks (2006), the indicators based on container positivity would have weak relationships with the risk of dengue occurrence, while the indicators based on pupae quantification would be more adequate to estimate that risk. However, in this study, the main difference regarding the capacity of the entomological indicators to indicate spatial dengue risk was not related to the use of pupae quantification but to the application of indicators calculated per hectare. Both of the indicators calculated per hectare, not only the one based on the quantification of pupae, were in agreement with the dengue risk maps.

This result is in agreement with the arguments put forward by several authors who noted that indices calculated by area may be more appropriate measures for assessing the risk of dengue (Barrera et al., 2006; Focks, 2003; Focks et al., 1993, 2000; Focks and Chadee, 1997; Getis et al., 2003; Morrison et al., 2004; Nathan and Focks, 2006; Romero-Vivas and Falconar, 2005).

One explanation for the differences found between the indicators calculated per property and those calculated per hectare was the presence of properties such as schools, churches, clubs and parks with large areas in the Jaguaré neighborhood; because of those large areas, there was a higher probability of hosting containers with immature forms of *Ae. aegypti* compared to smaller properties. Using those large areas to calculate indices per property led to artificial increases in those values compared to the indices calculated per hectare (Favaro et al., 2013). The indicators calculated per property might have similar performance to those calculated per hectare in areas where the properties had homogeneous sizes, but this homogeneity is not a common situation in intermediate-size and large municipalities in Brazil.

Therefore, in São José do Rio Preto and in other Brazilian cities with similar characteristics, changing the calculation of entomological indices by substituting the number of properties with their areas could provide these indices with a greater capacity to predict the spatial risk of dengue fever occurrence. Even then, the use of these indicators is restricted because the indicators should not be considered alone. The indicators make sense only if they are analyzed with other variables related to the dynamics of transmission, such as the degree of immunity of the population for the different serotypes of the dengue virus; the serotypes, genotypes and circulating clades; vector behavior; and weather, environmental and socioeconomic characteristics (Drumond et al., 2013, 2012; Focks et al., 1995; Ghouth et al., 2012; Kuno, 1977; Lee et al., 2012; Morrison et al., 2008; Teixeira et al., 2005; Yamanaka et al., 2011).

Even though the indices calculated for property have proved worse predictors of the spatial risk of occurrence of dengue than those calculated per hectare, BI, lagged two months, was a good predictor of temporal risk of dengue, as another study conducted in the region of São José do Rio Preto had already shown (Dibo et al., 2008).

The degree interference of immunity in the relationship between entomological indices and dengue became evident in this study, since it was found higher OR values for 2006 than 2007, in Jaguaré, and higher correlation coefficients between them for the

