Abstract

Introduction: Mosquito-borne diseases are currently the biggest public health problem globally. Dengue fever infection is one of the most important arboviral diseases in humans. *Aedes aegypti* is the most efficient vector for arboviruses because it is highly anthropophilic, frequently bites, and thrives in close proximity to humans. Stored water in the containers for long period, extended rainfall during the rainy season, and ambient relative humidity and temperature may favor the breeding of *Ae. aegypti* and other mosquitoes. Objectives: We assessed potential container breeding sites of *Ae. aegypti* in a peripheral area of a medium-sized municipality of the State of Maranhão, Brazil. Methods: In this study, *Ae. aegypti* breeding sites were investigated in the dry and rainy seasons, in 800 properties, located in peripheral area with poor urbanization and sanitation. Results: During the dry season 35 (4.37%) residences showed the presence of immature forms of *Ae. aegypti* and in the rainy season we have found 82 positive residences (10.25%). The group of containers with higher positivity to immature forms of *Ae. aegypti* was water storage containers, with values of 100% and 94.05% in the dry and rainy seasons, respectively. We found 3,529 immature forms in the dry season and 17,827 in the rainy. Conclusion: Storage containers are the main recipients found with *Ae. aegypti*, and the most important for immature forms, markedly during the rainy season, and contribute to the maintenance of this vector in high rates in this period, in addition to providing the right conditions for vector survival during the dry period, in this semi-arid region.

Keywords: *Aedes aegypti*. Arboviruses. Dengue.

Introduction

*Aedes aegypti* mosquitoes have great importance in public health. In Brazil, this species is responsible for the transmission of several arboviruses highlighting Dengue virus (DENV), Zika virus (ZIKV), Chikungunya virus (CHIKV) and Yellow Fever virus (YFV). From January to December 2016, the Ministry of Health reported 1,500,535 probable dengue cases across the country, with the confirmation of 844 cases of severe dengue and 8,237 cases of dengue with alarm signals. Coupled with the increasing reports of this arbovirus, the autochthonous transmission of the ZIKV occurs in the country since April 2015. Chikungunya fever has also concerned the public health authorities, because it was notified 3,748 indigenous suspected cases of the disease in 18 Federation units in the country, in the same period.

This scenario points to the need for massive studies about the interaction pathogens-vector, including those related to monitoring the movement of such viruses and the vector presence. In particular, the unique clinical and epidemiological characteristics of dengue, and in recent years Zika and Chikungunya in Brazil, have attracted the interest of institutions and researchers of public health. This happens because of the importance of identifying factors that determine the different forms of individual and collective expression of these infections to the improvement of their treatment and control. In terms of number of cases and...
impact on public health, represent the most important vector diseases in the world. It is considered that certain types of containers can assume different roles in production of adult Ae. aegypti. In different regions of the country, domestic containers have been incriminated as the main breeding sites for Ae. aegypti. Permanent containers for human consumption provide excellent conditions for the vector proliferation, thus contributing to the maintenance of populations of this mosquito, not even in good times, as in the months of low rainfall. Furthermore, Ae. albopictus, a dengue secondary vector, although not still being incriminated with this role in Brazil, has been increasingly found in intra regions and peridomicile of large Brazilian cities.

In the municipality of Caxias, State of Maranhão, 314 dengue cases were recorded in 2011, 340 in 2012, 39 in 2013, 80 in 2014 and 247 cases in 2015. However, it is known that there are many cases without notification and the municipality remains classified as high risk for transmission. In Caxias, the neighborhoods are increasing the population process and suffer intense environmental impact with vegetation destruction and housing construction. These factors contribute to the pathogens dispersion. The Western zone of the city, presented in recent years high incidence of dengue cases having large area of vegetation and the presence of several streams. Next to these places is observed the waste accumulation, providing that local perfect way to oviposition and consequently the proliferation of Ae. aegypti. It is therefore essential to conduct studies aimed at knowing the frequency, types and characteristics of artificial breeding and contribute in the production of immature in different seasons (dry and rainy) in this location.

Methods

Study area

The study was carried out in the urban zone of the municipality of Caxias, from 2007 to 2008. Caxias is located in the eastern part of the State of Maranhão (4°51'32"S; 43°21'2"W), and has a mean altitude of 67 m above sea level and an area of 5,196,771 Km². According to the Brazilian Institute of Geography and Statistics, the population of Caxias in 2010 totaled 155,129 inhabitants, including 118,534 in the urban zone and 36,595 in the rural zone, with a demographic density of 30.12 inhabitants/Km² and an annual growth rate of 1.06%. The climate is tropical, with a mean annual temperature of 28.4°C and a mean annual precipitation of 1,454.6 mm, with 2 distinct seasons: a rainy period from January-June and a drier one from July-December. The main temperature varies between 26.1 °C in the rainy season, to 35.6 °C during the dry season. The mean annual relative humidity is 70%, the maximum occurs in March and April (rainy season), with value of 83%, while in the dry season the value could reach 57%

In this study, Ae. aegypti breeding sites were investigated in the dry (August-October 2007) and rainy (February-April 2008) seasons in 4 neighborhoods of Caxias: Fazendinha, Salobro, Pirajá and Caldeirões, located in the Western zone of the city. This zone of the city was selected taking into account the fact that the Western zone had a higher incidence of dengue cases between the years 2000 and 2006.

