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Circulation and molecular characterization of *Chikungunya virus* (*Togaviridae*) reveals a new mutation (E2-N207D) in Espirito Santo, Brazil

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ABSTRACT. *Chikungunya virus* (CHIKV) is an RNA virus from the family *Togaviridae* transmitted primarily by *Aedes* mosquitoes. The first report of CHIKV infection in Brazil dates to 2014; since then, the virus has become a major public health challenge. The main goal of this study was to perform a phylogenetic analysis of CHIKV isolates from febrile patients from Espirito Santo (ES) state during the 2017 outbreak to identify the genetic diversity of circulating CHIKV strains. CHIKV RT-qPCR confirmed cases were sequenced for phylogenetic relationship inferences and molecular characterization. Phylogenetic results showed that the virus in the 2017 ES outbreak belongs to the ECSA genotype. Molecular characterization revealed a new mutation in ES strains (E2-

N207D). This variation generated an amino acid substitution by exchanging asparagine (N) for aspartate (D) at position 207 and it is associated with an anchoring groove, possibly interfering in viral envelope assembly and associated interaction with the target cell. Here we report a CHIKV-ECSA IIa outbreak that demonstrates the ES population's vulnerability to an Asian strain of this virus circulating elsewhere in Brazil. Despite the small sample size, this study describes phylogenetic data about CHIKV in ES state that helps expand of the virus genotype database, and reveals a new E2 protein CHIKV variant (E2-N207D). This data helps improve understanding of chikungunya fever in order to design efficient public health control strategies.

Key words: Chikungunya virus; Phylogeny; Molecular characterization; Espírito Santo state; Brazil

INTRODUCTION

Chikungunya virus (CHIKV) is a single-stranded RNA virus from the family *Togaviridae*, genus *Alphavirus,* species Chikungunya virus*,* transmitted by infected females of the mosquitos *Aedes aegypti*, *Aedes albopictus* and *Culex quinquefasciatus* (White et al., 2018). It causes an acute febrile disease characterized by high fever, polyarthralgia, headache, skin rash and severe joint pain. Chronic arthralgia can last from months to years in approximately 40% of the infected people (Feldstein et al., 2017).

CHIKV infection was first reported in 1952, in Tanzania (Ross, 1956). Since then, sporadic cases were observed in other countries in Africa and Asia during the 1960s and 70s. However, between 2005 and 2006 an outbreak of CHIKV in Indian Ocean islands caused 1.2 million cases (Schuffenecker et al., 2006). Since 2015, CHIKV has spread to several countries on most continents, and it is estimated that about 1.3 billion people are now living in Chikungunya-endemic areas (Nsoesie et al., 2016). These many outbreaks have occurred across the globe due to exposure of naive human populations or in areas with previous circulation of other genotypes.

Phylogenetic studies have revealed three major CHIKV genotypes: East/Central/South African (ECSA), Asian and West African (WA). Within ECSA, there are three subgroups, two derived from Africa (ECSA I and II) and another isolate from the Indian Ocean region (IOL; ECSA III) (White et al., 2018). A recent study has shown a clear distinction within the ECSA II isolate, with a clade containing strains from Brazil and Haiti (ECSA IIa) and another including strains from various regions (ECSA IIb) (White et al., 2018).

From late 2014, the introduction of the ECSA genotype in the Brazilian northeast (Bahia state) and an Asian genotype in the north and northeast (Amapá, Pará, Roraima and Pernambuco states) were reported (Nunes et al., 2015; Naveca et al., 2019). During the last years, the ECSA genotype was notified as predominant in the country (Machado et al., 2019; Naveca et al., 2019; Xavier et al., 2021), while the Asian genotype was only reported in Pernambuco state in 2016 (Machado et al., 2019).

Despite approximately 200,000 CHIKV infection cases in Brazil during the outbreak of 2016, the distribution of viral lineages isolated from patients infected in different regions is unknown (Machado et al., 2019), indicating a need for continuous virological surveillance. Thus,

the main goal of this study was to perform a phylogenetic analysis of CHIKV isolates from Espírito Santo (ES) state febrile patients during the 2017 outbreak to identify circulating CHIKV strains genetic diversity. Additionally, a molecular analysis was carried out to identify mutations previously described in the literature associated with viral transmission augmentation. The results revealed a new mutation in ES strains.

