Original Article

Use of ovitraps for the seasonal and spatial monitoring of *Aedes* spp. in an area endemic for arboviruses in Northeast Brazil

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Abstract

Introduction: Due to recent outbreaks of Dengue and Chikungunya and an absence of effective monitoring of the mosquito *Aedes* spp. in the municipality of São Raimundo das Mangabeiras, State of Maranhão, we aimed to demonstrate the potential of ovitraps used together with mathematical models and geotechnology to improve control of this mosquito.

Methodology: From January to December of 2017, ovitraps were set up in five different neighborhoods (Centro, Vila Cardoso, Nazaré, São José e São Francisco). Positivity indices were calculated for each ovitraps, besides the egg density and average number of eggs. Some of the eggs were used for species identification. Mathematical models of correlation and logistic regression were used to evaluate the influence of abiotic factors on egg distribution during each month. Spatial analysis was carried out using georeferencing.

Results: A total of 4,453 eggs were counted, with *A. aegypti* and *A. albopictus* present in each month and neighborhood. The mathematical models show that rainfall can result in a significant increase in the number of eggs. Entomological calculation indicates that there is a high risk of dissemination of arboviruses in the area. Spatially, it was possible to indicate sites with the largest number of collected eggs, which may facilitate future interventions.

Conclusions: As such, ovitraps have proven to be an effective and low cost method for the monitoring of *Aedes* spp., and that its use may help in arboviruses prevention campaigns.

Key words: traps; arboviruses; mosquitoes; vectors.

Introduction

The species *Aedes aegypti* and *A. albopictus* belong to the order Diptera and the family Culicidae. They originated in Egypt and India respectively, and are widely distributed across the world, especially occurring in Asia and the Americas [1-4]. These species of mosquito are worldwide vectors of arboviruses, such as Zika, Chikungunya and Dengue [5, 6, 7].

The drastic increase in the incidence of these arboviruses in recent years is generally attributed to the rise in the world population of *A. aegypti* and *A. albopictus* and to the fact that there is still no effective vaccines; epidemiological surveillance and vector control are therefore still the best ways of preventing potential outbreaks [8-10]. The use of oviposition traps (ovitraps) is seen as an appropriate method of verifying the population density of *Aedes* spp. in different

environments [11]. The advantages of using this type of trap are related to the shorter set-up time in the field, greater sensitivity in detecting the presence of *Aedes* spp. (even at low densities) and low maintenance costs [12-15]. Another approach that has increased the efficiency of monitoring *Aedes* spp. using ovitraps is linking them to geographical information systems (GIS), as these allow spatial and temporal visualisation and analysis of fluctuations in the mosquito population, providing digital situational maps of the problem, identifying critical places and risk areas [16].

Nevertheless, there are still too few studies employing ovitraps in the State of Maranhão. Faced with the recent epidemic of Dengue, Zika and Chikungunya that occurred in the State, with respectively 7,049, 6,416, and 516 probable cases in 2017 [17], there is an urgent need for inexpensive and

sensitive methodologies that would allow the prediction of future epidemics. The aim of this work, therefore, was to demonstrate ovitrap potential for monitoring spatial and seasonal infestation of *Aedes* spp. mosquitoes in an area endemic for arboviruses in the State of Maranhão, Brazil.

Methodology

Study area

The study was carried out in the municipality of São Raimundo das Mangabeiras, in the State of Maranhão, Brazil (Figure 1), during a 12-month period (January to December 2017). The municipality is inserted in the Cerrado biome, presenting a varied climate regime with a mean annual temperature and rainfall of 26.4°C and 1157mm, respectively [18]. According to the Municipality Department for Epidemiological Surveillance of São Raimundo das Mangabeiras, there were 714 confirmed cases of Chikungunya and 83 cases of Dengue during 2017 [19].

