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Natural Vertical Transmission of Dengue Virus Serotype 4 in *Aedes aegypti* Larvae from Urban Areas in Sinaloa, Mexico

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Abstract

Dengue virus (DENV) is transmitted to humans by the bite of the vector *Aedes aegypti*. Several researchers have suggested that the mechanism of vertical transmission of DENV in the vector is a key aspect for the prevalence of the virus in the environment and the potentiation of epidemic outbreaks of the disease. In this context and as part of an integrated study of DENV serotypes in mosquitoes of urban areas in Sinaloa, Mexico, the presence of DENV-4 in larval stages of *Ae. aegypti* was evaluated to demonstrate the vertical transmission of this serotype. In total, 672 larvae of *Ae. aegypti* were collected in 16 sectors and were grouped into 36 pools, of which 41.66% (15/36 pools) tested positive for DENV-4, with a minimum infection rate = 22.32. The analysis of the obtained sequences showed a 98% similarity to the DENV-4 with sequences previously reported in GenBank. These results show that *Ae. aegypti* acts as a natural reservoir for DENV-4 in this region.

Keywords: vertical transmission, dengue virus 4, *Aedes aegypti*, urban areas, natural reservoir

Introduction

DENGUE VIRUS (DENV) is the etiological agent of dengue, a disease that affects ~40% of the world-wide population with greater impact in tropical and subtropical countries (Whiteman et al. 2018). The four serotypes of DENV (1, 2, 3, and 4) are transmitted to humans by the bite of hematophagous females of the genus *Aedes*, the main vector being *Aedes aegypti* (Campbell et al. 2019). In addition to horizontal transmission of DENV (mosquito to person), two routes of virus transmission between mosquitoes have been reported: sexual transmission (ST) and vertical transmission (VT) (Sánchez-Vargas et al. 2018). The last of these routes has been studied the most in *Ae. aegypti* populations and consists of the transmission of DENV from an infected female to her progeny (Grunnill et al. 2016, Sánchez-Vargas et al. 2018).

It has been reported that VT of DENV in mosquitoes is a factor that favorably influences the maintenance of the virus during periods when human cases of viremia are scarce (Gutiérrez-Bugallo et al. 2018). Also, VT-infected adults of *Ae. aegypti* could initiate the cycle of horizontal transmission of DENV (mosquito to human), thus enhancing the transmission of the disease (Joshi et al. 2002).

Previously, our research group reported the VT of DENV-2 in *Ae. aegypti* larvae in Sinaloa, Mexico (Apodaca-Medina et al. 2018). In addition, according to the Dirección General de Epidemiolgía (DGE), from 2016 to 2018 in the state of Sinaloa, 8, 317 and 789 probable and confirmed cases of dengue have been reported respectively, and only the circulation of serotypes 1, 2, and 3 has been recorded in humans (DGE 2017, 2019). However, until now there is no evidence of the presence of DENV-4 in Sinaloa. Therefore, this study followed up on the monitoring of DENV-4 in *Ae. aegypti* larvae that could be useful to generate molecular epidemiology data to help guide and empower vector control strategies.

Materials and Methods

In total, 672 larvae of *Ae. aegypti* were collected in the period from September to January of the years 2016–2018 in

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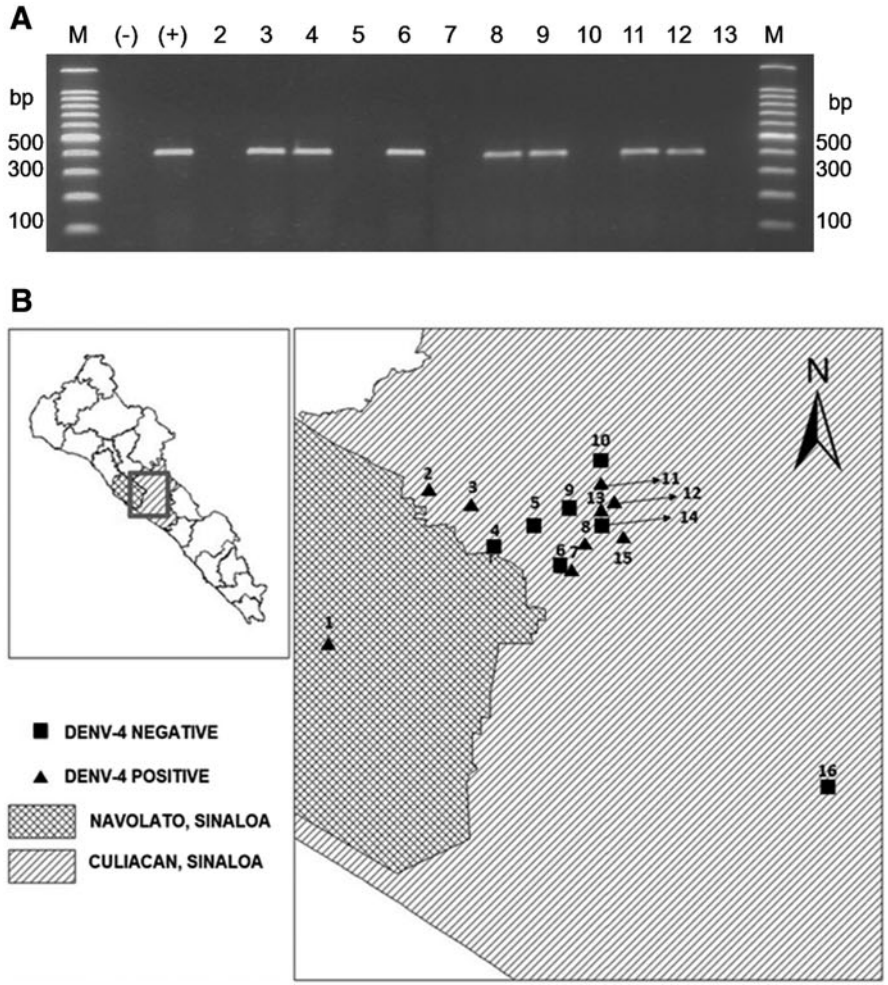

