



Variation in *Aedes aegypti* (Linnaeus) (Diptera, Culicidae) infestation in artificial containers in Caxias, State of Maranhão, Brazil

Variação da infestação de *Aedes aegypti* (Linnaeus), (Diptera, Culicidae) em recipientes artificiais em Caxias, Estado do Maranhão, Brasil

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ABSTRACT

Introduction: Dengue is a serious public health problem worldwide, with cases reported annually in tropical and subtropical regions. *Aedes aegypti* (Linnaeus, 1762), the main vector of dengue, is a domiciliary species with high dispersal and survival capacities and can use various artificial containers as breeding sites. We assessed potential container breeding sites of *A. aegypti* in the municipality of Caxias, Maranhão, Brazil. **Methods:** In the initial phase, we analyzed 900 properties in 3 neighborhoods during the dry and rainy seasons (August-October 2005 and February-April 2006, respectively). During the second sampling period, September 2006-August 2007, we used 5 assessment cycles for 300 properties in a single neighborhood. **Results:** During the dry and rainy seasons, water-storage containers comprised 55.7% (n = 1,970) and 48.5% (n = 1,836) of the total containers inspected, and showed the highest productivity of immature *A. aegypti*; we found 23.7 and 106.1 individuals/container, respectively, in peridomicile sites. In intradomicile sites, water-storage containers were also the most important breeding sites with 86.4% (n = 973) and 85.6% (n = 900) of all containers and a mean of 7.9 and 108.3 individuals/container in the dry and rainy seasons (October 2006 (1,342)). The highest number of positives (70) was recorded in May, mostly (94%) in storage containers. **Conclusions:** Storage containers are the principal and most productive *A. aegypti* breeding sites and are a major contributing factor to the maintenance of this vector in Caxias.

Keywords: Dengue. Vector. Prevention. Control. Containers.

RESUMO

Introdução: A dengue constitui de um sério problema de saúde pública mundial, com casos sendo registrados anualmente nas regiões tropicais e subtropicais. O *Aedes aegypti* (Linnaeus, 1762) é o principal vetor da doença, um mosquito domiciliado de fácil dispersão e sobrevivência, com capacidade de utilizar variados recipientes artificiais como criadouro. Em vista disso, neste trabalho investigou-se os tipos de recipientes potenciais criadouros de *A. aegypti* na Cidade de Caxias, Maranhão, Brasil. **Métodos:** Inicialmente, a pesquisa foi desenvolvida em três bairros e 900 imóveis analisados no período da seca e das chuvas (agosto a outubro de 2005 e fevereiro a abril de 2006, respectivamente). Um segundo período de amostragem ocorreu de setembro de 2006 a agosto de 2007 em que inspeções de cinco ciclos foram desenvolvidas em um dos bairros com 300 imóveis amostrados. **Resultados:** Os recipientes de armazenamento de água representaram 55,7% (n=1.970) e 48,5% (n=1.836) do total dos inspecionados, e maior produtividade de imaturos de *A. aegypti*, com proporção de 23,7 e 106,1 por recipiente no peridomicílio, nas estações seca e chuvosa respectivamente. No intradomicílio, também foi mais representativo, 86,4% (n=973) e 84,6% (n=900), apresentando média de 7,9 e 108,3 indivíduos, nas duas estações. O maior número de recipientes com água foi registrado em setembro e outubro 2006 (1.342), obtendo-se maior número de positivos (70) no mês de maio, sendo (94%) pertencentes ao grupo armazenamento. **Conclusões:** Armazenamento são os principais criadouros de *A. aegypti*, contribuindo na manutenção do vetor na cidade de Caxias, Maranhão.

Palavras-chaves: Dengue. Vetor. Prevenção. Controle. Recipientes.

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INTRODUCTION

In recent years, Brazil has reported a high proportion of the world's dengue cases^{1,2}. In the Americas in 2008, Brazil recorded the highest incidence of this disease, as well as the largest number of associated deaths¹. Taking into account the lack of a vaccine against the disease, the most viable way to control dengue is to eliminate its main vector, the mosquito *Aedes aegypti* (Linnaeus, 1762) (Diptera, Culicidae)^{2,3}.

