A clinical-ecological study of a triple viral epidemic: Zika, Dengue and Chikungunya

Um estudo clínico-ecológico de uma tripla epidemia viral: Zika, Dengue e Chikungunya

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ABSTRACT

In Brazil, since 2015, co-circulation of three arboviruses, Dengue (DENV), Chikungunya (CHIKV) and Zika (ZIKV), have presented diagnostic challenges, due to their similar clinical manifestations. Our goal was to analyze cases of arboviral illness using key clinical features and to ascertain house infestation indices (HII) in the study area. A total of 28,064 medical records were analyzed by clinical-epidemiological criteria for DENV, ZIKV and CHIKV in 2015 and 2016 at the public health unit in Xerem, Duque de Caxias, Rio de Janeiro. The collection of vectors at home breeding sites in Xerem was performed to determine the HII, in March and June 2015. The total number of cases of suspected arboviral illness in 2015 was 969, of which 444 (45.8%) were due to DENV, 146 (15.1%) to ZIKV, and 11 (1.1%) to CHIKV. In 2016, the number of suspected cases of arboviral illness was 2019, of which 324 (16.0%) were classified as DENV, 779 (38.6%) as ZIKV, and 53 (2.60%) as CHIKV. The clinical manifestations prevalent in ZIKV were rash (67.8% to 79.5%) and pruritus (63.7% to 71.4%). The HII for the immature stages of *Aedes* in the study area, in March and June 2015, was 11.8% for *Ae. aegypti* and 8.1% for *Ae. albopictus*, both very high. There was a strong positive correlation observed for precipitation and HII for both vectors (*Ae. aegypti* and *Ae. albopictus*), but not for temperature levels. We conclude that a triple epidemic occurred in the studied area probably due to the high infestation rates and a naive population for the two newly introduced arboviruses; whilst there are no available specific laboratory tests a practical clinical diagnosis workout is crucial.

Keywords: arboviruses, dengue, zika, chikungunya, clinical manifestations, Brazil

RESUMO

No Brasil, desde 2015, a co-circulação de três arbovírus, Dengue (DENV), Chikungunya (CHIKV) e Zika (ZIKV), tem apresentado desafios diagnósticos, devido a suas manifestações clínicas semelhantes. Nosso objetivo era analisar casos de doença arboviral usando características clínicas chave e verificar índices de infestação domiciliar (HII) na área de estudo. Um total de 28.064 prontuários médicos foram analisados por critérios clínico-epidemiológicos para DENV, ZIKV e CHIKV em 2015 e 2016 na unidade de saúde pública em Xerem, Duque de Caxias, Rio de Janeiro. A coleta de vetores em locais de reprodução domiciliar em Xerem foi realizada para determinar o HII, em março e junho de 2015. O número total de casos de suspeita de doença arboviral em 2015 foi de 969, dos quais 444 (45,8%) eram devidos ao DENV, 146 (15,1%) ao ZIKV, e 11 (1,1%) ao CHIKV. Em 2016, o número de casos suspeitos de doença arboviral foi 2019, dos quais 324 (16,0%) foram classificados como DENV, 779 (38,6%) como ZIKV, e 53 (2,60%) como CHIKV. As manifestações clínicas prevalecentes em ZIKV eram erupção cutânea (67,8% a 79,5%) e prurido (63,7% a 71,4%). O HII para as fases imaturas do Aedes na área de estudo, em março e junho de 2015, foi de 11,8% para Ae. aegypti e 8,1% para Ae. albopictus, ambos muito elevados. Observou-se uma forte correlação positiva para a precipitação e HII para ambos os vetores (Ae. aegypti e Ae. albopictus), mas não para os níveis de temperatura. Concluímos que uma tripla epidemia ocorreu na área estudada provavelmente devido às altas taxas de infestação e uma população ingênua para os dois arbovírus recém-introduzidos; enquanto não há testes laboratoriais específicos disponíveis, um trabalho prático de diagnóstico clínico é crucial.

