Complete Genome Sequence of a 2016 Bluetongue Virus Serotype 3 Isolate from Louisiana

Erin E. Schirtzinger, a Mark G. Ruder, b David E. Stallknecht, b William C. Wilson a

a USDA Agricultural Research Service, Arthropod-Borne Animal Diseases Research Unit, Manhattan, Kansas, USA
b Southeastern Cooperative Wildlife Disease Study, Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, Georgia

ABSTRACT The full-genome sequence of bluetongue virus serotype 3 (BTV-3) USA2016/LA CC16-564, isolated from a white-tailed deer in East Feliciana Parish, Louisiana, is reported here. Nine genomic segments of this virus have 99% identity with a 2013 BTV-3 isolate from Florida, while segment 10 has 97% identity with 2003 BTV-5 and 2006 BTV-2 isolates from Florida.

Bluetongue disease is an economically important disease of wild and domestic ruminants caused by the arthropod-transmitted bluetongue virus (BTV; family Reoviridae; genus Orbivirus) (1, 2). Five BTV serotypes are currently endemic to the United States, BTV serotype 2 (BTV-2), BTV-10, BTV-11, BTV-13, and BTV-17 (3–5). BTV-3 was isolated for the first time in Florida in 1999, and it was recently isolated in Mississippi, Arkansas, South Dakota, and Texas (6–10). Phylogenetic analyses of U.S. BTV-3 isolates have identified evidence of reassortment between recent U.S. BTV-3 isolates and serotypes endemic to the United States (11). These analyses and a 2016 outbreak of BTV-3 in white-tailed deer in Virginia and West Virginia (12) suggest that BTV-3 may be establishing itself as the sixth BTV serotype endemic to the United States. To understand the process of an exotic serotype becoming endemic to the United States, additional isolates of BTV-3 need to be sequenced and their phylogenetic relationships analyzed.

Here, we report the full-genome sequence of BTV-3 USA2016/LA CC16-564, isolated from a lung sample from a euthanized white-tailed deer in East Feliciana Parish, Louisiana, in 2016. The virus was initially isolated and passaged on cattle pulmonary artery endothelial (CPAE) cells (ATCC CCL-209) maintained in minimal essential medium (MEM) supplemented with sodium bicarbonate, 10% fetal bovine serum (FBS), and penicillin, streptomycin, and amphotericin B (PSF) (Sigma) at 37°C and 5% CO₂. To produce enough RNA for sequencing, the virus was then passaged twice in baby hamster kidney (BHK-21) cells (ATCC CCL-10) (Eagle’s minimal essential medium [EMEM; Sigma], 10% FBS, 2 mM glutamine plus, 1× PSF [Atlanta Biologicals], and 1× nonessential amino acids [Corning]) in a humidified 37°C incubator with 5% CO₂. When cytopathic effect was at 80 to 90%, the cells were pelleted by centrifugation. Total RNA was extracted using Trizol LS reagent (Invitrogen) according to the manufacturer’s instructions. The RNA was precipitated with an equal volume of isopropanol, incubated at −20°C for 10 min, and centrifuged at 12,000 × g for 30 min at 4°C. The pellet was washed with 1 ml of 75% ethanol and centrifuged at 7,500 × g for 10 min. The RNA was resuspended in RNase-free water warmed to 55°C. Double-stranded RNA (dsRNA) was isolated from total RNA by lithium chloride differential separation and subjected to whole-genome sequencing using the sequence-independent amplification procedure with modifications as described previously (14, 15). In brief, a 5’ phosphorylated primer (PC3) was ligated to the 3’ end of the dsRNA template. cDNA was produced from the ligated RNA with cloned avian myeloblastosis virus reverse transcriptase (AMVRT;
Invitrogen) and the PC2 primer, which was complementary to the 5′ end of the PC3 primer. Excess RNA was removed with NaOH, and single-stranded cDNA was annealed. The double-stranded cDNA was further amplified using Ex Taq DNA polymerase (TaKaRa) and the PC2 primer. The library (16 total samples) was prepared with the Nextera XT DNA library prep kit and Nextera XT index kit (Illumina) according to the manufacturer’s instructions. The library was normalized manually and sequenced on an Illumina MiSeq instrument using a MiSeq reagent kit (300 cycles) v2 (Illumina).

Reads were demultiplexed, and indexes and adaptors were removed automatically in the MiSeq Reporter software (Illumina) as fastq files were created. Low-quality bases were trimmed from the 588,278 paired-end reads in CLC Genomics Workbench 11.0 (Qiagen), and reads shorter than 50 nucleotides were discarded. The resulting 465,178 reads were de novo assembled into contigs. The contigs were compared to the NCBI GenBank nucleotide database using the BLAST at NCBI function in CLC Genomics Workbench to identify a suitable reference genome for reference-based assembly.

Based upon high nucleotide identity to the contigs and expected (E) values of 0.0, the BTV-3 USA 2013/FL N13-03980 genome was chosen as the reference. The reference-based assembly resulted in 10 contigs with an average fold coverage of 388 to 10,279 and 8,219 to 88,499 reads.