Field installation of the ovitraps

Two houses from five neighborhoods were chosen at random to set up the traps: Centro (C1 and C2), Vila Cardoso (VC1 and VC2), Nazaré (BN1 and BN2), São José (SJ1 and SJ2) and São Francisco (SF1 and SF2). Each point was georeferenced using an Etrex 20® GPS (Global Positioning System). The model of ovitrap used in this study was adapted as described by Regis et al. [15], comprising a black plastic container, filled with 500mL of a 30% grass infusion [*Panicum maximum* (Poaceae)], able to attract preferentially females of *Aedes* spp. [15]. Three pieces of white, non-woven fabric of approximately 2.0×12.0cm were used in each trap, identified with the place and date of their installation in the field. Installation was carried out near or around the houses in a shaded area protected from rain, at a height of approximately 15 cm from the ground. The ovitrap remained in the field for seven days. To avoid the possibility of the traps becoming artificial breeding sites, Pyriproxyfen larvicide (Sumilarv®, Sumitomo Chemical, São Paulo, Brazil) was also added to the trap, using the dosage proposed by the manufacturer.

Calculating the entomological indices and morphological identification of the species of Aedes spp.

The pieces of non-woven fabric were removed from the traps and individually packed in plastic containers for drying at room temperature. They were then observed un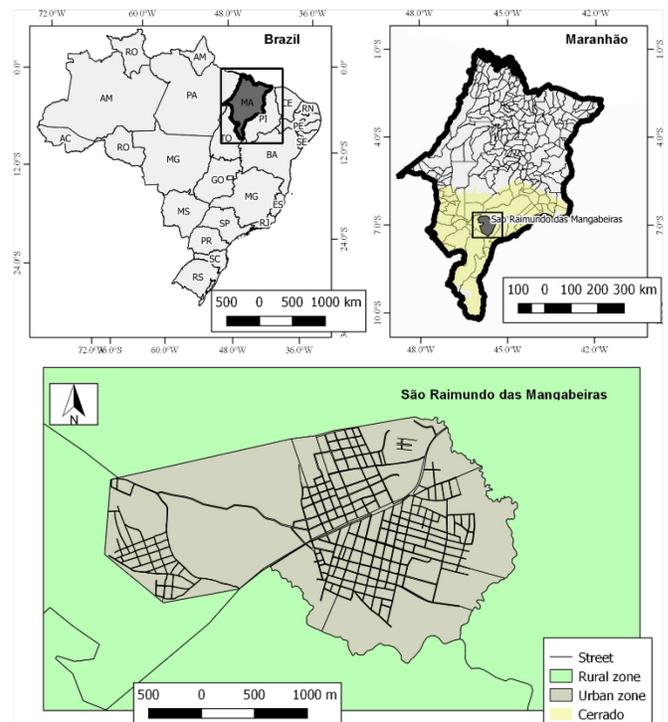der a stereoscopic microscope (40x magnification) to verify the presence and to count the mosquito eggs. The following parameters were estimated from the egg count: a) Ovitrap Positivity Index (OPI); b) Egg Density Index (EDI); and c) average number of eggs per ovitrap (ANE) [20]. The presence of eggs from other mosquitoes was also occasionally verified in ovitraps.

A total of 10% of the eggs collected at each collection point were submerged in individual beakers (600mL) containing a 10% grass infusion (*P. maximum*) to stimulate hatching of the larvae. After 24 hours, the L1 obtained were transferred to breeding boxes. Upon reaching the fourth stage of development, the larvae were fixed with 10% formalin and mounted on slides, to identify the species based on morphological characteristics [2].

Meteorological information of the region and geoprocessing of the data

Rainfall and temperature data for the municipality of São Raimundo das Mangabeiras (MA) for the exact time the ovitraps remained in the field were obtained from Accuweather website (https://www.accuweather.com). From the coordinates obtained with the GPS Etrex 20®, a location map was generated using the QGIS v 2.18 software.

Figure 1. Location of São Raimundo das Mangabeiras municipality, Cerrado region of the Maranhão State, Brazil.

