Chapter

Characterization of the Proliferation Sites of *Aedes aegypti* (Diptera: Culicidae) in the Artificial Breeding Sites of Caxias, Maranhão, Brazil

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Abstract

The *Aedes aegypti* (Diptera: Culicidae) is dispersed throughout the Brazilian territory, being a transmitter of dengue and other arboviruses. This study analyzes the main breeding of *A. aegypti* in semi-arid tropical region. Samples have been collected for 12 months in the dry and rainy periods to watch the main breeding and characteristics of the proliferation sites. Most of the positive containers have been from the storage group (75.96%) found with immature forms mainly in the rainy season, with a predominance of containers having a height superior to 50 cm, protected from the sun and with organic matter. Theses breeding sites, where *A. aegypti* performs oviposition and survives in the dry season too.

Keywords: mosquitoes, oviposition, control, tropical climate, semi-arid, vector

1. Introduction

Aedes aegypti (Linnaeus) and *Aedes albopictus* (Skuse) are mosquitoes of the genus *Aedes*, belonging to the family Culicidae, which stand out as the two main species of vectors transmitting viral diseases, such as dengue, which has four serotypes (DENV 1–4), viruses Chikungunya, Zika virus, among other arboviruses [1–4].

Dengue is currently the most prevalent arboviruses in the world. According to the World Health Organization, about half of the world's population lives at risk of infection [5]. The *A. aegypti* is incriminated by the transmission of dengue virus and has been circulating in several continents such as Asia, Africa, the Americas and Europe [6].

In Brazil, *A. aegypti* is the most important vector of dengue virus. Since the twentieth century, campaigns to combat this vector have been intensified; however, they have not been successful. The *A. aegypti* continues to disperse in various parts of the world where it transmits several arboviruses. This fact can also be seen throughout the Brazilian territory [6, 7].

The *A. aegypti* is a diurnal, anthropophilic mosquito with development of holometable, passing through the egg phase, four larval stages (L1, L2, L3 and L4), pupa and adult forms. The increase in the availability of human-made containers associated with climatic factors such as temperature rise, and variations in rainfall and relative humidity favor the vector development [8–11].

Among the control methods, the most important are the chemicals, which have been used since the 1940s with the discovery of synthetic insecticides. Although several studies prove the resistance of mosquito populations to different classes of insecticides, they are still widely used for the control of *Aedes*. We also have the mechanical control that aims at the removal of breeding grounds, and the biological control that uses living organisms considered ecologically safe for the environment, as an attempt to reduce the population density of the vector [12, 13].

In order to combat *A. aegypti*, it is essential to regularly implement public water supply systems, basic sanitation and the adequate packaging of municipal solid waste, because a large part of the population is not aware of the problems generated by poor packaging of household waste and containers [14, 15].

The present work aims to characterize the breeding sites most frequented by *A. aegypti*, to analyze the physical conditions in which these breeding sites are found to contribute for the actions to combat vector proliferation. As a consequence, outcome of this work will promote improvement in health and the environment, avoiding the disposal of waste in inappropriate places and an adequate packaging of domestic containers.

2. Materials and methods

2.1 Characterization of the study area

The study has been conducted in the city of Caxias - MA, located between the coordinates 04°51′32 "south latitude and 43°21'22" longitude west. Its estimated population in 2018 is 164,224 inhabitants [16]. The climate is hot and humid with an average temperature of 28.2°C, having averages of the maximum of 27°C and averages of the minimum of 17°C, with two defined seasons, like rainy (January to June) and dry (July to December). The pluviometric index is between 1100 and 1500 mm/year, being the Itapecuru basin the main municipality, since it occupies 3567.40 km^2 (68.4%) of the territory, in which there is a diversity of biomes and vegetation formation inserted in an ecotone area [17].

The area has been chosen randomly, where five zones have been selected according to the listing of districts of Caxias - MA, with records of dengue cases in the last 5 years.

2.2 Immature collections

2.2.1 Sampling

The city of Caxias presents a total of 55,769 properties. The zones drawn have a total of 6973 real estate [18]. They have been inspected 80 properties by neighborhood, chosen of random form. This sampling has been defined according to the formula for the Calculation of the Minimum Sample Size, proposed by [19] tolerating the margin of error of 5%.

The surveyed neighborhoods are among the most populous, have poor sanitation conditions, the sewers are open, some streets without adequate pavement, there is a regular lack of water from the public supply. Most homes have large backyards with trees and the presence of domestic animals such as chickens, cats, dogs and even pigs. It is common to find accumulated garbage and water containers that serve as drinking fountains for the animals.

