Virologica Sinica 40 (2025) 680–683

Contents lists available at ScienceDirect

Virologica Sinica

journal homepage: www.keaipublishing.com/en/journals/virologica-sinica
www.virosin.org

Letter

Co-circulation of all four DENV serotypes during 2016 outbreak in Sinaloa, Mexico: First report of DENV-4 in patients

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Dear Editor,

Dengue virus (DENV) is a positive-sense single-stranded RNA virus belonging to the *Flaviviridae* family, which causes dengue—a disease affecting over 400 million people annually worldwide. DENV is transmitted through the bite of mosquitoes from the *Aedes* genus, primarily *Aedes aegypti*, and has a wide distribution in tropical and subtropical areas (de Souza et al., 2022). The virus is classified into four antigenically distinct serotypes (DENV-1 to DENV-4) (Sirisena et al., 2021), which differ by approximately 30%–35% in their amino acid sequences. Each serotype is further divided into genotypes, with up to 10% variation in the amino acid sequence of the viral envelope (E) protein (Phadungsombat et al., 2024). Although all four serotypes can cause the full range of clinical manifestations, evidence suggests that DENV strains vary in virulence. For example, a Southeast Asian strain of DENV-2 was linked to a severe dengue (SD) outbreak in Cuba in 1981, later spreading to Venezuela, Mexico, Colombia, and Brazil, where it caused SD outbreaks (Gardella-García et al., 2008). By contrast, the American genotype of DENV-2 was associated with dengue fever (DF) outbreaks in Peru, but did not lead to SD (Kochel et al., 2002).

The World Health Organization (WHO) considers dengue fever (DF) a serious public health issue, whose incidence has increased over the past decade, even in non-endemic areas. In 2024, the WHO reported 14,284,310 cases of dengue worldwide, resulting in 10,554 deaths (WHO, 2025). Mexico recorded a historic number of cases in 2024, totaling 558,846 dengue cases and 478 deaths, involving all four DENV serotypes, with a higher incidence of DENV-3. Sinaloa was among the top ten affected states, with 20,394 cases and 34 deaths (DGE, 2024).

To strengthen epidemiological data and contribute to the understanding of the mechanisms underlying the emergence of dengue outbreaks, this study carried out molecular epidemiological surveillance on samples from 150 patients during the 2016 outbreak in Sinaloa to identify DENV serotypes. Initially, we detect the DENV NS1 antigen by ELISA test in the 150 serum samples included in the study. Of the ELISA-positive simples (n = 29), 62% were from female patients (n = 18) (Fig. 1A). Subsequently, RT-PCR was performed on all 150 serum samples to amplify the CprM region of the DENV genome using D1 and D2 primers (Utama et al., 2019). As a result, 48 samples (32%) tested positive for one or more DENV serotypes. The positive rates in different gender groups showed that the female group had a higher positivity proportion, with 66% (Fig. 1B). Among the samples analyzed, the age range of patients was 11 and 58 years. The age group with the highest DENV RT-PCR positivity rate was 20–29 years, comprising 37.5% of the positive cases, followed by the 10–19 year group with 27%. The 30–39 and 40–49 year groups each represented 12.5% of the positive cases, while the 50–58 year group constituted 10.5% (Fig. 1C). Of the 29 NS1 ELISA-positive samples, 65% were also positive for DENV by RT-PCR. In contrast, among the 121 NS1-negative samples, 21.48% were positive by RT-PCR (Fig. 1D). These findings underscore the importance of conducting molecular diagnostics even when NS1 serological results are negative.

The serotypes with the highest incidence in this study were DENV-2 (73.33%), followed by DENV-4 (64.44%) (Fig. 1E). This is the first report of DENV-4 in the region, as it had not been previously identified in Sinaloa until 2022 (DGE, 2022). Our findings align with those

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https://doi.org/10.1016/j.virs.2025.07.010
Received 1 March 2025; Accepted 16 July 2025
Available online 24 July 2025

