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Effect of heavy-equipment aided environmental nebulization on *Aedes aegypti* vectors of Dengue, Zika and Chikungunya in São Paulo, Brazil

R. Piovezan[1]*, J. Paulo O. Acorinthe[2], A. Visockas[2], T.S. de Azevedo[3] and C.J. Von Zuben[1]

[1]Universidade Estadual Paulista, Departamento de Zoologia, Rio Claro, SP, Brazil: [2]Department of the Environment-Prefeitura Municipal de Santa Bárbara d'Oeste, Santa Bárbara d'Oeste, SP, Brazil: [3]Universidade de São Paulo, Faculdade de Saúde Pública, São Paulo, SP, Brazil

Abstract

The control of dengue constitutes a great challenge for public health; however, the methods normally used have shown themselves to be insufficient to keep the indices of infestation of *Aedes aegypti* under control. Recently, beyond the large number of cases and deaths associated with dengue, new risks have arisen such as those represented by chikungunya fever and Zika. In the light of the great significance of these problems within the public health context, two areas in a municipality in the interior of the State of São Paulo, Brazil were selected in 2014. One of them, Bairro Cidade Nova, was submitted to the conventional method of nebulization with portable equipment, and the other, Bairro Jardim Europa, received the application of the insecticide by means of heavy-equipment coupled to the vehicle. During the project, 1355 mosquito eggs were collected, 1105 of them in Bairro Cidade Nova and 205 in Bairro Jardim Europa. After the applications with heavy-equipment in the months of March and April, the number of cases of the disease reported in the month of April for Bairro Jardim Europa was less than half that of Bairro Cidade Nova, which had received the conventional treatment. The nebulization with the heavy-equipment may constitute a viable and effective strategy for achieving better results in the control of *Ae. aegypti*.

Keywords: *Aedes aegypti*, vector control, dengue, Zika, chikungunya

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Introduction

The determining factors associated with the maintenance of the transmission of the four dengue viruses in the municipalities are due to the difficulties involved in the combat of the mosquito vector and the lack of control of the infectious agents. As a rule, the control of the vector is reduced, in large measure, by the occasional nature of the actions taken. The interruption of the control programs leads to mistakes in the estimates of the infestation of the municipalities. Concomitantly, the difficulty and the precarious character of the public health surveillance actions favor, still further, the scenarios conducive to epidemic events (Brasil, 2001, 2002; SUCEN, 2001).

The control of *Aedes* (*Stegomyia*) *aegypti* Linnaeus, 1762 represents one of the greatest public health challenges in Brazil (Câmara *et al*., 2007). The financial investments set aside for programs for the vector's control and for the care of patients suffering from dengue exceed R$ 1 billion (Brasil, 2015*a*). Despite this, the mosquito has shown itself sufficiently well adapted to maintain populations in the urban environment and cause negative impacts on human health. The most severe

*Author for correspondence
Phone : (55)19-43595900
Fax: (55)19-34595921
E-mail: piovezan.rafael@gmail.com

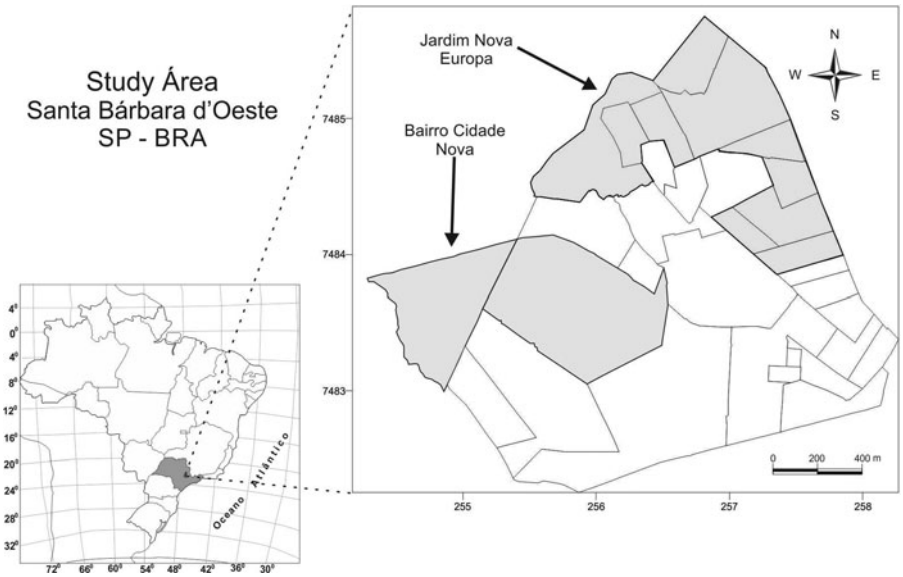

Fig. 1. Location of the Study Areas in Santa Bárbara d'Oeste, São Paulo state – Brazil.

manifestations of the disease can be linked to several factors, such as the type of virus responsible for the infection, the patient's age, chronic diseases and immune status, genetic factors of affected populations, and pre-exposure to other serotypes, among others (Forattini, 2002). Also according to PAHO-WHO (2016), there were, in 2015, more than 2.3 million cases of dengue, with 10,000 severe manifestations of the disease and 1181 deaths worldwide.

In Brazil, in spite of the millions of cases and of the countless deaths caused by dengue, the great appeal in favor of the combat of this vector is at the present time concentrated on the dissemination of the Zika virus. This arbovirus belongs to the Flaviviridae family, which also includes the Dengue and Yellow fever viruses. Despite the symptoms of infection by the Zika virus's being relatively light, its neuropathy can lead to serious syndromes, such as that of Guillain Barré (Marcondes & Ximenes, 2015), and at the present time this virus has been associated with an increase in some regions of the country in cases of congenital anomalies of the central nervous system (Brazil, 2015*b*, 2016; CDC, 2016; Mlakar *et al*., 2016; Rasmussen *et al*., 2016).