Palavras-chave: arbovírus, dengue, zika, chikungunya, manifestações clínicas, Brasil

1 INTRODUCTION

Arboviral diseases are a group of entities caused by viruses transmitted by arthropod vectors (*arthropod-borne* virus). These have been recognized by the World Health Organization (WHO) as a global health problem due to their growing territorial spreading and the need for ever more complex prevention and control strategies. The predominantly circulating arboviruses in Brazil today are dengue (DENV), Chikungunya (CHIKV) and Zika (ZIKV)(1). DENV has been recognized as circulating in Brazil since 1845(2); more recently, in the 1980's, serotypes 1 and 4 (DENV-1 and DENV-4) were responsible for the Roraima and Rio de Janeiro epidemics, and over 1 million people were affected by DENV-1 in Rio(3). Subsequently, between 1990 and 2000, DENV-2(4) and DENV-3 caused epidemics throughout the country(5). In 2014, cases of CHIKV infection were reported in Bahia and Amapá, and cases of ZIKV in Bahia. In 2016, there were 13.236 laboratory confirmed cases and 38.332 clinico-epidemiological probable cases of CHIKV(6). Regarding ZIKV, in 2015 an outbreak of congenital microcephaly was noted in the Northeast of Brazil(7) and this was later strongly related to maternal ZIKV infection(8). Since then these viruses have continued to be epidemic in most cities and states in Brazil.

Clinically differentiating these arboviral illnesses is a challenge in low and middle- income countries. These illnesses share common clinical features such as fever, myalgia and skin rash which, in turn, also occur in other febrile illnesses, such as measles, coxsackievirus infections, rubella, leptospirosis, Brazilian spotted fever and malaria for example. Laboratory diagnosis is often unavailable, and to the present date, the most used tool, which is an ELISA test (*enzyme linked immunosorbent assay*), has low specificity, as cross reactions do occur with other flavivirus (between ZIKV and DENV more importantly). In the course of an epidemic, the confirmation of the cases can be made through clinical-epidemiological criteria, except for the first cases in the area, which should have laboratory confirmation, which may include ELISA, viral isolation, polymerase chain reaction (PCR) and immunohistochemistry(13-15).

Regarding the *Aedes* mosquitoes, climate factors, such as temperature, rainfall and altitude, influence their life cycle(9); besides, the high urbanization rate, mass events, insufficient waste collection, and deficient water supply all contribute to the propagation of the mosquito vectors and to their inefficient control(10).To add on to this complex scenario, in December 2016, an outbreak of yellow fever, so far still sylvatic, occurred in Brazil.

Epidemiological surveillance of arboviral diseases in Brazil follow the guidelines of the Dengue National Control Program which includes control strategies for the *Aedes* mosquitoes, mainly the so called LIRAa (Larval Index Rapid Assessment for *Aedes aegypti,* recently extended to include the assessment of *Ae. albopictus* larvae, which are carried out by local [state] health authorities[(11)]) One in every 5 dwellings are systematically sampled for larval forms of the vectors, and the house infestation index (HII) is calculated for municipalities and their subareas, representing the percentage number of houses that harbor foci containing immature forms of *Aedes*.

In the present report, our first objective was to describe the clinical features of patients with acute febrile illness compatible with DENV, ZIKV or CHIKV who were seen at the local public health clinic in Xerem, Duque de Caxias, state of Rio de Janeiro, from March to June 2015 and 2016, and secondarily to analyze the relationship of larval density in this same area with available meteorological data.

2 METHODS

Patients and study area

The city of Duque de Caxias has a Human Development Index of 0.711 and is divided into four districts: Duque de Caxias, Campos Eliseos, Imbariê, and Xerem. Xerem is located within the geographic coordinates 22°34'55.7"S 43°18'16.1"W and was our study area. It is a semi-rural district with 21,880 inhabitants[(12)], and it has 8000 households distributed in the urbanized area and its residents are provided prehospital care at the Álvaro Santos S. Figueira Unit, which is the reference unit in basic health care where about 300 patients per day are seen. In this unit, 28,064 medical notes from March to June 2015 (14,495) and 13,569 in the same period in 2016 were identified, photographed and saved in a computer for posterior analysis. The medical notes were randomly selected to cover all weeks in this time frame, and all patients seen in a total of 15 days per month were included. Patients who did not reside in the study area (280 or 0.98%) were excluded. Medical notes that were illegible or incomplete (84 or 0.29%) were not analyzed. The 28,064 medical notes of the patients involved in the research were anonymized.

