Short Communication

Chikungunya virus ECSA lineage reintroduction in the northeasternmost region of Brazil

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ARTICLE INFO

ABSTRACT

The Northeast region of Brazil registered the second-highest incidence proportion of Chikungunya fever in 2019. In that year, an outbreak consisting of patients presenting with febrile disease associated with joint pain was reported by the public primary health care service in the city of Natal, in the state of Rio Grande do Norte, in March 2019. At first, the aetiological agent of the disease was undetermined. Since much is still unknown about chikungunya virus' (CHIKV) genomic diversity and evolutionary history in this northeasternmost state, we used a combination of portable whole-genome sequencing, molecular clock, and epidemiological analyses that revealed the reintroduction of the CHIKV East-Central-South-African (ECSA) lineage into Rio Grande do Norte. We estimated that the CHIKV ECSA lineage was first introduced into Rio Grande do Norte in early June 2014, while the 2019 outbreak clade diverged around April 2018, during a period of increased Chikungunya incidence in the Southeast region, which might have acted as a source of virus dispersion towards the Northeast region. Together, these results confirm that the ECSA lineage continues to spread across the country through interregional importation events, likely mediated by human mobility.

Introduction

Since its emergence in East Africa, the chikungunya virus (CHIKV) has caused more than 70 epidemics worldwide, mainly in Southeast Asian and Latin American countries (Mascarenhas et al., 2018).

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Table 1
Characteristic and clinical symptoms of patients with Chikungunya fever.

	Patients (n = 13)
Mean year of age (SD)	42 ± 22
Gender	
Female	4 (31%)
Male	9 (69%)
Symptoms onset date range	19/Mar/2019–29/Mar/2019
Presenting symptoms	
Fever	13 (100%)
Myalgia	8 (62%)
Cephalea	7 (54%)
Skin rash	8 (62%)
Vomiting	2 (15%)
Arthralgia	12 (92%)
Retro-orbital pain	4 (31%)
Conjunctival hyperemia	2 (15%)
CHIKV RT-qPCR mean Ct value (cycle threshold)	28.15 ± 6.69

Data is presented as a number (%) unless otherwise indicated. SD, standard deviation. RT-qPCR, quantitative reverse transcription PCR.

Northeast Brazil presented with the second-highest incidence (59.4 cases per 100,000 population) of Chikungunya reported in 2019 in the country (Ministério da Saúde, 2020). In that year, an outbreak of 13,713 Chikungunya cases was recorded in the state of Rio Grande do Norte, in the far northeastern tip of Brazil, where a previous outbreak of almost 25,000 probable cases was reported in 2016 (Ministério da Saúde, 2018). Despite the high number of cases, much remains unknown about CHIKV genomic diversity and the evolutionary history in this northeastern state. We generated eight CHIKV genomes from the 2019 outbreak registered in Natal, the capital city of Rio Grande do Norte, and provide a genomic epidemiological report of the virus circulating in that state.

Methods

An outbreak consisting of patients presenting with febrile disease associated with joint pain was reported by the public primary health care service in the city of Natal, Rio Grande do Norte, in March 2019. Since the aetiological disease agent was undetermined at first, serum samples were provided to the State Central Laboratory of Public Health (Dr. Almino Fernandes) and then sent to the Laboratory of Flavivirus at the Oswaldo Cruz Foundation (Fiocruz) to confirm the laboratory diagnosis by RT-qPCR (Lanciotti et al., 2007). CHIKV-RNA positive samples were submitted to a MinION sequencing protocol (Quick et al., 2017), and newly generated genomes were used for phylogenetic analysis (more details in Supplementary files).

