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# Genome characterization of two bluetongue virus serotype 5 strains isolated from white-tailed deer: first detected cases in Florida after 19 years

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**Abstract**

Hemorrhagic disease caused by bluetongue virus (BTV) significantly impacts Florida's deer farming industry each year. In 2022, we investigated mortality events in white-tailed deer on two separate farms. Necropsies were performed on affected animals, and tissues were subjected to RT-qPCR screening and molecular diagnostics, confirming BTV infection. Virus isolation in C6/36 cell culture and whole-genome sequencing identified BTV serotype 5 (BTV-5) infection in both fawns—the first cases reported in Florida since 2003. Our analyses further reveal that key serotype-defining genome segments (2 and 6) remained conserved, while other segments exhibited evidence of reassortment with co-circulating BTV strains in the region. This suggests that BTV-5 may have persisted undetected at low levels, undergoing genetic exchange with co-circulating strains. These findings highlight the need for improved surveillance programs to monitor genetic changes and mitigate impacts on deer and other ruminants in Florida.

## 1 Introduction

Bluetongue virus (BTV; species *Orbivirus caerulinguae*) is an arthropod-transmitted pathogen that causes a hemorrhagic disease, particularly in certain susceptible domestic and wild ruminants such as sheep and some species of deer, while other species—including cattle, goats, buffalo, and antelope—may experience mild or asymptomatic infections [1]. This virus can lead to significant economic losses in livestock industries worldwide [2]. In Florida, the white-tailed deer (WTD; *Odocoileus virginianus*) are economically valuable, with farming and hunting generating $95 million in state and local taxes and supporting over 14,000 jobs in 2011 alone [3]. Although BTV infections in cattle, goats, and camelids are typically self-limiting and asymptomatic, sheep and farmed deer show high susceptibility to the virus [4]. Combined with the humid-subtropical climate in Florida—which enables the year-round activity of *Culicoides* midges, the primary

vectors of BTV—contributes to seasonal epidemics, particularly during the warmer months when midge populations peak, and severely impacts the deer farming industry [5]. As a result, BTV continues to pose a significant threat to this sector.

Bluetongue virus is classified under the genus *Orbivirus* within the *Sedoreoviridae* family and contains 10 linear double-stranded RNA segments [6]. Like other segmented viruses, BTV undergoes genetic reassortment during co-infections, allowing genome segment exchange between strains [7]. This process, coupled with the high mutation rate of RNA viruses, drives BTV evolution, potentially altering virulence, transmission dynamics, and host range [8]. Genome segments 2 and 6 encode the VP2 and VP5 proteins, respectively, which form the outer capsid of the virion [9]. These proteins are highly variable and are key determinants of the host immune response, serving as the primary factors that determine the serotype of BTV strains [1, 9]. Of the 36 known BTV serotypes identified thus far [10], BTV-5 has exhibited sporadic yet persistent occurrences across different countries worldwide [11–15].

Bluetongue virus serotype 5 was first identified in South Africa in 1960 [16] and has since been reported in several other countries, including Australia [12], Cameroon [17], China [13], the French Caribbean Island of Guadeloupe [18], Israel [15], Nigeria [19, 20], and the United States [21]. In the U.S., BTV-5 was first detected in 2003 in cattle from Manatee County, Florida [21] and later in Nebraska in 2020 [22]. However, based on updated guidelines, BTV-5 is classified as a "reported but not established" serotype, as it has not been detected continuously for more than two years in the U.S. [23]. According to annual reports from the United States Animal Health Association (https://usaha.org/meetings/) and the University of Florida Cervidae Health Research Initiative (CHeRI), no BTV-5 cases had been documented in Florida since 2003, highlighting a critical gap in monitoring efforts.

To date, only one complete genome sequence from the 2003 BTV-5 case in Florida and a segment 2 (VP2) sequence from a case in Guadeloupe are available for this serotype in the Americas. Herein, we report the clinical findings, ancillary diagnostics, complete genome sequencing, and phylogenetic analyses of gene segments from two novel reassortant BTV-5 strains detected in deceased WTD from Florida farms in 2022.