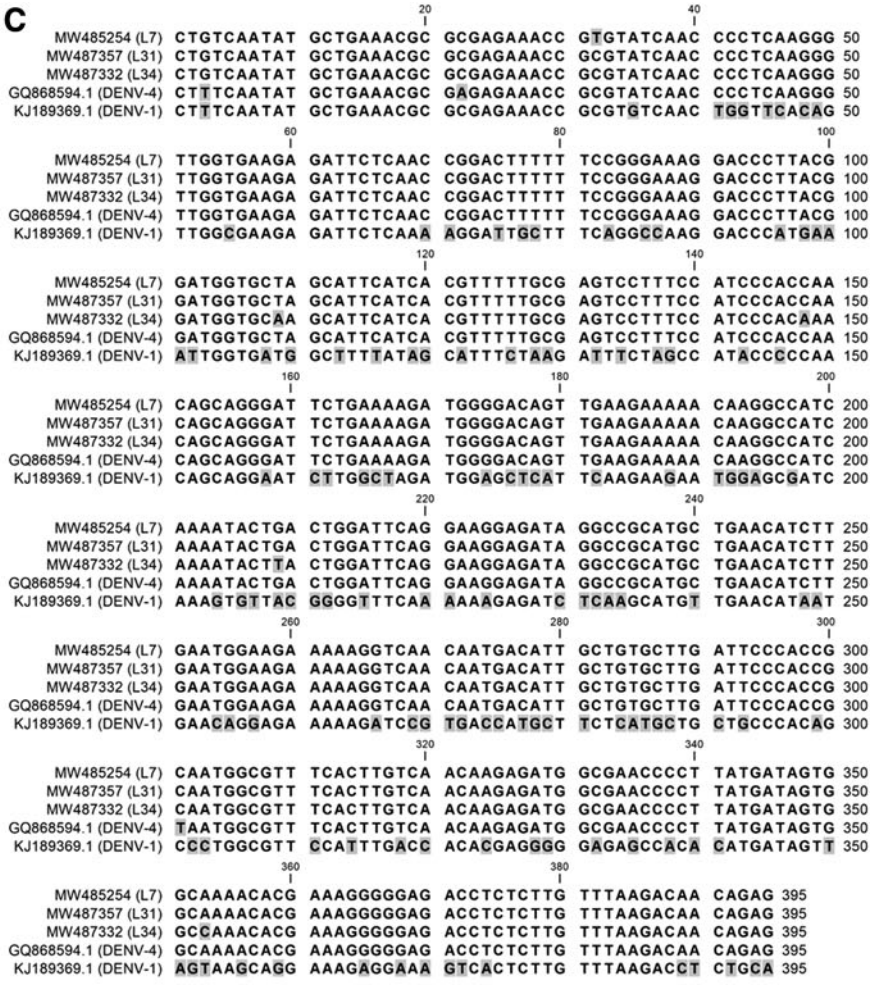

FIG. 1. Vertical infection of DENV-4 in *Aedes aegypti* larvae. **(A)** Molecular identification of DENV-4 in *Ae. aegypti* larvae; M: 100 bp molecular marker, (–): PCR negative control, (+): PCR positive control, 2–13: samples. In lanes 3, 4, 6, 8, 9, 11, and 12, a band of ~400 bp is observed corresponding to the expected molecular size for DENV-4. **(B)** Capture areas of *Ae. aegypti* larvae. Each point represents the sampling sector of ~2 km^2. **(C)** Sequence alignment of samples L7 (MW485254) (sector 3, 2016), L31 (MW487357) (sector 15, 2017), and L34 (MW487332) (sector 7, 2018) shows a 99% similarity with sequences previously reported in GenBank of DENV-4 (GQ868594.1) and 67% for DENV-1 (KJ189369.1). DENV, dengue virus.