Patients were defined as having arboviral illness if they presented acute fever and no localizing signs of infection, such as those of the respiratory, urinary and gastrointestinal tracts or from the soft tissues.

Study variables included age, gender, address, and clinical features, and were inserted into spreadsheets. Signs and symptoms selected followed the clinical criteria listed in the Brazilian Ministry of Health guidelines for the classification of arboviral illnesses DENV, ZIKV e CHIKV[13-15]. In short, cases were classified as ZIKV when there was pruritus and/or, pruritic rash and/or non-purulent conjunctivitis; as DENV when there were minor bleeding and/or high fever and/or intense myalgia and/or retroorbital pain and/or systemic events, and as CHIKV when there was severe arthralgia or arthritis. When cases did not fit into any clear pattern, they were grouped as DENV/ZIKV or DENV/CHIKV. Systemic events noted were most often diarrhea, vomiting, fainting or abdominal pain, and were written as free text whenever present in the medical notes. No serological results were available.

Ethical approval for this data collection was obtained from the Ethics Committee from Unigranrio University under number CAAE 54544316.3.0000.5283.

Home visits

Between March and June 2015, home visits were made to 20% of the residences located in the study area, as recommended by the National Guidelines on Dengue Outbreak Prevention and Control[11], where the suspected cases of arboviruses are located. Immature forms of the vectors were collected from domiciliary deposits and stored in 70% alcohol, and sent for laboratory identification. *Aedes* were identified to species level and other Culicidae were identified to the genus level with the help of a dichotomic identification key proposed by Consoli & Lourenço-de-Oliveira.[16]Information from the study area, such as classification of the dwelling (entry not permitted, vacant lot, visited dwelling), types of deposits/foci, and vector forms collected were fed into a mobile equipment (a smartphone) utilizing a MECE® System - Add Technologies, RJ, Brazil (MECE stands for strategic monitoring for epidemic control). Informed consent was obtained from all study participants whose residences were visited.

Depots which may serve as breeding sites for mosquitoes were classified according to the Brazilian Ministry of Health (2013)[17]: Group A -depots of water for human consumption such as water tanks on roofs or at ground level; including smaller containers such as barrels or buckets, water depots for agriculture; Groups B and C -home depots such as empty flower pots, flower or other plants with pot saucers, storm drains, fountains, pools, cemetery urns; Group D - depots which may be removed, such as trash containers, tires, cans, bottles, litter; Group E -natural deposits, such as tree hollows, leaves, bromeliad plants, holes in rocks.

The home infestation index for *Aedes* larvae (HII) was calculated as the ratio between the number of positive dwellings and the total number of visited dwellings. Based on the HII the area was classified according to the vector transmission risk as satisfactory (HII<1%); alert status (HII between 1 and 3.9%); and high risk (HII>3.9%)[(17)].

Rainfall and temperature indices were obtained for the study periods (March to June 2015 and 2016) through the site of the Instituto Nacional de Meteorologia (INMET) http://www.inmet.gov.br/portal/. The monthly average temperatures were calculated. For rainfall, average was calculated as the monthly precipitation divided by the number of rainy days.

Treatment of mosquito breeding sites

Breeding sites found during visits to the dwellings were treated on the same day with the larvicide *Bacillus thuringhiensis israelenses* (Bti), as recommended by the Ministry of Health. The adult vectors were treated with low volume insecticidal electrical equipment (ULV) containing malathion aqueous emulsion, in a concentration of 44%, according to the dengue control program[(11)].