The collections have been carried out during a year with a survey of 400 residences in the rainy season with repetition of the same ones in the dry season. All artificial containers that showed potential for *A. aegypti* proliferation have been analyzed, following the methodology of the National Health Foundation with inspection of the internal and external residences [20, 21].

The vessels inspected have been classified into groups according to following [22–25].

TR1 – vase (plant pots, and pot dishes);
TR2 – bottle (bottles, cans, plastic);
TR3 – tire (all types of tire);
TR4 – building materials and car parts (materials used for construction and parts of cars);
TR5 – storage (tank, barrel, filter, pot, bucket, drum);
TR6 – fixed (drains, grease box and wells);
TR7 – others (all containers that do not fit the others).

The positive containers, which have been found in the presence of immature classified as to height (separated into three categories, 0–50, 51–100 and 100 cm above), exposure of breeding (total or partial exposure to the sun and/or shadow), and the presence or absence of organic matter.

In the containers with larvae/pupae all specimens have been collected. In the deposits where it is not possible the total withdrawal of the immature ones was made in a numerical calculation of the area multiplied by the volume of the breeding place that measured with the aid of millimeter vial and tape measure. This calculation is used by endemic agents to estimate the population density of large breeding sites [21].

The larvae and pupae have been captured using plastic pipettes, and collected and conditioned in 10 ml hemolysis tubes containing 70% alcohol. Each tube stipulated a maximum number of 20 specimens, and the flasks have been identified with label containing the location data and type of deposit where they collected.

Subsequently, they have been sent to the Laboratory of Medical Entomology (LABEM) to identify the specimens, and quantify and record the results in a bulletin for evaluation of larval density.

The identification has been made through the direct observation of the morphological characters evidenced to the stereoscopic and the light microscope using the dichotomous key proposed [8, 9].

The number of immatures collected in the five zones of the municipality of Caxias, Maranhão, has been evaluated for the normal distribution, using the Shapiro-Wilk test. As these do not fit to the normal distribution, corresponding non-parametric analyzes have been used.

The Kruskal-Wallis test (H) has been used to verify the differences between the number of immatures between zones, months and collection containers. The value of H calculated for each analysis has been compared to the value of H defined in the quantis table for Kruskal-Wallis test statistics. Whenever the calculated value is greater than that presented in the table at a certain degree of freedom, considered the number of groups compared, and where the value of p is less than or equal to 0.05, the hypothesis of medium males used, and continue with the Dunn test comparison of medians posteriori [26].

All analyzes have been performed using the Statistical Package for Social Sciences version 20.0 (SPSS Inc., Chicago, IL), the information compiled into database, mounted on Excel spreadsheets (2013) and Graph Pad Prism version 5.03 (La Jolla CA, USA), and from these obtained the percentages for the elaboration of tables and figures.

The values of p, significant at the mentioned level (5%), have been highlighted in the text, tables and graphs with an asterisk (*). For $p \leq 0.05$ an asterisk (*) has been used, for $p \leq 0.01$ two asterisks (**) have been used and for $p \leq 0.0001$ three asterisks (***) adopted (26).

3. Results

3.1 Collection cycles

When comparing the seven groups of containers surveyed, there is no significant difference between the number of containers found in the peri ($H = 2399$; $p = 0.6628$) and intradomicile ($H = 1275$; $p = 0.8656$) of residences. As well as there is no difference between the medians of vessels surveyed among the five zones throughout the study period ($H = 2026$; $p = 0.7309$).

The western zone has the highest percentage of containers in the storage group, with 637 (27.33%), of these 436 (68.44%) have been found in the peridomicile and 201 (31.55%) in intradomicile. It followed the Central zone with 530 (22.74%), North zone with 444 (19.05%) and South zone with 397 (17.03%) containers. The lowest percentage of inspected containers has been registered in the eastern zone with 322 (13.81%), of these 268 (83.23%), belongs to the peridomicile and 54 (16.77%) in the intradomicile.

When analyzing the presence of immatures in the positive containers of the seven groups, a difference is observed between the medians of *A. aegypti* in these groups ($H = 30.74$; $p < 0.0001$). According to the Dunn's test the storage group has predominant in relation to the vessels group ($p < 0.01$), construction material ($p < 0.01$), fixed ($p < 0.01$) and others ($p < 0.01$) (**Figure 1**).