16 sectors of the municipalities of Culiacan and Navolato, Sinaloa; Mexico, and were identified according to the criteria of Farajollahi and Price (2013). A total of 36 larval pools were used for RNA extraction (SV Total RNA Isolation System Kit; Promega, Madison, WI), followed by a retro transcription reaction using random primers (GoScript Reverse Transcription System; Promega). DENV identification was performed by PCR using D1 and D2 primers that amplify a 511 bp fragment (Lanciotti et al. 1992), and 3 μL of complementary DNA under the following conditions: 94°C for 4 min, 35 cycles (94°C for 1 min, 55°C for 1 min, and 72°C for 1 min), and 72°C for 5 min. A second amplification (seminested PCR) was performed using 1 μL of the initial amplification and the first D1 with the first specific TS4 for DENV-4 (Lanciotti et al. 1992), under the following conditions: 95°C for 1 min, 25 cycles (95°C for 30 s, 60°C for 30 s, and 72°C for 30 s), and 71°C for 2 min. The PCR products were purified (SV Gel and PCR Clean-Up System; Promega) and sequenced at Macrogen™ (Korea). The sequences were analyzed using the software CLC Sequence Viewer 7 and NCBI's BLAST. The minimum infection rate (MIR) value for DENV was calculated as MIR = (positive pools per PCR)/(total number of individuals tested) (1000). This study was reviewed and approved by the ethics and biosafety committee of the Biological Sciences Postgrad of the Faculty of Biology of the Autonomous University of Sinaloa.

Results

Of the 36 pools evaluated, 15 (41.66%) tested positive for DENV-4, evidenced in a PCR product of ~400 bp (Fig. 1A), with an MIR = 22.32. Ten of the positive samples correspond to 2016, four to 2017, and one to 2018. All positive samples came from nine sectors distributed among the municipalities of Culiacan and Navolato, Sinaloa (Fig. 1B). The sequences obtained were recorded in GenBank with accession numbers (MW485254) for sample from 2016, (MW487357) for sample from 2017, and (MW487332) for sample from 2018. The sequences showed a 99% similarity with sequences reported for DENV-4 (GenBank acc. no.GQ868594.1), and a 67% similarity when compared with other serotypes (DENV-1, GenBank acc. no. KJ189369.1) (Fig. 1C).

Discussion

VT has been proposed as a mechanism responsible for the persistence of DENV during periods of low dengue transmission (Joshi et al. 2002). Infected eggs can be viable for long periods, and female mosquitoes that emerge from larval and pupal development can transmit the virus to humans (Mourya et al. 2001). Molecular identification of DENV-4 in *Ae. aegypti* larvae coming from Culiacan and Navolato, Sinaloa, evidence a VT route. Recently in this area, our research group has reported the VT of DENV-2 in *Ae. aegypti* larvae under similar methodological parameters (Apodaca-Medina et al. 2018). In Mexico, VT of DENV-4 has been shown to occur in wild populations of *Ae. aegypti* originating from Oaxaca, as well as DENV-2 and DENV-3; the overall MIR was lower compared with our results (Günther et al. 2007).

The MIR value for DENV-4 obtained in this study was higher than the values reported in different Mexican states, such as Acapulco, Guerrero with a value of 0.41 for DENV-1 (Martínez et al. 2014), and Cancun, Quintana Roo, with a value of 0.33 for DENV-2 (Sanchez-Casas et al. 2016). Currently, VT of DENV-1, DENV-2, and DENV-3 is reported in *Ae. aegypti* adults bred from eggs collected in central and southern Mexico: the MIR corresponded to 2.52 (Danis-Lozano et al. 2019). These data indicate the importance of *Aedes* as a natural reservoir of the DENV. The public health implications of this mechanism of transmission should be further studied in future research.

Conclusions

This is the first report of the circulation of DENV-4 in *Ae. aegypti* larvae collected in anthropogenic environments of Culiacan and Navolato, Sinaloa, Mexico, suggesting that the mosquito could have a role as a virus reservoir in nature. Further studies are required to determine the epidemiological impact of VT of DENV-4 in the region studied.

Author Disclosure Statement

No competing financial interests exist.

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