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Occurrence of *Aedes aegypti* (Diptera, Culicidae) in a Dengue Transmission Area at Coastal Maranhão State, Brazil

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Abstract: This study aimed to estimate the occurrence of *Aedes aegypti* adults at distinct climatic seasons at neighborhoods from the municipality of São Luís, Maranhão State, Brazil, as well as to verify the presence of Dengue virus (DENV) in the specimens collected. A total of 320 properties were visited in eight neighborhoods that were previously randomly chosen. Samplings were made at three periods: dry season/2008, rainy season/2009 and dry season/2009. A total of 563 *Ae. aegypti* mosquitoes were collected, with 141 of them collected during dry season/2008, 272 during rainy season/2009 and the remnant, during dry season/2009. Specimen were divided into lots and subjected to RT-semi-nested-PCR analysis and virus isolation was carried out using cell culture (C6/36 clone) of *Ae. albopictus*. The number of adults significantly varied at the neighborhoods of Coréia de Baixo, Lira, João Paulo, and Conjunto Cohatrac I. Molecular analyses of specimens showed no positivity for DENV. At the municipality of São Luís, seasonal climate variation might influence the density of *Ae. aegypti* adults, however, other factors such as population density, sanitation conditions, and the presence of mosquito breeding sites ought to be assessed as important parameters influencing vector dispersion.

Keywords: *Ae. aegypti*, climatic factors, dengue, seasonality.

INTRODUCTION

Ae. aegypti is the main vector of dengue fever (DF) and dengue hemorrhagic fever (DHF) and is spread in the majority of world's tropical and subtropical regions [1, 2]. The wide dispersion of this vector causes dengue epidemics in many parts of the world [3, 4]. Taking into consideration *Ae. aegypti* adaptive complexity, many different measures must be adopted to get rid of containers that accumulate water and serve as breeding sites for their immature stages, avoiding mosquito reproduction and dispersion. Amongst them are included those actions related to virological and entomological surveillance, health education, and community participation [5-10].

The State of Maranhão is located in the transitional region between the Amazon and semi-arid areas of Northeast Brazil. There, the association amongst climatic conditions, low socioeconomic status and marked sanitary deficiencies favors *Ae. aegypti* reproduction and also hampers the implementation of vector control measures [11, 12]. In the municipality of São Luís, capital of the State, dengue epidemiological situation is characterized by the presence of the serotypes DENV-1 and DENV-2, since 2000, and DENV-3, since 2002. From 2006 until today, an increase in the number of cases recorded in children aged under 15, as well as in the number of severe forms and deaths have been observed. In the years 2008 and 2009, 1,409 and 994 dengue cases, re-

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spectively have been confirmed in the country, out of which 24 and 16 were DHF, in this order [13, 14].

After the reintroduction of serotype 4, in 2010, the circulation of these four dengue serotypes within the country increased the incidence of hemorrhagic forms [15-17], especially during periods where vector proliferation is favored. Thus, in order to contribute with vector control in São Luís, State of Maranhão, this study surveyed of *Ae. aegypti* population density in some neighborhoods of the municipality. Samples during different seasons of the year were collected, in order to assess the areas which had greater risk of *Ae. aegypti* maintenance, relating the factors involved in this process, to investigate the presence of DENV in the specimens collected.

MATERIAL AND METHODS

Study Area

The study was carried out in the county of São Luís, situated at the São Luís Island, Northern Maranhão State, Brazil (02º 31'47" S; 44º 18' 10" W). It has an altitude of 24.39 m above the sea level, an area of 827 km^2 (around 0.24% of the State territory), and a population of 957,515 inhabitants [18]. The climate tropical, hot and humid, being divided into two periods: a rainy season (from January to June), where mean precipitation is around 1,900 mm, and a dry season (from July to December), where monthly precipitation levels might drop to less than 50 mm. The annual mean temperature ranges between 28-30º C [19].