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Statistical analysis

All the data were initially analysed with the Shapiro-Wilk normality test. To compare the mean with standard deviation values between more than two groups, the ANOVA (One-way) test was used, followed by Tukey's test. When only two groups were compared, Student's *t*-test was carried out. The groups with nonparametric data had the medians with interquartile range compared by the Kruskal-Wallis test (more than two groups) or Mann-Whitney test (between two groups). Spearman's correlation was used to verify the influence of rainfall and temperature on the number of eggs obtained throughout the year. Results with a *p* value of < 0.25 were selected to construct the multivariates in the final model (logistic regression), using the number of eggs as a response variable. In addition, variables of low frequency and that showed collinearity were excluded from the multivariate analysis. The variables that remained in the final model were statistically significant, adopting p ≤ 0.05. All the data were analysed, and the graphs constructed using the software GraphPad-Prism 6 (Prism Software, Irvine, California, USA) and STATA version 11.1 (Stata Corporation).

Results

A total of 4,453 eggs were counted for the 120 samples (January to December of 2017), with a median of 210.5 and interquartile ranges varying between 63.25 and 619.0. The species *A. aegypti* and *A. albopictus* were identified for each of the months and neighborhoods under analysis. The presence of eggs from other mosquitoes species, such as *Culex* spp., were also frequently detected in traps. Distribution of the number of *Aedes* spp. eggs collected at each point, as well as the variation in temperature and rainfall, are shown in Figure 2.

During the rainy season (January to April, and November to December of 2017), a total of 3,821 eggs were counted (approximately 86% of the total), with the greatest number obtained in Vila Cardoso (n=1,344), considered one of the most populous neighborhoods in the area. During this period, 53 traps (88.33%) were positive. The smallest number was seen in Nazaré (n= 459).

For the dry period (May to October of 2017), there was a reduction in the total number of eggs obtained (n= 632, approximately 14%), which was statistically lower than the number of eggs counted during the rainy period (Figure 3). Interestingly, the neighborhood with the highest egg count was São Francisco (n= 260), since it is one of the least-populated areas when compared to

Figure 2. Number of *Aedes* spp. eggs distributed monthly among the neighborhood of the São Raimundo das Mangabeiras during the January to December of 2017. The values for rainfall (mm) and temperature (°C) are oriented based on the secondary axis (right y-axis). **C.** Centro, **VC.** Vila Cardoso, **BN.** Nazaré, **SJ.** São José, **SF.** São Francisco.
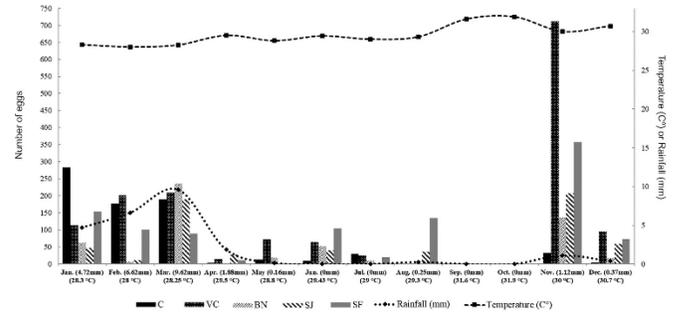
the other collection points. No eggs were found during September or October, considered the hottest months of 2017 and with no rainfall. In general, 21 (35%) positive traps were found during the dry season. There were no statistically significant differences when comparing the number of eggs obtained between neighborhoods (Supplementary Figure 1).

By using the total of eggs collected monthly in the neighborhoods under analysis, it was possible to obtain mean values for OPI, EDI and ANE (Table 1). When analysing the influence of abiotic factors (temperature and rainfall) on egg distribution and entomological indices over the months, it was found that the rainfall had a significant positive influence on the number of eggs collected, as well as on the values for the OPI, EDI and ANE (Figure 4).

