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Running title: DENV-3 Genotype I in Rio de Janeiro

Title: Imported case of Dengue virus 3 Genotype I in Rio de Janeiro state, Brazil

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Abstracts:

The dengue virus (DENV), of genus *Flavivirus* (*Flaviviridae*), has four antigenically distinct serotypes, of which DENV-3 is classified into five genotypes. Here, we describe the detection of DENV-3 genotype I in sera of a Brazilian patient returning from Singapore to Rio de Janeiro, Brazil by using multiplex real-time RT-PCR, Sanger sequencing of the whole envelope protein, and phylogenetic analysis. The virus shares

ancestry with those identified in Bali, Indonesia in 2015. Because of tourism and mass events occurring in the last four years (World Youth Day, World Cup, Olympics), arboviruses, such as Chikungunya genotype ECSA, DENV-4 Genotype I, and Zika, from other continents have been introduced in Brazil.

Key words: Dengue, Sequencing, Genotyping

Sponsorships: CNPq, FAPERJ

Dengue virus (DENV) is considered to be the most important arbovirus worldwide and approximately two-fifths of the world's population live in areas endemic for dengue (Guo et al. 2017). DENV is classified into family *Flaviviridae*, genus *Flavivirus*, and comprises four antigenically distinct serotype, Dengue virus 1 (DENV-1), Dengue virus 2 (DENV-2), Dengue virus 3 (DENV-3), and Dengue virus 4 (DENV-4), transmitted to humans by mosquitoes belonging to genus *Aedes*, mainly *Aedes aegypti*, prevalent in tropical and subtropical areas (Lanciotti et al. 1994, Whitehead RH et al. 1971).

Phylogenetic studies focusing the envelope protein proposed the further classification of four genotypes of DENV-3 (genotypes I, II, III, and IV). The first discovered genotype of DENV-3, Genotype IV, identified in Caribbean and Puerto Rico in 1963 (Lanciotti et al. 1994), was the only one circulating in different countries in the Americas, but may be currently extinct. Viruses classified as Genotype I were found in Colombia in 2003 (Usme-Ciro et al. 2008). In Brazil, similar viruses were detected in 2003, but the authors proposed a new genotype (genotype V) for the correct classification of these viruses owing to their topology in the phylogenetic tree. According to this classification, Genotype V viruses were present in Brazil and Colombia between 2003 and 2006. They were closely related to viruses from Asian samples isolated in around 1956, and until that date there was no record of their presence in nature (Aquino et al. 2009). Genotype III was introduced in Latin America in around 1991 (Araújo et al. 2009) and in Brazil in 2001 (Miagostovich et al. 2002), becoming the predominant genotype till date. To our knowledge, there is no record of the circulation of genotype II in the Americas.

Out of the 733 acute sera tested for dengue by real time RT-PCR using the protocol described by Santiago et al., only one sample was positive for DENV-3

presenting a Ct value of 23. The sample was obtained from a 36-year-old Brazilian, who lives in Singapore and toured Indonesia and other Asian countries during a 30-day vacation. On July 3rd, 2017, the patient arrived in Brazil with symptoms of dengue. The patient sought medical assistance and stayed at home, in an urban area of Rio de Janeiro state, for two weeks until the symptoms disappeared.

The Qiagen Onestep RT-PCR Kit was used to generate a 1795-bp fragment, which contains the entire coding region for the envelope protein. Ten primers were used for Sanger sequencing reactions (Data not shown; Acknowledgements). The MEGA 7 software package was employed for choosing the best model of nucleotide substitution and construction of the phylogenetic trees.

The tree shown in figure A was constructed using 2,040 sequences, obtained by Blast, of the envelope protein coding region from all five genotypes. In figure A, the virus from the municipality of residence of the patient, identified as Brazil-RJ/2017/LABFLA (MG812331), belongs to genotype I among samples from Indonesia and Singapore. It is in a cluster supported by 86 of bootstrap with four other samples, which are described in a study with samples from Bali, Indonesia, collected in 2015 (Megawati et al. 2017) Therefore, viruses from Bali, Indonesia and those from the patient (Brasil-RJ/2017/LABFLA) in this study have common ancestry.

A second tree (B) was constructed because there were no complete envelope protein sequence deposits from the Colombian samples classified as genotype I, reclassified as genotype V. The second tree was assembled with 224 3'-terminal nucleotides of the envelope protein coding region. It is evident by the phylogenetic tree that its introduction in Colombia has no correlation with the virus sample found in Rio de Janeiro. The patient probably acquired the DENV-3 infection during his stay in

Indonesia, arriving to Brazil infected. The surveillance in the area around the patient's residence during the viremic phase is very important, to identify other probable cases.

Again, our results show that Brazil has become an important gateway to arboviruses from other continents, as seen with Zika, Yellow Fever, Chikungunya genotype ECSA, DENV-4 Genotype I, and maybe DENV-3 Genotype I recently. This may be hiding other arboviruses or new genotypes. The identification of DENV-3 genotype I in this patient is an indication for the Brazilian health authorities to verify possible changes in DENV-3 infection pattern.

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Authors' contribution

Marcos César Lima de Mendonça, carried out DNA Sequencing, phylogenetic analyses, and wrote drafted the manuscript.

Maria Angelica Mares-Guia, Cintia Damasceno dos Santos Rodrigues, Carolina Cardoso dos Santos, Flavia Lowen Levy Chalhoub, Eliane Saraiva Machado Araújo, Alexandre Otávio Chieppe, carried out the laboratory experiments.

Rita Maria Ribeiro Nogueira, Ana Maria Bispo de Filippis designed the study.

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