



# Complete Genome Sequences of Identical *Zika virus* Isolates in a Nursing Mother and Her Infant

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**ABSTRACT** Complete genome sequences were obtained for Zika viruses isolated from the breast milk of a Venezuelan patient and her child, who was exclusively breastfeeding at the time. These sequences are the first to be reported from a presumptive autochthonous postnatal transmission case from mother to child in Venezuela.

*Zika virus* (ZIKV) is spreading widely in South and Central America, the Caribbean, and the Pacific (1). Although transmission of ZIKV is primarily mosquito-borne, direct transmission (e.g., through sexual contact and during pregnancy) is more common than previously expected (<https://www.cdc.gov/zika/transmission/>). We report here two complete genome sequences of ZIKV from two Venezuelan patients, where breastfeeding was the most likely mode of transmission.

The first isolate (VEN/UF-1/2016) is from the breast milk of a mother who developed symptoms of Zika fever (ZF) on 22 March 2016. The second isolate (VEN/UF-2/2016) is from the urine of her 5-month-old child who was exclusively breastfeeding and, interestingly, did not develop ZF. Breast milk, serum, and urine specimens were collected from the mother, and serum and urine specimens were collected from the child on 25 March 2016 at the Hospital Internacional Barquisimeto in Cabudare, Venezuela. All specimens tested positive for ZIKV genomic RNA (vRNA) by real-time PCR (RT-PCR). To determine whether the virus was infectious, aliquots of the specimens were inoculated onto LLC-MK2 cell cultures. Cytopathic effects (CPE) characteristic of ZIKV infection (2) were observed in all cell culture inoculations. To obtain sequencing templates, vRNA was extracted from the spent medium of cells inoculated with the mother's milk or child's urine using the QIAamp viral RNA minikit (Germantown, MD). Sanger Sequencing was completed using a genome walking strategy, as described previously (2). Briefly, cDNA was produced using AccuScript high-fidelity reverse transcriptase (Agilent Technologies, Santa Clara, CA) and sequence-specific primers. The resulting cDNA was amplified by PCR with Phusion polymerase (New England Biolabs)

Received 1 March 2017 Accepted 7 March 2017 Published 27 April 2017

**Citation** Blohm GM, Lednicky JA, Márquez M, White SK, Loeb JC, Pacheco CA, Nolan DJ, Paisie T, Salemi M, Rodríguez-Morales AJ, Morris JG, Jr, Pulliam JRC, Carrillo AS, Plaza JD, Paniz-Mondolfi AE. 2017. Complete genome sequences of identical *Zika virus* isolates in a nursing mother and her infant. *Genome Announc* 5:e00231-17. <https://doi.org/10.1128/genomeA.00231-17>.

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and gene-specific primers. The 5' and 3' ends of the viral genome were determined using a Rapid Amplification of cDNA Ends (RACE) kit (Life Technologies, Inc., Carlsbad, CA, USA). The sequences were assembled with Sequencher DNA sequence analysis software version 2.1 (Gene Codes, Ann Arbor, MI, USA). For phylogenetic analyses, ZIKV full-genome sequences were aligned using ClustalW (3) and BioEdit (4). The maximum likelihood phylogenetic tree was inferred from the full-genome alignment using the best fitting substitution model with IQ-TREE (<http://www.cdc.gov/zika/transmission/>) (5). Statistical robustness and reliability of the branching order within the tree were assessed by bootstrapping (1,000 replicates) and fast likelihood-based Shimodaira-Haswagawa (SH)-like probabilities (6) with IQ-TREE.

Full-genome comparison of the two ZIKV isolates revealed >99% identity between the two strains, with only two synonymous nucleotide substitutions at the third codon positions. The ZIKV sequences of the mother and child cluster with high bootstrap support (99%) within a larger clade of Colombian sequences. Both strains were different from the genomic sequences of ZIKV strains in the laboratory. The subjects of this report live in Barquisimeto, which is located along a major trade route between Colombia and Venezuela. The presence of infectious virus in the mother's breast milk is consistent with the findings of other recent studies (7–10) and suggests that breastfeeding could be an additional mode of direct transmission for ZIKV. We report here the first complete genome sequences of ZIKV isolated from a clinical breast milk sample in a patient from Venezuela.

**Accession number(s).** Sequences have been deposited in GenBank under the accession numbers [KX702400](#) (mother) and [KX893855](#) (child).

## ACKNOWLEDGMENTS

This work was supported by National Science Foundation Grant no. 1515734. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of National Science Foundation.

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