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Short Communication

Spatial distribution of *Aedes aegypti* (Diptera: Culicidae) in vulnerable areas for the transmission of arboviruses

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Abstract

Introduction: Areas at risk of transmission of arboviruses have been monitored using ovitraps. This study aimed to evaluate the spatial distribution of *Aedes aegypti* in vulnerable areas for the transmission of arboviruses and assess the influence of climatic conditions on the infestation of these culicids. **Methods:** Ovitraps were installed in Agrestina, Pernambuco, Northeastern Brazil. **Results:** Overall, 44,936 eggs were collected, and the indexes of infestation varied. Relative humidity was significantly associated with the infestations. **Conclusions:** Using ovitraps, entomologic indexes and analysis of climatic factors might be good strategies for monitoring vulnerable areas for the transmission of arboviruses.

Keywords: Culicids. Ovitrap. Dengue. Chikungunya. Zika.

Culicids are considered important vectors of many pathogens of medical and veterinary concern[1]. Among these Diptera, the species *Aedes aegypti* and *Aedes albopictus* can transmit many arboviruses responsible for diseases such as Dengue, Chikungunya, Zika, and Yellow Fever[2,3,4]. It is known that Northeastern Brazil is endemic for many of these arboviruses, and recently several cases have been reported. For example, in 2017 the Brazilian Health Ministry (HM) registered an incidence mean rate of 151.3 cases per 100,000 inhabitants for Dengue, 249.6 for Chikungunya and 9.3 for Zika in the Northeastern region. Conversely, in the same period, the state of Pernambuco reported incidence rates of 95.5 cases per 100,000 inhabitants for Dengue, 20.8 for Chikungunya and 0.4 for Zika[4].

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The increase in the number of cases of arboviruses is related to the vector population. In fact, it is well known that socioeconomic factors such as uncoordinated urbanization, lack of basic sanitation, irregular distribution of water, incorrect management of garbage, and climatic conditions have favored the reproduction and dispersion of these vectors[5].

In Brazil, the HM has proposed many strategies for monitoring the distribution of *Ae. aegypti*. For instance, the Building Infestation Index (BII) that identifies the larval amount per visited domicile and the Breteau Index (BI) that identifies the main types of breeding sites in the visited domiciles help determine the situation of a specific area. Furthermore, other methods of entomological research based on ovitraps have been used to determine the dispersion, density, and seasonality of *Ae. aegypti*[6].

Currently, georeferencing techniques have been widely employed worldwide to predict the dispersion of vectors[7]. The georeference information system (GIS) and spatial analysis provide important data for understanding the occurrence of diseases and for the developing measures of prevention[6,7].

In recent years, an increase in the number of cases of arboviruses has been observed throughout Brazil. Particularly, in the municipality of Agrestina, located in the state of Pernambuco, an increase of these viruses of about 90% was observed from 2014 to 2016[4]. Therefore, the aim of the present study was to evaluate the spatial distribution of *Ae. aegypti* in vulnerable areas for transmission of arbovirus, and to assess the influence of climatic conditions on the infestation of these culicids.

The study was conducted from May 2016 to April 2017 in the municipality of Agrestina (latitude 08º27'29" South and longitude 35º56'41" West), Pernambuco, Northeastern Brazil. The municipality has a population of 22,679 inhabitants and a territorial extension of 200,581 Km^2. The annual average temperature, relative humidity, and rainfall are of 24.3 ºC, 83.7% and 4.5 mm, respectively. The municipality has seven neighborhoods, from which samples were collected. Each neighborhood was considered an area of collection with five traps (points), totaling 35 traps for collection. These areas will be referred to in the text from now on as Area 1 (A1) to Area 7 (A7) (**Figure 1**).

Climatic data of temperature, relative humidity, and rainfall were obtained from the database by the Agronomic Institute of Pernambuco [*Instituto Agronômico de Pernambuco* (IPA)] and the Pernambuco Agency for Water and Climate [*Agência Pernambucana de Águas e Clima* (APAC)].

The ovitraps (black plastic vials containing 300 mL of water and a rough wooden palette (15 × 5 cm) partially submerged) were installed in the peridomicile, 5 m from the house, in a not covered place, at 1.00 to 1.50 m from the ground. All ovitraps were georeferenced through the satellite remote sensing global positioning system Garmin Etrex20. The areas of installation of the ovitraps were determined after the georeferencing of the whole extension of the municipality. In addition, the neighborhoods and buildings for installations were selected according to the larval indexes registered in the last 4 years available in the *Sistema de Informação do Programa Nacional de Controle da Dengue* (SISPNCD). Each ovitrap was inspected weekly and replaced every 15 days for a period of 12 months, corresponding to 6 cycles of entomological investigation according to the National Dengue Control Program [*Programa Nacional de Controle da Dengue*][8]. After collection, palettes were placed in plastic boxes (50 × 50 cm) and maintained at room temperature for 24 hours until drying.