The great emphasis placed on the Zika fever epidemic and the association of the infection in pregnant women with the increase in the incidence of microcephaly in new-born infants should be used to create greater awareness among the population as to the means by which the mosquito could be combated. However, dubious information regarding the control of the mosquito is disseminated, with little emphasis on the safe methods of elimination of the immature forms and their habitats, while ineffective and unsafe measures are widely publicized. We do not wish here to minimize the importance of alternative methods of control or of individual protection, but these should not take the place of integrated management techniques for the control of the mosquito vector, which are of recognized efficacy for the protection of the community (Andrade & Cabrini, 2010). Another relevant aspect which ends up by having its focus dissipated in this context is the relationship established between the information publicized and the content necessary to assist in the greater involvement of society as a whole.

Within this context, the validation of the methods usually employed and available for the control of *Ae. aegypti* is fundamental to guaranteeing the effectiveness of the measures used for the control of the vector and also the safety of society with regard to the various arboviruses related to this vector (Marcondes & Ximenes, 2015). The objective of this study was to compare the application of the insecticide Malathion GT 96% by the method of nebulization with portable equipment with that using heavy-equipment. There are municipalities that have adopted one or other of these methods for their programs of integrated control of the mosquito *Ae. aegypti*.

Methods

Study area

The present study was undertaken in the municipality of Santa Bárbara d'Oeste located in the interior of the São Paulo state, southeastern Brazil (fig. 1) (22.75°S; 49.38°W) at an average altitude of 560 m. The total area of the municipality is of approximately 271 km^2, with a population of about190 thousand inhabitants, 98% of whom are concentrated in the urban nucleus and only 2% in the rural zone (IBGE, 2013). The municipality is situated among the foothills of the Peripheral depression of the State of São Paulo (Depressão Periférica Paulista), with relief of gently undulating hills of intermediate height. The remaining vegetation of the area belongs to an area of transition between the biomes of the Atlantic Forest and the Savannah (Cerrado). In accordance with the classification proposed by Koeppen (Strahler & Strahler, 1989), Santa Bárbara d'Oeste possesses a climate defined as Cwa that is characterized as high-level tropical, with summer rains and dry winters. The average temperature of the hottest and coldest months is, respectively, above 22 and 12°C. The average annual rainfall is of 1466.1 mm (IBGE, 2013; CEPAGRI, 2014).

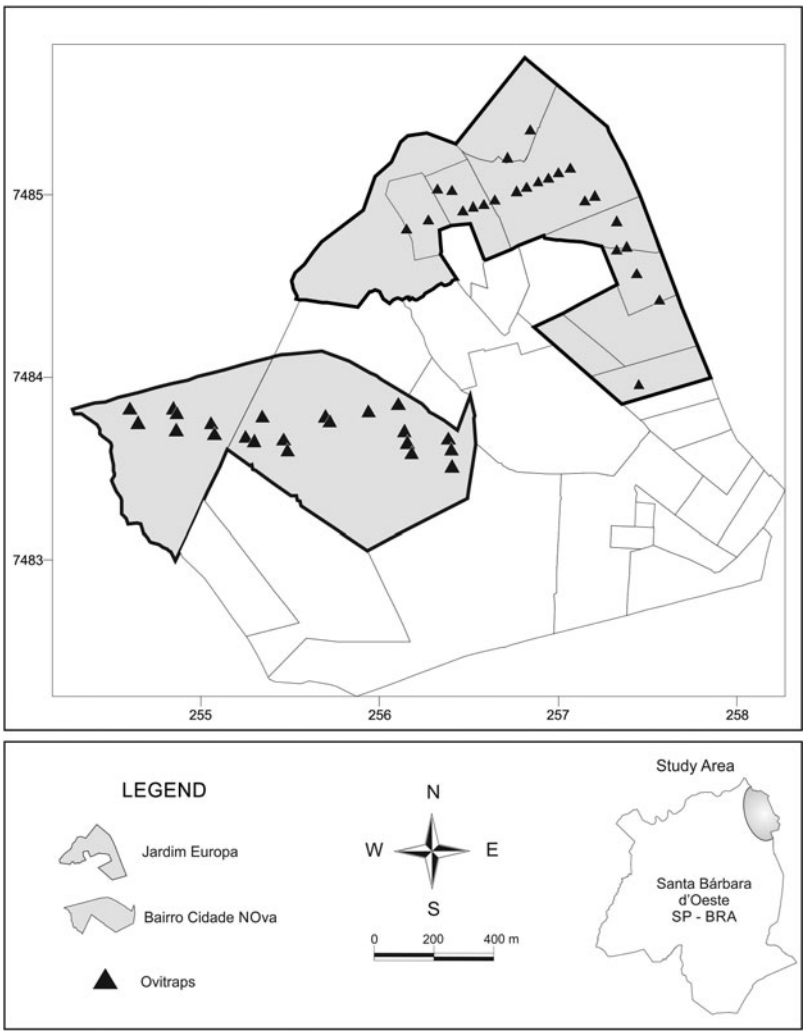

Fig. 2. Location of the traps in the suburbs of Jardim Europa and Cidade Nova, Santa Bárbara d'Oeste, São Paulo state – Brazil.

The municipality of Santa Bárbara d'Oeste has been infested by the vector since 1986, the first cases of dengue having been notified in 1995 (Lima *et al*., 1999).

Suburbs selected

For the choice of the study areas, two sources of information were considered. The first was based on the study of Piovezan *et al.* (2012), which indicated the northeastern area of the city as that where there is a great probability of finding larvae of *Ae. aegypti*. Also consulted was the study of Piovezan *et al.* (2014), which presents the history of the occurrences of dengue in the municipality. Thus were selected the two suburbs of Cidade Nova and Jardim Europa, both of which present a history of serious epidemics and which are characterized as the main foci of the dissemination of the disease in the eastern area of the city. Between 2013 and 2015, these two suburbs alone accounted for10.01% of the total number of cases of dengue in the city, 319 of them being confirmed, in this same period, in Jardim Europa and 269 in Cidade Nova. The resident population of these same districts was similar, being estimated at 14,143 in the former and 14,392 in the latter.