## 2 Material and methods

In 2022, we received reports from farmers regarding cases of deceased WTD on separate farms in Florida in August and September. The first case involved a 3-month-old buck fawn (Animal ID: OV1688), which was found in Hernando County on August 16. A field necropsy was promptly performed later that day by UF IFAS CHeRI technicians. The second case involved a 9-week-old buck fawn (Animal ID: OV1714), which was found in Clay County on September 7, with a necropsy also performed the same day. Lung tissue samples were submitted to the University of Florida, College of Veterinary Medicine Diagnostic Laboratories—Microbiology, Parasitology, and Serology Laboratory (Gainesville, FL, USA) for bacterial isolation and identification. Additional tissue samples (i.e., blood, kidney, liver, lung, heart, and spleen) were collected and stored at – 80 °C for future analyses. All procedures performed in the study were in compliance with the approved protocols granted by the University of Florida Institutional Animal Care and Use Committee (IACUC Protocols 201609390 and 201909390). Additionally, permission was obtained from the clients for the use of their animals in this study.

Total RNA was extracted from spleen samples collected from two animals, OV1688 and OV1714, using a RNeasy Mini kit (Qiagen, Valencia, CA, USA) in accordance with the manufacturer's instructions. The RNA extracts were then routinely screened for BTV [24], Bovine Viral Diarrhea Virus (BVDV) [24, 25], Epizootic Hemorrhagic Disease Virus (EHDV) [24], West Nile Virus (WNV) [26], and Eastern Equine Encephalitis Virus (EEEV) [27] using the VetMAX Plus One-Step RT-qPCR kit (Applied Biosystems, Waltham, MA, USA). Following the confirmation of BTV infection in the samples from OV1688 and OV1714, the spleen tissues collected from these animals were thawed and aseptically minced with forceps. The tissues were then homogenized to create a 10% w/v cell-free suspension in sterile phosphate-buffered saline (PBS) using a sterile manual tissue grinder (Fisher Scientific, Thermo Fisher Scientific, Waltham, MA, USA). The resulting homogenates were cleared of debris through low-speed centrifugation (5 min at 1500 × *g*) and filtered through a sterile 0.45 µm pore-size polyvinylidene fluoride filter (Fisher Scientific). A 0.5 mL aliquot of the filtrate of each sample was separately inoculated onto confluent C6/36 cells (*Aedes albopictus* [Asian tiger mosquito, ATCC CRL1660]) which were maintained in Advanced Dulbecco's Modified Eagle's Medium (Invitrogen Corp. Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 2 mM L-alanyl-L-glutamine (Invitrogen), antibiotics (PSN; 50 µg/mL penicillin, 50 µg/mL streptomycin, 100 µg/mL neomycin [Invitrogen]) and 10% low-antibody, heat-inactivated, gamma-irradiated fetal bovine serum (HyClone™, Marlborough, MA, USA). The cells were re-fed every 3 days and monitored daily for virus-induced cytopathic effects (CPE) over a period of 30 days. Non-inoculated cells were maintained in parallel as negative controls.

Once cells showed cytopathic effects (CPE), RNA was extracted from the scraped cells together with spent cell growth media and floating dead cells using the QIAamp Viral RNA Mini kit (Qiagen) as per the manufacturer's instructions and stored at − 80 °C prior to next-generation sequencing. cDNA libraries were prepared using the NEBNext Ultra RNA Library Prep kit (New England Biolabs, Ipswich, MA, USA) and sequenced on an Illumina MiSeq sequencer (Illumina, San Diego, CA, USA). Cell culture host sequences (*A. albopictus*; GenBank accession number MNAF00000000.2) were filtered out from the sequencing data using Kraken v2.0 [28]. The remaining paired-end reads were assembled de novo using the CLC Genomics Workbench version 20.0.4. Assembled contigs were screened for orbiviruses and other possible viral sequences using Diamond Blast (BLASTX) searches in OmicsBox v1.2 (BioBam Bioinformatics, Cambridge, MA, USA), against the National Center for Biotechnology Information (NCBI) non-redundant protein database.

For the phylogenetic analyses, ten coding sequences from 113 BTV strains, selected to represent the diversity of strains circulating in North America, as well as all reported BTV-5 strains available in GenBank. One EHDV-6 strain was included as an outgroup. Additionally, for the segment 2 phylogenetic analysis, seven more coding sequences of BTV-5 strains were included to broaden the representation of this serotype, although sequences for the other segments are unavailable. The accession numbers for all sequences are provided in Supplemental Table S1. Each coding sequence was aligned with the BTV strains from OV1688 and OV1714 using MAFFT in Geneious Prime v2022.2.2. Maximum-likelihood trees for each aligned coding sequence were generated using IQ-TREE v1.6.12 with 1000 replicates of ultrafast bootstrap and SH-like

approximate likelihood ratio test (SH-aL RT) to determine branch support [29]. Additionally, the nucleotide identity of all coding sequences from OV1688 and OV1714 was compared to each other and against the NCBI non-redundant nucleotide database through BLASTN analyses.