Statistical analysis

A qualitative and quantitative analysis was done with the *Bioestat*® Package version 5.0, using the Chi square test and Fisher's exact tests and *Pearson's* correlation. Median and mean values and other descriptive data (age, gender, symptoms) were calculated using the Microsoft Office Excel® 2007 program.

3 RESULTS

Sample Population

Age and gender distribution, as well as clinical features, are presented in Table 1. Analysis of the medical notes showed that the total number of cases of suspected arboviral illness in 2015 was 969, of which 444(45.8%) were classified as DENV, 146 (15.1%) as ZIKV, and 11 (1.1%) as CHIKV. Cases were classified as ZIKV/DENV for 312 (32.2%) and as CHIKV/DENV for 56 (5.8%). In 2016, the number of suspected cases of arboviral illness was 2019, of which 324 (16.0%) were classified as DENV, 779 (38.6%) as ZIKV, and 53 (2.60%) as CHIKV. Cases were classified as ZIKV/DENV for 856 (42.4%) and as CHIKV/DENV in 7 (0.3%). Mean age of patients considering all arboviral diseases was 30 years in 2015 and 35 in 2016. Medical notes that were illegible or incomplete added up to 1% and were not analyzed.

The incidence of arboviral illness in the district of Xerem was greater in women in both years, but this it was even higher in 2016, amounting to 3.353/100,000 female inhabitants, compared to 1.741/100,000 male inhabitants(12), including all cases of DENV, ZIKV e CHIKV, but excluding the cases classified as doubtful (DENV/CHIKV and DENV/ZIKV). When considering age strata, the incidence within each stratum was also higher for females, especially in the 20-29 (140; 7.45%) and the 30-39 (150;8.30%) years stratum, in 2016.

The median age of these cases in 2015 was 23 years and in 2016 was 31 years in both the interval was of (range 0 to≥60). The majority of the ZIKV cases consisted of females (528/777-68.0%), and among these, 19.25% were aged 30-39 years (150/777), in 2016.

When comparing the distributions of suspected arboviruses cases in the different age strata in the years 2015 and 2016, the behavior of the suspected ZIKV cases were significantly different ($p = 0.0009$), however, there was no significant difference for the suspected cases of DENV ($p = 0.1818$). In 2016, in relation to ZIKV, a higher frequency was found for those included in the 30-39 years stratum (144; 18.5%) (Table 1).

Regarding the clinical features presented by the patients, in the years 2015 and 2016, amongst the 146 cases suspected of ZIKV, rash occurred in 99 (67.8%) and 619 (79.5%) respectively while pruritus occurred in 93 (63.7%) and 556 (71.4%) respectively. Conjunctivitis occurred in 7.4 and 13% of cases (Table 1). Headache was more frequent in DENV bothin 2015 (370/444; 83.3%) as in 2016 (210/324; 64.8%). In patients suspected of having CHIKV, moderate to severe arthralgia occurred in 9/11(81.8%) in 2015. Bleeding phenomena occurred in low frequency (2.7%), as did neurological features (1.9%).

Table 1. Clinical features of suspected cases of arboviral illness in Xerem. Duque de Caxias. Brazil, 2015/2016.