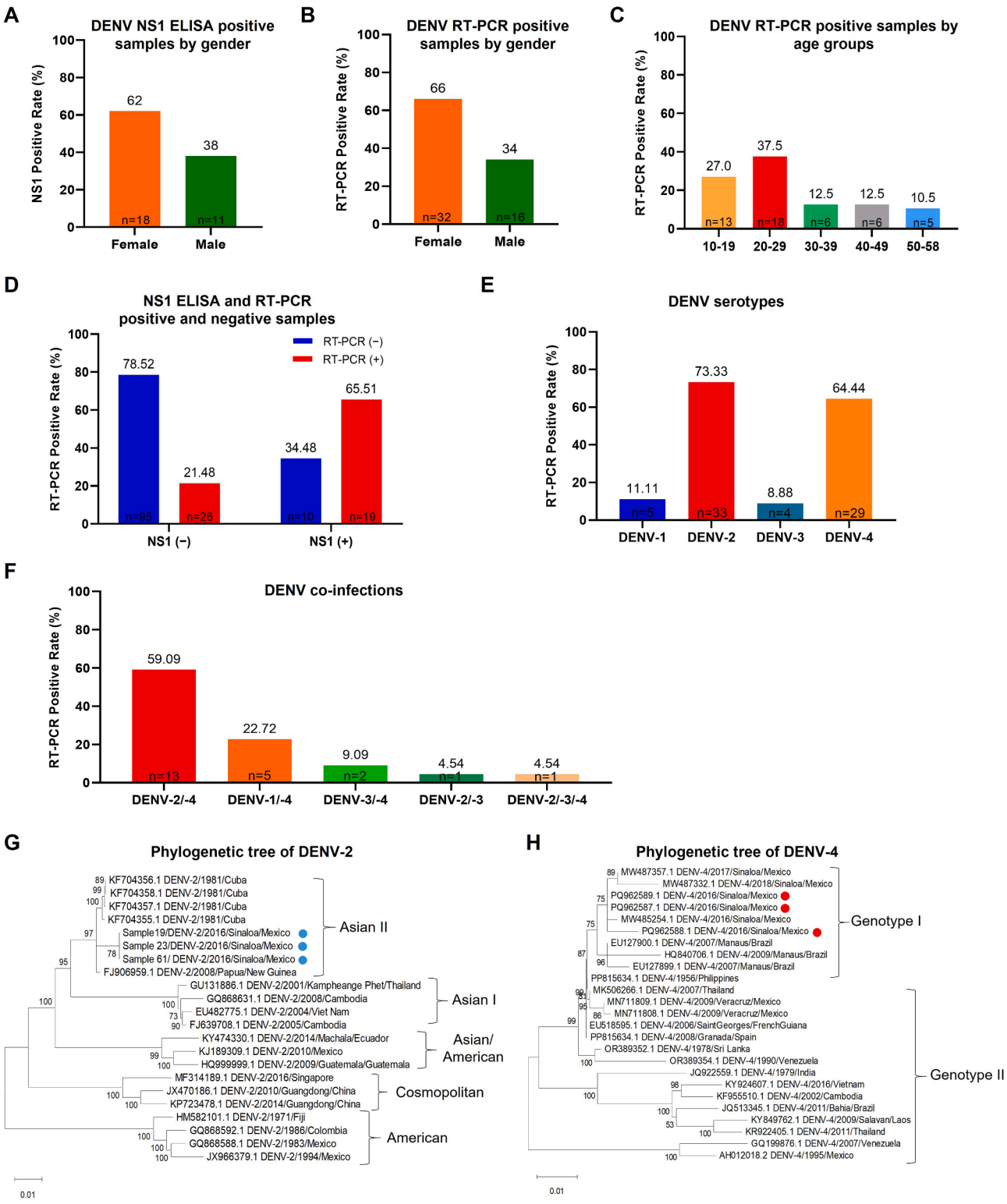

Fig. 1. DENV serotypes and genotypes in 150 patients during the 2016 epidemic outbreak in Sinaloa, Mexico. **A** –**C** The DENV seropositive and nucleic acid positive rates in these patients were detected by NS1 antigen ELISA and RT-PCR. The highest proportion of DENV positivity by ELISA and RT-PCR was found in the female group, with 62% and 66%, respectively. **C** The DENV RT-PCR positivity rate in different age groups. **D** Comparison of DENV detection by NS1 antigen ELISA and RT-PCR. Of the NS1-negative samples (n = 121), 21.48% were RT-PCR positive, while 65.51% of the NS1-positive samples were confirmed by RT-PCR. **E** Distribution of DENV serotypes. The predominant serotype was DENV-2, followed by DENV-4, DENV-1 and DENV-3. **F** DENV multiple infections. The predominant DENV serotype combination was DENV-2/-4, followed by DENV-1/-4, DENV-3/-4, DENV-2/-3 and DENV-2/-3/-4. **G** Phylogenetic analysis of DENV-2 sequences from patients of the 2016 Sinaloa outbreak. Samples for DENV-2 (19, 23 and 61, blue dot) were grouped closely with the Asian II genotype. **H** Phylogenetic analysis of DENV-4 sequences from patients of the 2016 Sinaloa outbreak. Samples for DENV-4 (19, 56 and 61, red dot) were closely grouped with genotype I. Evolutionary history was inferred using the Maximum Likelihood method based on the Kimura 2-parameter model. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test is shown next to the branches. Evolutionary analyses were conducted in MEGA 12.

reported by Torres-Avendaño, who identified DENV-4 in *Ae. aegypti* mosquito larvae collected in Sinaloa in 2016 (Torres-Avendaño et al., 2021), reinforcing the notion that epidemiological surveillance of DENV in the vector can be a valuable tool for predicting the serotypes circulating in the population.