## 3 Result and discussion

Sample locations for both cases are shown in Fig. 1A, and gross examination of fawns OV1688 and OV1714 revealed pallor of the mucous membranes in the mouth and eyes, signs of dark diarrhea, and severe hemorrhage in the lungs (Fig. 1B and C). Both animals were in good body condition prior to death. No pathogenic bacteria were isolated from the lungs of OV1688 and OV1714. Molecular diagnostics identified BTV infection in spleen samples from both fawns by RT-PCR, while tests for other viruses were negative. Following inoculation of spleen homogenates onto C6/36 cells, cytopathic effects (CPE) characterized by widespread cell death were first observed 8 days post-inoculation. By day 11, extensive CPE resulted in the destruction of more than 90% of the cell monolayer.

The next-generation sequencing of RNA extracts from infected cell cultures, followed by de novo assembly, successfully generated complete coding sequences for all segments of BTV (GenBank accession numbers PQ625392-PQ625411). The segment 2 sequences of OV1688 and OV1714 shared the highest identity (93.58% and 93.44%, respectively) with the BTV-5 strain BTV5/10.01 (5119) from Guadeloupe. Meanwhile, the segment 6 sequences exhibited the highest identity (91.02% and 90.96%, respectively) with the BTV-5 strain USA2003/FL 280559-7. Further analysis indicated that segment 1 (*VP1* gene) and segment 3 (*VP3* gene) from both fawns were closely related to the BTV-1 strain OV1706 previously identified in Florida. Other segments (4 (*VP4* gene), 5 (*NS1* gene), 7 (*VP7* gene), 8 (*NS2* gene), 9 (*VP6* gene), and 10 (*NS3* gene)) showed varying identity with strains from Florida, Cuba, and Louisiana (Tables 1 and 2). Comparison between BTV-5 strains OV1688 and OV1714 revealed that segments 2 and 6

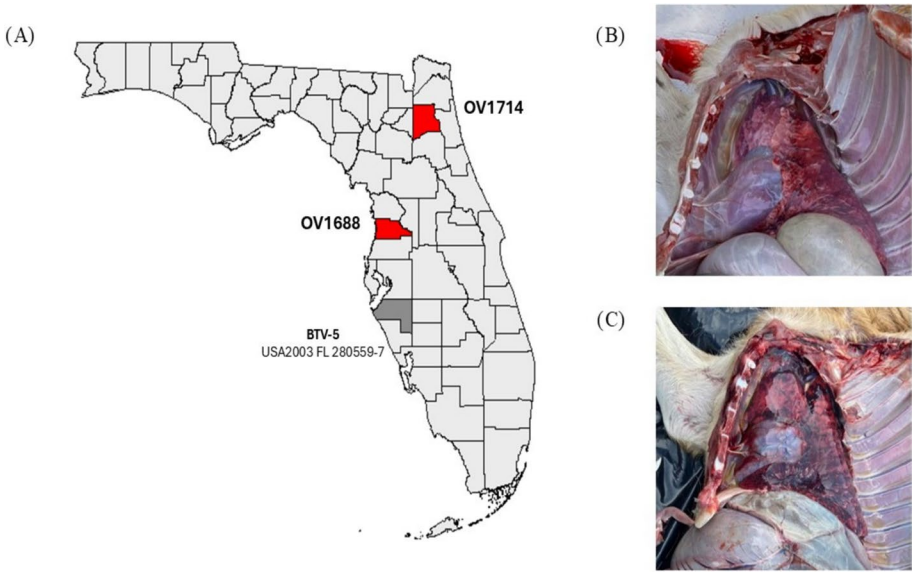


**Fig. 1** **A** Map of Florida showing Hernando and Clay Counties, where BTV-5 was detected in farmed white-tailed deer in 2022, highlighted in red, and Manatee County, the location of the initial 2003 BTV-5 detection highlighted in dark grey. **B**, **C** Gross observations of fawns OV1688 (**B**) and OV1714 (**C**) displaying severe pulmonary hemorrhage and congestion