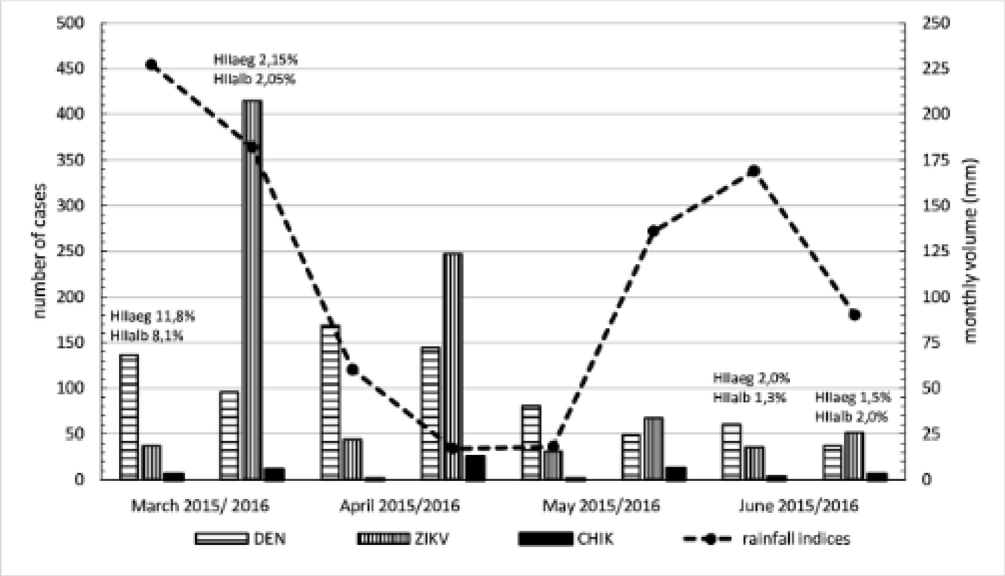
	DENV		ZIKV		CHIKV	
Gender	**2015**	**2016**	**2015**	**2016**	**2015**	**2016**
	n = 444 (%)	n = 326 (%)	n = 146 (%)	n = 777 (%)	n = 11 (%)	n = 52 (%)
Male	229 (51.6)	114 (35.0)	61 (41.8)	249 (32.0)	2 (18.2)	13 (25.0)
Female	215 (48.4)	212 (65.0)	85 (58.2)	528 (68.0)	9 (81.8)	39 (75.0)
p-values (α=0.05)	0.0001		0.0223		0.482	
Age stratum						
0-9 years	61 (13.7)	34 (10.4)	37 (25.3)	89 (11.5)	1 (9.1)	0 (0.0)
10-19 years	108 (24.3)	64 (19.66)	27 (18.5)	134 (17.2)	0 (0.0)	2 (3.8)
20-29 years	108 (24.3)	80 (24.5)	20 (13.7)	135 (17.4)	3 (27.3)	7 (13.5)
30-39 years	71 (16.0)	55 (16.9)	18 (12.3)	144 (18.5)	4 (36.4)	11 (21.2)
40-49 years	41 (9.2)	35 (10.7)	20 (13.7)	109 (14.0)	2 (18.2)	14 (26.9)
50-59 years	26 (5.9)	33 (10.1)	12 (8.2)	83 (10.7)	0 (0.0)	10 (19.2)
≥60 years	29 (6.5)	25 (7.7)	12 (8.2)	83 (10.7)	1 (9.1)	8 (15.4)
p-value (α=0.05)	0.1818		0.0009		*	
Clinical features						
Fever <38.5 C	94 (21.2)	77 (23.8)	75 (51.4)	457 (58.7)	4 (36.4)	15 (28.3)
Fever >38.5 C	350 (78.8)	247 (76.2)	71 (48.6)	320 (41.1)	7 (63.6)	38 (71.7)
Rash	0 (0.0)	42 (13)	99 (67.8)	619 (79.5)	0 (0.0)	2 (3.8)
Pruritus	0 (0.0)	39 (12)	93 (63.7)	556 (71.4)	0 (0.0)	2 (3.8)
Myalgia	300 (67.6)	222 (68.5)	43 (29.5)	340 (43.6)	5 (45.5)	37 (69.8)
Mild arthralgia	30 (6.8)	93 (28.7)	11 (7.5)	280 (35.9)	1 (9.1)	27 (50.9)
Moderate to severe arthralgia	1 (0.2)	2 (0.6)	0 (0.0)	8 (1.0)	9 (81.8)	14 (26.4)
Arthritis	0 (0.0)	1 (0.3)	0 (0.0)	3 (0.4)	2 (18.2)	7 (13.2)
Conjunctivitis	0 (0.0)	2 (0.6)	19 (13.0)	58 (7.4)	0 (0.0)	1 (1.9)
Headache	370 (83.3)	210 (64.8)	51 (34.9)	245 (31.5)	3 (27.3)	27 (50.9)
Lymphadenopathy	0 (0.0)	0 (0.0)	7 (4.8)	9 (1.2)	0 (0.0)	0 (0.0)
Bleeding	11 (2.5)	5 (1.5)	4 (2.7)	3 (0.4)	0 (0.0)	0 (0.0)
Neurological signs	8 (1.8)	1 (0.3)	2 (1.4)	3 (0.4)	0 (0.0)	1 (1.9)