In the light of the fact that the two areas selected had undergone the same kind of treatment historically, a different method for the application of insecticide was applied exclusively in the Jardim Europa suburb. In this suburb, four applications of insecticide were undertaken by means of nebulization (ULV – Ultra Low Volume) with Malathion GT 96% using heavy-equipment coupled to a vehicle. The dates on which these applications were made were 19 and 26 March and 2 and 9 April. In the other area analyzed, Cidade Nova, the nebulization was undertaken in accord with the technical norms (Sucen, 2001), that is to say, as the cases of dengue were confirmed the nebulization was undertaken with light, portable equipment.

Assessment of infestation of the vector

In each of the two areas chosen, 30 traps of the ovitrap type (for the capture of eggs) were installed, positioned on a transect in the center of each of the suburbs and distributed, spatially, in the residences of the blocks drawn by lot (fig. 2), both

in the peridomicile and also in the intradomicile at approximately 1.5 m above ground level. The ovitraps consist of a black plant pot with an approximate volume of one liter and awooden pallet 14 cm long, 3.5 cm wide and 0.5 cm thick. The traps were inspected by endemic control agents, the pallets removed from the ovitraps and immediately replaced by new ones of the same dimensions and characteristics.

When they were removed, the pallets were transported to the laboratory and then dried, thereafter the eggs on each substrate were counted under a microscope and the values obtained were entered into a data bank. After they had been counted, the eggs were systematically removed from the pallets by means of brushes and disposed of in the sewage network. The eggs thus disposed of in the sewage system were taken, for this purpose, to a point at a distance from the urban area of the municipality, their final destination being the River Piracicaba.

The collections occurred during two periods, in the first of which the traps were installed on 27 March 2014 and retrieved on 1 April of that same year and in the second they were installed on 10 April 2014 and retrieved on 16 April of that same year.

The egg collections undertaken by means of the ovitraps make use of the standardized methods routinely employed for the monitoring of *Ae. aegypti*. Other species may also sometimes lay their eggs in the same receptacle, however, the presence of the vector of dengue, Zika and chikungunya is much more abundant under urban conditions and its presence has already been demonstrated in the area studied by Piovezan *et al.* (2012).

Vector control

After the collection and examination of the pallets, the mosquito control activities were undertaken. These control activities were defined on the basis of three axes, namely: active search for dengue cases, active search for breeding places with their subsequent removal or destruction and the elimination of adults by nebulization with insecticide using portable equipment (SUCEN, 2001).

Briefly, the activities of which the active search for dengue suspects consists are mainly undertaken by the endemic control agents during domiciliary visits. During these visits information is collected on possible cases of infection by the dengue virus and is then passed on to the competent epidemiological surveillance unit for insertion into the data-bank. It was thus that the same activity was undertaken in both the areas studied, covering the periods, which preceded the research project, the research period itself and right through to the end of the transmission of that year.

The control actions regarding the breeding places were undertaken together with the active search for suspected cases of dengue. They were characterized by the active search for and the removal of the places which favored the development of the immature forms of *Ae. aegypti* in the properties, which lay within the area of transmission. This condition, i.e. working in the search for confirmed cases of dengue, often imposes distinct efforts in the different areas of transmission.

The present study considers that the historical record of cases that dates back to the 90s in the municipality studied and the control actions which have been applied in the same way in all the suburbs during that period, fulfilling the existing norms of the period, are factors which strengthen the methodology here adopted. Thus one may demonstrate that these areas always received the same methods of treatment and experienced various epidemics of different serum types.

The control of adults, to block the transmission of the dengue virus, was undertaken throughout the city by means of nebulization with portable equipment, as the cases of dengue were confirmed. This procedure, as has already been said, was applied uniformly throughout the municipality, with the exception of the area of Jardim Europa, which, on four different occasions (19 and 26 March; 2 and 9 April), received the application of insecticide by nebulization with heavy-equipment.

Nebulization for the control of adult mosquitoes requires spraying with the insecticide organophosphate (Malathion GT 96%) by means of either light or heavy-equipment, the Ultra Low Volume (ULV) dispersing micro drops with diameters of between 5 and 30 micronsin the air (SUCEN, 2001). In the area of the Jardim Europa suburb, the insecticide was applied with the use of heavy pulverizing equipment coupled to the body of the vehicle – which circulated along the streets of the suburb at a speed of between 10 and 16 Km h^{-1}, between16.00 and 20.00 hours. The portable equipment was checked by officials of the Municipality and underwent periodic inspection by the Endemic Control Superintendency of the State of São Paulo (SUCEN), while the equipment for pulverization coupled to the vehicle was lent by SUCEN to assist in the control activities undertaken in the municipality.

All the control activities are described in the manuals for the control of dengue (Brasil, 2001; SUCEN, 2001, 2010). The activities for vector control were carried out in accordance with the same procedure in the various areas of the municipality, by the same group of professional personnel using the same operational and technical skills. The ovitraps installed in the two suburbs mentioned above and the use of nebulization with heavy-equipment for the control of adult mosquitoes in the Jardim Europa suburb are exceptions to the above general description.

Spatial and temporal analysis of the quantity of eggs

After the collections, during the next step, the study of the spatial variability of the quantity of eggs was carried out by means of variographic analysis. According to Gringarten & Deutsch (2001), the determination of the semivariogram is the first and most important stage, in view of the fact that this kind of approach analyzes the spatial dependence of the values of a variable $V(x)$, separated by $a\Delta h$ vector. The semivariogram may be defined as the variance of the error committed by the estimate of an unknown content in $(x + h)$, with the help of a particular point in (x) (Andriotti, 2004). The semivariograms express the spatial distribution of continuous variables, demonstrating the zone of influence and the anisotropic aspects of the same (Pfeiffer *et al.*, 2008).