The Western zone has an extensive vegetation area and the presence of several streams: Sulina, Sanharó, Fazendinha, Grota, Ponte and Salobro. Among the neighborhoods that have contributed in recent years to the high number of dengue cases in this area, we can highlight Salobro and Campo de Belém neighborhoods. These have a poor sanitation system, with open sewers, unpaved streets, and waste lands with waste accumulation. The 4 neighborhoods that we visited in this study are characterized by the existence of buildings located on a slope with vegetation. The homes are brick and clay tiled roof, but we can also find many homes of mud and covered with straw in these neighborhoods. In some places are observed streams near the houses. There are many streets without asphalt. In the houses is common the meeting of domestic animals, like chickens and dogs. The roads and the sewage network are very precarious.

Immature collection and containers research

The immature collection was conducted between August 2007 and April 2008, with the inspection of 800 properties, been 200 properties visited in each district. All properties were visited twice: once in the dry season and another in the rainy season. The method adopted to inspect the properties was made according to the National Dengue Control Program.

The house inspections were made in order to record potential Ae. aegypti breeding sites, i.e., containers, and to determine whether or not they were filled with water at the time of the visit and, thus, might be able to shelter the vector, in the intradomicile and peridomicle areas. We researched immature forms of the vector in different groups of containers: group 1 – disposable containers and bottles (bottles, cans, plastic, cans and cups), group 2 – tires (car or bicycle tires), group 3 – pieces and materials (materials used for realty construction and car parts), group 4 – water storage containers (vats, filters, pots, buckets, drums, water tanks and gas), group 5 – fixed containers (drains, grease trap and wells) and group 6 – other containers (other containers that do not fit in the other groups, for example peel fruits and containers that animals use to drink), according to previously established methodology. The breeding sites were classified by the water capacity in: containers with less than 1 liter, 1 liter to 50 liter, 51 liter to 100 liters, and above 100 liters.

The containers in which there was a meeting of larvae and pupae of Ae. aegypti, immature forms were collected with the aid of pipettes and or plastic screens. The samples were placed in test tubes containing 70% ethanol and were taken to the Medical Entomology Laboratory of the State University of Maranhão, where it was made the count and identification to the species level by using specific identification keys.

Statistical analysis

The analyses of positive containers were made through database construction with the daily values of
collections, using immature productivity data and the location (peridomicile and intradomicile) of the container in 2 seasons, dry and rainy. To test the normality we used the Kolmogorov-Smirnov normality test and to investigate the homogeneity of variance was used Levene test. Positivity properties (between dry and rainy season), containers situation (without or with water) and the quantity of immature forms (between dry and rainy season) were examined according to the Chi-square test ($\chi^2$), and quantity of immature forms (between intradomicile and peridomicile areas) and positive containers (between intradomicile and peridomicile areas) according to Tukey test (t) and Mann-Whitney test (U). The statistical analyzes were performed in software SAS (Cary, North Carolina) and BioStat 5.0 (Belém, Pará).

Results

Regarding the 800 houses surveyed, 35 (4.37%) showed the presence of immature forms of *Ae. aegypti* (positive properties) during the dry season. Salobro neighborhood had the highest number of positive residences ($n = 11$; 31.44%) and Fazendinha neighborhood the lowest ($n = 5$; 14.28%), followed by Salobro with 27 (32.92%) and Caldeirões, 9 (25.71%). In the rainy season we found 82 positive residences (10.25%), a higher value compared to the dry season. Fazendinha neighborhood had the most positive residences in the rainy season ($n = 28$; 34.16%), while Pirajá neighborhood had 14 (17.07%), followed by Salobro with 27 (32.92%) and Caldeirões with 13 (15.85%). Analyses of the positive properties between the seasons had significant difference ($\chi^2 = 20.37; df = 1; P < 0.0001$) (Table 1).