Home visits

A total of 2,902 residences were visited in the area where the suspected cases of arboviruses occurred (urban area of Xerem) with positive properties containing immature forms (larvae and pupae) of *Aedes* in 283/1462 or 19.3% (March 2015) and 55/1440 or 3.8% (June 2015). In March 2015, 907 larvae/pupae were collected (583 or 64.3% of *Ae. aegypti* and 324 or 35.7% of *Ae. albopictus*) while in June 2015 there was a reduction in the number of immature forms found: 197 larvae/pupae (121 or 61.4% of *Ae. aegypti* and 76 or 38.6% of *Ae. albopictus*).

The house infestation index (HII) was high for both insect vectors, 11.8% for *Ae. aegypti* and 8.1% for *Ae. albopictus*. March was considered a month of heavy rainfall, as is expected during summer, with a total of 16 rainy days and the accumulated rainfall of 227 mm. In June, there were only three peaks of rainfall above 28 mm, with a total of 169 mm (Fig. 1). Average temperature in March was 24.7°C (maximum and minimum temperature of 31ºC and 20ºC) and in June, 20.8ºC (maximum 31ºC; minimum 13ºC). In March 2016 the HII was 3.9% for both *Aedes* spp and rainfall index was high, as well as the numbers of suspected ZIKV cases (779), as shown in Fig. 1.

Fig 1. Distribution of suspected cases of arboviral illness according to the months of the year and to rainfall indices, 2015/2016, Xerem. Duque de Caxias. Brazil.

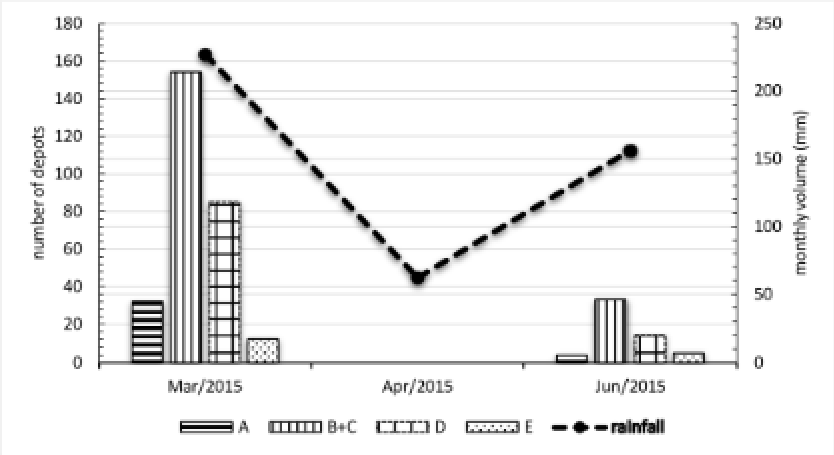


A strong positive correlation was found between rainfall and HII, both for *Ae. aegypti* (0.931) as for *Ae. albopictus* (0.942); on the other hand, there was a weak positive correlation between temperature and HII (of 0.457 and 0.439 for each vector, respectively). These data show that, despite the important role of temperature in the development of the immature stages of the vectors, rainfall plays a more decisive role in this respect.

Treatment of breeding sites

During the home visits, health agents applied *Bacillus thuringiensis israelensis* (Bti) in the depots where immature forms of the vectors were found, 1 g/50 L of water, according to the Brazilian Ministry of Health guidelines[(11)]. As shown in Fig. 2, in March, a high number of positive depots were found for groups B e C (154/283; 54%) and D (85/283; 30%) categories, and there was a high rainfall index in this month (227mm); on the other hand, in June 2015, an expressive reduction in the number of positive depots (in absolute numbers) in these groups were seen, group C (33/56; 59%) and D (14/56; 25%). In the areas where HII was above 3.99% measures to eliminate the adult mosquitos were also taken with the ULV insecticide electrical equipment[(13)].

