Heliyon 10 (2024) e39246

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Genomics-based timely detection of dengue virus type I genotypes I and V in Uruguay

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ARTICLE INFO

Keywords:
Dengue virus
Genomic monitoring
Uruguay
South America

ABSTRACT

This study details a genomics-based approach for the early detection of mosquito-borne pathogens, marked by Uruguay's first ever complete genomic sequencing of Dengue Virus type I genotypes I and V. This pioneering effort has facilitated the prompt identification of these genotypes within the country, enabling Uruguayan public health authorities to develop timely and effective response strategies. Further integrated into this approach is a climate-driven suitability measure, closely associated with Dengue case reports and indicative of the local climate's role in the virus's transmission in the country within the changing climate context. The detection of multiple DENV-1 genotypes co-circulating in Uruguay underscores the necessity for proactive surveillance, particularly at borders, to prevent the introduction and dissemination of novel viral strains within

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https://doi.org/10.1016/j.heliyon.2024.e39246
Received 16 February 2024; Received in revised form 2 October 2024; Accepted 10 October 2024
Available online 6 November 2024

the country and the region. This approach aids in facilitating prompt public health responses and intervention strategies, which are crucial in mitigating the impact of dengue outbreaks.

1. Introduction

Dengue virus (DENV), a member of the *Flavivivridae* family (genus *Flavivirus*), is a single-stranded RNA virus with a genome size of approximately 11,000 bp. This viral pathogen is transmitted by *Aedes aegypti* and *Ae. albopictus* mosquitoes, resulting in frequent outbreaks that have a significant impact on public health [1]. These outbreaks have been observed across the Americas, with both mild and severe dengue cases becoming more prevalent in recent decades. DENV exhibits diversity through the presence of four distinct antigenic serotypes (DENV-1 to DENV-4), which are often named based on their geographic origins [2]. There is a nucleotide variation of around 30 % among these serotypes. Furthermore, each serotype can be further divided into different genotypes. Starting from the year 2023, the Americas have experienced numerous and substantial dengue epidemics [2,3]. This year alone, there have been nearly three million suspected and confirmed cases, surpassing the 2.8 million cases reported in 2022. Despite the Uruguayan Meteorological Institute's identification of March 2023 as the warmest month in the previous 42 years [4], and with the widespread presence of DENV's vector mosquitoes throughout the country, Uruguay has thus far experienced sporadic and brief outbreaks rather than the continuous transmission observed in neighboring countries. One such outbreak, involving a total of 20 cases, was identified in 2016. Subsequently, in 2020, three cases were reported. In 2023, a larger, yet restricted outbreak with 35 cases detected, all attributed to the DENV1 serotype.

Given the ongoing changes and expansion of transmission in the Americas, including frequent cross-border viral movements [5], continuous and active surveillance remains indispensable for promptly identifying and managing potential outbreaks in Uruguay. Recognizing this imperative, we established a partnership with Montevideo's Central Public Health Laboratory to undertake a genomics-based investigation.

2. Material and methods

Clinical specimens (serum sample) were obtained from patients who tested positive for DENV (with a Ct value $\leq$ 35) and were reported and sampled during the time frame spanning from January 2016 to May 2023. The extracted RNA was initially converted into cDNA by employing the SuperScript IV Reverse Transcriptase kit (Invitrogen) and subsequently submitted to sequencing multiplex PCR (35 cycles) in accordance with previously outlined methods [6]. The DNA library preparation was executed employing the Ligation Sequencing kit (Oxford Nanopore Technologies) and the Native Barcoding Expansion 1–96 kit (Oxford Nanopore Technologies), following the reaction conditions as previously described [1, adeli6]. Sequencing was carried out over a period of 24 h using a MinION device and consensus sequences were obtained by using the Genome Detective software [7]. The arbovirus genotyping tool was employed to examine the sequence genotypes [8].