The county is currently divided into seven sanitary districts: Centro, Itaqui-Bacanga, Coroadinho, Cohab, Bequimão, Tirirical, and Vila Esperança. From those, two were randomly selected for the development of the study – Centro and Cohab. Moreover, four neighborhoods were also chosen for sampling.

For this study we used a simple random sample. The sortition was made for two of the seven sanitary districts. In each district, we made a sortition of four neighborhoods, according to the list given by the Health Department of São Luís. We preferred using simple random samples because all the districts and neighborhoods had an equal probability of being chosen for the sample. The sortition of only two districts and eight neighborhoods were taken into account, due to the limitations of the work.

The Centro District is mainly located at the historic portion of São Luís, and is characterized by the presence of old neglected homes – with some of them being even abandoned. Its neighborhoods have open sewers or small streams next to the dwellings, which favors the accumulation of garbage that is thrown by local population. In this district, samples were collected from the neighborhoods Coréia de Baixo, Lira, Goiabal, and João Paulo.

The Cohab District is located at a more peripheral portion of the county and comprises of an area with extensive vegetation. It faces great sanitary deficiencies, such as irregular system of water supply, and some unpaved streets of the neighborhood. Herein, samples were collected from the neighborhoods of Itapiracó, Residencial Canudos, Conjunto Cohatrac I, and Vila Luisão.

Collection of adult mosquitoes

In each neighborhood, a total of 40 properties were visited, summing up 320 properties at the eight visited neighborhoods. Collections were made from November 2008 to August 2009, with an interval of two months between each sample, as following: 1) November-December 2008 (dry season/2008); 2) March-April 2009 (rainy season/2009); and 3) July-August 2009 (dry season/2009).

Mosquitoes were collected with Nasci [20] mechanical aspirator, which was moved in every extent of indoor and outdoor. Later on, aspirations were made next to plant pots and bushes. 15 minutes were used as a standard time for aspiration at each property, both indoor and outdoor, where the equipment remained attached throughout the aspiration. Collected specimens were identified at species level in the laboratory, by means of Forattini [5] dichotomous key of identification.

At each station, two trained students were responsible to catch the mosquitoes who were accompanied by dengue control agents of the Health Department of São Luís. Collections were made on the same property in the three seasons studied, and followed the same criteria of time, place and duration of the effort. All this work was done from Monday to Friday, in four weeks per month.

Molecular Analyses

At the Arbovirology Section and Hemorrhagic Fevers of the Instituto Evandro Chagas (county of Belém, State of Pará, Brazil), collected specimens were firstly sent to the Entomology Laboratory, for confirming previous identifications. Thereafter, they were sent to the Cell Culture Laboratory and to the Molecular Biology Laboratory, in order to attempt virus isolation and the conduction of semi-nested-RT-PCR analysis for viral detection. *Ae. aegypti* specimens were divided into lots containing from one to 37 mosquitoes.

A total of 13 specimens were included in the dry season/2008 lot; 23 in the rainy season/2009 lot; and 15, in the dry season/2009 lot. Thereafter, they were ground in a phosphate buffered saline (PBS) solution, pH 7.4, with 0.75% bovine serum albumin (BSA), penicillin (100 IU ml^{-1}), and streptomycin (100 mg ml^{-1}), according to the protocol proposed by Reynes [21].

Supernatant was filtered before being used for RNA extraction and viral isolation. Suspensions were inoculated into adult *Ae. albopictus* cell cultures, clone C6/36 (American Type Cell Culture Colection/ATCC), and observed for 14 days to assess cytopathic effect (CPE). This material was also analyzed for DENV detection, by means of indirect immunofluorescence using monoclonal antibodies. The method herein used was previously described by Gubler *et al.* [22]. RNA was extracted using the Trizol LS reagent protocol (Invitrogen, San Diego, CA, USA), according to the manufacturer's instructions. Extracted RNA was eluted by RNAse-free water.