Fig 2. Types of depots/breeding sites and rainfall indices in March and June 2015, Xerem, Duque de Caxias, Brazil.



Discussion

Our study analyzes a large number of medical consultations in an area where there was an outbreak of ZIKV in the State of Rio de Janeiro, Brazil, in the years 2015 and 2016, utilizing clinical and epidemiological criteria to characterize the disease caused by co-circulating arboviruses.

The first cases of ZIKV in Brazil were reported in 2015(1,18) and in 2016 the incidence of the disease was very high, reported as 416.6 cases/100,000 inhabitants(6). More importantly, during this very same year an association with neonatal microcephaly was noted(8) CHIKV, which had been circulating in Brazil since 2014(19), also presented higher number of cases in 2015 and 2016. Our study showed a higher number of cases of ZIKV, similar to the national trends, when 2015 and 2016 were compared.

Clinical diagnosis

A great challenge in areas which are endemic for arboviruses is to make a specific diagnosis quickly and at low cost, and for this purpose one has to rely essentially on clinical-epidemiological features, so as to manage patients adequately(13-15,20). Clinical criteria are the primary tool used when dealing with an epidemic, and they must be carefully applied by health professionals who are in the frontline(20). Besides, the similar clinical syndromes produced by DENV, CHIKV and ZIKV, plus the inconclusive laboratory tests available for their diagnosis justify a populational study such as ours. In Rio de Janeiro, during the 2015 ZIKV outbreak, the most frequently described signs and symptoms were rash, pruritus and conjunctivitis(18), and these were the features given the highest consideration for the classification of cases in the present study. More recently, Braga and colleagues (2017)(20) evaluated clinical criteria for the arboviral diseases, in a study where only patients who had RT-PCR results for DENV, ZIKV and CHIKV available for confirmation of diagnosis during the epidemic period (2014 to 2016) were included. Symptoms among suspected cases such as rash (86%) or conjunctival hyperemia (78%), without other general clinical manifestations such as fever, petechiae or anorexia, were the bestcase definition of ZIKV. We note less than half of our patients classified as presenting ZIKV had a fever above 38.5º C.

Regarding CHIKV, a significantly greater number of suspected cases occurred in 2016, with a more than 10-fold increase in absolute numbers. Although we have the limitation of clinical diagnosis, and some of the cases might be actually ZIKV (where painful periarticular swelling of hands, feet and ankles is not rare), it is interesting to note that the most frequently affected group were older women (80% of cases were in women 15-64 years of age), as has been described in other reports(18,21).

Age and gender distribution

The age distribution in the suspected cases of arboviral illness showed a tendency to predominantly affect adults in 2015 and 2016, similarly to other studies on ZIKV and CHIKV (18,19).

Arboviral illness was seen most often in female patients in our study, and this has been described elsewhere(18). Incidence rate was 3,353/100,000 inhabitants in the year 2016 for females in our study area. This may be because women are more exposed to home vectors, as they usually spend more time in the household than men due to domestic chores. Another explanation may be different ways of dressing, with more exposure of the lower limbs and ankles by women, facilitating mosquito bites(22). Moreover, a higher incidence in the 30-39 years stratum in women suspected of ZIKV was noted; this stratum includes the most sexually active years (20-49 years) and this finding may indicate other non-vector forms of transmission, such as the sexual route(23) and/or contact with other bodily fluids such as saliva and urine(24). Coelho et al(20) using data on the incidence of ZIKV in 2015 to 2016, and DENV in 2015 to 2016, and 2013 for the city of Rio de Janeiro, Brazil, concluded that women of reproductive age were 90% more likely to acquire ZIKV than their male counterparts. This was an important epidemiological study in an area of active ZIKV transmission, which ruled out that female health seeking behavior or systematic testing of pregnant women were the cause of more reporting of ZIKV in women of childbearing age.

