



Complete Genome Sequence of Dengue Virus Serotype 2, Asian/American Genotype, Isolated from the Urine of a Venezuelan Child with Hemorrhagic Fever in 2016

Gabriela M. Blohm,^{a,b,c} Alberto E. Paniz-Mondolfi,^{c,d,e} Marilianna C. Márquez,^{c,e,f} Julia C. Loeb,^{a,b} Carlos Pacheco,^g John A. Lednicky,^{a,b} Juliet R. C. Pulliam,^h J. Glenn Morris, Jr.^{b,i}

^aDepartment of Environmental and Global Health, College of Public Health and Health Professions, University of Florida, Gainesville, Florida, USA

^bEmerging Pathogens Institute, University of Florida, Gainesville, Florida, USA

^cThe Venezuelan Science Research Incubator, Zoonoses and Emerging Pathogens Collaborative Network, Barquisimeto, Lara, Venezuela

^dDepartment of Infectious Diseases and Tropical Medicine, IDB Biomedical Research Institute/IDB Clinic, Cabudare, Lara, Venezuela

^eDirectorate of Health, Department of Research and Academic Affairs, Instituto Venezolano de los Seguros Sociales (IVSS), Caracas, Venezuela

^fHealth Sciences Department, College of Medicine, Universidad Centrocidental Lisandro Alvarado, Barquisimeto, Lara, Venezuela

^gDepartment of Pediatrics, Policlínica Barquisimeto, Barquisimeto, Lara, Venezuela

^hDST-NRF Centre of Excellence in Epidemiological Modelling and Analysis (SACEMA), Stellenbosch University, Stellenbosch, South Africa

ⁱDepartment of Medicine, College of Medicine, University of Florida, Gainesville, Florida, USA

ABSTRACT The complete genome sequence was obtained for a *Dengue virus 2* isolate from the urine of an 8-year-old girl who was hospitalized with dengue hemorrhagic fever in 2016 in Venezuela.

Dengue virus serotypes 1 to 4 (DENV-1 to DENV-4) are distributed throughout Central and South America and the Caribbean (1). Symptoms of infection with DENV range from inapparent infection to dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (see <https://www.cdc.gov/dengue/symptoms/index.html>). DENV-1 to DENV-4 cocirculate in Venezuela (2), with the Asian/American (AS/AM) genotype of DENV-2 being associated with an increased incidence of DHF (3, 4). The AS/AM genotype of DENV-2 has been present throughout South America since the late 1970s (5). Several genetic subtypes of DENV-2 have been identified in Venezuela, suggesting that the virus has continued to evolve (6).

In April 2016, an 8-year-old girl presented to the hospital with a 2-day history of generalized petechiae, ecchymoses with acute onset, rectal bleeding, and occasional epistaxis. A week earlier, she had developed high-grade fever (38.5 to 39°C) followed by chills lasting 48 h, followed 3 days later by the appearance of a pruritic maculopapular rash. On admission, she was thrombocytopenic (platelet count, 25,000/ μ l), with a total leukocyte count of 9,200/ μ l, an elevated serum lactate dehydrogenase (LDH) level (443 IU/liter), and normal prothrombin time. She was conscious and well hydrated with stable blood pressure. In the hospital, there was continued spread of the ecchymoses, including ecchymotic skin lesions at venipuncture sites, and additional epistaxis, which prompted platelet transfusions. Hydration status was maintained, with no evidence of development of a shock syndrome. Her symptoms gradually resolved, with persistence of a few ecchymotic lesions that faded, and resolved uneventfully after 4 weeks.

Received 9 May 2018 Accepted 14 May 2018 Published 14 June 2018

Citation Blohm GM, Paniz-Mondolfi AE, Márquez MC, Loeb JC, Pacheco C, Lednicky JA, Pulliam JRC, Morris JG, Jr. 2018. Complete genome sequence of dengue virus serotype 2, Asian/American genotype, isolated from the urine of a Venezuelan child with hemorrhagic fever in 2016. *Genome Announc* 6:e00529-18. <https://doi.org/10.1128/genomeA.00529-18>.

Copyright © 2018 Blohm et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to J. Glenn Morris, Jr., jgmmorris@epi.ufl.edu.

Samples of blood and urine were collected 5 days after hospitalization. Serological tests revealed that she was positive for DENV IgG. Cryopreserved aliquots of the patient's plasma and urine were shipped on dry ice to the University of Florida (UF), where the urine but not plasma tested positive for DENV-2 genomic RNA by reverse transcriptase-PCR (RT-PCR) (7). For verification purposes and to produce sufficient virus for sequence analyses, both plasma and urine were inoculated into LLC-MK2 (CCL-7), MRC-5, and Vero E6 (CRL-1586) cells, with mock-infected cells were kept in parallel. Cells inoculated with urine developed cytopathic effects (CPEs) 14 days postinoculation or later. No CPEs were formed in cells inoculated with plasma. Viral genomic RNA (vgRNA) was extracted from virions in spent cell growth medium using a QIAamp viral RNA minikit (Qiagen, Inc., Valencia, CA), and DENV-2 vgRNA was detected by reverse transcription quantitative PCR (qRT-PCR) (7). Dengue virus 2 vgRNA from the Vero cells was Sanger sequenced as previously described (8, 9), and the complete virus genome sequence was obtained and designated DENV-2 strain Homo sapiens/VEN-HUPAZ-1/2016.