As proposed by Lanciotti *et al.* [23], the semi-nested-RT-PCR included the prM/M protein genes. The reaction was carried out in two steps, starting with the synthesis of cDNA from extracted viral RNA. Later, Semi-nested-RT-PCR amplification products were analyzed in agarose gel, with respective molecular weight markers and positive and negative controls of reaction [23].

Statistical Analyses

The data of adult frequency were compared by means of a Chi-square adherence test ($p < 0.05$). All neighborhood data of the adults frequencies between seasons were also tested with a Chi-square adherence test ($p < 0.05$), except for the neighborhood Residencial Canudos. This neighborhood was submitted to a Yates' correction prior to the same analysis, due to their low mosquito frequency [24, 25].

RESULTS

A total of 563 *Ae. aegypti* adults were collected at the sampled districts, 368 of them being females and the remnant males. The largest number of specimens were obtained during the rainy season/2009 (n = 272), whilst the lowest number (n = 141) was obtained at dry season/2008 (Table **1**). The highest frequency was observed at March 2009 (rainy season/2009), with the capture of 173 specimens.

When adult frequency was compared amongst sampled neighborhoods and season (Table **2**), a significant variation was observed during rainy season at the neighborhoods Coréia de Baixo ($p = 0.0012$), Lira ($p < 0.0001$), João Paulo ($p < 0.0001$) – located at the central portion of the municipality, as well as at Conjunto Cohatrac I ($p < 0,0001$) – located at the Cohab District. In general, the samples collected at the rainy season had higher adult frequency than the others two collected at dry season ($p < 0.0001$). No variation in adult frequency amongst sampled periods was observed at the neighborhoods Goiabal, Itapiracó, Residencial Canudos, and Vila Luisão.

The lowest and highest mean temperature was observed during April 2009 (rainy season/2009) and November 2008 (dry season/2008), respectively. These months also had the highest and lowest mean relative humidity and rainfall, in the same order (Table **3**).

All *Ae. aegypti* exemplars subjected to virus isolation and semi-nested-RT-PCR showed no positivity for DENV infection. One of the factors that might have influenced this lack of DENV detection was the high case records observed during the periods prior (n = 3,162 cases, in 2007) and subsequent (n = 4,909 cases, in 2011) to the development of this study, but there was a decrease in the number of cases during study period (Table **4**).

Table 1. Frequency of *Aedes aegypti* Adults Collected in the Years of 2008 and 2009, at the County of São Luís, State of Maranhão, Brazil

Period	Month	Total of Adults	Adult	
			Female	Male
Dry season/2008	November – December	141	94	47
Rainy season/2009	March – April	272	182	90
Dry season/2009	July – August	150	92	58

Table 2. Number of Adults Collected Per Sampled Period at the Neighborhoods of the County of São Luís, State of Maranhão, Brazil

Neighborhood	Period			*p – value*	Total
	Dry Season/2008	Rainy Season/2009	Dry Season/2009		
COR	3 (2.12)	21 (7.72)	12 (8.00)	0.0012	36 (6.39)
LIR	6 (4.25)	58 (21.32)	21 (14.00)	0.0001	85 (15.09)
GOI	39 (27.66)	42 (15.44)	50 (33.35)	0.4769	131 (23.26)
JP	56 (39.75)	84 (30.91)	35 (23.33)	0.0001	175 (31.13)
ITA	8 (5.67)	12 (4.41)	10 (6.66)	0.6703	30 (5.32)
CAN	4 (2.83)	5 (1.83)	5 (3.33)	0.9311*	14 (2.48)
COH	4 (2.83)	36 (13.23)	3 (2.00)	0.0001	43 (7.63)
VLU	21 (14.89)	14 (5.14)	14 (9.33)	0.3679	49 (8.70)
Total	141 (100.00)	272 (100.00)	150 (100.00)	0.0001	563 (100.00)

p = Adherence test. *Using Yates' correction.
COR = Coréia de Baixo. LIR = Lira. GOI = Goiabal. JP = João Paulo. ITA = Itapiracó. CAN = Residencial Canudos. COH = Conjunto Cohatrac I. VLU – Vila Luisão.