With the use of the Variowin software (Pannatier, 1996), temporal semivariograms of the mosquito egg abundances in the areas studied were constructed. After the calculation of the modeling of the experimental semivariograms, a mathematical model was used to present the variety of the data set. This procedure was used for the fit of the model, thus allowing the estimation of the surface generated more precisely and trustworthily. In this way, the method of the interpolation of the data adopted was that of ordinary kriging, which allows the generation of maps of the temporal distribution of the quantity of eggs. This method of interpolation was therefore used for the construction of four isoplethic quantitative maps. This procedure was carried out using Surfer software (Golden Software, 1995).

Table 1. Distribution of eggs collected in ovitraps in theCidade Nova and Jardim Europa suburbs of the municipality of Santa Bárbara d'Oeste, SP in April 2014.

	Collection 1 (27 March 2014–01 April 2014)		Collection 2 (09–16 April 2014)	
	Cidade Nova	Jardim Europa	Cidade Nova	Jardim Europa
No. eggs	584	104	521	146
No. eggs intradomicile	220 (37.7%)	12 (11.5%)	254 (48.8%)	48 (32.9%)
No. eggs peridomicile	364 (62.3%)	92 (88.5%)	267 (51.2%)	98 (67.1%)
No. positive traps	14 (46.6%)	10 (33.0%)	23 (76.6%)	4 (13.3%)
No. positive traps (intradomicile)	4 (13.3%)	3 (10.0%)	11 (36.6%)	2 (6.6%)
No. positive traps (peridomicile)	10 (33.3%)	7 (23.0%)	12 (40.0%)	2 (6.6%)

* No., number.

We used the equation to calculate the mean variation of positive traps at the time intervals defined by different collections and months, respectively. The same procedure was applied for the number of cases found in the study areas:

$$\Delta A = \frac{\alpha_2 - \alpha_1}{T}$$

where ΔA is the mean variation of positive traps during the time period studied, α_1 is the number of positive traps in the initial period; α_2 is the final number of positive traps after that same period; T is the duration of the study period.

We used Fisher's exact test to analyze a contingency table and test whether the row variable and column variable are independent. For the Santa Bárbara dengue fever cases this test was based on the hypergeometric distribution. This procedure determines whether two population proportions are equal. For this application, the null hypothesis states that the two populations' proportions are equal (H0: $p\,1 = p2$). The alternative hypothesis may be either (H1: $p1 \neq p2$). Fisher's exact test was also used as it is accurate for all sample sizes, when the number of trials minus the number of events is <5 (Zar, 2010).

General considerations

During the actions for the control of dengue undertaken in the municipality of Santa Bárbara d'Oeste since the first cases reported in 1995, all the suburbs have received the same kind of treatment involving the actions for the control of the vector described below. In this present study, the only difference occurred in the nebulization, which was undertaken with heavy-equipment coupled to a vehicle in the Jardim Europa suburb.

Beyond the use of the number of eggs found on the pallets as an ecological indicator, the number of confirmed cases of dengue, which occurred in the two areas during two distinct periods was taken into consideration. In the Jardim Europa suburb, after the first two applications with heavy-equipment (on 19 and 26 March) the ovitraps, which remained at the sites from 27 March to 1 April, were installed. Similarly, after the two following applications (on 2 and 9 April), once again the traps, which remained at their sites between 10 and 16 April, were installed. For the Cidade Nova suburb, the traps remained at the sites chosen for the same period of time as those described above.

The cases of dengue were counted during two periods, those which were confirmed, the date of whose first symptoms occurred between 1 January and 31 March and those which presented their first symptoms between 1 and 30 April. The choice of these periods took into consideration that, although the entomological aspect indicated by the number of eggs collected in the ovitraps might suffer the rapid and direct influence of the applications made by the two kinds of nebulization equipment used in this study, the count of the number of dengue cases should be made after the consideration of the period of the latency of the virus in the contaminated organism. Therefore, as the first application with heavy-equipment occurred on 19 March, its final results, in terms of the impact on the number of confirmed cases of dengue, should only be considered as from 1 April.

Results

During the project, 1355 eggs were removed from the traps, 534 of them being found in the intradomiciliary environment and 821 in the peridomiciliary. In the Cidade Nova suburb, 1105 eggs were found and in the Jardim Europa suburb 250 eggs (table 1). The peridomiciliary collections presented a greater number of eggs caught in the ovitraps, as the number of positive traps installed in the peridomiciles was also greater than that of the traps installed inside the residences.

Overall, the first collection yielded 688 eggs, 84.9% of this total coming from the Cidade Nova suburb, and 15.1% from Jardim Europa. In the second collection, the numbers found were not so different from those of the first, being 78.1 and 21.9%, coming, respectively, from the suburbs of Cidade Nova and Jardim Europa.

The first collection of eggs, for the Jardim Europa suburb, occurred after two applications of insecticide using the heavy nebulization equipment. On this first occasion, the positive traps were 33% of the total, 10% being the value found for the traps in the intradomicile and 23% that for those installed in the peridomicile. At the second collection undertaken in this same suburb, the positive traps were13.3% of the total, being 6.6% for both the types of distribution of the traps.

In the Cidade Nova suburb, in which the insecticide was applied with light equipment, the positive traps increased from 46.6 to 76.6% from the first to the second collection. A larger number of positive traps were found in the peridomicile in both collections, however, there was a considerable increase in the number of positive traps installed inside the properties – increasing from 13.3% on the first collection to 36.6% on the second.