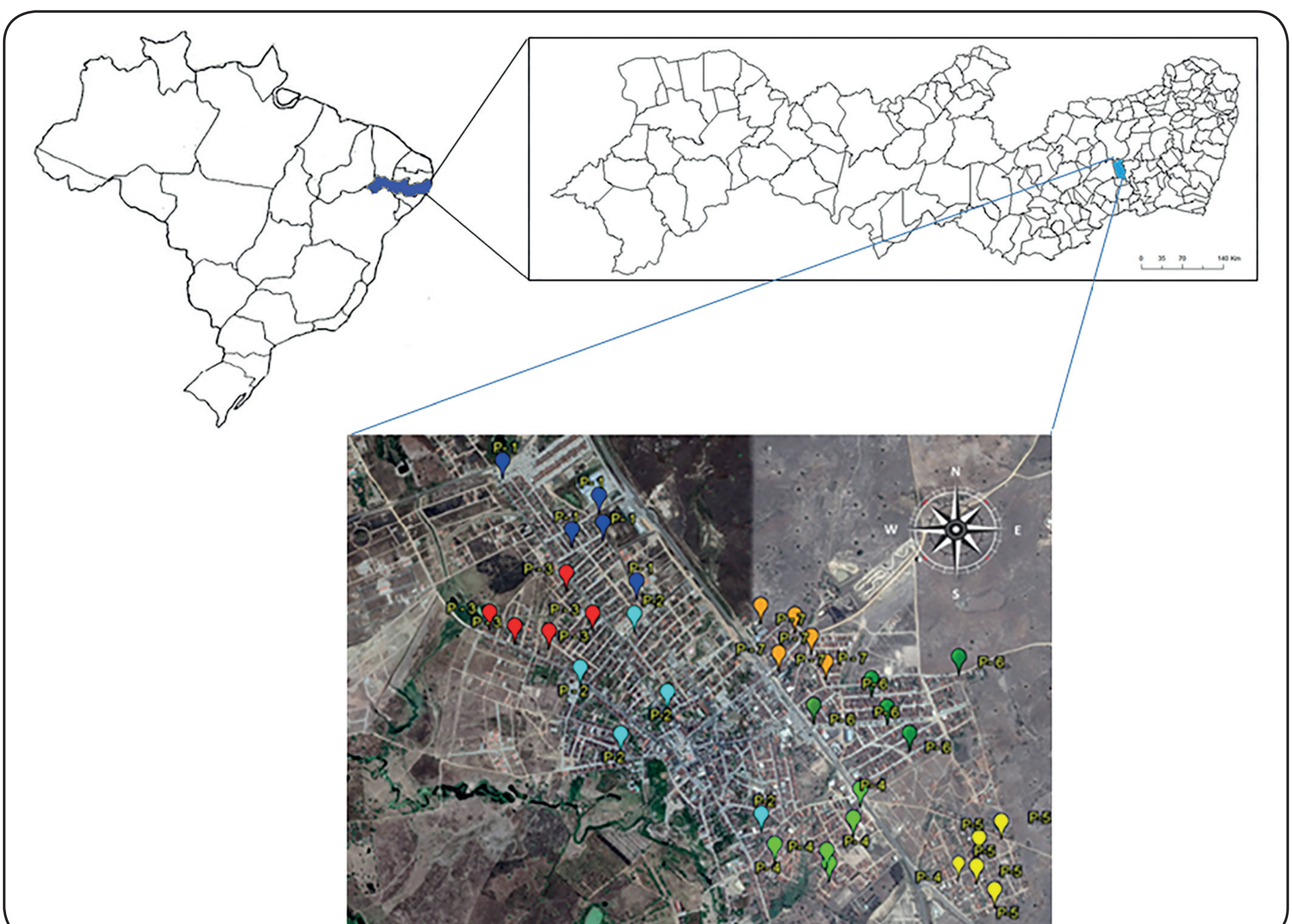

FIGURE 1: Spatial distribution of collection areas in Agrestina, Pernambuco, Brazil. Each area with five traps (points) represented by different colors and numbers.

Eggs were quantified, and their viability was evaluated using a stereomicroscope. Afterward, palettes were submerged in plastic vials (30 × 40 × 10 cm) containing tap water and maintained at 28°C until larvae hatching. Third-stage larvae (L3) were fixed in 70% alcohol and identified using dichotomy keys[9].