To put the newly sequenced DENV1 genotypes I and V in a global perspective, we built phylogenetic trees using other isolates of the same genotypes from GenBank gathered up to October 30, 2023. Sequences that lacked a sample date and location, as well as those that had less than 50 % coverage of the virus genome, were excluded. MAFFT [9] was used to align the sequences, and AliView [10] was used to modify them. The HKY + G4 substitution model was used to create a maximum likelihood phylogeny using the IQ-TREE 2 program [11].

Time series data for confirmed, suspected, and probable infections were downloaded from the PAHO website [12]. Additionally, climate-driven suitability for DENV (averaged across Uruguay) was estimated using the Index P, as described by Nakase and colleagues [13].

3. Results

A total number of 24 positive samples tested contained sufficient DNA (2 ng/L) for library preparation. The average cycle threshold (Ct) values for PCR ranged between 15 and 32. The sequencing procedure resulted in the generation of the first 24 DENV1 genome sequences of Uruguay, which included the initial documented case (sample ID OR494342). Novel genome sequences presented an average coverage of 94.5 %, with a range of 88 %–98.0 %. Cases were reported between January and July, peaking in April, following the natural variation in climate-driven suitability with monthly Pearson's correlation of 0.92.

Four districts were sampled: Canelones (n = 4), Maldonado (n = 2), Montevideo (17) and Rocha (1). Genotyping was accomplished utilizing the Dengue virus typing tool (https://www.genomedetective.com/app/typingtool/dengue/) along with preliminary phylogenetic analysis. The results from this analysis confirmed that among the samples, one (n = 1) was classified as belonging to Genotype I, while the remaining 23 genomes were categorized as Genotype V. The age of sampled patients ranged from 16 to 66 years, with a median of 39. Among the patients sampled, 70 % (n = 17) were male. Among the cases examined, ten were classified as autochthonous, while the remaining fourteen individuals reported recent travel to various locations, including Argentina (n = 1), Asia (n = 1), Brazil (n = 9), and Paraguay (n = 3).

To explore the phylogenetic history of DENV1 genotype I, we combined our newly generated sequence with other DENV1 genotype I genomes available on GenBank (n = 3004). Our analysis revealed that the sample obtained from a patient who had traveled to Asia clustered together with other genome sequences from Asia. This suggests that the genotype was introduced to Uruguay and the broader

Americas region by a traveler returning from Asia.

At the same time, genotype V was shown to be the most common genotype circulating in the country. To investigate the phylogenetic history of DENV1 genotype V independently, we combined our generated sequences with other DENV1 genotype V genomes available on GenBank (n = 782). Our analysis revealed that the novel isolates belonged to three distinct clades (clade I, III, and IV), which clustered with viral strains isolated from different Brazilian regions and countries in South America, including Paraguay. This result strongly suggested that multiple introductions have taken place within Uruguay further underscoring the intricate dynamics of viral transmission across geographical boundaries. These observations emphasize the crucial importance of a genomics-based approach in promptly detecting emerging viral strains.