The storage group has the largest number of containers surveyed in all study areas, with 2330 (55.17%) containers, of which 1653 (70.95%) have been found in the peridomicile and 677 (29.05%) in intradomiciliary. Followed by the bottle group with 1028 (24.34%), tires with 244 (5.77%) and others with 232 (5.49%). The lesser containers belong to the group pots 145 (3.43%), building material with 126 (2.98%) and fixed 118 (2.79%).

Positive containers exposed to the sun/shade showed a difference between the medians ($H = 12.15$, $p = 0.0023$). The containers have been positioned in the shade predominantly in relation to those in the shade ($p < 0.01$) – Dunn's test (**Figure 2**).

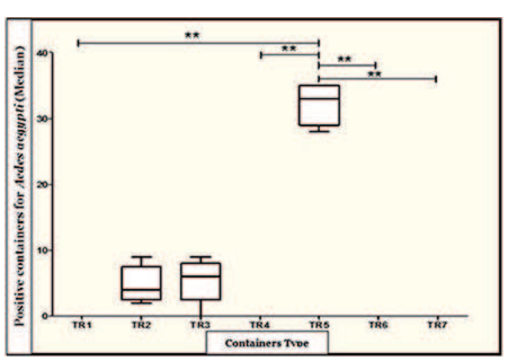

Figure 1.
Positive containers in the five collection zones in the municipality of Caxias-Maranhão, from January to December, 2016. Subtitle. TR1: Vessels, TR2: Bottles, TR3: Tires, TR4: Building Materials, TR5: Storage, Tr6: Fixed, TR7: Others.

When the exposure of the containers to the environment has been analyzed, it is observed in the rainy season predominance of immatures placed in containers in the sun with 1365 (75.74%) and shade with 437 (24.25%). In the dry season, the largest number of specimens is found in the shade with 602 (51.89%) and sun with 558 (48.10%). No immature is found in partially covered containers for the storage group (**Figure 3**).

When analyzing the positive containers with presence or absence of organic matter, a greater quantity of specimens in containers with organic substrates, have been registered in the rainy and dray seasons 1629 (90.55%) and 869 (74.91%), respectively. For containers without the presence of organic matter has been registered in the rainy season 170 (9.44%) immature and 291 (25.08%) in the dry season (**Figure 4**).

The containers with height between 51 and 100 cm recorded the highest number of *A. aegypti* in both rainy and dry seasons, with 1299 (72%) and 781 (67%), respectively. Following the containers with height between 0 and 50 cm, in the rainy season with 431 (23%) specimens and 238 (21%) in the dry season.

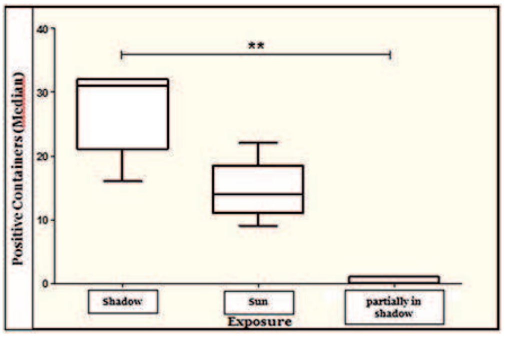

Figure 2.
Place of exposure of positive containers in the five collection zones from January to December 2016.

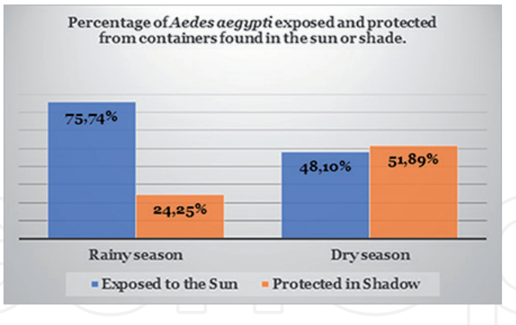

Figure 3.
Percentage of Aedes aegypti *found in containers exposed to the environment during the rainy and dry seasons, from January to December 2016.*

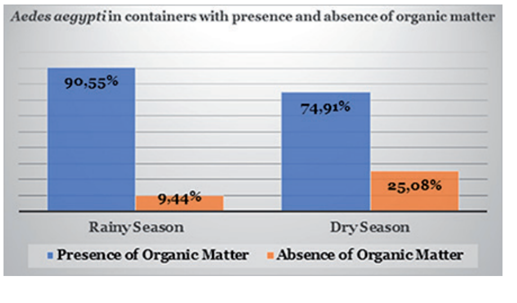

Figure 4.
Number of Aedes aegypti *found in containers with presence or absence of organic matter during the rainy and dry seasons, from January to December, 2016.*