The entomological indexes were obtained through the Ovitraps Positivity Entomologic Index (OPI), Eggs Density Index (EDI), and Vector Density Index (VDI). All parameters described above were calculated based on the National Dengue Control Program [*Programa Nacional de Controle da Dengue*][8].

According to the values obtained for OPI, EDI and VDI, the areas were classified as: i) Risk zone: OPI ≥ 60%, EDI > 60 eggs and VDI >40 eggs; ii) Alert zone: OPI from 41 to 60%, EDI from 41 to 60 eggs and VDI from 21 to 40 eggs; and iii): Control zone: OPI ≤40%, EDI ≤ 40 eggs, and VDI ≤ 20 eggs[8].

Data were analyzed through descriptive statistics where absolute and relative frequencies were obtained. Then, the Lilliefors test was used to verify the normality of the data. In addition, the Chi-square test ($\chi 2$) with Yates correction was used to compare the number of eggs per cycle at different collection points. The number of eggs per cycle and climatic variables were assessed by the cross-correlation coefficient. The significance level was set at 5%. The analyses were performed using the statistical software BioEstat version 5.3[10]. The cross-correlation coefficient was calculated using the software R version 3.4.3[11].

The address of each collection point was manually geocoded using QGIS version 2.8. The kernel density estimation (KDE) is a smoothing and interpolating technique for generalizing point location for the whole study area. KDE is a simple alternative to analyze focal patterns, in which the outputs are easily readable and understood[12]. For the study, an adaptive bandwidth was used, and the adaptive kernel was chosen because the distribution of the domiciles in the study area was not homogeneous. The results were plotted using QGIS software.

A total of 44,936 culicid eggs were collected during the whole study period at all collection points and from all ovitraps. *Ae. aegypti* was the only species herein identified, and among all collected eggs, 55.44% (24,897/44,936) were considered viable.

No statistical difference was observed regarding the number of eggs obtained at each collection area ($p < 0.01$). It is important to note that a minimum of 4,056 eggs (at A3) and a maximum of 8,583 eggs (at A6) were observed.

A high number of eggs were obtained in May (third cycle) and August 2016 (fourth cycle), with 13,999 and 15,046 eggs, respectively ($p < 0.01$). Conversely, in November 2016 (sixth cycle) and February 2017 (first cycle), a low number of eggs were detected, with 2,429 and 2,685 eggs, respectively ($p < 0.01$) (**Figure 2**).

Interestingly, from May (87.7%) to August 2016 (89.7%), high levels of relative air humidity (RH) were detected. This parameter strongly influenced the number of eggs during the study (cross-correlation = 0.99). Conversely, a weak correlation was observed between the oviposition and rainfall (cross-correlation = 0.44). It is important to highlight that a negative and strong correlation (cross-correlation = - 0.90) was observed between the temperature and oviposition during the period from July to August 2016 (22.6°C; fourth cycle) (**Figure 2**).