Aedes aegypti multiplies in artificial containers, such as tanks, water-storage tanks, disused tires, vases, and various kinds of flasks⁴⁻⁶. As a consequence, *A. aegypti* is mainly controlled by assessing its breeding sites and sampling larvae and pupae in urban areas. This method is used to assess the levels of infestation in order to reduce the larvae and pupae and consequently minimize the risk of epidemics⁷. This strategy is adopted because the vector uses artificial containers that are often found within dwellings or in the peridomicile area as breeding sites⁸. This behavior assures the survival of the vector and reduces the efficiency of dengue control³. In regions where the water supply may be interrupted and people have low purchasing power, storage containers are the most important breeding sites for *A. aegypti*. In contrast, in large urban centers, the occurrence of artificial mosquito breeding sites is related to the enormous amounts of unusable, mainly disposable, materials⁹.

One of the most striking features of Brazil is the heterogeneity of its regions, with widely varying climate and socio-cultural conditions. Consequently, the prevention of dengue becomes complex because its occurrence is intimately related to the local conditions¹⁰. Therefore, studies at different sites throughout the country are useful to assess the process of *A. aegypti* adaptation in various situations. The understanding of these conditions may facilitate the determination of the most suitable control measures for each area of the country.

In recent years, the State of Maranhão has recorded an increase in the number of dengue cases, including the hemorrhagic forms¹¹; however, only a few studies have investigated *A. aegypti* and its reproductive mechanisms in this region. In view of this and of the eclectic behavior of *A. aegypti* in the use of domestic containers as breeding sites, and because of the different social factors, the aim of this study was to assess *A. aegypti* breeding sites and its productivity in the municipality of Caxias, Maranhão. This assessment included consideration of the effects of the dry and rainy seasons in this region.

METHODS

The study was carried out in the urban zone of the municipality of Caxias, from 2005 to 2007. 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 5,313.2 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 25 inhabitants/km² and an annual growth rate of 1.06%¹². 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-December¹³.

In general, the municipality of Caxias has a low socioeconomic level, with a human development index of 0.614, demonstrating the state's penultimate place in the national ranking scale. The low quality of life in the city is apparent in the substandard basic sanitation, with an irregular water supply and garbage collection and disposal services¹⁴.

In the first phase of the study, *A. aegypti* breeding sites were investigated in the dry (August-October 2005) and rainy (February-April 2006) seasons in 3 neighborhoods of Caxias: Cangalheiro, Campo de Belém, and Conjunto Habitacional Pampulha (CHP). These neighborhoods were selected because of their locations in different areas of the municipality and also because of differences in their physical aspects, in order to provide a representative sample of the study area.

The Cangalheiro neighborhood is located in the central part of the municipality, with a population of 4,134 and 1,627 buildings, mostly of masonry construction. The streets are paved, but deficiencies in sanitation include open sewage ditches, almost daily interruptions in the water supply, and irregular garbage collection services that do not reach all areas of the neighborhood.

The Campo de Belém neighborhood, located in the eastern part of the municipality, has a dense population of 6,297 and 2,478 houses, mostly of masonry construction but several (20%) of cob (*taipa*) construction. The majority of houses have outdoor bathrooms, with large numbers of water-storage containers. Most of the streets are paved but some dirt roads remain, which turn to mud in the rainy season. Poor sanitation includes open sewage ditches and irregular water supply and trash collection.

The CHP neighborhood, in the southern part of the municipality, has 1,134 inhabitants and 562 buildings, all of masonry, with the homes constructed in the same pattern. The dwellings have better conditions than the other 2 neighborhoods, with indoor bathrooms and water tanks present in each home. The streets are unpaved and graveled. The water system is very irregular, with water supplied

only during the night. Trash is collected regularly, but the sewage system is open, similar to that in the other neighborhoods. Of the 3 neighborhoods, CHP has the lowest population density, since it is the most recently built.

Between August 2005 and July 2006, a total of 900 properties were inspected, with 300 properties from each neighborhood. Each house was visited twice, once in the dry season (August-October 2005) and again in the rainy season (February-April 2006).