Between the two collections undertaken, there was a growth in the proportion of eggs found in the intradomicile in the Cidade Nova suburb, from 37.7 to 48.8%, and in the peridomicile the proportions were 62.3% in the first collection and 51.2% in the second. For the Jardim Europa suburb, in the

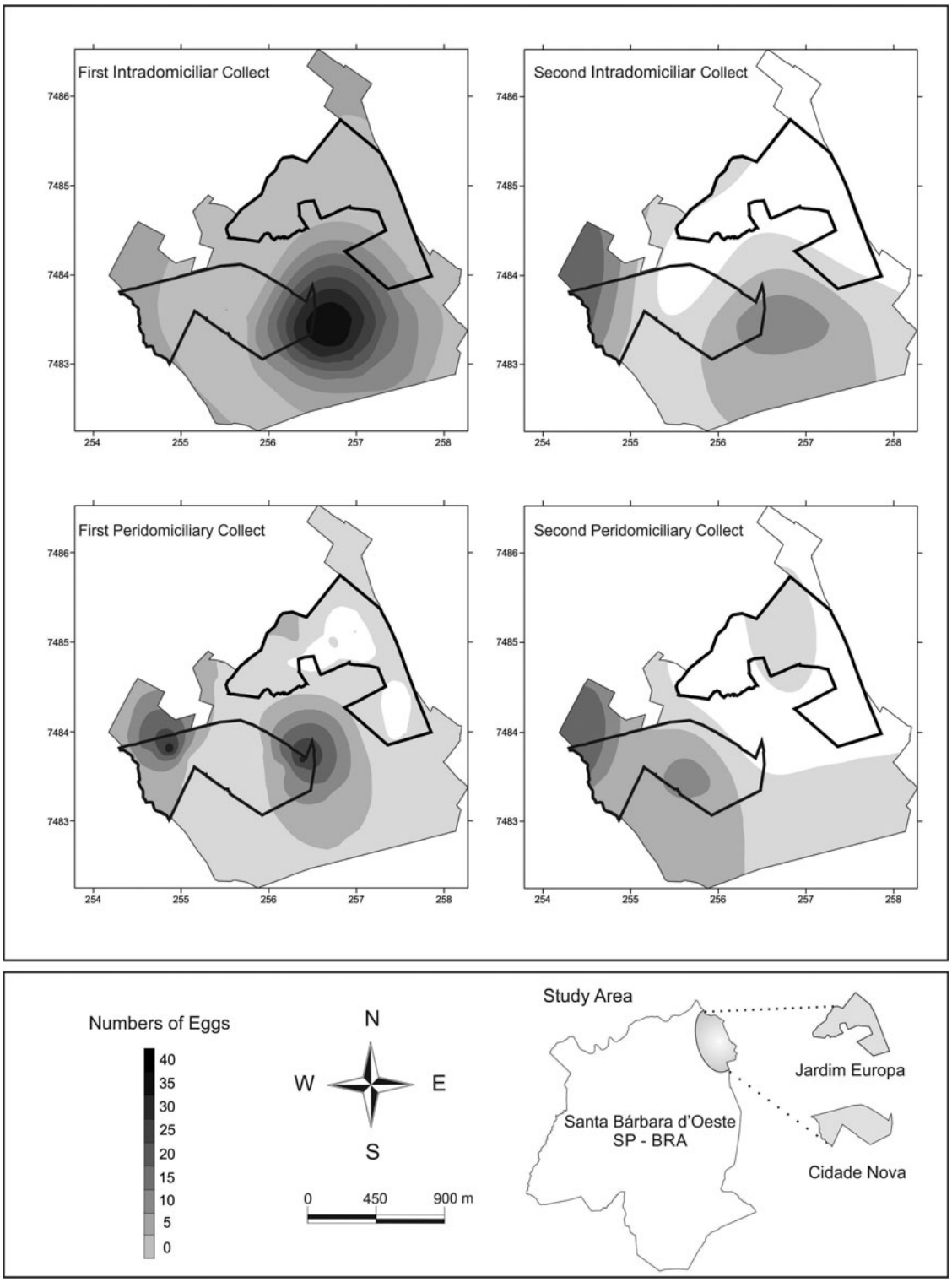

Fig. 3. Spatial distribution of eggs collected in the areas of the Cidade Nova and Jardim Europa suburbs.

intradomicile, the values were 11.5% of eggs found in the first collection and 32.9% in the second, and 88.5 and 67.1%, in the first and second captures, respectively, in the peridomicile.

The results of the spatial analysis (fig. 3) demonstrated that in the intradomiciliary collections the eastern region of the Cidade Nova suburb and the southern region of the Jardim

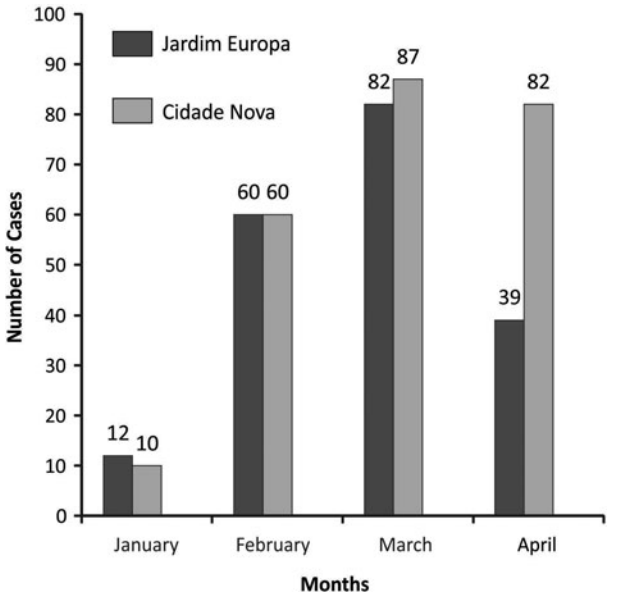

Fig. 4. Distribution of the cases of dengue from January to April in the suburbs studied.

