

Prevalence of dengue, Zika and chikungunya viruses in *Aedes (Stegomyia) aegypti* (Diptera: Culicidae) in a medium-sized city, Amazon, Brazil

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ABSTRACT

Aedes aegypti is associated with epidemic diseases in Brazil, such as urban yellow fever, dengue, and more recently, chikungunya and Zika viruses infections. More information about *Ae. aegypti* infestation is fundamental to virological surveillance in order to ensure the effectiveness of control measures in use. Thus, the present study aims to identify and compare infestation and infectivity of *Ae. aegypti* females in Macapa city, Amapa State (Amazon region), Brazil, between the epidemiological weeks 2017/02 and 2018/20. A total number of 303 *Ae. aegypti* females were collected at 21 fixed collection points, 171 at the 10 collection points in the Marabaixo neighborhood and 132 at the 11 collection points in the Central neighborhood. Among the collected samples, only two were positive for dengue virus, with a 2.08% (2/96 pools) infectivity rate for Marabaixo. The difference between the medians of *Ae. aegypti* females captured in Central and Marabaixo sites was not statistically significant. The findings indicate similar mosquito infestation levels between the neighborhoods, and a low-level of mosquito infectivity, although dengue virus was found only in Marabaixo. Virological surveillance of *Ae. aegypti* was important to identify sites of infection and determine possible routes of transmission to enable health surveillance teams to adopt preventive strategies where infected mosquitoes are present and act faster.

KEYWORDS: Arboviruses. Chikungunya. Dengue. Entomology. Infectivity. Infestation. Prevention. Surveillance in public health. Zika.

INTRODUCTION

Introduction of exotic vectors into new areas are associated with human epidemics throughout history¹. In Brazil, *Aedes (Stegomyia) aegypti* (Diptera: Culicidae), a vector from Africa², is a known transmitter of the viruses responsible for urban yellow fever, dengue and more recently chikungunya and Zika epidemics³.

The prevention and control of these arboviral diseases can be performed through immunobiologicals (vaccines against yellow fever⁴ and dengue⁵), by disruption of host-vector contact or vector control actions. Dengue, chikungunya and Zika prevention depends mainly on effective vector control measures, unlike yellow fever, for which vaccination is the most effective method⁶.

The main vector control action is to adopt measures to eliminate *Ae. aegypti* breeding sites. In addition, in places where probable cases of dengue, chikungunya and/or Zika infections are reported, measures to break the chain of transmission in

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the surrounding area with insecticide application must be considered (Ultra Low Volume)⁷.

Surveillance of diseases transmitted by *Ae. aegypti* is based on entomological indices and mandatory reporting of suspected/confirmed cases, which is often late⁸.

Macapa, the State capital of Amapa, has high infestation rates for *Ae. aegypti* in all the urban areas of the municipality. Summer monsoon brings rain from December to May, with the latter being the rainiest month with the highest recorded density of mosquitoes⁹. The city is 14 meters above sea level and has a humid tropical climate, with small temperature variations (average of 27° C), an average annual relative humidity of 81% and an average rainfall of around 2,600 mm. The driest period is from September to November (quarterly rainfall below 200 mm) and the wettest period from March to May (quarterly rainfall higher than 1,000 mm)¹⁰.

In places where there is a high infestation of *Ae. aegypti*, it is essential to establish a virological surveillance to determine more effective control measures. Thus, the present study aims to identify and compare the infestation and infectivity of *Ae. aegypti* females in two neighborhoods

in the city of Macapa, between the epidemiological weeks 2017/02 and 2018/20.

MATERIALS AND METHODS

Study area

The research was conducted in the urban zone of the municipality of Macapa, the State capital of Amapa, located in the Brazilian Amazon, with an estimated population of 474,706 inhabitants in 2017¹¹.