Results

Laboratory diagnostics confirmed thirteen positive samples of CHIKV RNA with a mean Ct value of 28.15. We generated eight CHIKV genomes from patients with an average age of 42 years. We
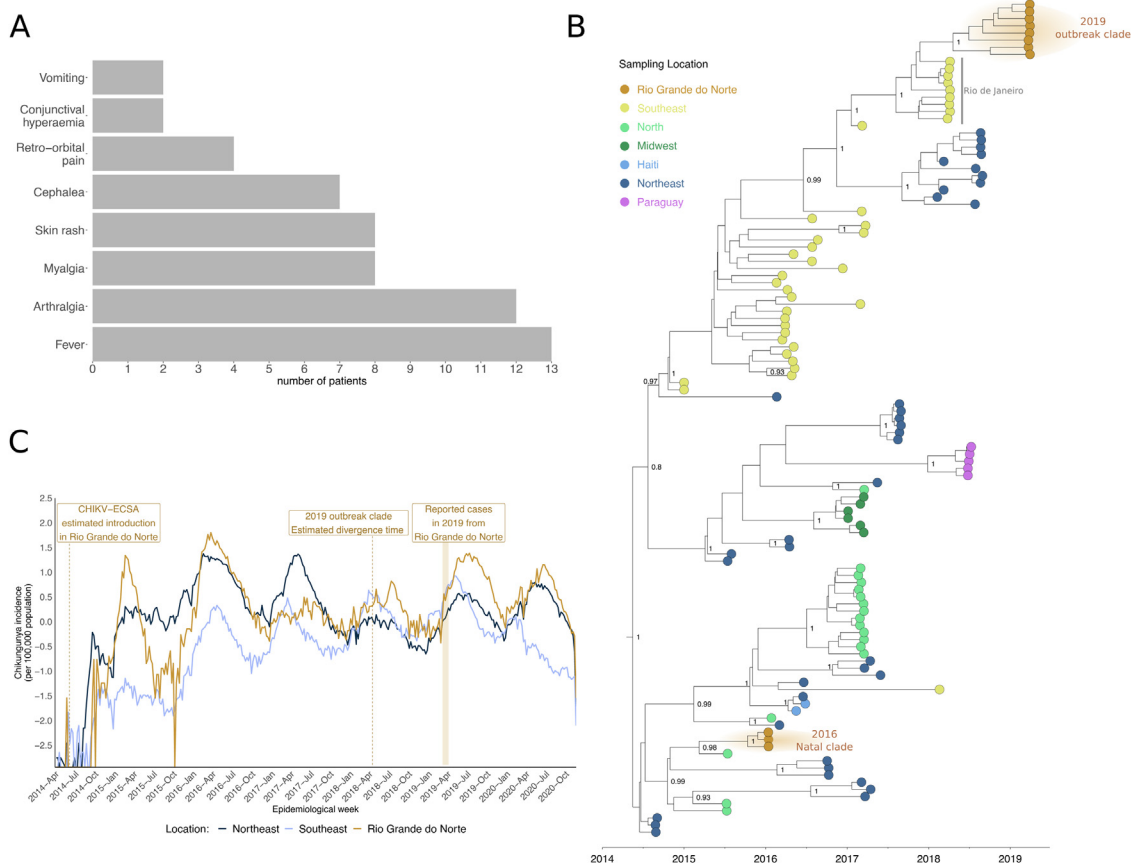
Figure 1. Symptoms frequency, epidemic curves, and phylogenetic analysis from Chikungunya outbreaks reported in Rio Grande do Norte, northeast Brazil. (A) Symptom frequency among thirteen patients with confirmed laboratory diagnosis of chikungunya virus infection. (B) Time-scaled maximum clade credibility tree of chikungunya virus East-Central-South African lineage in Brazil, including the eight new genomes generated in this study. Tips are colored according to the sample source location. Values around nodes represent posterior probability support of the tree nodes inferred under Bayesian Evolutionary Analysis using a molecular clock approach. (C) Time-series plot of Chikungunya incidence per 100,000 population calculated from notified cases from Rio Grande do Norte, Northeast, and Southeast regions of Brazil. Y-axis values were log-transformed.

found five (38%) patients who presented with fever associated with myalgia, arthralgia, and rash (see Table 1 and Figure 1A). The eight CHIKV genomes generated have an average genome coverage of 93% (ranging from 92% to 94%); they belonged to the East-Central-South-African (ECSA) lineage as indicated by our Maximum Likelihood (ML) phylogeny (see Supplementary files).