<table>
<thead>
<tr>
<th>Neighborhood</th>
<th>Dry season</th>
<th>Rainy season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Fazendinha</td>
<td>05</td>
<td>14.28</td>
</tr>
<tr>
<td>Salobro</td>
<td>11</td>
<td>31.44</td>
</tr>
<tr>
<td>Pirajá</td>
<td>10</td>
<td>28.57</td>
</tr>
<tr>
<td>Caldeirões</td>
<td>09</td>
<td>25.71</td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>100.00</td>
</tr>
</tbody>
</table>

$x^2 = 20.37; df = 1; P < 0.0001$

Positive properties were examined during the dry season. The research made in 4 neighborhoods of West zone of Caxias, in dry and rainy seasons, allowed the existence of tanks and barrels in almost all properties, with values of $P = 0.001$ and $P = 0.0001$ (Table 2). There was found in peridomicile and in intradomicile areas of the properties, 28 (80.00%) and 7 (20.00%) positive containers, respectively, in the dry season. Fazendinha neighborhood had only 2 positive containers in the intradomicile area, but had also the highest percentage of immature forms of *Ae. aegypti* (76.17%). In relation to the peridomicile area, Salobro neighborhood got 9 positive containers and 66.21% of immature forms of *Ae. aegypti* found in the 4 regions. In this season, the values of positive containers ($X^2 = 0.89; df = 1; P = 0.34$) did not have difference between peridomicile and intradomicile. However, the number of immature forms was significant in peridomicile area ($X^2 = 19.63; df = 1; P < 0.0001$) (Table 4).

<table>
<thead>
<tr>
<th>Neighborhood</th>
<th>Without water</th>
<th>With water</th>
<th>With IF</th>
<th>Immature forms in CWW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without IF</td>
<td>Immature forms in CWW</td>
<td>Without water</td>
<td>With water</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Fazendinha</td>
<td>352</td>
<td>44.74</td>
<td>309</td>
<td>28.62</td>
</tr>
<tr>
<td>Salobro</td>
<td>198</td>
<td>25.15</td>
<td>310</td>
<td>28.70</td>
</tr>
<tr>
<td>Pirajá</td>
<td>109</td>
<td>13.85</td>
<td>247</td>
<td>22.87</td>
</tr>
<tr>
<td>Caldeirões</td>
<td>128</td>
<td>16.26</td>
<td>214</td>
<td>19.81</td>
</tr>
<tr>
<td>Total</td>
<td>787</td>
<td>100.00</td>
<td>1,080</td>
<td>100.00</td>
</tr>
</tbody>
</table>

$x^2 = 38.27; df = 1; P < 0.0001$

$x^2 = 21.92; df = 1; P < 0.0001$
In relation to the water capacity of the positive containers, most of them had above 100 liters (42.87%) in the dry season and 11 to 50 liters (34.54%) in the rainy season. In these cited containers there was found an expressive number of immature forms of the vector, with 73.42% (dry season) and 38.53% (rainy season) of larvae and pupae, in containers with above 100 liters and with 11 to 50 liters, respectively (complementary data).

**Discussion**

The research made in 4 neighborhoods of Western zone of Caxias, in dry and rainy seasons, allowed the observation of properties with containers and immature forms of *Ae. aegypti*. The data showed a significant difference in positive containers in the rainy season and this occurrence was also reported by other authors. In this context we should consider the importance of the containers in residences in the maintenance of *Ae. aegypti*. This vector search for favorable places, where finds food and after uses containers to ovipositing. The home environment perfectly fits in the conditions necessary for mosquito survival.

All 4 districts had an increase in the number of positive households in the rainy season. Salobro neighborhood had the highest number of positive residences and Fazendinha neighborhood the lowest in the dry season. However, in the rainy season, Fazendinha was the neighborhood with the highest number of positive residences for *Ae. aegypti*. It is interesting to highlight that the greater number of containers with water was found in Fazendinha neighborhood, becoming fine breeding to mosquito oviposition. Seasonality has been indicated with one of the main factors that influence the *Ae. aegypti* productivity.

Our results confirm the considerable increase in productivity rates of *Ae. aegypti* immature forms in the rainy season. These results are similar to those observed in other Brazilian cities. In São Paulo city, there was a record of the highest proliferation of eggs and larvae of *Ae. aegypti* during the rainy season. A similar situation was reported, with a higher number of positive breeding in the rainy season in Brasília city. In Roraima city the dynamics of mosquito population was influenced by rainfall. A research in São Luis, State of Maranhão shows a trend for the development of mosquitoes during the temperature reduction periods, increase in relative humidity and precipitation. *Aedes* mosquitoes have a strong seasonal pattern associated with temperature and rainfall. The occurrence of heavy and continuous rain, interspersed with short periods without rainfall, is associated with the outbreak of mass eggs and an increase in the number of mosquitoes.