For this research, two neighborhoods were chosen: Central and Marabaixo, located in Central and Western Macapa, respectively (Figure 1). The former is the most urbanized neighborhood in the city, where the commercial and administrative areas are concentrated, and the largest coverage of water supply and sewage occurs. Houses are mostly built with both brick and stone masonry. Marabaixo is an unofficial neighborhood with deficient basic urban infrastructure, lacking coverage of water and sewage networks. The majority of the houses are made of brick and cement plaster, and there are also numerous wooden houses.

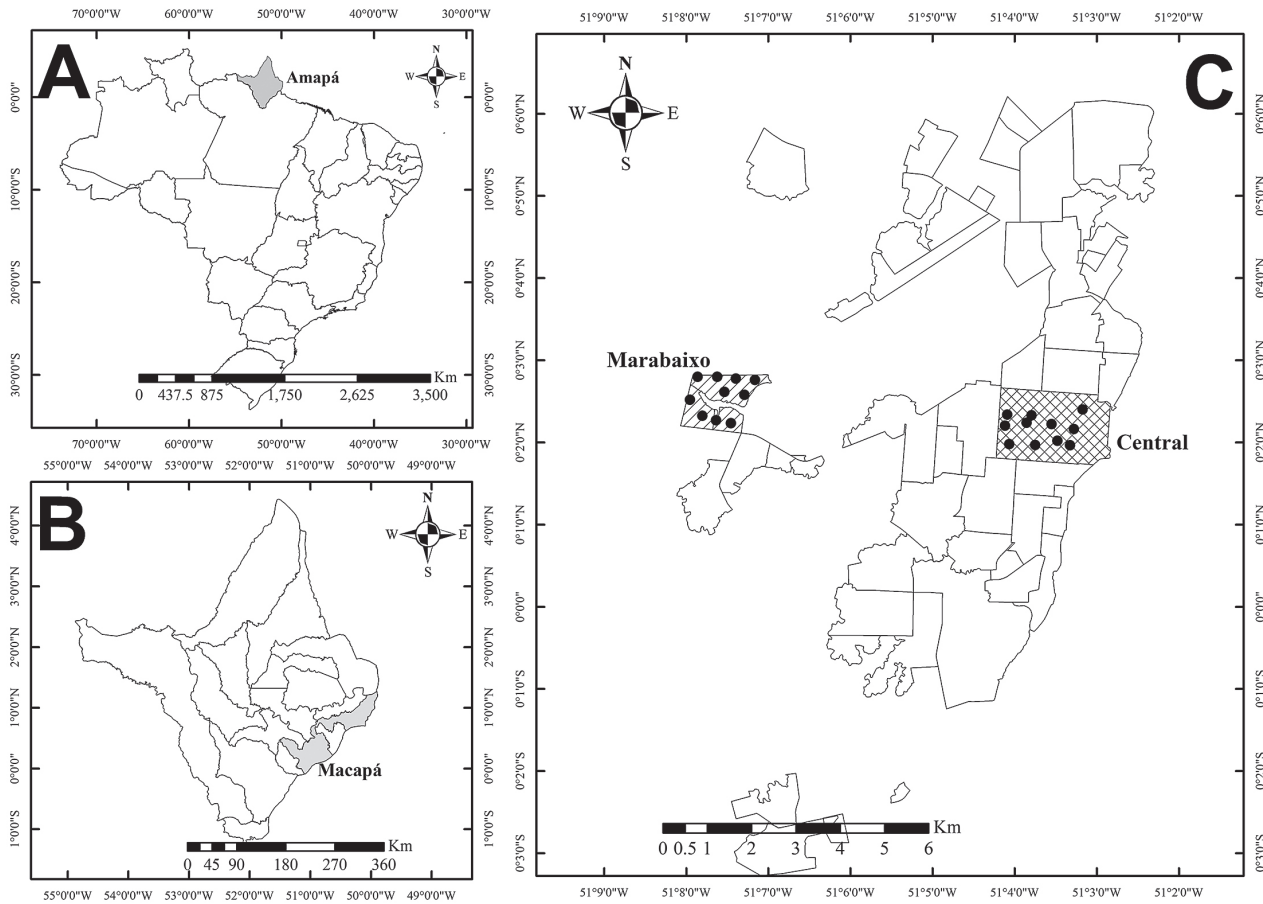


Figure 1 - A) Map of Brazil, highlighting the Amapa State in gray; B) Map of the Amapa State, showing the Macapa city in gray; C) Marabaixo and Central neighborhoods are highlighted in gray and the sites of collections are black dotted.