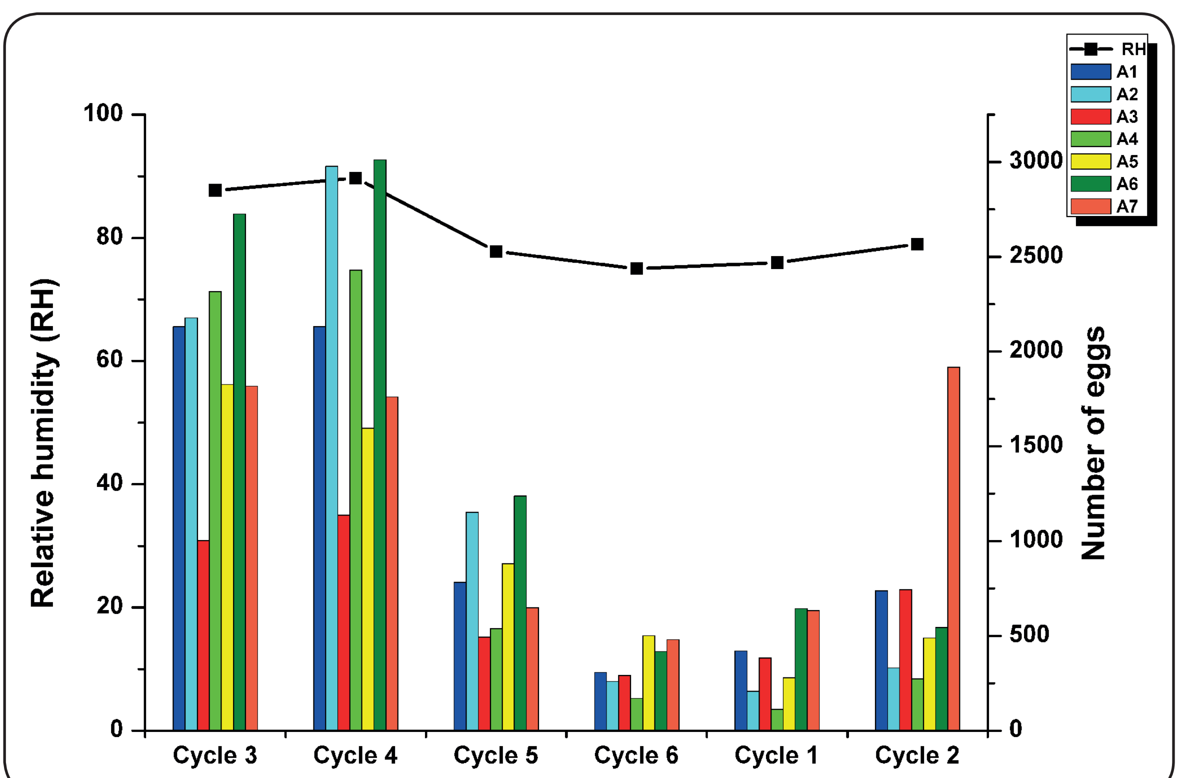

FIGURE 2: Number of eggs collected per cycle and relative humidity observed in Agrestina, Pernambuco, Brazil.

The entomological indexes (OPI, EDI, and VDI) demonstrated a spatial and temporal distribution of infestation by *Ae. aegypti*. In all cycles of the study, the classification of collection areas varied from control to risk zones. According to the OPI, all areas were classified from alert zone to risk zone, except A7 that remained a risk zone during the whole study period. In the sixth cycle, a reduction of oviposition was observed. Coincidentally, during this cycle, A1, A2, A3, A4, and A6 were classified as alert zones. It is important to highlight that according to IDO, A1 and A5 were classified as control zones, and that according to IDV, A1 and A4 were classified as control zones in the sixth and seventh cycles, respectively.

The kernel map (**Figure 3**) showed a heterogeneous spatial distribution of *Ae. aegypti* in the municipality of Agrestina, with hotspots occurring from May to October 2016 (third to fifth cycles) and January to February 2017 (first cycle). During the study, a reduction in egg density, as well as the dispersion and high concentration of eggs at A7 during March and April 2017 (second cycle), was observed (**Figure 3**).

The study results highlight the presence of *Ae. aegypti* in the whole urban territory of the municipality of Agrestina, state of Pernambuco, Northeastern Brazil. The exclusivity of this species in the area may be related to its predilection for domestic and urban environments, which presents many similarities with the *Ae. albopictus* species regarding its adaptation to the domestic and urban environments. A study performed in the state of Rio de Janeiro evaluated the frequency of culicid species in different environments and observed that *Ae. aegypti* is the most frequent species observed in urban areas[13].

The predominance of eggs in A2, A4, and A6 in the third and fourth cycles of 2016 is most likely related to the high population density of this area, as well as to the lack of basic sanitation, which is a common problem in Brazilian urban areas. A study performed in the municipality of Caxias demonstrated that localities with increasing human population process, associated with the intense environmental impact owing to the destruction of vegetation and house construction, lead to the dispersion of pathogens transmitted by mosquitoes[14]. In addition, the presence of several culicid breeding sites due to the absence of regular water supply in these areas has contributed to the spreading of these vectors. Interestingly, at the third and fourth cycles of 2016, a higher oviposition was observed. This period overlaps a time in which high relative humidity was registered (**Figure 2**).

After comparing the number of eggs captured in the ovitraps according to the climatic factors registered during all cycles of the study, the results showed that RH is directly related to the