It was found that the most frequent breeding was the water storage containers (group 4), which had the highest percentage of immature forms, larvae and pupae, in both seasons. It should be noted that in the dry season that immature forms were found only in this group and the percentage of them was above 90% in the rainy season. During the home visits, it was observed the existence of tanks and barrels in almost all properties, which occur due to the constant lack of water in the public water supply system, which makes these deposits, preferred breeding of *Ae. aegypti* in the city.
Another highlight is the importance of this container type in vector maintenance in dry period. These deposits are permanently supplied with water by the population. Data from other surveys in Caxias reported that the storage group is the main container in 2 seasons\(^5\). Other works in different cities of Brazil, also show the importance of the storage group and its contribution to the spread of \textit{Ae. aegypti}\(^7\). In general, in northeastern Brazil, the driest region of the country, where lack of water is constant, the population custom observed is to store water to be used in daily activities, which facilitates the larvae production\(^1\). A different situation occurs in the southeastern region of the country, as seen in the State of Rio de Janeiro, where most breeding sites are thin and plastic packaging\(^26\). In other region of Brazil, there is an occurrence of breeding sites belonging to other groups, such as bottles\(^21\). More recently, studies have shown the presence increasingly frequent of \textit{Ae. aegypti} in rudimentary cesspits in the northern region of the country\(^13\).

The data from this study show that the containers predominated in peridomicile area, both in the dry and in the rainy season, in all sampled neighborhoods. It was verified that the number of immature forms in peridomicile differed significantly. A study in Manaus neighborhoods, State of Amazonas, showed similar results, with a predominance of containers, seen as potential breeding sites, outside the homes. The intradomicile containers are especially the vessels. This is because the population keeps potted plants for ornamental inside the homes, contributing to the proliferation of \textit{Ae. aegypti}\(^25\).

Regarding the water capacity of the containers, our research showed that there was a variation between the 2 seasons studied. In the rainy season containers with 11 to 50 liters were more productive while in the dry season the most productive were those containing 51 to 100 liters. This aspect may be related to the permanent presence of these containers, like tanks, with constant replenishment of water by the population. Emergency and frequent interruption in that supply water mobilizes the population affected to store water for domestic consumption, and in turn, the careless way in keeping the storage containers, mostly uncapped, may facilitate the provision of sites for proliferation of \textit{Ae. aegypti} and \textit{Ae. albopictus}, as in some States of the Northeast region of the country\(^13\). In São Paulo\(^5\), these containers considered permanent produced more larvae and pupae of \textit{Ae. albopictus}, further emphasizing the permanent breeding can play an important role in keeping mosquito populations because their volume is usually maintained by several factors.

This research, therefore, allows observing the rainy season positive influence on productivity of immature \textit{Ae. aegypti}. This study also showed the importance of storage group in the \textit{Ae. aegypti} reproduction in these areas and the need to implement measures to control made by the population, such as proper management of deposits to prevent the proliferation of immature forms of the vector.

Due to the limited time for the study, the small size of the work team, as well as the resistance of some residents to the entrance in their respective properties, it is suggested that a new approach be taken in the continuation of the study for the control of \textit{Ae. aegypti} and more recently \textit{Ae. albopictus} in the municipality of Caxias. It is important to continue this work in other areas of the city, to know the prevalent containers groups.

This type of study can help the health public organs to target control measures to \textit{Ae. aegypti} facing the containers, thus preventing the mosquito proliferation and consequently the epedemics occurrence. It is important to highlight the need to carry out health education activities in these communities, considering that adequate water supply for the whole population will not be realized until the reduced rainfall and increased consumption. It is crucial to adopt cleaning habits and proper handling of the permanent deposits, such as water-holding deposits in homes to reduce the density of vectors.

References


constant replenishment of water by the population. The permanent presence of these containers, like tanks, with water in the two seasons studied. In the rainy season containers peridomicile differed significantly. A study in Manaus showed the importance of the storage group and its containment type in vector maintenance in dry period. These findings suggest that storage of water to be used in daily activities, such as water-holding deposits in homes to reduce the risk of infestation by Aedes aegypti and Ae. albopictus for domestic consumption, and in turn, the careless way of disposing of waste can facilitate the proliferation of vectors. This type of study can help the health public health authorities to control made by the population, such as water-holding deposits to prevent the proliferation of immature forms of the vector. It is crucial to adopt cleaning habits and proper handling of the permanent deposits, to prevent the proliferation of immature forms of the vector and consequently the epidemics occurrence. It is important to highlight the need to carry out health education and prevention activities. The data from this study show that the container type in vector maintenance in dry period. These findings suggest that storage of water to be used in daily activities, such as water-holding deposits in homes to reduce the risk of infestation by Aedes aegypti and Ae. albopictus for domestic consumption, and in turn, the careless way of disposing of waste can facilitate the proliferation of vectors. This type of study can help the health public health authorities to control made by the population, such as water-holding deposits to prevent the proliferation of immature forms of the vector. It is crucial to adopt cleaning habits and proper handling of the permanent deposits, to prevent the proliferation of immature forms of the vector and consequently the epidemics occurrence. It is important to highlight the need to carry out health education and prevention activities.