Table 3. Temperature, Air Relative Humidity and Precipitation Monthly Averages Recorded from November 2008 to August 2009, at the County of São Luís, State of Maranhão, Brazil

Month/Year	Mean Monthly Temperature (ºC)	Mean Monthly Air Relative Humidity (%)	Mean Monthly Precipitation (mm)
Nov/2008	29.14	68.16	0
Dec/2008	28.61	72.93	40.6
Mar/2009	26.68	85.19	442.2
Apr/2009	26.22	87.43	537.6
Jul/2009	27.23	82.35	82.4
Aug/2009	27.88	78.67	23.2

Source: Meteorology and Geoenvironmental Center, State University of Maranhão (UEMA), São Luís, State of Maranhão, Brazil.

Table 4. Frequency of Dengue Records between the Years 2002 and 2012, at the County of São Luís, State of Maranhão, Brazil. Cases are Divided According to the Clinical Form

Clinical Form	Year											
	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	Total
DF	297	404	111	66	388	2,737	1,019	974	2,454	4,801	1,284	14,535
DHF	11	14	4	30	24	315	24	16	63	89	31	621
DC	4	21	4	38	126	110	6	4	45	19	14	391
Total	312	439	119	134	538	3,162	1,049	994	2,562	4,909	1,329	15,547
%	2	2.82	0.76	0.86	3.46	20.33	6.74	6.39	16.47	31.63	8.54	100.00

DF = Dengue Fever. DHF = Dengue Hemorrhagic Fever. DC = Dengue with Complications. Source: Brazilian National Notifiable Diseases Information/ Health Department of São Luís, State of Maranhão, Brazil.

DISCUSSION

At São Luís, State of Maranhão, Brazil, the highest *Ae. aegypti* population density was observed during the rainy season, when the most elevated precipitation levels were recorded. These results indicated that the climatic factors might influence vector population density in different periods of the year, by influencing the man-vector contact and hence, the incidence of dengue at the county studied.

Glasser and Gomes [26] reported the influence of climatic factors on *Ae. aegypti* and *Ae. albopictus* population distribution at the State of São Paulo, observing that precipitation levels influenced *Ae. aegypti* geographic expansion only. Additionally, in the county of Manaus, State of Amazonas, Brazil, Pinheiro and Tadei [27] detected a higher frequency of containers positive for the vector during rainy season.

The differences observed in the adult frequency amongst localities might be related to the variation in socioeconomic conditions of the neighborhoods herein studied. At the neighborhoods Coréia de Baixo, Lira, João Paulo, and Conjunto Cohatrac I, a high density of *Ae. aegypti* adults was observed, mainly during the rainy season. Except Conjunto Cohatrac I, all the other neighborhoods are located at the Centro District, where demographic density is high and urbanization problems are present where many properties are of old construction. Besides, sanitation is poor and commercial activity is intense – factors that certainly favor the creation of a great number of *Ae. aegypti* breeding sites.

A similar relationship between socioeconomic factors and infestation rates of *Ae. aegypti* larvae was observed by Ferreira and Chiaravalotti Neto [28] at the county of São José do Rio Preto, São Paulo State, Brazil.

During the visits to the neighborhood properties of São Luís, it was also verified that the presence of containers being used for water storage where present, which might contribute for vector infestation. Gonçalves Neto *et al*. [12] detected that the majority of the municipality population still has the habit to keep water stored, since the water supply is present, but frequently disrupted.

In a research carried out at the county of Caxias, State of Maranhão, Brazil, the findings of Soares-da-Silva *et al.* [10] indicated that containers used for water storage were those with the largest number of *Ae. aegypti* immatures. Such results were observed during both dry and rainy season, indicating that this practice favors vector reproduction and the risk of dengue transmission.