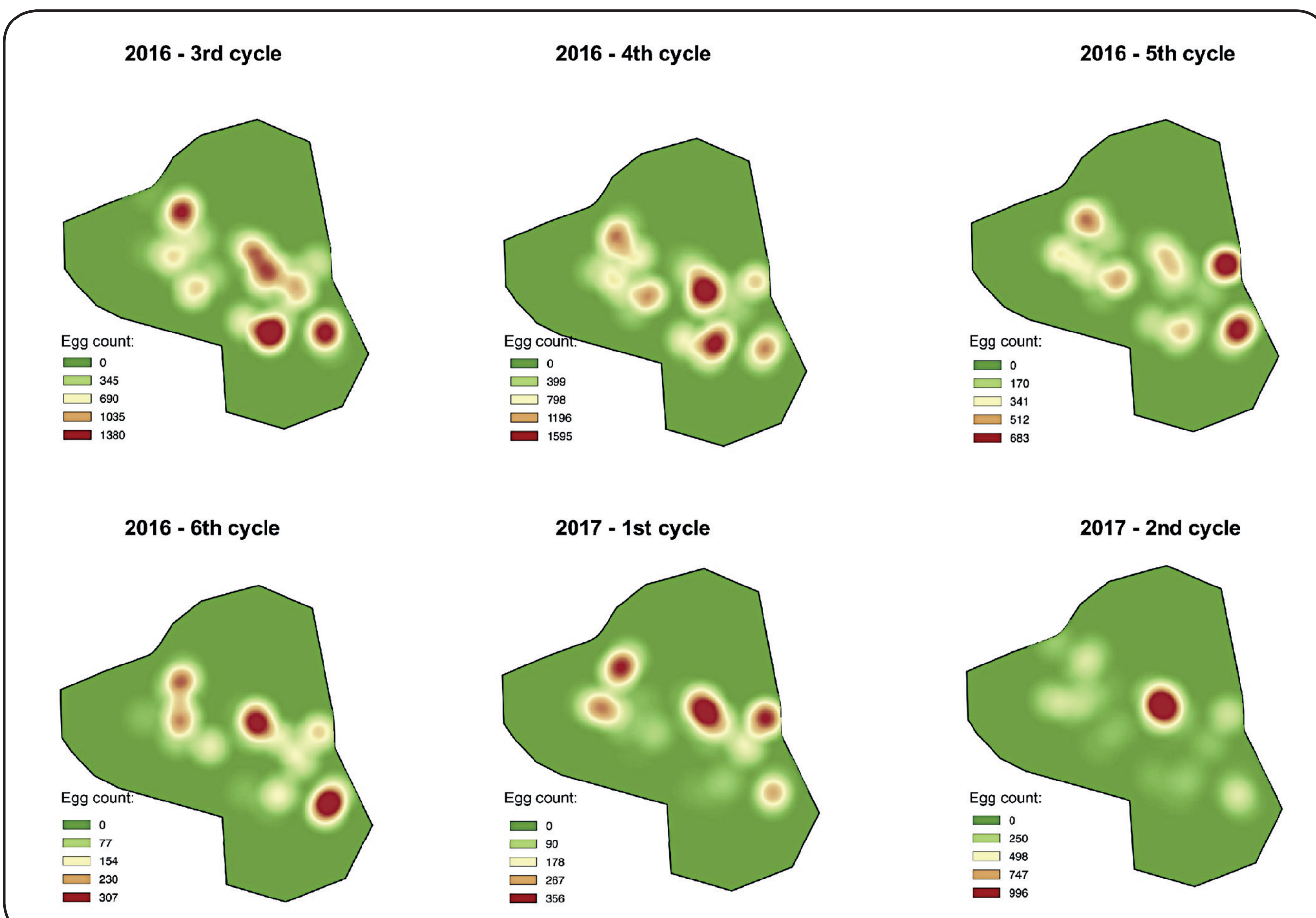

FIGURE 3: Kernel estimation map of eggs in ovitraps in the municipality of Agrestina, Pernambuco, Brazil.

increase in the number of eggs. This finding corroborates with a previous study conducted in Nepal, in which the authors reported that high relative humidity contributes to the hatching of these eggs. In addition, the same study found that temperature, relative humidity, and rainfall significantly affected the abundance of the *Ae. aegypti* population[15].

The entomologic indexes OPI, EDI, and VDI revealed that many areas of the municipality were at a risk. This finding has been very frequently observed in Brazilian urban areas with high population density, and the absence of basic sanitation and incorrect garbage disposal contribute to *Ae. aegypti* dispersion. The oviposition traps demonstrated sensitivity to capture culicid eggs and consequently to detect areas with a risk for transmission of arboviruses. The methods used in this study, ovitraps, entomologic indexes, the correlation of climatic factors and georeferencing show that it is necessary to implement and monitor these tools in association with better actions in health education to effectively control *Ae. aegypti*.

This study demonstrated that the municipality of Agrestina presents several areas in risk for transmission of arboviruses, and that climatic conditions influence the infestation of *Ae. aegypti* during wet months. Moreover, ovitraps associated with entomologic indexes, correlation of climatic factors and georeferencing tools should be used to assess vector dispersion in a given area, contributing to the development of preventive measures against culicid vectors.

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Conflict of interest

The authors declare that there is no conflict of interest.

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