Figure 3. Comparison of the number of *Aedes* spp. eggs captured by ovitraps during the dry and rainy seasons. Result obtained from Student's t-test. *statistically significant.
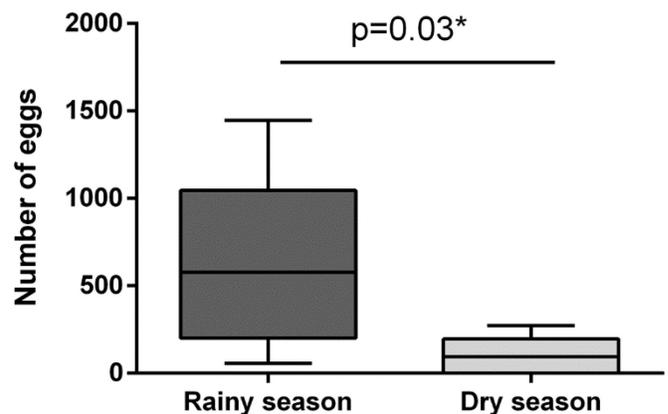
Table 1. Values of entomological indices obtained for São Raimundo das Mangabeiras, Maranhão, Brazil.

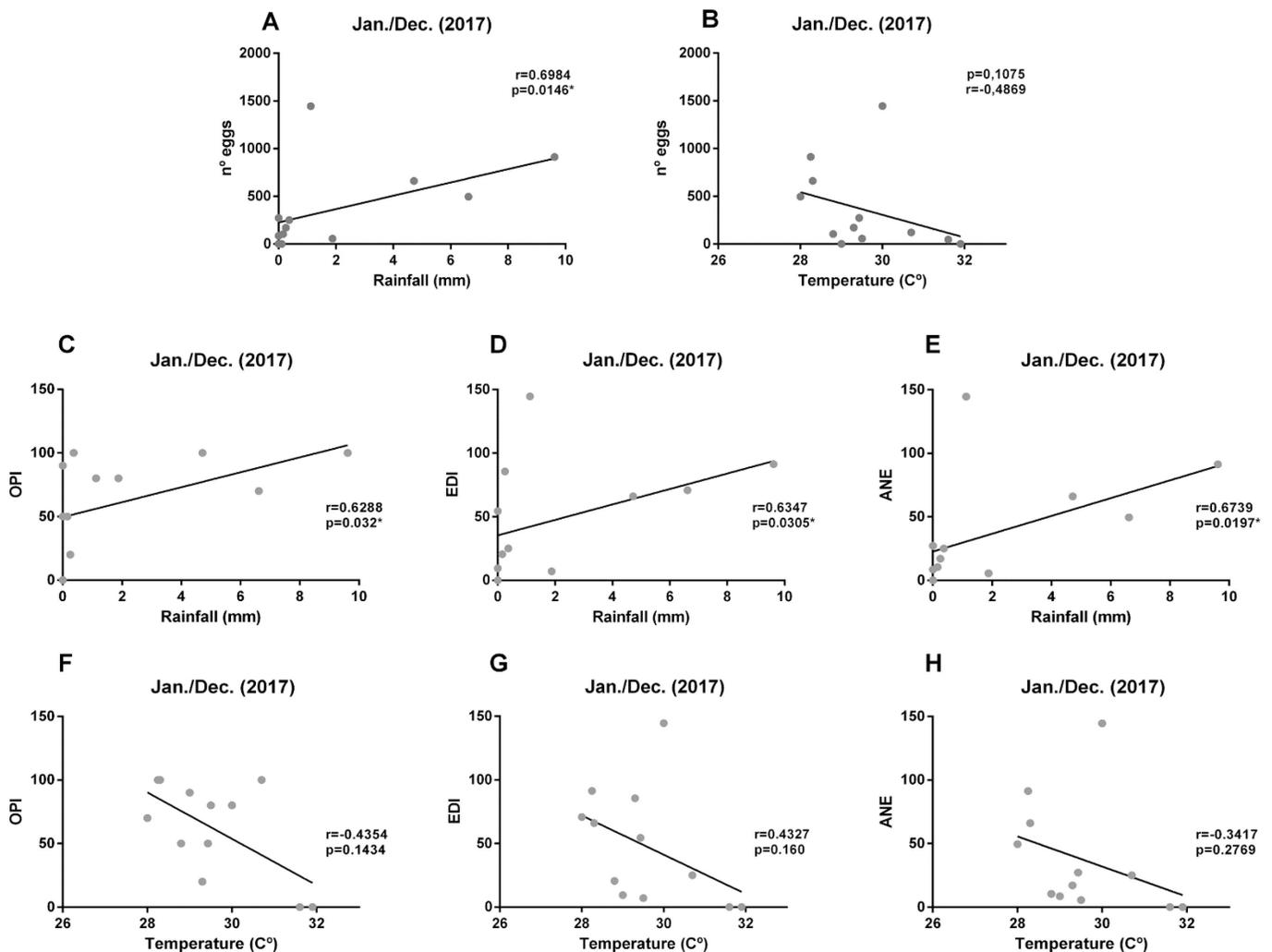| Entomological Parameters	Values
OPI [a]	61.6%
EDI [b]	47.8
ANE [c]	37.1