Table 1 lists the length of each contig/segment, including the lengths (in base pairs) of the 5′ and 3′ untranslated regions (UTRs) and the open reading frame (ORF) and the size in amino acids of each of the seven viral proteins (VP1 to VP7) and three nonstructural proteins (NS1 to NS3) (16, 17). BLAST searches of the complete segments of BTV-3 USA2016/LA CC16-564 showed 99% nucleotide identity with BTV-3 USA2013/FL N13-03980 for segments 1 to 9. Segment 10 instead showed 97% nucleotide identity with BTV-5 USA2003/FL 280559-7 and BTV-2 USA2006, also from Florida, but only 84.4% nucleotide identity with BTV-3 USA2013/FL N13-03980. When put into the larger phylogenetic context, BTV-3 USA2016/LA CC16-564 segment 10 is found in a clade containing Central American and Caribbean isolates and multiple invasive serotypes from Florida. Segment 10 of USA2013/FL N13-03980, however, is found in a clade with serotypes endemic to the United States, indicating that this isolate was a reassortant for segment 10 (data not shown). The relationships of the newly sequenced segments suggest that BTV-3 USA2016/LA CC16-564 is most likely an invasive strain of BTV-3, as opposed to evolving from BTV-3 strains circulating in Florida. This conclusion is not definitive, however, since sequences from recent Central American and Caribbean BTVs are not available. Continued sequencing and analysis of U.S. BTV-3 isolates will enable investigators to understand the complex process of virus invasion and establishment.

Data availability. The complete genome sequence of BTV-3 USA2016/LA CC16-564 has been deposited in GenBank under the accession numbers MH778118 to MH778127.

ACKNOWLEDGMENTS

Funding was provided by the USDA Agricultural Research Service (project 3020-32000-010-00D).
The views and conclusions contained in this document are those of the authors and should not be interpreted as necessarily representing the official policies, either expressed or implied, of the USDA.

REFERENCES

1. Hoar BR, Carpenter TE, Singer RS, Gardner IA. 2004. Probability of introduction of exotic strains of bluetongue virus into the US and into California through importation of infected cattle. Prev Vet Med 66:79–91. https://doi.org/10.1016/j.prevetmed.2004.08.006.

2. Schwartz-Cornil I, Mertens PPC, Contreras V, Hemati B, Pascale F, Bréard E, Mellor PS, MacLachlan NJ, Zientara S. 2008. Bluetongue virus: virology, pathogenesis and immunity. Vet Res 39:46. https://doi.org/10.1051/vetres:2008023.

3. Barber TL, Collisson EW. 1983. Implications of a new bluetongue serotype for the US livestock industry, p 90–104. In Proceedings of the 87th Annual Meeting. United States Animal Health Association, St. Joseph, MO.

4. Barber TL. 1979. Temporal appearance, geographic distribution and species of origin of bluetongue virus serotypes in the United States. Am J Vet Res 40:1654–1656.

5. Gibbs EP, Greiner EC, Taylor WP, Barber TL, House JA, Pearson JE. 1983. Isolation of bluetongue virus serotype 2 from cattle in Florida: serotype of bluetongue virus hitherto unrecognized in the Western Hemisphere. Am J Vet Res 44:2226–2228.

6. Ostlund EN. 2000. Report of the Committee on Bluetongue and Bovine Retroviruses: bluetongue virus (BTV) and epizootic hemorrhagic disease virus isolations/PCR positives, p 160–163. In Proceedings of the 104th Annual Meeting. United States Animal Health Association, St. Joseph, MO.

7. Ostlund EN. 2007. Report of the Committee on Bluetongue and Bovine Retroviruses: bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) isolations/PCR positives—calendar year 2004, p 112–116. In Proceedings of the 119th Annual Meeting. United States Animal Health Association, St. Joseph, MO.

8. Ostlund EN. 2010. Report of the Committee on Bluetongue and Bovine Retroviruses: National Veterinary Services Laboratory update, p 155–156. In Proceedings of the 114th Annual Meeting. United States Animal Health Association, St. Joseph, MO.

9. Ostlund EN. 2015. Report of the Committee on Bluetongue and Related Orbiviruses: bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) isolations/PCR positives—calendar year 2014, p 112–116. In Proceedings of the 119th Annual Meeting. United States Animal Health Association, St. Joseph, MO.

10. Ostlund EN. 2016. Hemorrhagic disease update. SCWDS Briefs 32:1–2.

11. Ruder MG, Kienzle C. 2016. Hemorrhagic disease update. SCWDS Briefs 32:1–2.

12. 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.

13. Schirnigl EE, Jaspersn DC, Ostlund EN, Johnson DJ, Wilson WC. 2018. Recent US bluetongue virus serotype 3 isolates found outside of Florida indicate evidence of reassortment with co-circulating endemic serotypes. J Gen Virol 99:157–168. https://doi.org/10.1099/gv.0.000965.

14. Potgieter AC, Page NA, Liebenberg J, Wright IM, Landt O, van Dijk AA. 2009. Improved strategies for sequence-independent amplification and sequencing of viral double-stranded RNA genomes. J Gen Virol 90:1423–1432. https://doi.org/10.1099/vir.0.009381-0.

15. Ratinier M, Caporale M, Golder M, Franzoni G, Allan K, Nunes SF, Armezzani A, Bayoumy A, Rixon F, Shaw A, Palmarini M. 2011. Identification and characterization of a novel non-structural protein of bluetongue virus. PLoS Pathog 7:e1002477. https://doi.org/10.1371/journal.ppat.1002477.