



Complete Genome Sequence of *Epizootic hemorrhagic disease virus* Serotype 6, Isolated from Florida White-Tailed Deer (*Odocoileus virginianus*)

✉ Mohammad Shamim Ahasan,^{a,b} ✉ Kuttichantran Subramaniam,^a ✉ John A. Lednicky,^{c,d} Julia C. Loeb,^{c,d} Katherine A. Saylor,^e ✉ Samantha M. Wisely,^e ✉ Thomas B. Waltzek^a

^aDepartment of Infectious Diseases and Immunology, College of Veterinary Medicine, University of Florida, Gainesville, Florida, USA

^bFaculty of Veterinary and Animal Sciences, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh

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

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

^eDepartment of Wildlife Ecology and Conservation, University of Florida, Gainesville, Florida, USA

ABSTRACT Here, we report the complete genome sequence of *Epizootic hemorrhagic disease virus* (EHDV) serotype 6 (EHDV-6), isolated from a Florida white-tailed deer (*Odocoileus virginianus*) in 2016. To our knowledge, this is the first full genome sequence determined for an EHDV-6 isolate from Florida.

Epizootic hemorrhagic disease virus (EHDV) is a causative agent of hemorrhagic disease of farmed and wild cervids, including white-tailed deer (1, 2). This epitheliotropic double-stranded RNA virus is a member of the genus *Orbivirus* within the family *Reoviridae* (3). Biting midges of the genus *Culicoides* are confirmed vectors of EHDV (4). Clinical cases of EHDV infection vary from peracute death to a chronic disease course that includes respiratory distress, edema of the head and neck, oral erosions, and sloughing of the hooves (1). Ten EHDV serotypes (EHDV-1 through EHDV-8, 318, and Ibaraki virus) have been described (5), and of these, 7 (EHDV-1, EHDV-2, and EHDV-4 through EHDV-8) are globally distributed (6). Complete genome sequences of EHDV-1 and EHDV-2, isolated from Florida white-tailed deer, were recently reported (7). Although EHDV-6 has been reported in some parts of the United States (8, 9), the complete genome sequence of this serotype from white-tailed deer in Florida had not previously been determined.

A spleen sample was taken from a farmed white-tailed deer (animal no. OV208) suspected of having epizootic hemorrhagic disease (EHD) during a 2016 health assessment conducted by researchers from the University of Florida Cervidae Health Research Initiative. The presence of EHDV virus RNA (vRNA) in a homogenized spleen specimen was confirmed by reverse transcription-PCR (RT-PCR) (10) and followed by observation of virus-induced cytopathic effect (CPE) in Vero E6 cells (*Cercopithecus aethiops* [African green monkey] kidney, ATCC CRL-1586) 20 days postinoculation. The vRNA was then extracted from virions in spent medium using a QIAamp viral RNA minikit (Qiagen). A cDNA library was constructed using a NEBNext Ultra RNA library prep kit and sequenced on an Illumina MiSeq instrument. *De novo* assembly of the paired-end reads was performed in SPAdes v3.10.0 with the default parameters (11). All 10 EHDV segments were recovered from the Florida white-tailed deer isolate OV208. BLASTN analysis of the outer-coat protein (VP2) from isolate OV208 revealed the highest nucleotide identity (99%; 2890/2907) to an EHDV-6 isolate from the spleen of

Received 21 February 2018 Accepted 9 March 2018 Published 5 April 2018

Citation Ahasan MS, Subramaniam K, Lednicky JA, Loeb JC, Saylor KA, Wisely SM, Waltzek TB. 2018. Complete genome sequence of *Epizootic hemorrhagic disease virus* serotype 6, isolated from Florida white-tailed deer (*Odocoileus virginianus*). *Genome Announc* 6:e00160-18. <https://doi.org/10.1128/genomeA.00160-18>.

Copyright © 2018 Ahasan 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 Thomas B. Waltzek, tbwaltzek@ufl.edu.

a white-tailed deer in Ohio in 2012 (12). The OV208 VP2 sequence was aligned in MAFFT 5.8 (13) to 79 orthologous EHDV-1, EHDV-2, and EHDV-6 nucleotide sequences retrieved from the GenBank database. A maximum likelihood (ML) analysis was performed in IQ-TREE (version 1.4.4) using 1,000 nonparametric bootstraps to test the robustness of the clades (14). Similar to the BLASTN results, the ML analysis supported placement of isolate OV208 as a member of the EHDV-6 clade most closely related to an EHDV-6 isolate from a white-tailed deer in Ohio (12).