We inferred a time-measured phylogeny using a dataset comprising 110 Brazilian sequences plus two isolates from Haiti and five from Paraguay. Both our ML and Bayesian phylogenies showed the 2019 CHIKV isolates from Rio Grande do Norte formed a single well-supported clade (hereafter, the 2019 outbreak clade; posterior probability = 1.00), which was closely related to isolates from Rio de Janeiro (posterior probability = 1.00) (Figure 1B). Note that this 2019 outbreak clade did not cluster to the other three previously published isolates from Natal, Rio Grande do Norte, sampled in January 2016 (hereafter, the 2016 Natal clade; posterior probability = 1.00). We estimated the time of the most recent common ancestor (TMRCA) of the 2019 outbreak clade to be around mid-April 2018 (95% BCI: early December 2017 to late August 2018) (Figure 1B and C). Moreover, the TMRCA of the CHIKV-ECSA lineage in Rio Grande do Norte was estimated to be in early June 2014 (95% BCI: late December 2013 to early October 2014).

Weekly reported incidences revealed three major outbreaks in Rio Grande do Norte during early 2015, early 2016, and mid-2019. Smaller waves were observed throughout the years from 2017 to 2020, indicating the persistence of the virus in the state through year-round transmission cycles (see Figure 1C), which were also observed in other regions of Brazil (Supplementary files).

Discussion

In this study, we generated eight CHIKV genomes from symptomatic cases of Chikungunya fever reported during an outbreak in March 2019 in Natal, the capital city of the state of Rio Grande do Norte. We found fever, arthralgia, myalgia, and rash were the most common presenting symptoms, which are similar findings to those described in other studies of suspected cases during the 2016 Chikungunya outbreak in Natal (Monteiro et al., 2020) and on Chikungunya-associated neurological disease cases reported from 2014 to 2016 in another northeastern Brazilian state (Brito Ferreira et al., 2020).

Our phylogenetic analysis revealed the 2019 outbreak clade is distantly related to other isolates from the previous 2016 outbreak in Rio Grande do Norte. This suggests at least two importation events of CHIKV-ECSA lineage occurred in Rio Grande do Norte; the time of the first virus importation event estimated to be early June 2014. This estimate indicates the virus was circulating for several months before the first Chikungunya cases were confirmed in 2015 in Rio Grande do Norte, including one Chikungunya-associated death, as indicated by available epidemiological surveillance data from the State Health Department (Secretaria da Saúde Pública, 2017).

From the combined analysis of regional epidemic curves and time-scaled phylogeny, we hypothesize that the 2019 outbreak in Rio Grande do Norte was caused by a second event of CHIKV introduction imported from Rio de Janeiro. We estimate the divergence time of the 2019 outbreak clade to be mid-April 2018, which corresponds to a period of increased Chikungunya incidence in the southeast region, where Rio de Janeiro is located. This might be explained by virus interregional spread through people's movement, as discussed elsewhere (Candido et al., 2020; Churakov et al., 2019).

Our study increases the number of CHIKV genomic sequences and, consequently, provides more information on the evolution and genomic diversity of the ECSA lineage currently circulating

in Brazil's Northeast region, where this lineage was first introduced.

Ethics statement

This project was reviewed and approved by the Comissão Nacional de Ética em Pesquisa (CONEP) [National Research Ethics Committee], as part of the arboviral genomic surveillance efforts within the terms of Resolution 510/2016 of CONEP, by the Pan American Health Organization Ethics Review Committee (PAHOERC) (Ref. No. PAHO-2016-08-0029), and by the Oswaldo Cruz Foundation Ethics Committee (CAAE: 90249218.6.1001.5248).

Data availability

Newly generated CHIKV sequences have been deposited in GenBank under accession numbers MW260512-MW260519.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2021.01.026.

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