Sample collection

Permanent collection points were established randomly, at approximately 400 m from each other, and they were georeferenced using the Universal Transverse Mercator (UTM), by means of the Garmin Oregon 550 GPS (Garmin International, Inc., Olathe, Kansas, USA).

Between the epidemiological weeks 2017/02 and 2018/20, at each sampling point, mosquitoes were captured biweekly in peridomiciliary and intradomiciliary environments through electric aspirators¹², Castro aspirators¹³ and insect collection nets.

Immediately afterwards, the captured insects were transported to the Vector Laboratory of the Epidemiological Surveillance Department of Amapá.

Using the key of Consoli and Oliveira¹⁴, *Ae. aegypti* females were identified and classified as either non-blood fed females (without evidence of hematophagia) and blood fed females (females with blood in the abdomen). Following this classification, pools of up to five mosquitoes per tube, per epidemiological week, were prepared for each collection point. During the study, *Ae. aegypti* males and other trapped species of mosquitoes were discarded.

Sample preparation and Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) assay

Initially, wings and legs were removed and discarded from collected mosquitoes. Samples were placed in 2.0 mL

microcentrifuge tubes, then, they were stored at $-70\text{ }^{\circ}\text{C}$ until analysis.

The pools were macerated in 1 mL phosphate saline (PBS), pH 7.4, through the Retsch Model MM400 Vibratory Mill (Retsch-Allee, Haan, Germany). Then, the samples were centrifuged for 10 min at $16,128\text{ x g}$. For nucleic acids extraction, approximately 300 μL of the supernatant were pipetted and filtered (0.22 μm) using the PureLink™ RNA/DNA Mini Kit (Invitrogen, Carlsbad, CA, USA) to obtain 50 μL of purified nucleic acids, as recommended by the manufacturer.

Finally, the samples were aliquoted into qRT-PCR Fast plates along with the oligonucleotide sequences for dengue (DENV1, DENV2, DENV3 and DENV4), chikungunya and Zika viruses (Table 1), together with positive and negative controls for further interpretation of the results, as recommended by the manufacturer¹⁵⁻¹⁷.

Statistical analysis

For statistical analysis the software BioEstat version 5.3 (Mamirauá Institute, Manaus, Brazil)¹⁸ was used. The weeks during which collection in either site was not possible were discarded for statistical analysis.

Data were tested for Lilliefors normality, which proved to be significant, and for this reason nonparametric tests were used.

Then, to compare the total of females captured between the studied neighborhoods, the Mann-Whitney test was used. To verify the difference between non-blood fed

Table 1 - Oligonucleotide sequences used for the detection of dengue, chikungunya and Zika viruses.

Virus	5'-3' Sequence	Reference
DEN-1 Forward	CAAAAGGAAGTCGTGCAATA	Jonhson <i>et al.</i> ¹⁵
DEN-1 Reverse	CTGAGTGAATTCTCTACTGAACC	
DEN-1 Probe	CATGTGGTTGGGAGCACGC	
DEN-2 Forward	CAGGTTATGGCACTGTCACGAT	
DEN-2 Reverse	CCATCTGCAGCAACACCATCTC	
DEN-2 Probe	CTCTCCGAGAACAGGCCTCGACTTCAA	
DEN-3 Forward	GGACTGGACACACGCACTCA	
DEN-3 Reverse	CATGTCTCTACCTTCTCGACTTGTCT	
DEN-3 Probe	ACCTGGATGTCGGCTGAAGGAGCTTG	
DEN-4 Forward	TTGTCCATATGATGCTGGTCCG	
DEN-4 Reverse	TCCACCTGAGACTCCTTCCA	
DEN-4 Probe	TTCTACTCCTACGCATCGCATTCCG	
Chikungunya - Forward	CATCTGCACYCAAGTGACCA	Michlmayr <i>et al.</i> ¹⁶
Chikungunya - Reverse	GCGCATTTTGCCTTCGTAATG	
Chikungunya - Probe	GCGGTGTACTGCTGCTGTGACYGC	
Zika - Forward	CAGCTGGCATCATGAAGAAAYC	Waggoner <i>et al.</i> ¹⁷
Zika - Reverse	CACCTGTCCCATCTTTTCTCC	
Zika - Probe	CYGTTGTGGATGGAATAGTGG	