MATERIAL AND METHODS

The study used isolates from 10 patients infected by CHIKV, confirmed by RT-qPCR in the Central Laboratory of Espírito Santo of the Espírito Santo Health Department (LACEN/SESA-ES). The samples were collected from patient's resident in three municipalities in the state of Espírito Santo. Those isolates were sequenced for phylogenetic relationship inferences with other 56 Brazilian and global sequences retrieved from GenBank. These sequences were chosen to sample the main genotypes phylogenetic trees, as well as the subclusters within ECSA. Strain lineage was identified by analysis of a partial *E2* gene region (432 nt) using primers forward E2 5'TGGAACAATGGGACACTTC 3' and reverse E2 5'AGCTCCTGGTGTCAGTTCRT 3'. All reactions were performed using *GoTaq® Probe 1-Step RT-qPCR System kit* (Promega, Wisconsin, United States) and sequencing reactions were carried out using *BigDye™ Terminator v3.1 Cycle Sequencing Kit* (Applied Biosystems, California, United States) at *Genomic Platform of DNA Sequencing- PDTIS/FIOCRUZ*.

A Maximum likelihood tree was constructed based on GTR +G +I model with 4 gamma categories by use of the Akaike Information Criterion with 1000 bootstrap values of the dataset in MEGA 7 (Kumar et al., 2016). Sequences obtained were deposited in GenBank under accession numbers: MH455339-MH455344 and MN752695-MN752698.

To infer phylogenetic relationships inside ECSA IIa clade, a Bayesian approach was conducted in BEAST v.1.10.4 (Suchard et al., 2018). A GTR +G +I with four gamma categories and a 1+2,3 codon partition model was used. The tree was constructed with a relaxed lognormal molecular clock model and the Bayesian Skyline tree prior. Fifty million chains were run and parameters convergence (effect sample size >200) were evaluated in Tracer v.1.7.1 (Rambaut et al., 2018). A maximum clade credibility tree was achieved in TreeAnnotator v.1.10.4. Phylogeny formatting was performed on the Figtree v.1.4.3.

Ethics

This research was approved by the Federal University of Espírito Santo Research Ethics Committee, registry 1.819.673. All procedures performed in studies were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration. The informed consent was dispensed by the Ethics Committee.

RESULTS

The phylogenetic tree showed that all circulating CHIKV strains from ES belong to the ECSA genotype and ECSA IIa subgroup (Figure 1). Molecular characterization of partial E2 protein did not reveal the adaptive mutation E2-V264A, which is related to enhanced fitness in *A. aegypti* (Agarwal et al., 2016), the main vector in Brazil.