**Table 1** Comparison of genome segments from BTV-5 strain OV1688

| Gene name (segment number) | Length (bp) | Top BLASTN match | | | | | |
|---|---|---|---|---|---|---|---|
| | | Nucleotide identity to OV1714 (%) | Host | Serotype | Strain/isolate | Identity (%) | GenBank accession no. |
| VP1 (1) | 3909 | 98.93 | WTD | BTV-1 | OV1706_ FL_BTV1 | 98.98 | OQ847485.1 |
| VP2 (2) | 2868 | 99.02 | Cattle | BTV-5 | BTV5/10.01 (5119) Guadeloupe | 93.58 | HQ241072.1 |
| VP3 (3) | 2706 | 98.97 | WTD | BTV-1 | OV1706_FL_BTV1 | 99.11 | OQ847487.1 |
| VP4 (4) | 1935 | 98.91 | WTD | BTV-11 | USA2013/FL 13–037190 | 99.59 | KM580476.1 |
| NS1 (5) | 1659 | 88.91 | WTD | BTV-1 | OV1049_FL_BTV1 | 99.46 | OQ847479.1 |
| VP5 (6) | 1581 | 99.56 | Cattle | BTV-5 | USA2003/FL 280559–7 | 91.02 | KX164064.1 |
| VP7 (7) | 1050 | 95.62 | Cattle | BTV-6 | USA2006/01 | 98.1 | GQ506542.1 |
| NS2 (8) | 1065 | 97.09 | WTD | BTV-2 | OV1681_FL_BTV2 | 99.62 | OR672568.1 |
| VP6 (9) | 990 | 95.96 | Cattle | BTV-10 | Cuba BTV10-23.02 (3037) | 98.18 | OR611784.1 |
| NS3 (10) | 690 | 92.61 | WTD | BTV-11 | USA2013/FL 13–037190 | 99.57 | KM580474.1 |

This table summarizes the BLASTN results for each gene segment, detailing sequence identity with top-matching strains and nucleotide identity with BTV-5 strain OV1714

*WTD* white-tailed deer

**Table 2** Comparison of genome segments from BTV-5 strain OV1714

| Gene name (segment number) | Length (bp) | Top BLASTN match | | | | |
|---|---|---|---|---|---|---|
| | | Host | Serotype | Strain/isolate | Identity (%) | GenBank accession no. |
| VP1 (1) | 3909 | WTD | BTV-1 | OV1706_FL_BTV1 | 98.98 | OQ847485.1 |
| VP2 (2) | 2868 | Cattle | BTV-5 | BTV5/10.01 (5119) Guadeloupe | 93.44 | HQ241072.1 |
| VP3 (3) | 2706 | WTD | BTV-1 | OV1706_FL_BTV1 | 99.26 | OQ847487.1 |
| VP4 (4) | 1935 | WTD | BTV-11 | USA2013/FL 13–037190 | 99.33 | KM580476.1 |
| NS1 (5) | 1659 | Sheep | BTV-22 | USA2005/FL 402286 | 99.28 | KX164143.1 |
| VP5 (6) | 1581 | Cattle | BTV-5 | USA2003/FL 280559–7 | 90.96 | KX164064.1 |
| VP7 (7) | 1050 | WTD | BTV-3 | USA2016/LA CC16-564 | 96.67 | MH778124.1 |
| NS2 (8) | 1065 | WTD | BTV-18 | USA2014/FL 15–008010 | 99.15 | KX164126.1 |
| VP6 (9) | 990 | Cattle | BTV-19 | Cuba BTV19-23.01 (3100) | 96.67 | OR611874.1 |
| NS3 (10) | 690 | WTD | BTV-24 | USA2007/FL 520518 | 98.99 | KX164158.1 |

This table summarizes the BLASTN results for each gene segment, detailing sequence identity with top-matching strains

*WTD* white-tailed deer

were mostly conserved, with identities of 99.02% and 99.56%, respectively. However, the presence of genetic differences suggests that these strains likely share a common ancestor but have evolved separately over time, while the other segments exhibited identities ranging from 88.91 to 98.97%.

Phylogenetic analyses of segments 2 and 6 further confirmed the BTV serotype 5 classification for strains OV1688 and OV1714, as these clustered with other BTV-5 representatives (Fig. 2). These analyses further indicated that OV1688 and OV1714 are closely related to BTV-5 strains reported in Florida in 2003 and Guadeloupe in 2010 (BTV-5 strain USA2003/FL 280559-7 and BTV5/10.01 (5119), respectively (Fig. 2). Due to the lack of a complete genome sequence for the Guadeloupe strain BTV5/10.01 (5119), a comprehensive comparison with Florida BTV-5 strains was limited. However, our