[a] OPI: ovitrap positivity index; [b] EDI: egg density index; [c] ANE: average number of eggs per ovitrap.

Table 2. Logistic regression model demonstrating the relationship between number of *Aedes* spp. eggs and rainfall in São Raimundo das Mangabeiras, Maranhão state, Brazil.

Variable response: > 250 eggs	Odds Ratio	β	p*	95% CI
Co-variable: > 1mm of rainfall	24	2.04	0.041	1.14-505.19

β: coefficient estimates; CI: confidence interval; *statistically significant.

Figure 4. Influence of the abiotic factors (temperature and rainfall) on the number of eggs collected (A and B) and on the values obtained for the entomological indices (C, D, E, F, G and H). Result obtained from Spearman's correlation. *statistically significant.

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Although the temperature had a negative influence on all these variables, it was not statistically significant (Figure 4).

When the number of eggs was categorised into values greater or smaller than 250 and compared with the categorised values for rainfall (greater or smaller than 1 mm), the frequency of values greater than 250 eggs of *Aedes* spp. was significantly higher during periods when the rainfall was greater than 1 mm (p < 0.05). Later, using the multivariate model to specifically investigate this association, the results obtained in the Spearman's correlations were confirmed, showing that there were 24-fold more chance of this number of *Aedes* spp. eggs occurring (n= 250) in months with rainfall values above this range (1 mm) (Table 2).

Using the collection-point coordinates, it was possible to construct a map of the municipality of São Raimundo das Mangabeiras (Figure 5), showing the number of eggs collected during 2017 at each point. In order to help locate these points, the streets of the municipality were shown with their names. In this way, it was possible to summarise the principal data of this study.

Discussion

From the data on the number of eggs of *Aedes* spp. obtained with the ovitraps, it was possible to efficiently characterise the pattern of seasonal and spatial distribution of mosquito infestation in the municipality of São Raimundo das Mangabeiras, an area endemic for Dengue and Chikungunya in Northeastern Brazil.

Distribution of the oviposition of these mosquitoes between the neighborhoods in the area seems to show a homogeneous pattern, since there were no significant differences in the number of eggs obtained at the different collection points. However, a clear increase was seen in the most populous places, such as Vila Cardoso and the Centro. Although the species *A. aegypti* and *A. albopictus* could be detected in every month and every neighborhood, several studies show that species *A. aegypti* displays a more anthropophilic behaviour, being adapted to the daily life of humans, and preferring to inhabit the city centers [21, 22]. According to Li et al. [23], the process of urbanisation favours an increase in the prevalence and abundance of species of *Aedes*, as it increases the number of artificial breeding sites, such as tyres, cans or water-storage containers.

On the other hand, less populous neighborhoods, such as São Francisco, had the greater number of eggs during the dry period. A possible explanation for this