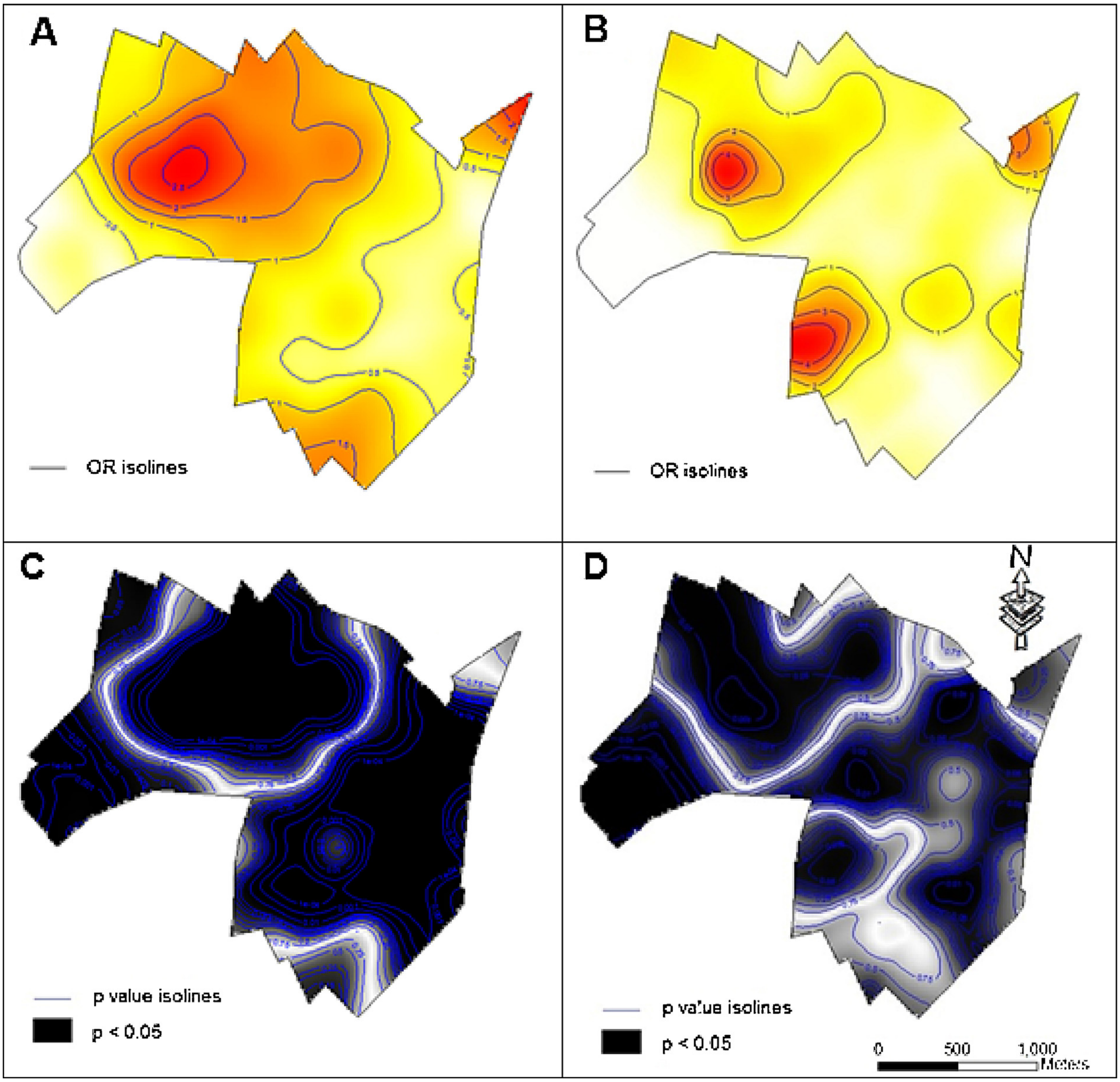

Fig. 6. Spatial risk map for dengue with odds ratio (OR) from January to May of 2006 (A) and from January to May of 2007 (C) and p value map for the first period (C) and for the second period (D), Jaguaré neighborhood, municipality of São José do Rio Preto, state of São Paulo, Brazil.

entire city in 2005/6 than in 2006/7. These discrepancies are probably due to the higher degree of immunity against DENV3 in 2006/7 than in 2005/6. Ghouth et al. (2012) and Morrison et al. (2008) pointed out the decrease in the entomological indicator's accuracy to pinpoint dengue risk would be expected in partially immunized areas.

According to the results of this work and of the work of Niño (2008), the use of geostatistical techniques, such as semivariograms and kriging, to estimate the values of entomological indicators in a geographic area allows for the identification of sites with greater infestation by *Ae. aegypti* without being restricted to regions where an outline has been previously established. The use of GIS together with spatial analyses also allows for the assessment of the relationship between dengue and the entomological indicators, which was observed in both this study and the study by Sanchéz et al. (2010, 2006).