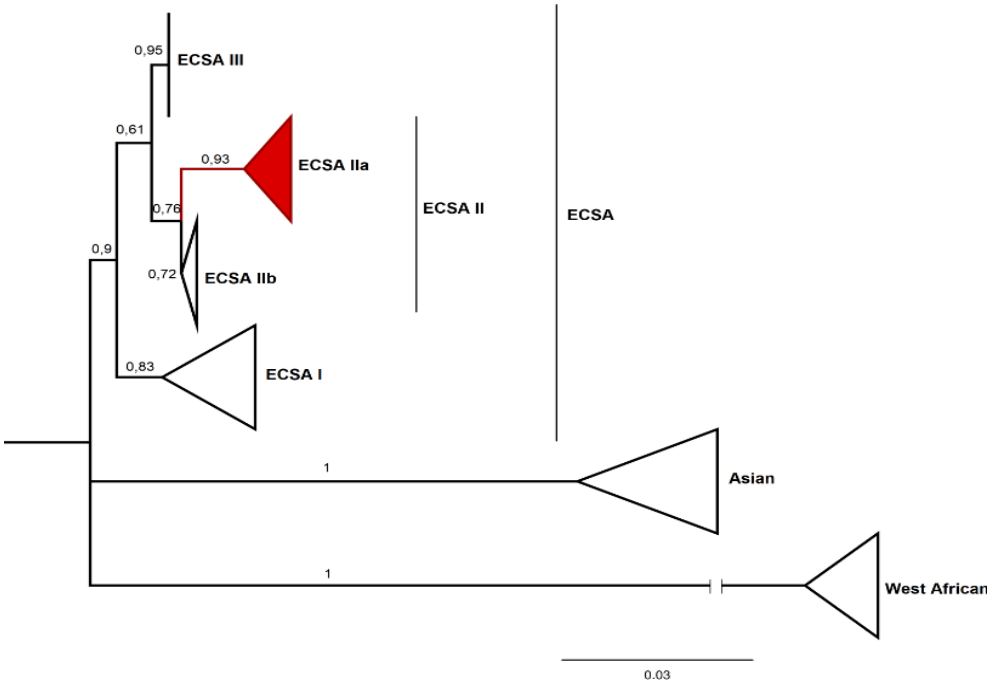

Figure 1. *Chikungunya virus* phylogeny performed in the MEGA 7 software by Maximum likelihood and GTR+G+I with four gamma distribution parameters. ECSA IIa subgroup is in red. A 1,000-replicate bootstrap was used and support above 60 are shown.

The molecular approach showed that five ES sequences shared a synonymous alteration and form a monophyletic clade in ECSA IIa subgroup (Figure 2). Furthermore, in one sample (MH455339) was observed a non-synonymous variation (E2-N207D). This variation generated an amino acid substitution by exchanging asparagine (N) for aspartate (D) at position 207. Phylogenetic analysis revealed that MH455343 sequence did not clustered with others ECSA IIa sequences. Relaxed molecular clock analysis estimated the CHIKV entrance in ES state between May/June 2016 with a 95% highest posterior density between February 2016 - January 2017.

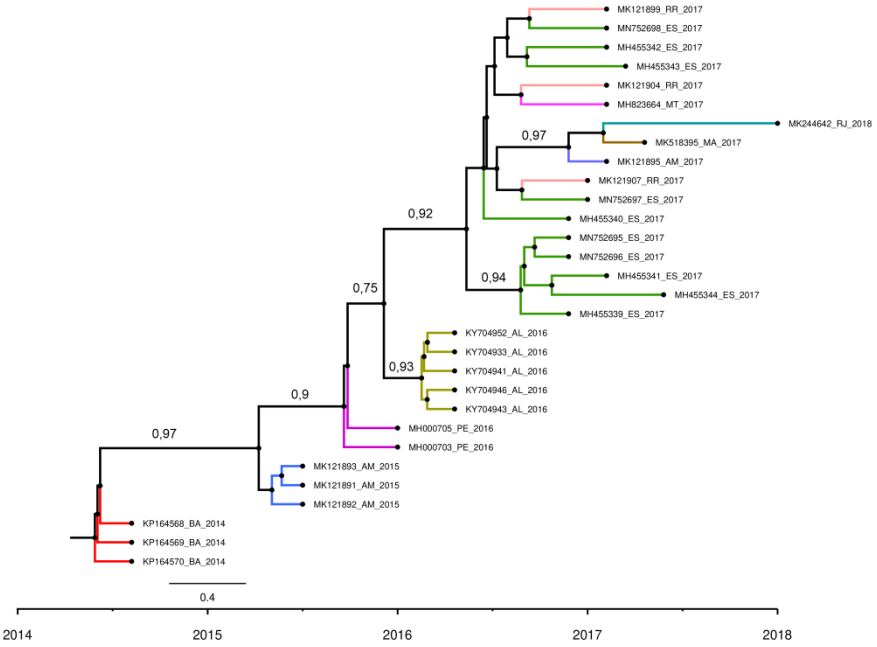

Figure 2. Bayesian reconstruction with relaxed molecular clock of ECSA IIa genomes found in Brazil (states of: Roraima (RR), Rio de Janeiro (RJ), Maranhão (MA), Alagoas (AL), Pernambuco (PE), Mato-Grosso (MT), Amazonas (AM)) with the index case from Feira de Santana-Bahia (BA). Study samples are in green. Support above 75 is shown.

DISCUSSION AND CONCLUSIONS

Since the first CHIKV-ECSA confirmed cases in Bahia state in 2014 (Nunes et al., 2015), other Brazilian states reported CHIKV-ECSA outbreaks, so that the presence of the virus is now confirmed in all states of the country (Ministério da Saúde, Secretaria de Vigilância em Saúde, 2021b). These data point to the great power of dissemination of ECSA strain in Brazil and highlight the importance of further and continued studies about newly CHIKV genomic alterations associated with clinical responses.

