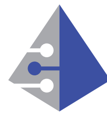
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GENOME SEQUENCES

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

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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% CO_2 (13). 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% CO_2. 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;

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TABLE 1 Sequencing and length information for each segment of BTV-3 USA2016/LA CC16-564

					Length of[a]:				
GenBank accession no.	Segment	Protein	No. of reads	Coverage (×)	Segment (bp)	5′ UTR (bp)	ORF (bp)	3′ UTR (bp)	Protein (aa)
KY091945	1	VP1	36,804	1,220	3,944	6	3,906	32	1,302
KY092168	2	VP2	16,612	738	2,935	21	2,880	34	959
KY092134	3	VP3	8,219	388	2,772	17	2,703	52	901
KY092107	4	VP4	16,359	1,069	1,981	8	1,932	41	644
KY092079	5	NS1	14,530	1,066	1,771	34	1,656	81	552
KY092055	6	VP5	33,507	2,665	1,637	15	1,578	44	526
KY092026	7	VP7	31,817	3,505	1,156	17	1,047	92	349
KY091999	8	NS2	88,499	10,279	1,125	19	1,062	44	354
KY091956	9	VP6	75,746	9,186	1,046	15	984	47	328
KY091918	10	NS3	42,487	6,617	822	19	687	116	229

[a] UTR, untranslated region; ORF, open reading frame; aa, amino acids.

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) encoded by each segment (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.

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