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A localized outbreak of Chikungunya virus in Salvador, Bahia, Brazil

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Abstract

A localized chikungunya virus (CHIKV; East/Central/South African genotype) outbreak (50 cases, 70% laboratory-confirmed; attack rate: 5.3 confirmed cases/100 people) occurred in a Salvador, Brazil neighborhood, between Apr-Jun/2017. Highly clustered cases in space and time, mostly along a single street, highlight an increased risk of CHIKV transmission among pockets of susceptible populations. This finding underscores the need for ongoing local level surveillance for arboviral outbreaks.

Keywords: Chikungunya virus, Alphavirus, Outbreak

Chikungunya virus (CHIKV), an alphavirus transmitted mostly by *Aedes* mosquitoes, has become a serious public health problem in Brazil. It was first detected in the country in September 2014 and spread rapidly, joining the long-established Dengue virus (DENV) and the nearly concomitantly introduced Zika virus (ZIKV). [1, 2] Salvador, the fourth largest city of Brazil, where DENV has been transmitted endemically since 1995, experienced concomitant outbreaks of CHIKV and ZIKV in 2015. [2, 3] However, since 2016, CHIKV transmission in Salvador has been very low with only sporadic cases reported. [4] Herein, we describe the investigation of a localized CHIKV outbreak that occurred in suburban Salvador in 2017.

Between 15 April and 25 June 2017, we investigated a cluster of 39 cases reported due to fever, arthralgia and other symptoms compatible with an arboviral infection. [3] All initially reported cases lived in a small area within Coutos, a poor suburban neighborhood of Salvador (Figure 1), characterized by a disorganized spatial distribution of self-built houses, without regular water supply or closed sewage pipes. The area is adjacent to the sea and borders the train tracks, where garbage and various mosquito-producing containers were abundant.

We visited all 230 households in the area to detect additional cases presenting fever and arthralgia during the previous 30 days, and among the 662 residents that were counted, 11 additional cases were detected, totaling 50 arboviral suspected cases, living in 33 households. Clinical data, as well as blood samples, were collected from 45 (90%) of these cases.

As the initial clinical suspicion was CHIKV or DENV infection, serum samples were first tested by IgM ELISA for CHIKV (Euroimmun, Germany) and DENV (Focus

Diagnostics, USA). In addition, RNA was extracted from 17 cases with an available sample stored at -70ºC and amplified by RT-PCR using primers for CHIKV, ZIKV, and DENV, as well as for Oropouche (OROV), Mayaro (MAYV), and Yellow Fever virus (YFV). [5-10] The PCR products were sequenced using the Sanger method.

To investigate mosquitoes species potentially involved in the outbreak, we surveyed the household of suspected cases. Pools of captured female mosquitoes from each species were tested by RT-PCR for the same arboviruses as described above. Virus isolation was also attempted in C6/36 (*Ae. albopictus*) cell cultures.

Of the 45 patients tested by CHIKV IgM ELISA, 35 (77.8%) were positive, 8 (17.8%) negative, and 2 (4.4%) equivocal. IgM ELISA testing for DENV was performed for 31 (68.9%) of the 45 samples and 3 (9.7%) were positive, 25 (80.6%) negative, and 3 (9.7%) equivocal. Of the three patients IgM-positive for DENV, two were also positive for CHIKV. Of the 17 patients tested by RT-PCR, 8 (47.1%) were positive for CHIKV. All of them were also positive by the CHIKV IgM ELISA. No other arbovirus was detected by RT-PCR.

We sequenced the PCR products of seven CHIKV confirmed cases and the consensus sequences (M6591452-M6591458) showed high nucleotide identity (98-100%) among them, and with sequences obtained from humans in Salvador during the 2015 outbreak (KU940225), all belonging to the East/Central/South African (ECSA) genotype. [11]

The 50 cases had a median age of 38 years, and 23 (46%) were females (Table 1). Clinical characteristics of the laboratory-confirmed and unconfirmed cases were similar, except for rash, which were 2.5-fold times more common among the confirmed group, but this difference was not statistically significant (P=0.06) (Table 1).

Arthralgia was present in all the 50 cases and 45 (95%) reported it to be symmetric and poliarticular. None of the patients was hospitalized.

The overall attack rate was 7.6 cases/100 persons (8.5 cases/100 men; 6.4 cases/100 women; 5.4 cases/100 children <15 years of age; 7.3 cases/100 persons 15-39 years of age, and 9.0 cases/100 adults ≥ 40 years of age). Considering only the laboratory-confirmed CHIKV cases, the attack rate was 5.3 cases/100 persons (5.7 cases/100 men; 4.9 cases/100 women; 0.7 cases/100 children <15 years of age; 5.4 cases/100 persons 15-39 years of age, and 5.6 cases/100 adults ≥ 40 years of age). Of the 50 cases, 45 (90%) resided in the same street (Figure 1).