The current study is the first to report the complete genome sequence of an EHDV-6 isolate from Florida white-tailed deer. A recent investigation (7) and the present study have sought to characterize the EHDV serotypes circulating in Florida white-tailed deer as the first step in the development of an efficacious vaccine to curb the impact of EHD on farmed deer populations.

Accession number(s). The genome sequence for EHDV-6, isolated from a Florida white-tailed deer (animal no. OV208), has been deposited in GenBank under the accession no. [MG886400](https://doi.org/10.1128/genomeA.01174-17) to [MG886409](https://doi.org/10.1128/genomeA.01174-17).

ACKNOWLEDGMENT

This study was funded by the University of Florida, Institute of Food and Agricultural Science Cervidae Health Research Initiative, with funds provided by the State of Florida legislature.

REFERENCES

- Savini G, Afonso A, Mellor P, Aradaib I, Yadin H, Sanaa M, Wilson W, Monaco F, Domingo M. 2011. Epizootic haemorrhagic disease. *Res Vet Sci* 91:1–17. <https://doi.org/10.1016/j.rvsc.2011.05.004>.
- Stevens G, McCluskey B, King A, O'Hearn E, Mayr G. 2015. Review of the 2012 epizootic hemorrhagic disease outbreak in domestic ruminants in the United States. *PLoS One* 10:e0133359. <https://doi.org/10.1371/journal.pone.0133359>.
- Attoui H, Mertens PPC, Becnel J, Belaganahalli S, Bergoin M, Brussaard CP, Chappell JD, Ciarlet M, del Vas M, Dermody TS. 2011. Family Reoviridae. In King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ (ed), *Virus taxonomy: ninth report of the International Committee on Taxonomy of Viruses*. Elsevier Academic Press, London, United Kingdom.
- Stallknecht DE, Howerth EW. 2004. Epidemiology of bluetongue and epizootic haemorrhagic disease in wildlife: surveillance methods. *Vet Ital* 40:203–207.
- Mertens PPC, Maan S, Samuel A. 2005. Genus *Orbivirus*, family Reoviridae, p 466–483. In Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (ed), *Virus taxonomy: eighth report of the International Committee on Taxonomy of Viruses*. San Diego, CA.
- Anthony S, Maan S, Maan N, Kgosana L, Bachanek-Bankowska K, Batten C, Darpel K, Sutton G, Attoui H, Mertens P. 2009. Genetic and phylogenetic analysis of the outer-coat proteins VP2 and VP5 of epizootic haemorrhagic disease virus (EHDV): comparison of genetic and serological data to characterise the EHDV serogroup. *Virus Res* 145:200–210. <https://doi.org/10.1016/j.virusres.2009.07.012>.
- Subramaniam K, Lednický JA, Loeb J, Saylor KA, Wisely SM, Waltzek TB. 2017. Genomic sequences of epizootic hemorrhagic disease viruses isolated from Florida white-tailed deer. *Genome Announcements* 5:e01174-17. <https://doi.org/10.1128/genomeA.01174-17>.
- Allison AB, Goekjian VH, Potgieter AC, Wilson WC, Johnson DJ, Mertens PPC, Stallknecht DE. 2010. Detection of a novel reassortant epizootic hemorrhagic disease virus (EHDV) in the USA containing RNA segments derived from both exotic (EHDV-6) and endemic (EHDV-2) serotypes. *J Gen Virol* 91:430–439. <https://doi.org/10.1099/vir.0.015651-0>.
- Ruder MG, Johnson D, Ostlund E, Allison AB, Kienzle C, Phillips JE, Poulson RL, Stallknecht DE. 2017. The first 10 years (2006–15) of epizootic hemorrhagic disease virus serotype 6 in the USA. *J Wildl Dis* 53:901–905. <https://doi.org/10.7589/2016-12-284>.
- Sun F, Cochran M, Beckham T, Clavijo A. 2014. Molecular typing of epizootic hemorrhagic disease virus serotypes by one-step multiplex RT-PCR. *J Wildl Dis* 50:639–644. <https://doi.org/10.7589/2013-11-302>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
- Anbalagan S, Cooper E, Klumper P, Simonson RR, Hause BM. 2014. Whole genome analysis of epizootic hemorrhagic disease virus identified limited genome constellations and preferential reassortment. *J Gen Virol* 95:434–441. <https://doi.org/10.1099/vir.0.059659-0>.
- Katoh K, Kuma K, Toh H, Miyata T. 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Res* 33: 511–518. <https://doi.org/10.1093/nar/gki198>.
- Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol* 32:268–274. <https://doi.org/10.1093/molbev/msu300>.