Figure 5. Map of São Raimundo das Mangabeiras municipality, Maranhão, Brazil, which shows the distribution of the number of *Aedes* spp. eggs captured during January to December of 2017 by ovitraps in the neighborhoods analyzed.
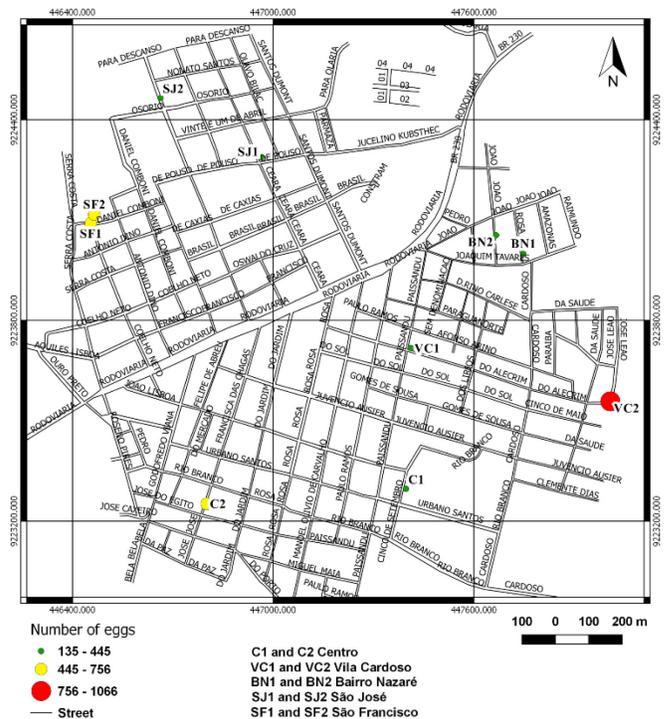
situation would be the fact of water supply in this neighborhood is insufficient, which favors the residents' habit of storing water in large concrete or plastic tanks (personal observation) without taking any necessary precautions. This may have determined an increase in oviposition during the dry period in the neighborhood, since it has been shown that *Aedes aegypti* can reproduce in both natural and artificial water reservoirs [24].

In addition to urbanisation, rainfall was another element involved in promoting *Aedes* spp. in the study area. The data demonstrated that rainfall exerted a strong, positive and significant influence on the number of eggs, determining high values for OPI, EDI and ANE. In field research with ovitraps, several studies report that rain is the only abiotic factor capable of interfering in a significant and positive way in the number of *Aedes* spp. [25, 26], suggesting a higher risk of arbovirus transmission during rainy periods. This intimate association between rainfall and the reproductive rate of *Aedes* spp. occurs since increased rainfall favours a greater variety of breeding sites. Our mathematical model was also able to associate rainfall with the number of eggs collected. It was shown that rainfall greater than 1 mm was able to determine a 24-fold increase in the chances of oviposition of these

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mosquitoes on a scale greater than 250 eggs. Thus, it is strongly recommended that models such as this be made for each locality, since they can provide more-concrete data on how any one factor can influence the seasonal and spatial distribution of *Aedes* spp.

It is also interesting to note that there was a sudden increase in the number of eggs in November (transition from the dry to the rainy season), mainly at points VC and SF, supposedly due to atypical precipitation during this period. It may have favored the hatching of latency eggs accumulated during the rainless months, consequently increasing the adult population and their reproduction rates. This reinforces the importance of mosquito control even during dry seasons.

In general, the municipality of São Raimundo das Mangabeiras was characterised as at risk or under alert for the dissemination of arboviruses, as shown by the values for the OPI and EDI [27]. According to Barrera et al. [28], the EDI must be reduced to a value below 10 for there to be no risk of Dengue being transmitted in any given region.

Besides that, it was possible to demonstrate spatially, exactly where the highest reproductive rates of *Aedes* spp. were located in the study area. The choice of using GIS together with the results of the ovitraps was to demonstrate that georeferencing may result in a more instructive perception of the differences found between the collection sites, facilitating decision-making in future interventions.

Conclusion

Generally, the data demonstrated that ovitraps were able to describe, reliably and at low cost, the infestation pattern of *Aedes* spp. in the municipality of São Raimundo das Mangabeiras. Rainfall is the main abiotic factor capable of influencing the reproductive rate of these insects, even in climatic conditions of the Cerrado biome. In addition, the constant presence of *A. albopictus* in the collection points suggests a probable participation of this species in the recent outbreaks of Zika and Chikungunya in the municipality. It is also worth mentioning that combining the data obtained from the ovitraps with geographic information systems was perfectly viable, and allowed the preparation of maps that describe, in a simple way, priority areas for intervention, which may facilitate decision-making by managers.