The first case initiated symptoms in April, but most cases occurred during May (Figure 2). The spatial and temporal distribution of all cases was analyzed with a k nearest neighbor (k-NN) statistic for space-time clustering using ClusterSeer software (Biomedware, Ann Arbor, MI). [12] The k-NN statistic is the number of case pairs that are k^{th} nearest neighbors when both space and time are considered. The null hypothesis is that nearest neighbor relationships in space and time are independent from each other. Cases were highly clustered in space and time, with proximal cases also the ones closest temporally ($P<0.01$ for the first nearest neighbors).

We performed entomological surveys in 27 of the 33 houses with suspected cases, between 14 June and 18 July 2017 (Figure 1). A total of 125 adult mosquitoes were collected in 21 houses, with *Cx. quinquefasciatus* the most abundant (99, 79.2%; 69 of them (69.7%) female), followed by *A. aegypti* (26, 20.8%; 8 of them (30.8%) female). The RT-PCR and culture isolation from all of them yielded negative results.

After 2.5 years of the first CHIKV detection in Brazil and the ensuing countrywide spread of the virus, we found that CHIKV maintains its potential to cause

highly localized outbreaks. It is likely that similar small, circumscribed outbreaks, which are not easily detectable, help explain the prolonged CHIKV transmission in Brazil, in contrast to the more widespread, explosive ZIKV transmission pattern that was observed. [2]

Most of the cases during this outbreak were residents of a single street, and the strong space-time clustering points to an outbreak that travelled from house to neighboring house. This is probably associated with transmission by multiple mosquitoes that became infected at about the same time and/or by a small number of infected mosquitoes feeding on multiple proximal hosts. [13]

Poor socioeconomic conditions in the neighborhood, such as unreliable water supply and waste collection services, and the accumulation of containers that serve as habitats for *Aedes* larvae may have facilitated this outbreak. In response, the Zoonosis Control Center, the municipal agency responsible for mosquito surveillance and control, intervened in the area aiming to reduce mosquito numbers by removing any sources of standing water, treating water-holding containers with larvicides, and outdoor spraying of insecticide in 5 cycles (Figure 2). In addition, the community was sensitized to act together to eliminate potential mosquito breeding sites. As many of these interventions were conducted after the outbreak peak, we were not able to determine their role in preventing additional cases. We also could not establish mosquito species implicated in CHIKV transmission during this outbreak because we did not detect infected mosquitoes. This is probably because the number of collected and tested mosquitoes was low due to the insecticide application in the region, which preceded our entomological surveys.

Given the complex epidemiological scenario in the Americas since CHIKV and ZIKV joined DENV as common etiologies of urban febrile diseases, we reinforce the importance of integrating data from clinics, entomological surveys, epidemiological surveillance, and laboratory testing during outbreak investigations and surveillance activities. Only with ongoing local level surveillance of arboviral diseases, outbreaks affecting pockets of susceptible population will be promptly detected, in order to guide timely control measures.

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Conflict of interest

The authors declare that they have no competing interests.

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Table 1: Demographic and clinical characteristics of patients suspected of chikugunya virus (CHIKV) infection during a community outbreak in, Salvador, Brazil, according to CHIKV laboratory test results, April to June 2017.

Reported characteristics	Total suspected cases (n=50)	Laboratory-confirmed cases[1] (n=35)	Unconfirmed cases[2] (n=15)	P value[3]
	Number (%) or median (interquartile range)			
Demographic				
Female	23 (46)	17 (48)	6 (40)	0.75
Median age	38 (23 – 48)	38 (23 - 48)	42 (28 - 48)	0.84
Clinical				
Fever	50 (100)	35 (100)	15 (100)	1.00
Arthralgia	50 (100)	35 (100)	15 (100)	1.00
Poliarticular[4]	45 (90)	32 (91)	13 (86)	0.62
Symmetric[5]	45 (90)	31 (88)	14 (93)	1.00
Myalgia	49 (98)	35 (100)	14 (93)	0.30
Prostration	43 (86)	31 (88)	12 (80)	0.41
Chills[6]	39 (79)	25 (74)	14 (93)	0.14
Headache	36 (72)	26 (76)	10 (66)	0.50
Retro-orbital pain	30 (60)	20 (57)	10 (66)	0.75
Pruritus[7]	27 (56)	18 (54)	9 (60)	0.76
Joint edema	24 (48)	17 (48)	7 (46)	1.00
Nausea	22 (44)	15 (42)	7 (46)	1.00
Rash	21 (42)	18 (51)	3 (20)	0.06
Conjunctival hyperemia	20 (40)	15 (42)	5 (33)	0.75
Vomit	12 (24)	8 (22)	4 (26)	1.00
Swollen lymph nodes	7 (14)	6 (17)	1 (7)	0.65

[1] All 35 laboratory-confirmed patients were positive by CHIKV IgM ELISA; 8 of them were also positive by CHIKV RT-PCR.