However, to conduct studies identifying areas with higher probabilities of the occurrence of disease or of *Ae. aegypti* with the goal of assessing the relationship between dengue, risk factors, and measures for vector control and to identify and select priority areas for vector-control activities (Eisen et al., 2009; Resendes et al., 2010; Thai et al., 2010), it is necessary to have geo-referenced maps of the assessment units of the sites studied, which is presently enabled by the use of increasingly user-friendly GIS and by the availability of geo-referenced databases (Eisen and Lozano-Fuentes, 2009).

Another aspect to be considered in the discussion about the relationship between entomological indicators and the risk of dengue is the level of vector infestation. São José do Rio Preto showed lower levels of infestation (Barbosa et al., 2010; Ferreira and Chiaravalloti-Neto, 2007) than sites used in studies in which other indicators based on pupae quantification were better predictors of dengue occurrence (Barbazan et al., 2008; Barrera et al., 2006; Focks, 2003). The lower levels of infestation are related to the almost universal and adequate water provisions and collection of solid residues and the low frequency of large water-holding containers (Favaro et al., 2013) that result from the lack of need for water storage by the population, as there is no

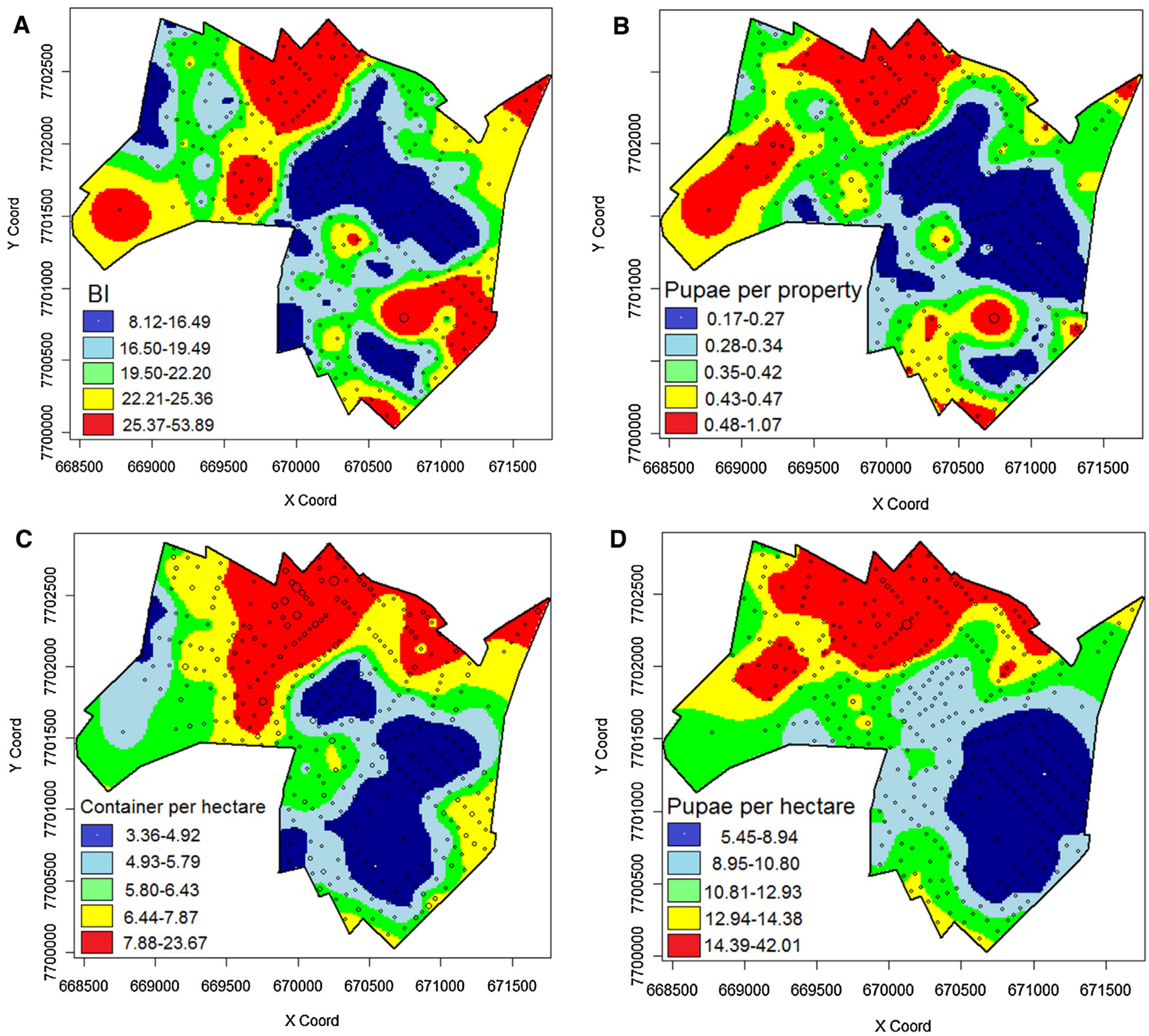

Fig. 7. Breteau Index (BI) (A), number of *Ae. aegypti* pupae per property (B), number of containers harboring *Ae. aegypti* larvae and pupae per hectare (C), and number of *Ae. aegypti* pupae per hectare (D) in the Jaguaré neighborhood, municipality of São José do Rio Preto, state of São Paulo, Brazil, from December 2006 to February 2007.

intermission in the water supply. Despite the low levels of vector infestation, dengue has endemic behavior in the municipality (Mondini et al., 2005) with the occurrence of epidemic peaks (CVE, 2014).

In addition to the lack of differences among indices based on pupae quantification and container positivity, the use of entomological indicators that employ pupae quantification in cities with characteristics such as the ones found in São José do Rio Preto would require sampling plans that use a larger number of properties than the sampling plans used to measure the indices based on container quantification, as finding containers with pupae can be considered a rare event (Favaro et al., 2013).