Authors' contributions
GSM conceived the idea. GSM, JVON, HLMNM, TML, JGMR, DTC, KCL and, RSSM performed the laboratory experiments and field collections. GSM, JGMR, DTC, and RSSM designed and analyzed the data. GSM, JGMR and, DTC wrote the manuscript with suggestions from all the other co-authors.

References
1. Forattini OP (2002) Medical Culicidology: Identification, Biology, Epidemiology. Vol. 2. São Paulo: Universidade de São Paulo publishing company, 860 p. [Book in Portuguese.]
2. Consoli RAGB, Lourenço-de-Oliveira R (1994) Main mosquitoes of sanitary importance in Brazil. Rio de Janeiro: Fiocruz publishing company, 228 p. [Book in Portuguese.]
3. Reinert JF, Harbach RE, Kitching IJ (2009) Phylogeny and classification of tribe Aedini (Diptera: Culicidae). Zool J Linnean Soc 157: 700–794.
4. Lambrechts L, Paaijmans KP, Fansiri T, Carrington LB, Kramer LD, Thomas MB, Scott TW (2011) Impact of daily temperature fluctuations on dengue virus transmission by *Aedes aegypti*. Proc Natl Acad Sci USA 108: 7460–7465.
5. Wong SS, Poon RW, Wong SC (2016) Zika virus infection-the next wave after dengue? J Formos Med Assoc 115: 226–242.
6. Vega-Rúa A, Zouache K, Girod R, Failloux AB, Lourenço-de-Oliveira R (2014) High level of vector competence of *Aedes aegypti* and *Aedes albopictus* from ten American countries as a crucial factor in the spread of Chikungunya virus. J Virol 88: 6294–6306.
7. Chouin-Carneiro T, Vega-Rua A, Vazeille M, Yebakima A, Girod R, Goindin D Dupont-Rouzeyrol M, Lourenço-de-Oliveira R, Failloux AB (2016) Differential Susceptibilities of *Aedes aegypti* and *Aedes albopictus* from the Americas to Zika Virus. PLoS Negl Trop Dis 10: e0004543.
8. Whitehead SS, Blaney JE, Durbin AP, Murphy BR (2007) Prospects for a dengue virus vaccine. Nat Rev Microbiol 5: 518–528.
9. Guzman MG, Halstead SB, Artsob H, Buchy P, Farrar J, Gubler DJ, Hunsperger E, Kroeger A, Margolis HS, Martínez E, Nathan MB, Pelegrino JL, Simmons C, Yoksan S, Peeling RW (2010) Dengue: a continuing global threat. Nat Rev Microbiol 8: S7–S16.
10. Achee NL, Gould F, Perkins TA, Reiner RC Jr, Morrison AC, Ritchie SA, Gubler DJ, Teyssou R, Scott TW (2015) A critical assessment of vector control for dengue prevention. PLoS Negl Trop Dis 9: e0003436.
11. Vezzani D, Velazquez SM, Schweigmann N (2004) Seasonal pattern of abundance of *Aedes aegypti* (Diptera: Culicidae) in Buenos Aires city, Argentina. Mem Inst Oswaldo Cruz 99: 351–356.

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12. Jakob WL, Bevier GA (1969) Evaluation of ovitraps in the US *Aedes aegypti* erradication program. Mosq News 29: 650–653.

13. Chiaravalloti-Neto F (1997) The population's knowledge concerning dengue fever, vectors, and control measures in São José do Rio Preto, São Paulo state. Cad Saude Publica 13: 447–453. [Article in Portuguese.]

14. Gama RA, Eiras AE, Resende MC (2007) Effect of lethal ovitrap on the longevity of females of *Aedes aegypti* (Diptera: Culicidae) (Diptera: Culicidae). Rev Soc Bras Med Trop 40: 640–642. [Article in Portuguese.]