We employed the domicile visit method used by the Brazilian National Program for Dengue Control¹¹. The house inspections were made in order to record potential *A. 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 intra- and peridomicile areas.

From the containers where immature forms of *A. aegypti* were found, all of the specimens were collected using plastic pipettes and sieves. The specimens were placed in test tubes containing 70% ethanol and were forwarded to the Laboratório de Entomologia Médica at the State University of Maranhão, where they were counted and identified at the species level with appropriate identification keys⁸.

From September 2006 through August 2007, collections were made in the Campo de Belém neighborhood to survey the containers and monitor the variation in larvae production during the course of 1 year, as well as the impact of control measures. A total of 300 properties were inspected in 5 periods of the year, termed cycles, with the following durations: 1st period, September-October; 2nd period, January; 3rd period, March; 4th period, May; and 5th period, July-August. These cycles were established by the Municipal Dengue Control Program, and the domicile visit method was also employed in this series¹¹. The previously described inspection method was used again.

The containers found on the properties were classified into 7 groups: I, storage (tanks, vats, water filters, pots, pails, and drums); II, flasks (bottles, cans, and plastics); III, fixed (drains, grease traps, and wells); IV, construction materials (construction materials and car parts); V, tires; VI, vases (plant pots and saucers); VII, others (every container that did not belong to the previous groups, such as Styrofoam ice chests and eggshells).

In order to determine the productivity (p) of the breeding sites, the following equation was used: $p = \frac{XI}{ZI}$, where X corresponds to the number of larvae and pupae found in a container in group I, and Z is the number of positive containers (containing larvae and/or pupae) from the same group. The same calculation was made for each type of container.

The chi-square test of independence was used for comparisons among the different types of containers to verify the independence of the data among the groups. The chi-square test of adherence was used to compare seasons to verify if the differences found between seasons indeed resulted from chance. The tests were performed using the statistical package BioEstat 5.0¹⁵.

RESULTS

In the survey of the 900 properties in the 2 seasons, a large number of potential *A. aegypti* breeding containers were found, with a total of 1,970 containers in the dry season and 1,836 in the rainy season. Among the different types of containers, those of

the storage group were the most numerous, with a total of 55.7% (n = 1,970) and 48.5% (n = 1,836) during the dry and rainy seasons, respectively (Table 1). Importantly, when the variation in the number of containers in each group in both seasons was analyzed, there was a trend toward an increase in the availability of containers from the tires, construction materials, storage, fixed containers, and "others" groups from the dry to the rainy season (Table 1).

Water-filled containers were significantly more abundant in the rainy season (n = 1,169; p < 0.05; Table 1). Storage containers were the predominant type in the dry season, comprising 73.8% (n = 1,105), which was approximately 3 times the total number of all other types of containers found; these also predominated in the rainy season, comprising 69.7% (n = 1,169) of all containers sampled (p < 0.05; Table 1).

With respect to the seasonal variation in the availability of water-filled containers, each group, except for the vases and storage groups, showed different patterns in each season. Flasks constituted the highest number of water-filled containers during the dry season; however, with the onset of the rains, the tires, fixed, and others groups gained importance as breeding sites, since their numbers doubled during this season (p > 0.05; Table 1).

With respect to the positive containers, during the dry season, although many containers were found filled with water, only the storage group contained larvae and pupae, with a mean of 23.72 larvae and/or pupae per container. In contrast, during the rainy

season, immature stages of *A. aegypti* were recorded in all types of containers, except plant pots. Storage containers were the most important, comprising 66.6% of the containers found (n = 99) (p > 0.05; Table 1). This number was more than double that in the dry season, and with 4 times as many larvae and/or pupae during this period (106.13; p < 0.05; Table 1). These numbers suggest that with the increase in precipitation, the productivity of *A. aegypti* increases considerably.

In the intradomicile area, 1,125 and 1,064 containers were recorded in the dry and rainy seasons, respectively; however, these totals did not differ between the seasons. Storage containers were more than 3 times as numerous as the other groups in both seasons, accounting for 86.5% (n = 1,125) during the dry season and 84.6% (n = 1,064) during the rainy season (p < 0.05; Table 2). A comparison of the remaining groups by season revealed that only the vase and others groups showed a seasonal relationship, being more numerous in the dry and rainy seasons, respectively (p < 0.05; Table 2).