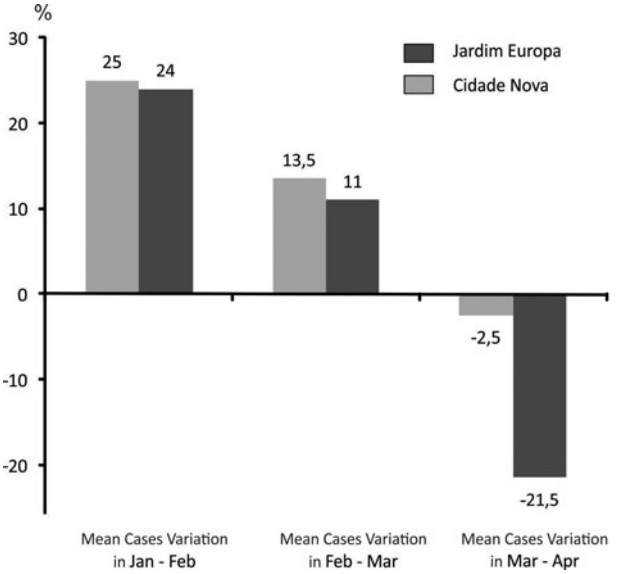

Fig. 5. Variation index of dengue cases, which occurred in the study areas between the months surveyed.

Europa suburb were the areas with the largest numbers of eggs during the first capture. In the second sampling period, the east and the west of the Cidade Nova suburb presented the higher densities of eggs, and in Jardim Europa the greatest quantity of eggs occurred in the southern region of the suburb.

In the collections made in the peridomicile, once again the western and eastern regions of the Cidade Nova suburb were those which presented the greatest infestation of eggs during the first survey. In the second collection the south-center and the west of the suburb were the most infested. For Jardim Europa, as also for the intradomiciliary collections, the two egg capturing events presented small variations with a reduced difference in the infestation as between the regions of the suburb.

The cases of dengue were surveyed in both the study areas. The notifications of the Epidemiological Surveillance and the geo-referenced data of the Center for the Control of Zoonoses were used for this, the date of the appearance of the first symptoms being noted for the temporal definition of the cases. Between the months of January and April, the two suburbs together presented a total of 432 cases of the disease, 193 of them in Jardim Europa and 239 in Cidade Nova. Fig. 4 shows the increase in the number of cases of dengue over the first 4 months of the year in the two places studied. The rate of variation found with regard to the cases occurring in March and April, for the Jardim Europa suburb, was greater than that observed in the Cidade Nova suburb (fig. 5).

The number of cases of dengue which occurred in the two suburbs studied, presented closely similar proportions during the first 3 months of the year, demonstrating an epidemiological similarity between the areas. In the month of April, a greater proportional difference was observed in the number of cases reported for the suburbs, the high number of positive notifications being maintained for the Cidade Nova suburb. In terms of participation, as for the total number of cases recorded, the month of April presented 34.3% for the Cidade Nova suburb, as compared with 20.2% for Jardim Europa. Fisher's test rejected the null hypothesis for the distribution of the cases of dengue between the two areas studied, presenting a value of F ($F = 0.9$) above the critical value ($F = 0.1$).

Discussion

The comparison of the results presented in table 1, for example, in the Cidade Nova suburb, demonstrated different behavior regarding the number of eggs found in the intradomicile and the peridomicile. In this suburb, a reduction of 11.1% was observed in the proportion of the eggs found in the peridomicile between the two collections. In the first collection, the percentage of eggs in the peridomicile, in relation to the total collected in the suburb, was 62.3%, while in the second, this proportion was reduced to 51.2%. In the eggs found in the peridomicile the proportional values underwent an increase of 11.1%, increasing from 220 (37.7%) eggs found in the first collection to 254 (48.8%) collected in the second. In the Jardim Europa suburb as between the two collections, a reduction was observed in the proportion of eggs collected in the peridomicile, falling from 88.5% ($N = 92$) of the total collected to 67.1% ($N = 98$), while the proportion of eggs found in the intradomicile rose from 11.5% ($N = 12$) to 32.9% ($N = 48$). In any case, for the Jardim Europa suburb, the values in terms of number of eggs found in the traps, are lower than those observed in the Cidade Nova suburb. The formula applied for the calculation of the rate of variation showed that in Jardim Europa, the reductions related to the number of cases in March and April, as also regarding the number of positive traps from one collection to the other, was smaller than that observed in Cidade Nova.

The analysis of the total number of eggs collected in the two areas confirms that the nebulization actions with heavy-equipment kept the values of the indices of infestation of the area that received the treatment with heavy-equipment lower. Andrighetti *et al.* (2013) compared the actions of nebulization with the light and heavy-equipment and their results

demonstrated the greater efficacy of the portable equipment, with a reduction in the number of adult and female mosquitoes in the areas studied. In that study, no count was made of the number of eggs found, as in this present study no research was undertaken into adult individuals, the results should, therefore be considered distinct. One point which should be made is that the application with heavy-equipment promotes the dispersion of the insecticide which, both under the effect of gravity and carried by the wind, enters the dwellings and thus affects the mosquitoes present in that environment. In view of the fact that during the period in which the insecticide was being applied with heavy-equipment a large number of the properties had their windows and doors open, this would seem to be an important factor that influenced the numbers of eggs found.

When the numbers of positive traps are analyzed, the influence and the difference between the methods of control applied may be demonstrated. While in the Cidade Nova suburb an increase in the number of positive traps from 14 to 23 was observed (the positive indices were 46.6 and 76.6%, respectively, of the total traps installed), in the Jardim Europa suburb the number of positive traps diminished from ten to four (33 and 13%, respectively). This suggests that the method of nebulization with heavy-equipment, despite its limitations, may be utilized as a strategy to avoid the dispersion of the females of the dengue vector in situations in which the control of the breeding places has not attained the necessary efficiency (Focks *et al*., 2000).