4. Discussion

The recent identification of multiple genotypes of DENV-1 co-circulating in Uruguay underscores the urgent need for enhanced genomic surveillance, particularly at border crossings. This observation aligns with studies conducted in South America, which highlight the importance of genomic monitoring in tracking viral diversity and preventing cross-border transmission of dengue virus variants [1,3,5,14]. The timely detection of these genotypes is essential for implementing proactive public health measures to control the spread of the virus. Furthermore, the significant correlation observed between local climatic conditions and dengue incidence in Uruguay mirrors broader findings from other regions where dengue is endemic. Numerous studies have demonstrated that climatic factors such as temperature, precipitation, and humidity are key drivers of vector-borne disease transmission dynamics, including dengue. For instance, Nakase et al. [13] analyzed global transmission suitability maps for dengue virus, revealing how climate variations influence vector transmission patterns over time. Similarly, López et al. [15] found a strong relationship between climate variables and dengue incidence in Argentina, underscoring the significant role of environmental conditions in driving outbreaks in South America. Additionally, Nakase et al. [16] conducted a retrospective analysis spanning four decades, further validating the influence of climate factors on dengue transmission suitability. The integration of climate data into surveillance strategies is vital, as it enhances the ability to predict outbreak timing and severity, thereby facilitating the implementation of targeted interventions. This perspective is supported by Messina et al. [17], who examined the global distribution of dengue and the populations at risk, emphasizing the importance of integrating climatic and epidemiological data for more effective dengue outbreak management. Collectively, these studies underscore the critical role that climatic variables play in shaping dengue transmission dynamics and reinforce the need for climate-based surveillance systems in Uruguay and beyond. By establishing comprehensive surveillance systems, including genomic surveillance at major entry points, Uruguay can significantly improve its ability to mitigate dengue outbreaks. Brazil has successfully implemented similar genomic surveillance strategies, which have been instrumental in enhancing outbreak detection and response [18]. Such systems are crucial for monitoring viral evolution and the emergence of new variants, especially in light of climate change, which is projected to expand the geographic range and intensity of dengue outbreaks [17,18]. The complexity of managing multiple DENV-1 genotypes further underscores the necessity of integrating genomic data with epidemiological and climatic information. Studies in Latin America have demonstrated that combining these data sources can enhance outbreak prediction models, enabling more timely and effective public health interventions [1]. Sustained national-level genomic surveillance will be pivotal in identifying regions that require prioritized surveillance and control efforts. Strengthening border surveillance and implementing preemptive mosquito control measures will be critical to preventing the introduction and spread of new DENV genotypes into Uruguay.

In conclusion, the integration of genomic surveillance with climate data offers a powerful tool for managing dengue outbreaks in Uruguay. These combined efforts are essential to maintaining public health security, especially in the face of increasing global mobility and climate change.

CRediT authorship contribution statement

Noelia Morel: Writing – review & editing, Investigation, Formal analysis. **Marta Giovanetti:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Vagner Fonseca:** Writing – review & editing, Methodology, Investigation. **Analía Burgueño:** Writing – review & editing, Methodology, Investigation. **Mauricio Lima:** Writing – review & editing, Methodology, Investigation. **Emerson Castro:** Writing – review & editing, Methodology, Investigation. **Natália R. Guimarães:** Writing – review & editing, Methodology, Investigation. **Felipe C.M. Iani:** Writing – review & editing, Methodology, Investigation. **Victoria Bormida:** Writing – review & editing, Methodology, Investigation. **Maria Noel Cortinas:** Writing – review & editing, Methodology, Investigation. **Viviana Ramas:** Writing – review & editing, Methodology, Investigation. **Leticia Coppola:** Methodology, Investigation, Formal analysis. **Ana I. Bento:** Writing – review & editing, Resources, Investigation. **Alexander Rosewell:** Writing – review & editing, Investigation, Funding acquisition. **Leticia Franco:** Writing – review & editing, Investigation, Funding acquisition. **Jairo Mendez Rico:** Supervision, Investigation, Funding acquisition. **José Lourenço:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Luiz Carlos Junior Alcantara:** Supervision, Investigation, Funding acquisition. **Hector Chiparelli:** Writing – review & editing, Methodology, Investigation.

Data availability

Newly generated sequences have been deposited in GenBank under accession numbers OR494329– OR494352.

Ethics statement

The Pan American Health Organization Ethics Review Committee (PAHOERC) reviewed and approved this project (Ref. No. PAHO-2016-08-0029). The samples used in this research were de-identified residual samples from the routine diagnosis of arboviruses at the Uruguayan public health laboratory, which is part of the Uruguayan Ministry of Health's public network.

Declaration of competing interest

None.

Acknowledgement

This study was supported by the National Institutes of Health USA grant U01 AI151698 for the United World Arbovirus Research Network (UWARN) and the CRP-ICGEB RESEARCH GRANT 2020 Project CRP/BRA20-03, Contract CRP/20/03. M. Giovanetti's funding is provided by PON "Ricerca e Innovazione '' 2014–2020. The authors would also like to acknowledge the Global Consortium to Identify and Control Epidemics – CLIMADE, (T.O., L.C.J.A., E.C.H., J.L., M.G.) (https://climade.health/).

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