The genome sequence of DENV-2 strain Homo sapiens/VEN-HUPAZ-1/2016 clusters within the AS/AM genotype and has high identity (99%) with various DENV-2 sequences obtained from viruses in Venezuela in 2007 (e.g., GenBank accession numbers HQ332185, HQ332190, and HQ332187). These genomes fall within Venezuelan AS/AM subcluster B (10).

Accession number(s). The complete genome sequence of DENV-2 strain Homo sapiens/VEN-HUPAZ-1/2016 has been deposited in the GenBank database under the accession number [MH215277](https://www.ncbi.nlm.nih.gov/nuccore/MH215277).

ACKNOWLEDGMENTS

This work was supported by National Science Foundation grant 1515734 and National Institutes of Health grant R01 AI126357-0151.

REFERENCES

- Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, Drake JM, Brownstein JS, Hoen AG, Sankoh O, Myers MF, George DB, Jaenisch T, Wint GRW, Simmons CP, Scott TW, Farrar JJ, Hay SI. 2013. The global distribution and burden of dengue. *Nature* 496:504–507. <https://doi.org/10.1038/nature12060>.
- Huhtamo E, Comach G, Sierra G, Camacho DE, Sironen T, Vapalahti O, Uzcátegui NY. 2013. Diversity and composition of dengue virus type 2 in Venezuela. *Epidemiol Infect* 141:1816–1822. <https://doi.org/10.1017/S0950268812002324>.
- Gubler DJ, Clark GG. 1995. Dengue/dengue hemorrhagic fever: the emergence of a global health problem. *Emerg Infect Dis* 1:55–57. <https://doi.org/10.3201/eid0102.952004>.
- Rico-Hesse R, Harrison LM, Salas RA, Tovar D, Nisalak A, Ramos C, Boshell J, de Mesa MTR, Nogueira RMR, da Rosa AT. 1997. Origins of dengue type 2 viruses associated with increased pathogenicity in the Americas. *Virology* 230:244–251. <https://doi.org/10.1006/viro.1997.8504>.
- Vasilakis N, Weaver SC. 2008. The history and evolution of human dengue emergence. *Adv Virus Res* 72:1–76. [https://doi.org/10.1016/S0065-3527\(08\)00401-6](https://doi.org/10.1016/S0065-3527(08)00401-6).
- Uzcátegui N, Camacho D, Comach G, Cuello de Uzcátegui R, Holmes E, Gould E. 2001. Molecular epidemiology of dengue type 2 virus in Venezuela: evidence for *in situ* virus evolution and recombination. *J Gen Virol* 82:2945–2953. <https://doi.org/10.1099/0022-1317-82-12-2945>.
- Santiago GA, Vergne E, Quiles Y, Cosme J, Vazquez J, Medina JF, Medina F, Colón C, Margolis H, Muñoz-Jordán JL. 2013. Analytical and clinical performance of the CDC real time RT-PCR assay for detection and typing of dengue virus. *PLoS Negl Trop Dis* 7:e2311. <https://doi.org/10.1371/journal.pntd.0002311>.
- Iovine NM, Lednicky J, Cherabuddi K, Crooke H, White SK, Loeb JC, Cella E, Ciccozzi M, Salemi M, Morris JG, Jr. 2017. Coinfection with Zika and dengue-2 viruses in a traveler returning from Haiti, 2016: clinical presentation and genetic analysis. *Clin Infect Dis* 64:72–75. <https://doi.org/10.1093/cid/ciw667>.
- White SK, Iovine NM, Nickels LC, Morris JG, Jr, Lednicky JA. 2017. Complete genome sequence of dengue virus type 2 from a resident of north-central Florida with locally transmitted dengue fever. *Genome Announc* 5:e00782-17. <https://doi.org/10.1128/genomeA.00782-17>.
- Rodríguez-Roche R, Villegas E, Cook S, Poh Kim PA, Hinojosa Y, Rosario D, Villalobos I, Bendezu H, Hibberd ML, Guzman MG. 2012. Population structure of the dengue viruses, Aragua, Venezuela, 2006–2007. Insights into dengue evolution under hyperendemic transmission. *Infect Genet Evol* 12:332–344. <https://doi.org/10.1016/j.meegid.2011.12.005>.