The analysis of the efficacy of the conventional method of nebulization used for the control of *Ae. aegypti* in urban areas presupposes the discussion of those situations in which refusals, or the non-application of the insecticide in residences with the use of portable equipment, may – for any one of several reasons – occur. This circumstance may become a determining factor in the process of the control and dispersion of this vector.

The control of breeding places also faces this same problem, that is to say, in the present Brazilian economic and social scene, some families use recyclable materials as a source of income, while others, aware of the need for sustainable action, seek to transport the waste correctly, stocking the recyclable material and sending it on to appropriate destinations. Both, however, as a general rule, store the materials in unsuitable ways thus creating the conditions favorable to the development of immature mosquitoes, thus frustrating the efforts made to control the vector by means of conventional methods. Focks *et al*. (2000) present as a condition for the avoidance of the dispersion of females within a particular area that almost the totality of existing breeding places be eliminated.

Following this same line of thought, it must be remembered that, on average, 35–45% of residences are left uninspected because they are closed or because the resident refuses admittance, and that an approximately similar percentage does not receive the control agent to apply nebulization with the portable equipment. This circumstance makes the use of the method employing heavy-equipment important, as it does not depend on the authorization of the resident.

Andrighetti *et al*. (2013) presented data on the mortality of female adults in the intradomicile, greatly superior when the portable rather than the heavy-equipment is used. In this present study, however, the absolute number of eggs collected in the intradomicile in the two areas studied is discrepant, 474 of them being in the Cidade Nova suburb as against 60 in the Jardim Europa suburb. These values, taken together with the reduction in the number of positive traps, suggest that nebulization with the heavy-equipment keeps intradomiciliary levels of *Ae. aegypti* down. The movement of females at the end of the afternoon in their search for places for development and feeding may be what determines the efficiency of the method studied here (Forattini, 2002).

Nebulization with heavy-equipment may thus be seen as an important strategy in the activities undertaken for the control of *Ae. aegypti*. The study presented consistent numbers for the reduction of infestation by the vector and, still more important, the area which received the treatment with heavy-equipment presented numbers of confirmed cases of dengue significantly smaller than those of the suburb, which received the conventional treatment. Santa Bárbara d'Oeste, as also various other municipalities, has been presenting cases of dengue uninterruptedly since 1995 (Lima *et al*., 1999). The areas studied present very similar epidemiological and entomological histories (Piovezan *et al*., 2014), which still further supports the interpretation of the results obtained by this study.

The present situation of the control of *Ae. aegypti*, in Brazil, calls for changes in the behavior and commitment of civil society and of the authorities. As a rule, occasional reductions in the number of cases are associated with dubious biases from the point of view of the epidemiology of the disease. When we consider the new diseases which are spreading in society such as, for example, chikungunya and Zika, as well as dengue, the urgency for us to rethink more effective methods of control is evident. The application of insecticide with heavy-equipment has shown itself to be efficient in keeping down the numbers of eggs found as also in obtaining better results as regards the number of patients suffering from the disease after their treatment.

Despite the two methods tested not having been used in one and the same area, the two suburbs are very close to each other and, as has already been stated, they have had the same history of treatment over the years in which the transmission of dengue has been occurring in the city, the demographic and socio-economic conditions are similar and, during the execution of this study, they underwent the same procedures related to the control of the vector, except for the nebulization, which was undertaken with different equipment. It is important to state these definitions as they do not affect possible occasional biases related to the results found. However, within the range of possibilities, the methodology employed sought to minimize these problems and, clearly, the number of cases observed in the month of April and the number of eggs collected on the pallets, as also the number of positive traps, make it clear that the nebulization with heavy-equipment exerted an influence on the indicators analyzed. Apart from the above observations, Fisher's test was used in the distribution of the cases of dengue between the two areas, thus reinforcing the hypothesis that the variation in the number of cases of dengue observed in the two suburbs studied is related to the methods of application of the insecticide used.

The results presented here show that this is a method that can be put into effect with greater frequency in the State of São Paulo, being used also prior to the control of breeding places to avoid the dispersal of adult mosquitoes to adjacent areas. It is also deserving of note that, despite the cost of nebulization with heavy-equipment being greater than that of portable nebulizers, the area treated per unit of time is much greater, and the costs with salaries and labor law expenses of the teams are thus greatly reduced.

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References

Andrade, C.F.S. & Cabrini, I. (2010) Estratégias inócuas, estrambólicas ou inseguras para o controle do vetor da dengue. *Revistas Vetores & Pragas* **27**, 22–27.

Andrighetti, M.T.M., Marcoris, M.L.G., Takaku, L., Galvani, K. C., Cardoso, R.P., Scandar, S.S., Glasser, C.M., Wanderley, D.M.V. & Yang, H.M. (2013) Avaliação do efeito do inseticida Malathion aplicado sob a forma de ultra e baixo volume com equipamentos portátil e pesado sobre *Aedes aegypti* (Diptera: Culicidae). *Rev Pat Tropical* **42**, 81–95.

Andriotti, J.L.S. (2004) *Fundamentos de Estatística e Geoestatística*. Editora Unisinos. São Leopoldo, Editora do Vale do rio dos Sinos (UNISINOS), 165 p.

Brasil (2001) Ministério da Saúde. Fundação Nacional de Saúde. Dengue, instruções para o pessoal de combate ao vetor: Manual de Normas Técnicas 3ª Edição. Ministério da Saúde.

Brasil (2002) Ministério da Saúde. Fundação Nacional de Saúde. Programa Nacional de Controle da Dengue. Ministério da Saúde.