Mosquito vectors

Aedes albopictus immature forms were consistently found in depots in the urban area studied, and corresponded to one third of the immature forms found both in the rainy season and in the dry season. The district of Xerem has an extensive green area surrounding the urban area which includes forested land. *Ae. albopictus* is the primary vector for CHIKV, is more sylvatic than *Ae. aegypti* and may be relevant in the perpetuation of this virus in this and in other similar areas of Brazil. *Ae. albopictus* has a better survival rate than *Ae. aegypti* as it is more tolerant to lower temperatures, and in this way it may be more prevalent in certain regions(25); the adaptation of the emerging Indian Ocean lineage (IOL), permitted its dispersal and caused a series of epidemics(26), despite this, this lineage was not definitively identified as the CHIKV epidemic lineage. We did not find an important relationship between HII and temperatures for both vectors, but a strong positive correlation was found for rainfall. A study from Taiwan has suggested moderate to high precipitation indices may increase the incidence of dengue cases(27).

Other studies have shown that egg laying behavior of *Ae. aegypti* and *Ae. albopictus* are associated to climatic conditions and different types of breeding sites(10). In our study we found that depots of the B and C groups, essentially home depots, predominated in March and June, suggesting that there is an urgent need to address these depots in combatting the mosquito vectors.

Although *Ae. aegypti* has a synanthropic and anthropophilic behavior(16), *Ae. albopictus* has shown adaptation to several habitats, both rural and urban, becoming more disperse(10). The adaptation of this vector to the urban environment in Brazil may bring serious public health issues, as it is an important vector for CHIKV as well as for DENV(28). In fact, a recent publication(29) comments on the fact that the distribution of CHIKV cases has progressively moved towards the inland and the forested areas in Brazil based on Pan American Health Organization maps on CHIKV. The authors found that other mosquitoes, such as *Haemagogus leucocelaenus* and *Ae. terrens* from the state of Rio de Janeiro, experimentally infected, were excellent vectors of all tested lineages of CHIKV and therefore they suggest there is a definite risk for CHIKV to establish a sylvatic cycle in the tropical Americas if local non-human primates can amplify the virus to infect wild primatophilic mosquitoes. Interestingly, another report showed that the proportion of both *Ae. aegypti* and *Ae. albopictus* experimentally infected mosquitoes capable of transmitting ZIKV on the total number of tested mosquitoes, was unexpectedly low suggesting that these two species were poorly competent to ZIKV[30]. These authors suggest that the ZIKV epidemic seen was due essentially to the naive population. Moreover, it is of note that in 2018, the sylvatic cycle of ZIKV was described in São Paulo and Minas Gerais[31] and that a previous study had shown the sylvatic cycle in the Northeast of Brazil[32]; this may lead to the long-term maintenance of the circulation of ZIKV in our country.

4 CONCLUSION

The clinical-epidemiological diagnosis of DENV, ZIKV and CHIKV suggested the co-circulation of these three arboviruses in the studied area, which showed an epidemic behavior in 2016, mainly for ZIKV and CHIKV. This is due to the wide dispersion of vectors and, in the case of ZIKV, probably to the other transmission routes, as suggested by the analysis of age and sex most affected, that is, sexually active women. Due to the ZIKV outbreak in Brazil, several cases of congenital syndrome, mainly associated with microcephaly in newborns, have been reported with the detection of viral RNA in the affected patients' amniotic fluid[6]. The threat posed by ZIKV has major implications not only for immediate public health, but also for the family economy and social resources, due to the persistent long-term sequelae of congenital ZIKV syndrome. Despite the data presented, the epidemiological clinical criteria has many weaknesses, which makes reliable and sensitive surveillance and diagnostic tools for these arboviruses desirable.

High precipitation rates favor the high infestation of houses by immature forms of *Ae. aegypti* and *Ae. albopictus*, allowing the co-circulation of DENV, ZIKV and CHIKV, which reinforces the importance of vector control measures mainly in the rainy season.

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