The CHIKV-Asian genotype firstly reported in Amapa state in 2014 is still circulating in Brazil (Nunes et al., 2015). Recently, an ECSA and Asian coinfection was reported in Pernambuco state, Brazilian northeast region (Machado et al., 2019). Most of CHIKV outbreaks in Brazil were related to ECSA strain, but the confirmation that the Asian strain is still circulating in the country suggests that most of the population might not have an effective immune response to this strain, which could lead a new outbreak wave in Brazil.

Interestingly, it is the first time that E2-N207D is described in CHIKV strains. This mutation is located in Domain B which is associated in anchoring groove for E1 protein, possibly interfering in viral envelope assembly and being associated of interaction with the target cell (Voss et al., 2010). However, more studies are needed to verify the effect of this alteration on vector fitness and clinical responses.

Considering that the first positive case of CHIKV in Espírito Santo, diagnosed by RT-qPCR at LACEN, occurred in May 2016, the analyses seem to present an accurate virus

date of introduction in the region. The similarity between genomic and epidemiological surveillance data evidence that molecular clock studies are a feasible alternative to help predicting new outbreak waves. Furthermore, genomic and epidemiological surveillance data provide alternative perspectives on the same process and can thus be validated against one another (Pybus and Rambault, 2009).

According to the Ministry of Health epidemiological bulletins, more than 18,000 probable cases have been reported in ES state since 2016, with a new outbreak in 2020 reporting 13,624 probable cases (Ministério da Saúde, Secretaria de Vigilância em Saúde, 2018; Ministério da Saúde, Secretaria de Vigilância em Saúde, 2019; Ministério da Saúde, Secretaria de Vigilância em Saúde, 2020; Ministério da Saúde, Secretaria de Vigilância em Saúde, 2021a; Ministério da Saúde, Secretaria de Vigilância em Saúde, 2021b). Compared to the 2017 (841 probable cases) (Ministério da Saúde, Secretaria de Vigilância em Saúde, 2019), the 2020 CHIKV outbreak infected 16 times more people, raising concerns about the genetic characteristic of the new ES CHIKV. In view of this elevated spread rate, it is possible that the 2020 ES CHIKV outbreak is related to a new ECSA genotype introduction or an infection by the Asian genotype. In entire 2021, 96,288 chikungunya probable cases were reported in Brazil, while in Espírito Santo (ES) state, 1,716 cases were registered (Ministério da Saúde, Secretaria de Vigilância em Saúde, 2021b). There is the hypothesis that the virus may have reached ES due to its territorial proximity to states in the Northeast region (ES is a neighbor of Bahia), including issues of trade relations and tourism. In addition, the transmitting agent - the *Aedes aegypti* mosquito - has a common occurrence in Brazil and is also found in ES. Thus, constant genomic surveillance studies are imperative to assist in the formulation of public health policies.

Despite the small sample size, this study describes the first phylogenetic data about CHIKV in ES state and a new E2 protein CHIKV variant. In addition, this works helps the expansion of the virus genotype database. Lastly, the data reveal a CHIKV-ECSA IIa outbreak in ES state (2017) which points out the population's vulnerability to Asian infection circulating in Brazil (Nunes et al., 2015; Machado et al., 2019; Naveca et al., 2019). Due to the lack of an effective vaccine and the difficulty in mosquito vector control, genomic surveillance studies are feasible alternatives for better understand the chikungunya fever and help design efficient public health control strategies.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Agarwal A, Sharma AK, Sukumaran D, Parida M, et al. (2016). Two novel epistatic mutations (E1:K211E and E2:V264A) in structural proteins of *Chikungunya virus* enhance fitness in *Aedes aegypti*. *Virology*. 497: 59-68.

Feldstein LR, Rowhani-Rahbar A, Staples JE, Weaver MR, et al. (2017). Persistent arthralgia associated with *Chikungunya virus* outbreak, US Virgin Islands, December 2014-February 2016. *Emerg. Infect Dis*. 23: 673-676.

Kumar S, Stecher G and Tamura K (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol*. 33: 1870-1874.

Machado LC, de Morais-Sobral MC, Campos TL, Pereira MR, et al. (2019). Genome sequencing reveals coinfection by multiple *Chikungunya virus* genotypes in a recent outbreak in Brazil. *Plos Negl. Trop. Dis*. 13: e0007332.