Table 2 - Number of *Ae. aegypti* females collected in the survey and the mosquito pools by neighborhood.

Mosquito collection sites	NBF	BF	Total	NBF Pool	EF Pool	Total Pool
Central	97	35	132	53	26	79
Marabaixo	107	64	171	61	35	96
Total	204	99	303	104	61	175

NBF= *Ae. aegypti* non-blood fed; BF= *Ae. aegypti* blood fed

females and blood fed females in each neighborhood, the same statistical method was applied. The results were considered significant when $p \leq 0.05$.

RESULTS

A total of 303 *Ae. aegypti* females were collected, of which 132 were from the Central neighborhood (97 non-blood fed females and 35 blood fed females) and 171 from the Marabaixo neighborhood (107 non-blood fed females and 64 blood fed females), resulting in 175 pools, of which 79 were from the Central neighborhood (53 of non-blood fed females and 26 of blood fed females) and 96 from the Marabaixo neighborhood (61 of non-blood fed females and 35 of blood fed females) (Table 2).

There was no statistically significant difference between the medians of the total *Ae. aegypti* females captured in the Central (3) and the Marabaixo (3) neighborhoods (Mann-Whitney Test $U = 353.5$; $Z (U) 0.1903$; $p = 0.8491$).

In the Central neighborhood, there was a higher frequency of non-blood fed females (73.48%) than blood fed females (26.52%), with a statistically significant difference between the median number of non-blood fed females (2) and blood fed females (1) (Mann-Whitney $U = 243$; $Z (U) 2.1019$; $p = 0.0356$).

In the Marabaixo neighborhood, 62.57% of the captured females were non-blood fed, while 37.43% were blood fed ones, but the difference between the medians of non-blood fed (1) and blood fed (0) females was not statistically significant (Mann-Whitney U test = 305.5 $Z (U) 1.0207$; $p = 0.3074$).

Regarding the infectivity, the presence of dengue virus was only detected in two samples, both from the Marabaixo neighborhood, with a total infectivity of 2.08% (2/96), one from non-blood fed females (1/61 – DEN-1) and one from blood fed females (1/35 – DEN-4). For Zika and chikungunya viruses, no virus was detected in any sample.

According to the Epidemiological Surveillance Department of Amapa, during the research in Central neighborhood, 14 cases of dengue, five of chikungunya and none of Zika virus were confirmed. In Marabaixo

neighborhood, 17 cases of dengue, three of chikungunya and one of Zika virus were confirmed.

DISCUSSION

The presence of vectors, the existence of cases of dengue, chikungunya and Zika viruses, and the population susceptibility are conditions that favor the occurrence of epidemics.

Ae. aegypti has a high predilection for human blood¹⁹. Thus, the high number of fed mosquitoes in both neighborhoods showed that vector control measurements have not prevented the host-vector contact.

The detection of a non-blood fed female pool highlights the possibility of dengue virus transmission, as the virus can be present in the insect salivary gland and can be transmitted during the blood meal. However, the presence of dengue virus in blood fed female does not distinguish whether the detected virus was present in the blood from the meal or from the mosquito salivary gland.