Our findings differ from those reported by the Ministry of Health (DGE), which identified only DENV-1 as the cause of the epidemic that led to 4214 dengue cases (SSA, 2016). These discrepancies may be attributed to the limited number of samples evaluated for serotype identification in the epidemiological surveillance program, where only a small percentage (10%) of confirmed dengue cases are assessed for serotype identification. Additionally, this study identified the presence of DENV-3, a serotype not reported by the DGE in 2016, despite its first identification in Sinaloa in 2014 (DGE, 2014). This serotype was the most prevalent in 2024 (DGE, 2024).

We also identified multiple DENV infections in 45.83% of the samples. The most frequent co-infection was DENV-2/DENV-4, observed in 13 positive samples (59.09%), followed by DENV-1/DENV-4 (5 samples, 22.72%), DENV-3/DENV-4 (2 samples, 9.09%), DENV-2/DENV-3 (1 sample, 4.54%), and a triple infection involving DENV-2/DENV-3/DENV-4 (4.54%) (Fig. 1F). In contrast, a study conducted on the Mexican population reported that the most common combination was DENV-1/DENV-2 (60%), followed by DENV-2/DENV-4 (13.33%) (Requena-Castro et al., 2017). Several researchers have suggested that the co-circulation of multiple DENV serotypes in the same region constitutes an important risk factor for the development of severe dengue (SD), as the population becomes more susceptible to heterologous reinfection, which is considered one of the main risk factors for the development of complications in dengue fever. Therefore, it is essential to maintain a DENV serotype surveillance program in endemic areas.

To address two key points—the novel detection of DENV-4 in Northwestern Mexico and the high prevalence of multiple DENV infections in our population—we sequenced six samples representing the most frequently detected DENV serotypes. Three of the six sequences corresponded to DENV-2, with 129-bp from samples 19, 23, and 61. These sequences were not registered in the GenBank database due to their short length. The remaining three sequences corresponded to DENV-4, with 393-bp from samples 19, 56, and 61. These sequences were registered in the GenBank database with the accession numbers PQ962587.1, PQ962588.1, and PQ962589.1, respectively. These results confirm that two of the evaluated samples (19 and 61) exhibited dual infections with DENV-2 and DENV-4, further validating the presence of DENV-4 in Sinaloa at least since 2016.

We then conducted a phylogenetic analysis, which revealed that DENV-2 belonged to the Asian II genotype (Fig. 1G), while DENV-4 belonged to genotype I (Fig. H). The Asian II genotype was first reported in Mexico in two DENV-2 isolates from the state of Guerrero (Loroño-Pino et al., 2004). In 2020, Hernández-García reported the Asian II genotype of DENV-2 and genotype I of DENV-4 in patient samples from the 2009 epidemic outbreak in Veracruz, where they found co-infection (Hernández-García et al., 2020), as observed in the present study. Phylogenetic analysis of DENV-4 revealed a close relationship between the strains isolated in this study (PQ962587.1, PQ962588.1, and PQ962589.1), as well as those previously reported by our research group in 2021 from *Ae. aegypti* mosquitoes (MW487357.1, MW487332.1, and MW485254.1) in Sinaloa, and strains isolated in Manaus, Brazil, in 2007 (EU127900.1 and EU127899.1) and in 2009 (HQ840706.1) (Fig. 1H). These findings suggest that the DENV-4 cases reported in Sinaloa in 2016 were likely the result of imported cases, potentially introduced years earlier from Brazil through cross-border mechanisms such as international trade, migration, and tourism, among others.

The results of this study demonstrate the co-circulation of the four DENV serotypes in human serum samples from Sinaloa, Mexico, marking the first report of DENV-4 in this population. The findings indicate that the most prevalent serotypes were DENV-2 and DENV-4. Multiple serotype infections (up to three serotypes) were also observed in some patients, which represent a significant risk factor for the development of severe dengue. Our data underscores the necessity for coordinated efforts to conduct thorough epidemiological monitoring, providing a comprehensive overview of the DENV serotypes circulating within the population during each epidemic. Such efforts are critical for providing evidence-based guidance in the formulation of effective dengue prevention and control strategies for the region.

FOOTNOTES

The authors would like to acknowledge and thank all participants for their valuable contributions in providing serum samples for this study. They also extend their gratitude to Dr. José Ángel López Valenzuela for his assistance with the writing and editing of the manuscript. The authors declare that they have no conflicts of interest. All procedures involving human samples were conducted in accordance with the 1964 Helsinki Declaration and its subsequent amendments, or comparable ethical standards. Informed consent was obtained from all participants involved in the study.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virs.2025.07.010.

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