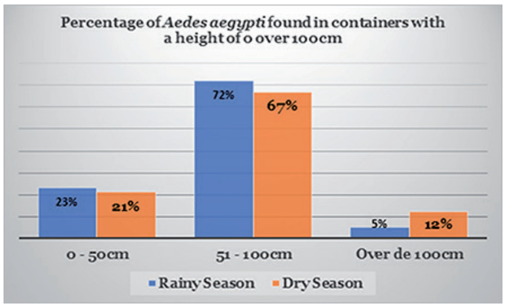

Figure 5.
Number of Aedes aegypti *distributed in containers with height between 0 and 100 cm, from January to December 2016.*

The containers above 100 cm registered 87 (5%) immature in the rainy season and 141 (12%) in the dry season (**Figure 5**).

4. Discussion

The data obtained in this research in relation to the main containers used by *A. aegypti* in urban area of tropical climate do not show statistical difference to the location in the peridomicile and the intradomicile of the residences, however, only the containers of the peridomicile have been found positive for immature forms, especially the storage group, which recorded the highest number of immature *A. aegypti*. Studies on the survey of potential *A. aegypti* breeding sites in the state of Ceará show that large containers such as water boxes hold *A. aegypti* larvae, and that containers found around the university such as cups, plastic bottles aluminum lunchboxes and small plastic containers contained *A. aegypti* eggs. In the present work we also found useless containers with foci of *A. aegypti* [27].

In the municipality of Caxias, Maranhão, other works have already been developed to characterize breeding sites most frequented by *A. aegypti* and *A. albopictus* [24, 25, 28] and also found preference for containers in the storage group, mainly tanks, barrels and basins, which are used by a large part of the local population to store water to be used in their daily activities, in view of the frequent lack of water in several districts of different municipal areas.

The routine of the residents to store water and other factors such as bad waste packaging and the local weather conditions, besides that dispersion of vector *A. aegypti* throughout the city, contribute to the occurrence of some diseases transmitted by *A. aegypti*, particularly Chikungunya and Zika, which is the target of an epidemic in this city during the accomplishment of this research (complementary information).

Artificial containers increasingly gain space in the urban environment, and inappropriate disposal favors conditions for vector mosquito breeding, among them *A. aegypti*, has demonstrated a high adaptive potential with the ability to colonize the most diverse breeding sites [29].

In relation to the exposure of *A. aegypti* breeding sites, the largest number of specimens has been collected in the rainy season in containers placed in the sun and in the dry season in shaded containers. These data demonstrate the adaptive evolution of *A. aegypti* that for decades according to [30], in Florida, [31] in the United States and by [32], in Brazil, has preferred only by shady containers, protected by trees, branches or slopes.

When analyzing the habitat of *A. aegypti*, it has been observed in a greater number of specimens in containers with organic matter presence, both in the rainy season and in the dry season [33]. When analyzing the effect of water quality on the life cycle of *A. aegypti,* there registered a significant decrease in the complete development of immature forms in relation to water and diet. This fact can justify the decrease in the number of specimens found in completely clean containers, without leaves, mosses, fruit residue or any other type of organic substrate.

Artificial containers are distinguished by their favorable conditions for proliferation of vector mosquitoes. The storage group is the main breeding site of *A. aegypti*, but other groups such as flasks and tires have also witnessed immature forms. It has been found that breeding sites between 0 and 50 cm deserve attention in the fight against *A. aegypti* because the accumulation of small containers such as bottles, glasses, sardine cans, soda cans and tires stacked in the peridomicile of homes or improperly discarded in the streets and corners contribute to the dispersion of this vector.

5. Conclusions

Most mosquitoes have a preference for the site of development, looking for conditions that help in their survival and proliferation. The residences offer ideal conditions for their growth and proliferation, since human blood that is part of their diet is easily found, as well as standing water and organic matter that enhances their growth.

The storage group was the most frequent breeding site for *A. aegypti*; these containers are often found in the municipality under study, and in rainy seasons deserve extra attention, as residents collect rainwater for use in domestic activities, significantly increasing the number of breeding sites in the municipality.

Disordering dump and inadequate maintenance of domestic containers mainly during periods of high rainfall, associated with favorable conditions of shade, height compatible with the ability to fly along with organic matter deposited in the bottom or edge of the breeding grounds favor the creation of these vectors. So, it becomes essential control measures and regular monitoring intra, peri and extra domicile residences, as well as information campaigns showing cases, notifications and mortality of diseases transmitted by *A. aegypti*.

The knowledge about the preferred sites for *A. aegypti* development is of fundamental importance as it contributes to the development of new strategies to control these mosquitoes.

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