Ministério da Saúde, Secretaria de Vigilância em Saúde (2018). Monitoramento dos casos de dengue, febre de chikungunya e febre pelo vírus Zika até a Semana Epidemiológica 52. 49:1-13. Available from: https://antigo.saude.gov.br/images/pdf/2018/janeiro/23/Boletim-2018-001-Dengue.pdf.

Ministério da Saúde, Secretaria de Vigilância em Saúde (2019). Monitoramento dos casos de dengue, febre de chikungunya e doença aguda pelo vírus Zika até a Semana Epidemiológica 52 de 2018. 50:1-14. Available from: https://antigo.saude.gov.br/images/pdf/2019/janeiro/28/2019-002.pdf.

Ministério da Saúde, Secretaria de Vigilância em Saúde (2020). Monitoramento dos casos de arboviroses urbanas transmitidas pelo *Aedes* (Dengue, Chikungunya e Zika), Semanas Epidemiológicas 01 a 52. 51: 1-16. Available from: https://antigo.saude.gov.br/images/pdf/2020/janeiro/20/Boletim-epidemiologico-SVS-02-1-.pdf.

Ministério da Saúde, Secretaria de Vigilância em Saúde (2021a). Monitoramento dos casos de arboviroses urbanas causados por vírus transmitidos por *Aedes* (dengue, chikungunya e zika), semanas epidemiológicas 1 a 53, 2020. 52: 1-31. Available from: https://www.gov.br/saude/pt br/assuntos/media/pdf/2021/fevereiro/01/boletim_epidemiologico_svs_3.pdf.

Ministério da Saúde, Secretaria de Vigilância em Saúde (2021b). Monitoramento dos casos de arboviroses urbanas causados por vírus transmitidos pelo mosquito *Aedes* (dengue, chikungunyae zika), semanas epidemiológicas 1 a 52, 2021. 53: 1-15. Available from: https://www.gov.br/saude/pt-br/centrais-de-conteudo/publicacoes/boletins/boletins-epidemiologicos/edicoes/2022/boletim-epidemiologico-vol-53-no1.pdf/view.

Naveca FG, Claro I, Giovanetti M, de Jesus JG, et al. (2019). Genomic, epidemiological and digital surveillance of *Chikungunya virus* in the Brazilian Amazon. *Plos Negl. Trop. Dis*. 13: e0007065.

Nunes MR, Faria NR, de Vasconcelos JM, Golding N, et al. (2015). Emergence and potential for spread of *Chikungunya virus* in Brazil. *BMC Med*. 13: 102.

Nsoesie EO, Kraemer MU, Golding N, Pigott DM, et al. (2016). Global distribution and environmental suitability for chikungunya virus, 1952 to 2015. *Euro Surveill*. 21: 30234.

Pybus OG and Rambault A (2009). Evolutionary analysis of the dynamics of viral infectious disease. *Nat. Rev. Genet*. 10: 540-550.

Rambaut A, Drummond AJ, Xie D, Baele G, et al. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Syst. Biol*. 67: 901-904.

Ross RW (1956). The Newala epidemic. III. The virus: isolation, pathogenic properties and relationship to the epidemic. *J. Hyg (Lond)*. 54: 177-191.

Schuffenecker I, Iteman I, Michault A, Murri S, et al. (2006). Genome Microevolution of Chikungunya Viruses Causing the Indian Ocean Outbreak. *Plos Med*. 3: e263.

Suchard MA, Lemey P, Baele G, Ayres DL, et al. (2018). Bayesian Phylogenetic and Phylodynamic Data Integration Using BEAST 1.10. *Virus Evol*. 4: vey016.

Voss JE, Vaney MC, Duquerroy S, Vonrhein C, et al. (2010). Glycoprotein organization of *Chikungunya virus* particles revealed by X-ray crystallography. *Nature*. 468: 709-712.

Xavier J, Fonseca V, Bezerra JF, Alves MM, et al. (2021). *Chikungunya virus* ECSA lineage reintroduction in the northeasternmost region of Brazil. *Int. J. Infect Dis*. 105: 120-123.

White SK, Mavian C, Salemi M, Morris-Jr JG, et al. (2018). A new "American" subgroup of African-lineage *Chikungunya virus* detected in and isolated from mosquitoes collected in Haiti, 2016. *Plos One*. 13: e0196857.