**Fig. 2** Maximum Likelihood cladogram illustrating the relationships of the *VP2* (**A**) and *VP5* (**B**) genes of bluetongue virus serotype 5 (BTV-5) strains OV1688 and OV1714, highlighted in red and bold to 113 other BTV strains and 1 EHDV strain. Additionally, seven more coding sequences of VP2 of BTV-5 strains were included to broaden the representation of this serotype. Each strain is identified by serotype, strain/isolate, host, country, state, and year of isolation. The SH-aLRT and ultrafast bootstrap support values ≥ 80% are displayed at each node, indicating robust support. The tree is rooted with the EHDV-6 strain OV1321. Additional metadata for each virus in the tree are provided in Supplemental Table S1

analyses show that these Florida BTV-5 strains form a distinct clade, setting them apart from BTV-5 strains reported in other regions. Additionally, evidence of reassortment was observed (SH-aLRT and bootstrap support values > 80%), particularly in the OV1688 strain's segment 8 (with BTV-2 strain OV1681 FL BTV2) and segment 9 (with BTV-1 strain OV1706 FL BTV1). Similarly, reassortment was detected in OV1714, involving segment 5 (with BTV-22 strain USA2005/FL 402286) and segment 10 (with BTV-24 isolate USA2007/FL 520518). This suggests that genetic exchanges between BTV-5

strains and other co-circulating BTV strains have occurred, resulting in the emergence of reassorted viruses. The remaining genome segments (1, 3, 4, 5, 7, 10 of OV1688; 1, 3, 4, 7, 8, 9 of OV1714) showed no evidence of reassortment based on the currently available sequences, and these segments clustered primarily with strains from Florida and Cuba. However, it is possible that this observation is influenced by the limited number of related sequences deposited in public databases, which may be a consequence of insufficient surveillance in some regions. Phylogenetic trees for each segment are shown in Supplemental Figures S1 to S4.

The detection of BTV-5 in Florida after nearly two decades raises critical questions about the persistence and evolutionary dynamics of this virus. Our analyses showed that while BTV-5 strains OV1688 and OV1714 exhibit high genetic similarity in segments 2 and 6, they have also acquired other genome segments from various BTV serotypes. This pattern suggests that OV1688 and OV1714 strains have followed separate evolutionary paths, with reassortment among co-circulating BTV serotypes contributing to the observed genomic complexity. These findings also imply that BTV-5 may have remained active in the region, potentially interacting with other circulating BTV strains. The genetic exchange likely occurred through co-infection events within individual hosts, facilitating the mixing of gene segments and the emergence of novel reassortant viruses. Prolonged infections in asymptomatic animals, particularly cattle, have also been shown to elevate the likelihood of BTV co-infections, which, in turn, can promote viral reassortment within the host [30]. Moreover, asymptomatic infections can facilitate undetected viral transmission within and between herds without exhibiting clinical signs of BTV [31]. The absence of data on this serotype in Florida over nearly two decades may also be due to limited surveillance programs and testing protocols. This discrepancy highlights a possible gap in robust monitoring efforts for BTV in this region. Furthermore, some animals may exhibit low viral loads, making the virus more difficult to detect using standard diagnostic methods [32]. Hence, implementing more comprehensive surveillance strategies will be essential for effectively monitoring and understanding the transmission dynamics of BTV-5 in farmed deer populations and other ruminants.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s44370-025-00023-y.

Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Supplementary Material 4.

Supplementary Material 5.

**Author contributions**

Conceptualization P.K.; J.A.L.; S.M.W.; K.S. Methodology P.K.; P.H.O.V.; K.N.W.; T.L.M.; J.M.C.K.; J.A.L.; S.M.W.; K.S. Formal analysis P.K.; K.S. Investigation P.K.; P.H.O.V.; K.N.W.; T.L.M.; J.M.C.K.; J.A.L.; S.M.W.; K.S. Resources J.M.C.K.; J.A.L.; S.M.W.; K.S. Writing—Original Draft P.K.; K.S. Writing—Review & Editing P.K.; P.H.O.V.; K.N.W.; T.L.M.; J.M.C.K.; J.A.L.; S.M.W.; K.S. Visualization P.K.; K.S. Supervision K.S. Project administration S.M.W.; K.S. Funding acquisition S.M.W.; K.S.

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**Data availability**

The complete gene coding sequences for all 10 segments of the genomes recovered in this study have been deposited in the NCBI GenBank database and are available under GenBank accession numbers PQ625392-PQ625411.

## Declarations

### Ethics approval and consent to participate

The study involves non-human vertebrates, specifically white-tailed deer, which are higher vertebrates. Clinical records and diagnostic samples were collected from veterinary clinical patients or symptomatic agricultural animals and were used with the owner's consent for this study. All procedures performed in the study were approved by the University of Florida Institutional Animal Care and Use Committee (IACUC Protocols 201609390 and 201909390) in accordance with the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals (OLAW Assurance #: D16-00244).

### Consent for publication

All the authors gave consent for the publication of this manuscript.

### Competing interests

The authors declare no competing interests.

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