With respect to the number of water-filled containers in the dry and rainy seasons, the storage group was again the most important, comprising 90.6% (n = 978) and 89.4% (n = 975) of the total containers inspected, respectively (p < 0.05; Table 2). Comparing the groups between seasons, flasks were the most abundant type of water-filled container during the rainy season, while tires were the most abundant type during the dry season (p < 0.05; Table 2). The remaining groups did not show a seasonal pattern.

TABLE 1 - Total amount of inspected containers in the peridomicile area and *Aedes aegypti* productivity by container group during sampling in the dry and rainy seasons in the municipality of Caxias, State of Maranhão, Brazil.

Container group	Inspected			Filled with water			Positives			Productivity		
	dry	rainy	P*	dry	rainy	P*	dry	rainy	P*	dry	rainy	pa
Vase	17	11	0.3447	6	10	0.317	0.00	0.00		0.00	0.00	-
Flasks	495	523	0.3974	150	101	0.002	0.00	7		0.00	11.71	-
Tires	94	112	0.2362	3	27	0.000	0.00	6		0.00	46.00	-
Construction materials	7	46	0.0001	0	19	-	0.00	1		0.00	7.00	-
Storage	1,097	890	0.0001	816	812	0.9407	29	66	0.0001	23.72	106.13	0.0001
Fixed	43	80	0.0012	43	64	0.042	0.00	1		0.00	6.00	-
Others	217	174	0.0337	87	136	0.001	0.00	18		0.00	48.11	-
Total	1,980	1,836	0.0206	1,105	1,469	0.0001	29	99	0.0001	23.72	83.20	0.0001
P**	<0.0001	<0.0001		<0.0001	<0.0001			<0.0001			<0.0001	

P*: adherence test; P**: independence test; pa: apply statistical test.

TABLE 2 - Total amount of inspected containers in the intradomicile area and *Aedes aegypti* productivity by container group during sampling in the dry and rainy seasons in the municipality of Caxias, State of Maranhão, Brazil.

Container group	Inspected			Filled with water			Positives			Productivity		
	dry	rainy	P*	dry	rainy	P*	dry	rainy	P*	dry	rainy	pa
Vases	60	98	0.0032	45	65	0.0701	0	3	na	0	4.6	na
Flasks	40	43	0.8262	10	24	0.0258	0	1	na	0	0	na
Tires	15	9	0.3074	14	2	0.0060	0	0	na	0	0	na
Construction materials	3	1	na	0	0	na	0	0	na	0	0	na
Storage	973	900	0.0962	886	872	0.7565	13	11	0.8383	7.9	108.36	0.0001
Fixed	1	1	na	1	1	na	0	0	na	0	0	na
Others	33	12	0.0029	22	11	0.0817	0	0	na	0	0	na
Total	1,125	1,064	0.1997	978	975	0.9639	13	15	0.8501	7.9	112.96	<0.0001
P**	<0.0001	<0.0001		<0.0001	<0.0001		na	<0.0001		na	<0.0001	

P*: adherence test; P**: independence test; na: statistical test not applied, pa: statistical test applied.

The positive containers showed no significant seasonal differences ($p < 0.05$; **Table 2**). During the dry season, only storage containers provided positive results, while they were the most important during the rainy season (73.3%; $n = 15$; $p < 0.05$; **Table 2**), with no seasonal difference in numbers. Although the number of storage containers did not differ between seasons, ~10 times more immature

A. aegypti individuals were found in the rainy than in the dry season ($p < 0.05$; **Table 2**). Notably, containers in the other groups also contained immature stages of *A. aegypti* in the rainy season, although in much lower numbers compared to the storage group.