The greatest part of *Ae. aegypti* mosquitoes was collected indoor, confirming its endophilic and endophagic behavior [5]. It was noticed that, during the collection, the majority of the properties has scarcely ventilated, dark and damp rooms. Such characteristics were also pointed out by Forattini [5], who described some factors such as temperature, shade, resting places and the availability of human blood favorable for vector reproduction and maintenance in the urban environment. Yet Caprara *et al*. [29] pointed out humidity as the main factor influencing the survival and permanence of *Ae. aegypti* adult forms within the dwellings. Other authors have also found similar results, such as Barata *et al.* [30], who collected more than 80 % of *Ae. aegypti* adults in the intradomicile, at the county of São José do Rio Preto, State of São Paulo, Brazil.

Conversely, Pinheiro and Tadei [27], however, observed a distinct result, reporting a large number of *Ae. aegypti* immatures in the peridomicile of dwellings at the county of Manaus, State of Amazonas, Brazil. They found such forms mostly in bottles and containers used for water storage, and also emphasized that these properties had backyards with great extent.

Different from such dwellings, the properties studied at São Luís have small areas, especially at the central portion of the county, influencing *Ae. aegypti* breeding sites. In most cases, population is unaware about the vector biology, an important factor to contribute for the reduction of potential breeding sites.

In this study, it was verified that temperature, air relative humidity, and precipitation are the major factors influencing vector's seasonal fluctuation at São Luís. The analyses showed a tendency of the development of mosquitoes during the periods of temperature reduction and increase in relative humidity, as well as on precipitation incidence. These abiotic factors are able to affect mosquito population dynamics, enhancing the supply of breeding sites that allow the raise in their population.

It is noteworthy that other factors ought to be considered in studies regarding *Ae. aegypti* population density. Some of them include the habits of population in relation to the disease prevention, the situation of properties, the frequency of insecticide application, and the community participation in campaigns for mosquito control [9].

Moreover, the unplanned urbanization, with the existence of a deficitary system of water supply and basic sanitation at São Luís neighborhoods, has created new opportunities for vector reproduction and its wide distribution at the county from the 90s up to the present day [14].

When observing dengue epidemiologic situation in the State, a high number of incidences have been noticed, with the increasing occurrence of cases with complications and hemorrhagic forms, favoring the high ratio of deaths, mostly in children, as seen in the last few years [31].

At the county of São Luís, a sum of 15,547 dengue records was confirmed between 2002 and 2012, 2011 being the year with the highest number of notifications. A low endemicity was observed during 2008 and 2009, in the period when the study was carried out, especially during the months that *Ae. aegypti* adults were collected. This pattern confirms the low viral circulation compared to the year 2010, where a sum of 2,562 dengue records was observed [31].

In our study we attempted to identify the circulation of DENV in *Ae. aegypti* using the virus isolation method in cell culture and molecular techniques had no positivity, this may be due to the decrease in the number of cases of DENV in the study population during the period investigated. However studies by Lucena *et al*. [32] collected *Ae. aegypti* mosquitoes at the counties of Caxias and São Luís, State of Maranhão, Brazil, and detected the circulation of two dengue serotypes: DENV-2 and DENV-3, respectively. Positivity was observed at the neighborhoods Castelo Branco and Cangalheiro, at Caxias, and Pirapora, at São Luís. Their results were supported by patients with positive dengue diagnosis, confirming the circulation of both serotypes at these sites.

Therefore, the control of vector population indices is an essential way to decrease viral circulation. This aim might be achieved with the implementation of different strategies to be applied in *Ae. aegypti* vigilance at the county. These strategies might take into consideration some local aspects of the neighborhoods, such as the characteristics of properties, most common breeding sites, and their commercial activities. Moreover, they should also consider seasonal factors that favor mosquito proliferation.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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