15. Regis L, Monteiro AM, Melo-Santos MA, Silveira JC Jr, Furtado AF, Acioli RV, Santos GM, Nakazawa M, Carvalho MS, Ribeiro PJ Jr, Souza WV (2008) Developing new approaches for detecting and preventing *Aedes aegypti* population outbreaks: basis for surveillance, alert and control system. Mem Inst Oswaldo Cruz 103: 50–59.

16. Bailey TC, Gatrell AC (1995) Interactive Spatial Data Analysis. Harlow: Prentice Hall publishing company, 414 p.

17. Brazilian Ministry of Health (2018) Monitoring of cases of dengue, chikungunya fever and fever by the Zika virus until Epidemiological Week 52, 2017. Available: https://portalarquivos2.saude.gov.br/images/pdf/2018/janeiro/23/Boletim-2018-001-Dengue.pdf. Accecced: 26 May 2019.

18. Brazilian Institute of Geography and Statistics (2010). Available: www.ibge.gov.br. Accecced: 19 April 2019.

19. Brazilian Ministry of Health (2017) Notifiable Diseases Information System. Consultation Report: Dengue and Chikungunya - São Raimundo das Mangabeiras, Maranhão. Available: http://www2.datasus.gov.br/DATASUS/index.php?area=0203&id=29878153. Accecced: 14 October 2019.

20. Gomes AC (1998) Measures of urban infestation levels for *Aedes* (Stegomyia) *aegypti* and *Aedes* (Stegomyia) *albopictus* in Entomological Surveillance programs. IESUS 7: 49-57. [Article in Portuguese.]

21. Costa FS, Silva JJ, Souza CM, Mendes J (2008) Population dynamics of *Aedes aegypti* (L) in an urban area with high incidence of dengue. Rev Soc Bras Med Trop 41: 309–312. [Article in Portuguese.]

22. Kamgang B, Yougang AP, Tchoupo M, Riveron JM, Wondji C (2017) Temporal distribution and insecticide resistance profile of two major arbovirus vectors *Aedes aegypti* and *Aedes albopictus* in Yaoundé, the capital city of Cameroon. Parasit Vectors 10: 469.

23. Li Y, Kamara F, Zhou G, Puthiyakunnon, Li C, Liu Y, Zhou Y, Yao L, Yan G, Chen XG (2014) Urbanization increases *Aedes albopictus* larval habitats and accelerates mosquito development and survivorship. PLoS Negl Trop Dis 8: e3301.

24. Dom NC, Ahmad AH, Ishak AR, Ismail R (2013) Assessing the risk of dengue fever based on the epidemiological, environmental and entomological variables. Procedia Soc Behav Sci 105: 183–194.

25. Almeida PS, Meotti C, Almeida GS, Nascimento J, Araújo AD, Faccenda O, Fernandes MG (2013) Infestation of *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae) determined by oviposition traps (ovitraps) in the city of Costa Rica, Mato Grosso do Sul State, Brazil. Rev Patol Trop 42: 331–339. [Article in Portuguese.]

26. Serpa LLN, Marques GRAM, Lima AP, Voltolini JC, Arduino MB, Barbosa GL, Andrade VR, Lima VL (2013) Study of the distribution and abundance of the eggs of *Aedes aegypti* and *Aedes albopictus* according to the habitat and meteorological variables, district of São Sebastião, São Paulo State, Brazil. Parasit Vectors 6: 321.

27. Avendanha JS (2007) Vectorial and Dengue virus monitoring, Belo Horizonte, Minas Gerais. Rev Inst Adolfo Lutz 66: 207-207. [Article in Portuguese.]

28. Barrera R, Amador M, MacKay AJ (2011) Population dynamics of *Aedes aegypti* and dengue as influenced by weather and human behavior in San Juan, Puerto Rico. PLoS Negl Trop Dis 5: e1378.

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Annex – Supplementary Items

Supplementary Figure 1. Total number of *Aedes* spp. eggs collected by neighborhood analyzed. Result obtained from the Kruskal-Wallis test. **C.** Centro, **VC.** Vila Cardoso, **BN.** Nazaré, **SJ.** São José, **SF.** São Francisco; **ns**= no significant.