In the peridomicile area, large numbers of water-filled containers were observed during the entire monitoring cycle, mainly in September-October when the highest number of filled containers was found, comprising more than 25.8% ($n = 2,579$) of the total number of containers ($p < 0.05$; **Table 3**). In the intradomicile area, this monitoring cycle was also the most important, with 24.3% of all the monitored containers. A comparison of both sites (i.e., peri- and intradomicile) revealed a significant difference between the domiciles in the 2nd (January 2007) and 5th (July-August 2007) cycles, with the greatest difference observed in the peri- and intradomicile areas ($p < 0.05$; **Table 3**). These observations indicate that the difference in the availability of containers in the peri- and intradomicile environments may be related to the time of year.

A total of 224 and 32 positive containers were found in the peri- and intradomicile areas, respectively, with significantly more containers in the peridomicile area ($p < 0.05$; **Table 3**). In the peridomicile area, although the highest number of water-filled containers was recorded in the 1st monitoring cycle, the highest number of immature *A. aegypti* was found in the 4th cycle (May 2007), with 29.4% ($n = 224$; $p < 0.05$; **Table 3**). The productivity of immature *A. aegypti* differed in the intradomicile area and was highest in the 2nd monitoring cycle (January 2007), i.e., 46.9% ($n = 32$; $p < 0.05$; **Table 3**). Positive containers predominated in the peridomicile area in all of the monitoring cycles.

Concerning the type of positive containers recorded during the inspection cycles of the breeding sites, the flasks and tires groups included low numbers of positive containers. Storage containers predominated, comprising 94% ($n = 256$) of the total number of positive containers. The highest proportion of positive containers was found during the 2nd monitoring cycle (January 2007), comprising 26.2% ($n = 241$; $p < 0.05$; **Table 4**). In the peridomicile area, the proportion of positive containers was significantly higher than in the intradomicile area, with 86.7% ($n = 241$; $p < 0.05$; **Table 4**). Positive storage containers predominated in the peridomicile area in all of the cycles ($p < 0.05$; **Table 4**).

TABLE 3 - Sampling cycles with the respective numbers of inspected and positive containers from peridomicile and intradomicile areas of 300 properties in the municipality of Caxias, State of Maranhão, Brazil, from September 2006 to August 2007.

Cycles (period)	Containers filled with water			Positive containers		
	peridomicile	intradomicile	P*	peridomicile	intradomicile	P*
1 st (Sep-Aug 06)	666	676	0.8059	18	4	<0.0056
2 nd (Jan 07)	533	423	0.0004	52	15	<0.0001
3 rd (Mar 07)	461	496	0.2717	48	7	<0.0001
4 th (May 07)	497	548	0.1219	66	4	<0.0001
5 th (Jul-Aug 07)	422	637	<0.0001	40	2	<0.0001
Total	2,579	2,780	0.0063	224	32	<0.0001
P**	<0.0001	<0.0001	P**	<0.0001	0.0025	

P*: adherence test; P**: independence test.

TABLE 4 - Sampling cycles, showing the number of positive storage containers from the peridomicile and intradomicile areas of 300 properties in the municipality of Caxias, State of Maranhão, Brazil, from September 2006 to August 2007.

Cycle	Dwelling site	Storage	P*
1 st	peridomicile	18	<0.0056
	intradomicile	4	
2 nd	peridomicile	48	<0.0001
	intradomicile	15	
3 rd	peridomicile	46	<0.0001
	intradomicile	7	
4 th	peridomicile	57	<0.0001
	intradomicile	4	
5 th	peridomicile	39	<0.0001
	intradomicile	2	
Total	peridomicile	208	<0.0001
	intradomicile	32	
P**	peridomicile	<0.0001	<0.0001
	intradomicile	<0.0001	

P*: adherence test; P**: independence test.

DISCUSSION

The occurrence of dengue is directly related to the presence of its vector *A. aegypti*. This mosquito can persist and disperse widely within Brazil as a result of the wide availability of artificial containers in urban areas, a phenomenon that is well described and discussed in the literature^{8,16,17}.

This study in the City of Caxias, Maranhão, showed that although dengue control campaigns emphasize the importance of water containers in the dispersal of the dengue vector, residences still contain large numbers of containers with the potential to serve as breeding habitats of *A. aegypti*. These containers were present in the intradomicile and peridomicile environments, in all of the neighborhoods studied. They provide perfect conditions to shelter the vector because they contain large amounts of exposed clean water with no protection, which facilitates the access of the mosquito to the water. These very numerous storage areas are the sites where larvae and pupae are most often found, and consequently produce high numbers of mosquitoes in the peri- and intradomicile areas and in the dry and rainy seasons.