Brasil (2015*a*) Ministério da Saúde. Available online at http://www.brasil.gov.br/saude/2015/04/orcamento-2015-para-acoes-de-combate-a-dengue-cresce-37 (accessed 28 December 2015).

Brasil (2015*b*) Ministério da Saúde. Available online at http://portalsaude.saude.gov.br/images/pdf/2015/dezembro/15/COESMicrocefaliasInformeEpidemiologico SE4915dez201510h.pdf (accessed 28 December 2015).

Brasil (2016) Ministério da Saúde. Available online at http://portalsaude.saude.gov.br/index.php/cidadao/principal/agencia-saude/21459-saude-divulga-dados-atualizados-de-microcefalia (accessed 08 February 2016).

Câmara, F.P., Theophilo, R.L.G., Santos, G.T., Pereira, S.R.F.G., Câmara, D.C. & Matos, R.R.C. (2007) Estudo retrospectivo (histórico) da dengue no Brasil: características regionais e dinâmicas. *RevSocBrasMedTrop* **40**, 192–196.

CDC – Centers for Diseases Control and Prevention (2016) Concludes Zika Causes Microcephaly and other birth defects. Available online at www.cdc.gov/media/releases/2016/s0413-zika-microcephaly.html (accessed: 06 June 2016).

CEPAGRI (2014) Centro de Pesquisas Meteorológicas e Climáticas Aplicadas à Agricultura. Clima dos Municípios Paulistas. Available online at: http://www.cpa.unicamp.br/index.html (accessed 17 April 2014).

Focks, D.A., Brenner, R.J., Hayes, J. & Daniels, E. (2000) Transmission thresholds for Dengue in terms of *Aedes aegypti* pupae per person with discussion of their utility in source reduction efforts. *American Journal of Tropical Medicine and Hygiene* **62**, 11–18.

Forattini, O.P. (2002) *Culicidologia médica: identificação, biologia, epidemiologia*. Vol. **2**. São Paulo, Editora da Universidade de São Paulo, 860 p.

Golden Software INC (1995) Surfer for Windows v. 6: user's guide. Colorado, Golden Software, 340 p.

Gringarten, E. & Deutsch, C.V. (2001) Variogram interpretation and modeling. *Mathematical Geology* **33**, 507–534.

IBGE (2013) Instituto Brasileiro de Geografia e Estatística. Censo Demográfico. Available online at http://cidades.ibge.gov.br/xtras/home.php (accessed 14 April 2014).

Lima, V.L.C., Figueiredo, L.T.M., Correa, F.H.R., Leite, O.F., Rangel, O., Vido, A.A., Oliveira, S.S., Owa, M.A. & Carlucci, R.H. (1999) Dengue: Inquérito Sorológico pós-epidêmico em zona urbana do Estado de São Paulo (Brasil). *Rev Saúde Pública* **33**, 566–74.

Marcondes, C.B. & Ximenes, M.F.F.M. (2015) Zika virus in Brazil and the danger of infestation by Aedes (Stegomyia) mosquitoes. *Rev Soc Bras Med Tropical* **49**, 4–10.

Mlakar, J., Korva, M., Tul, N., Popovic, M., Poljsak-Prijatelj, M., Mraz, J., Kolenc, M., Rus, R.K., Vipotnik, T.V., Vodusek, V. F., Vizjak, A., Pizem, J., Petrovec, M. & Zupanc, T.A. (2016). Zika virus associated with microcephaly. *New England Journal of Medicine* **374**, 951–958.

PAHO-WHO (2016) Pan American Health – World Health Organization. Dengue, PAHO/WHO data, Maps and Statistics. Available online at: http://www.paho.org/hq/index.php?option=com_topics&view=readall&cid=3273&Itemid=40734&lang=pt (accessed 08 February 2016).

Pannatier, Y. (1996) Variowin – Software for Spatial data Analysis in 2D. New York, Spring-Verlag.

Pfeiffer, D.U., Robinson, T.P., Stevenson, M., Stevens, K.B., Rogers, D.J. & Clements, A.C.A. (2008) *Spatial Analysis in Epidemiology (Eds)*. Oxford, Oxford University Press, 142 p.

Piovezan, R., Azevedo, T.S. & Von Zuben, C.J. (2012) Spatial evaluation of larvae of Culicidae (Diptera) from different breeding sites: application of a geospatial method and implications for vector control. *RevBra Entomologia* **56**, 368–376.

Piovezan, R., Azevedo, T.S., Acorinthe, J.P.O., Polizelli, N., Visockas, A., Canteiro, C.L. & Von Zuben, C.J. (2014) Perfil epidemiológico e análise espacial do risco da dengue da área urbana de Santa Bárbara d'Oeste – SP, durante o período de 1995 a 2010. *Geografia* **39**, 525–539.

Rasmussen, S.A., Jamieson, D.J., Honein, M.A. & Petersen, L.R. (2016). Zika virus and birth defects – Reviewing the Evidence for causality. *New England Journal of Medicine* **374**, 1981–1987.

Strahler, A.N. & Strahler, A.H. (1989) Geografia Física, Editora Ômega, 551 p.

SUCEN. (2001) Superintendência de Controle de Endemias. Vigilância e controle do *Aedes aegypti*: Normas, orientações e recomendações técnicas. Plano de Intensificação das ações de controle de dengue no Estado de São Paulo. Secretaria de Estado da Saúde de São Paulo Governo do Estado de São Paulo.

SUCEN. (2010) Superintendência de Controle de Endemias. Coordenadoria de Controle de Doenças Programa de Vigilância e Controle da Dengue. Secretaria de Estado da Saúde de São Paulo Governo do Estado de São Paulo. Available online at http://www.cve.saude.sp.gov.br/htm/zoo/pdf/Programa10_Estadual_dengue.pdf (accessed 30 December 2015).

Zar, J.H. (2010) *Biostatistical Analysis*. 5th edn. New Jersey, Pearson.