[2] Of the 15 unconfirmed patients suspected of CHIKV infection, 8 were negative, 2 equivocal, and 5 not tested by CHIKV IgM ELISA.

[3] Fisher exact test P values for the comparisons between confirmed and unconfirmed cases suspected of CHIKV infection.

[4] Polyarticular arthralgia defined by pain in more than one joint.

[5] Symmetric arthralgia defined by pain in at least one pair of joints.

[6] Data not available for one laboratory-confirmed CHIKV infection case.

[7] Data not available for two laboratory-confirmed CHIKV infection cases.

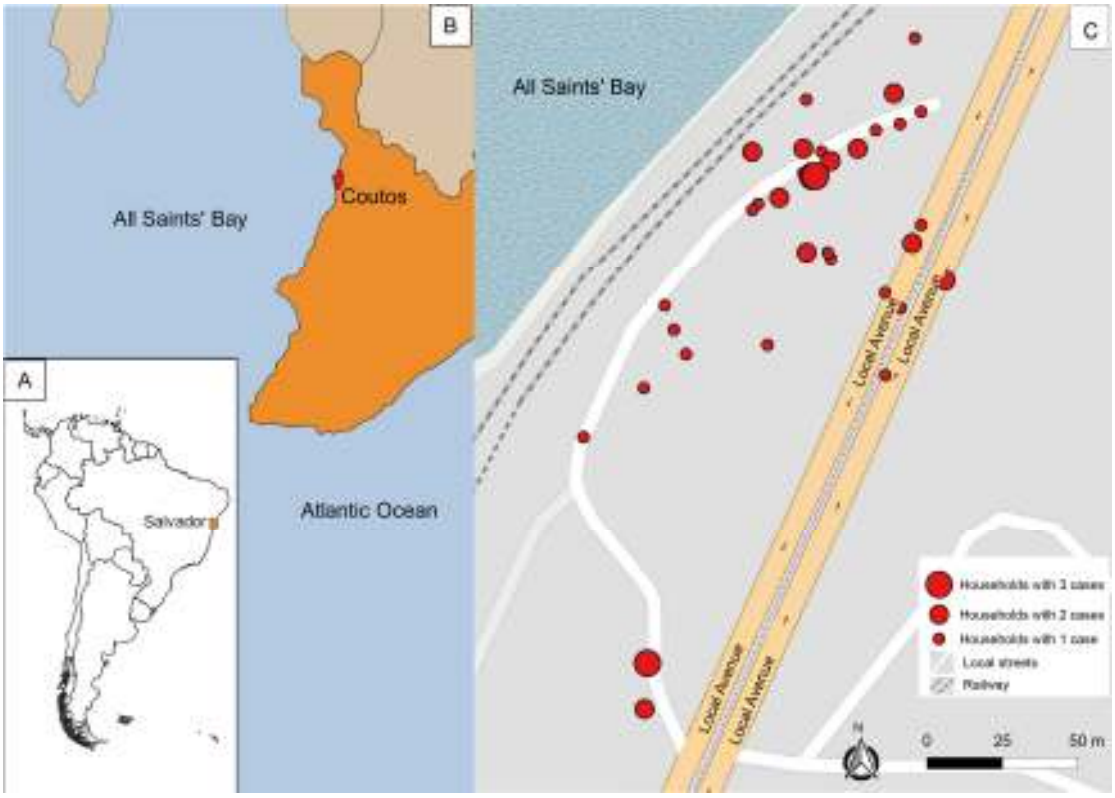

Figure 1: Spatial distribution of households of chikungunya cases during an outbreak in Coutos neighborhood, Salvador, Brazil. A. Location of Salvador in Brazil. B. Location of Coutos neighborhood in Salvador. C. Spatial distribution of the households of chikungunya cases (mostly along a side street situated between a railway line and a larger avenue.

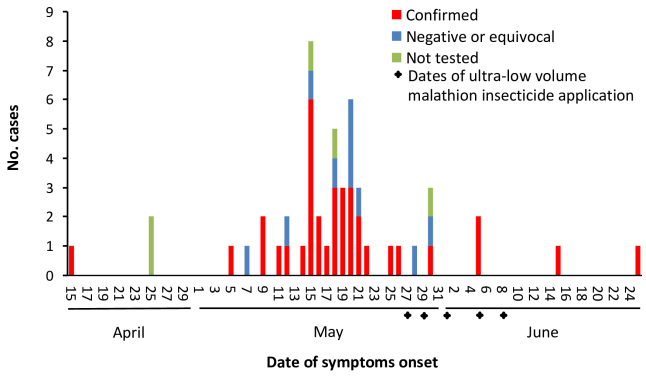

Figure 2: Number of cases per day during the chikungunya virus outbreak in Coutos neighborhood, Salvador, Brazil.