The potential limitations of the present study include the use entomological indicators that were measured for the summer of 2006/7, in Jaguaré, to be related with dengue occurrence in both study periods; the use of the dengue cases that were notified to the surveillance system, which is known to not include all occurrences; the consideration of the patient's residence as the probable site of infection; and the use of the clinical–epidemiological criterion to confirm some of the dengue cases. However, at the time of this outbreak, the clinical–epidemiological criterion was officially used by the government to quantify dengue cases. As a way to add more concrete data, we also used PCR to confirm dengue cases in our analysis.

Despite these limitations, some of the positive characteristics of this study include the performed analyses, such as the accomplishment of viral diagnostics using techniques of molecular biology on samples from patients with suspected dengue; the scope of the surveillance system of São José do Rio Preto, which includes units with primary, secondary and tertiary public and private care and is distributed throughout the municipality; and the achievement of conducting the entomological survey through the use of a census format and not by sampling. Even the use of the clinical–epidemiological criterion to confirm dengue cases could be minimized as a potential bias because in epidemic situations (high prevalence), the positive predictive value of this type of test could be considered high.

An aspect to be highlighted was the fact that the municipality's population had a high susceptibility to the DENV3 serotype before the period of development of this research and the almost complete predominance of the occurrence of this serotype between 2005 and 2007, which facilitated the analysis of the epidemiological situation of the disease and allowed us to connect it to the entomological indicators. Such aspect adding the extent and quality of the entomological survey and the plausibility of the results can in part minimize the cited limitations.

5. Conclusions

It is possible to conclude that, in the study neighborhood (Jaguaré), the entomological indices calculated by hectare were better predictors of the spatial distribution of dengue than the indices calculated by property, and the pupae quantification did not exhibit better prediction qualities than the indices based on the container positivity, in relation to the spatial risk of dengue occurrence. We also concluded that the BI, lagged two months, was a good predictor of the temporal distribution of dengue in the city of São José do Rio Preto. Even with the population partially immunized in 2007, it was possible to find association between higher infestation levels and an increased risk of dengue.

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