However, the tires, construction material, fixed, and others groups were also important potential breeding sites of *A. aegypti* in the rainy season, since immature stages of the vector were found in

these containers only in this season. These types of containers are discarded inappropriately, generally around residences and with no protection, and the regular rains in this period ensure that they retain water long enough for *A. aegypti* to breed.

This study showed that the large number of water-storage containers in Caxias is a result of the lack of water, a recurring situation in the 3 neighborhoods studied. Because of the water-supply problem, the residents must maintain large numbers of drinking-water containers at their residences for daily use, principally large-volume containers such as tanks, barrels, and buckets. Residents with a very low socio-economic level are unable to maintain adequate water-storage facilities and therefore store water in inappropriate ways, which creates ideal breeding conditions for *A. aegypti*. The presence of these storage containers is the determining factor for the maintenance of the vector in these areas.

Other investigators have described a similar situation in different locations in the northeast region of Brazil¹⁸⁻²⁰. In the State of Ceará, containers used to store water, such as tanks, drums, and rooftop water tanks, predominated as the principal breeding sites of *A. aegypti*^{18,21}. The problem of water supply was also mentioned as one of the aggravating factors in necessitating the large number of these containers in residences, as was their predominant role as breeding sites^{18,19}. In other countries, such as Cuba and Nicaragua, water-storage containers were most often found to contain the largest number of immature *A. aegypti*²¹⁻²³.

Different situations have been described for other regions of Brazil. In the southeast, the flasks and drains groups were most commonly found to have immature forms of the vector in domiciles in the City of São Paulo, and a similar result was noted for the grease traps in the City of Rio de Janeiro^{24,25}. Another study carried out in the north, in Manaus, Amazonas, found that the flasks and tires groups accounted for the largest number of larvae and pupae¹⁷. This shows that the potential types of breeding sites of *A. aegypti* vary according to the environmental conditions in each region.

With respect to the number of breeding sites present in the 2 seasons, in the rainy season, the number increased by ~3-fold and the productivity of larvae was ~14-fold higher.

One aspect of this study requiring emphasis is the existence of positive breeding sites that persist during the dry period. In spite of the high temperatures at this time of year, the immature forms continue to exploit the large water containers, assuring the continuity of the vector from one season to the next. Other studies have also noted this factor in helping to maintain the vector in the northeast region of the country^{24,26,27}.

The scenario described in this region shows that the effectiveness of control measures is impaired as a function of this dynamic of the breeding sites. On the other hand, the frequent lack of water means that the population must store sufficient water for each individual family unit.

The data from this study indicate the urgent need for investment in the region to address this controversial situation that has dogged the northeast of Brazil since the time that *A. aegypti* first infested the urban areas in this region. Storage tanks cannot continue to be manufactured by local artisans because, in this form, they are ideal breeding grounds for mosquitoes. It is imperative to introduce a model residential water-storage container that is completely screened, but still easy to use. The federal, state, and municipal governments

should invest in a fiberglass model with a screen cover that is practical for drawing water, in order to succeed in controlling dengue in this region.

The data from this study, together with a bibliographic review of the subject, indicate that the whole problem of dengue in the northeast of Brazil arises because of the lack of access to fresh water, with *A. aegypti* colonizing water-storage facilities in urban and peri-urban areas. This study also points to the need to educate the population in order to prevent the vector from breeding in and around their homes. These campaigns must be carried out during the entire year because, as observed here, some breeding containers are positive for immature *A. aegypti* throughout the year. The present scheduling of intermittent control campaigns contributes to the lack of success in controlling the disease²⁸.

Because the lack of fresh water is related to public policies that require a long period to implement, 1 way to reduce the dispersal of *A. aegypti* is to intensify vector-control campaigns. The population in areas affected by water shortages should be advised to clean their water-storage units appropriately, and thus prevent the vector from reproducing and developing.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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