When virological surveillance in *Ae. aegypti* is performed close to the occurrence of confirmed cases of arboviruses, the percentage of positive females is higher, as demonstrated by Perez-Perez *et al.*⁸ who found in Medellín, Colombia, 35.42% of dengue virus infected female pools. Pérez-Castro *et al.*²⁰ reported an infectivity of 62% of *Ae. aegypti* females in places where there were cases of dengue within 200 m of the collection point and 30 days before the collection date. In addition, in Aracaju city, the State capital of Sergipe, in Northeast Brazil, Costa-Silva *et al.*²¹ found 10% (1/10 pools) of *Ae. aegypti* infected by chikungunya virus. In contrast, in Rio de Janeiro city, in Southeast Brazil, Ferreira-de-Brito *et al.*²² detected a low natural infectivity in *Ae. aegypti* with Zika virus of 1.15% (3/198 pools).

The infectivity rates are lower when virological surveillance is independent from the reporting of positive cases, as is the case in the present study. This is corroborated by Baak-Baak *et al.*²³, who found 1.2% (2/166) positivity for dengue virus in churches. Meanwhile, in Belo Horizonte, the State capital of Minas Gerais, Eiras *et al.*²⁴ found 4.9% (4/82 pools) and 8.5% (7/82 pools) infectivity to dengue virus and Zika virus, respectively, at the university

campus representing a higher risk of transmission for these populations.

Moreover, the possibility of transovarian transmission of dengue²⁵, chikungunya²⁶ and Zika^{27,28} in *Ae. aegypti* shows that a varied percentage of mosquitoes can be infected with these viruses. Thus, cases may occur without the need of a previous source of infection.

Dengue, chikungunya and Zika are underreported diseases²⁹. Sometimes, these infections have subclinical manifestations. Additionally, some populations have less access to public health care, or, as is the case with Zika, laboratory examination is often required only for groups considered at higher risk, such as pregnant women.

In Brazil, *Ae. aegypti* entomological surveillance is based on the monitoring of immature forms (Larval Index Rapid Assay for *Ae. aegypti* - LIRA) as recommended by the Brazilian Health Ministry. Thus, according to the prevalence of *Ae. aegypti* immature forms, each stratum (area between 2,000 to 12,000 properties) receives a risk rating: low ($\leq 0.9\%$), medium (1% - 3.9%) or high ($\geq 4\%$)³⁰. This method depends on the accuracy of the inspector to collect immature forms. In addition, Macapa city does not have specialized staff equipped with stairs to inspect elevated deposits such as water boxes and gutters.

The epidemiological surveillance of dengue, chikungunya and Zika is performed by the follow-up of reported cases, thus helping the health surveillance teams to find sites for implementing vector control actions aimed at interrupting the transmission of the disease⁷.

In general, surveillance actions are reactionary rather than preventive. Monitoring the presence of these viruses in *Ae. aegypti* where there are high levels of vector density means a better guiding for the vector combat teams. Besides optimizing public resources, this process provides a transmission chain break while the virus is inside the mosquito.

Moreover, according to Pena-Garcia *et al.*³¹, density of mosquitoes is not a good predictor of dengue cases. Instead, mosquito infection rates can better explain the dengue heterogeneity, helping to predict infections up to six weeks before the onset of cases.

The current *Ae. aegypti* control program advocates that when a case of *Ae. aegypti* transmitted disease is identified, control actions should be performed at the probable site of infection, typically the patient's home. However, transmission often occurs in divergent locations, as the mosquito prefers feeding during the daytime³², and it is dispersed throughout the city.

The use of molecular methods to detect the presence of these viruses in *Ae. aegypti* is a powerful tool to perform a fast virological surveillance, allowing the adoption of

the most effective prevention measures to deal with these diseases.

CONCLUSION

The *Ae. aegypti* infestation rates were comparable for both Central and Marabaixo neighborhoods. However, the study only revealed the presence of the dengue virus in mosquitoes from the Marabaixo neighborhood, showing a low infectivity.

Virological surveillance for *Ae. aegypti* was important to identify infectivity sites and determine possible routes of transmission, driving the surveillance health team to adopt strategies to act faster in sites where infected mosquitoes were present.

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