Virus Genes (2023) 59:732–740
https://doi.org/10.1007/s11262-023-02019-6

ORIGINAL PAPER

Characterization of two novel reassortant bluetongue virus serotype 1 strains isolated from farmed white-tailed deer (*Odocoileus virginianus*) in Florida, USA

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Received: 10 May 2023 / Accepted: 27 June 2023 / Published online: 13 July 2023

Abstract

Hemorrhagic diseases caused by epizootic hemorrhagic disease virus or by bluetongue virus (BTV) are the most important orbivirus diseases affecting ruminants, including white-tailed deer (WTD). Bluetongue virus is of particular concern for farmed WTD in Florida, given its lethality and its wide distribution throughout the state. This study reports the clinical findings, ancillary diagnostics, and genomic characterization of two BTV serotype 1 strains isolated from two farmed WTD, from two different farms in Florida in 2019 and 2022. Phylogenetic and genetic analyses indicated that these two novel BTV-1 strains were reassortants. In addition, our analyses reveal that most genome segments of these strains were acquired from BTVs previously detected in ruminants in Florida, substantiating their endemism in the Southeastern U.S. Our findings underscore the need for additional research to determine the genetic diversity of BTV strains in Florida, their prevalence, and the potential risk of new BTV strains to WTD and other ruminants.

Keywords Cervidae · Bluetongue virus · Hemorrhagic disease · White-tailed deer · Orbivirus · Reovirus

Edited by Zhen Fu.

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Introduction

Deer farming is one of the fastest-growing industries in rural areas of the U.S. It is a significant contributor to the economy and vitality of rural areas, generating an annual revenue of approximately $7.9 billion (https://nadefa.org/). While multiple cervid species are farmed in the U.S. (https://nadefa.org/), white-tailed deer (WTD; *Odocoileus virginianus*) is the most farmed deer [1]. In Florida, there are over 300 registered game farms, and most of them breed WTD for the purpose of producing animals that are sought after by hunters for their impressive antlers, size, and appearance.

White-tailed deer in Florida are exposed to disease risks due to the humid subtropical climate and prime environmental conditions for arthropod vectors and pathogens [2]. Multiple orbiviruses within the genus *Orbivirus* (Family *Reoviridae*) have been isolated and identified from diseased Florida WTD, including Big Cypress orbivirus, bluetongue virus (BTV), CHeRI orbiviruses 1–3, epizootic hemorrhagic disease virus (EHDV; serotypes 1, 2, and 6), mobuck orbivirus, and Yunnan orbivirus [3–12]. BTV is of particular concern to farmed WTD in Florida, given its severe impacts and its wide distribution throughout the state. A recent surveillance study involving 539 post-mortem specimens collected from 55 deer farms across Florida between 2016 and 2020 showed the prevalence of BTV and BTV/EHDV coinfection at 16% and 10%, respectively, by RT-qPCR. Additionally, BTV was found in 62% (18/29) of the counties sampled, extending from north to south Florida [12].

Bluetongue virus is the causative agent of bluetongue disease and is mainly transmitted by *Culicoides* biting midges [13]. Historically, the epidemiological cycle involved wild African ruminant species and local *Culicoides* spp. in sustaining the virus cycle. However, the expansion of agriculture and the introduction of African cattle into other regions, including the Americas, Europe, the Middle East, Southern Asia, and Oceania, led to the expansion of its host range, including highly susceptible species such as WTD [14]. Ruminant species susceptible to BTV infection include antelope, buffalo, cattle, deer, elk, goat, and sheep [14]. The clinical signs of bluetongue disease vary between species, from inapparent to fatal. Sheep and deer are the most prone to developing severe clinical signs, including acute hemorrhagic disease and death [15].

The BTV genome consists of 10 linear double-stranded RNA segments that encode seven structural (VP1 - VP7) and at least four nonstructural (NS1, NS2, NS3/3a, and NS4) proteins [16–18]. The virus core consists of nonstructural proteins that are associated with transcriptase complexes, including VP1 (RNA-dependent RNA polymerase), VP4 (capping enzyme), and VP6 (virus helicase), which are enclosed in layers of VP3 and VP7 proteins [16, 19]. The BTV core is surrounded by the outer capsid layer consisting of VP2 and VP5 proteins. Of the virus proteins encoded by the BTV genome, VP2 and VP5 are the most variable and are used to determine the serotype of various BTV strains [16]. To date, at least 32 distinct BTV serotypes have been identified [20–26]. The status of BTV serotypes in the U.S. has recently been updated, and they are classified as either established (3, 6, 10, 11, 12, 13, and 17), reported (1, 2, 5, 9, 14, 15, 18, 19, 22, and 24), or not reported (4, 7, 8, 16, 20, 21, 23, 25, and 26) [15]. In the Southern U.S., 11 established or reported BTV serotypes (1, 3, 5, 6, 9, 12, 14, 18, 19, 22, and 24) have been detected since 1999 [15, 27].

Genetic diversity among BTV strains can be attributed to virus reassortment and mutation [28, 29]. Reassortment is a genetic process that occurs when two or more virus strains infect the same host cell and exchange their gene segments to generate virus progeny with unique genome combinations [28, 30]. The emergence of new BTV strains through reassortment likely stems from interactions between different endemic BTVs or the importation of novel BTVs from other regions. For example, BTV-2, a serotype previously confined to the Southeastern U.S., was isolated in a California heifer in 2010 and was found to be a reassortant of BTV-2 and BTV-6 [27, 31]. Similarly, Schirtzinger et al. [32] analyzed 22 strains of BTV originating from the U.S., the Caribbean, and South Africa. Their findings indicate the presence of at least two distinct lineages of reassortant BTV-3 strains circulating in the U.S. Given the significant mortality and economic losses attributed to BTV disease, specifically in farmed animals such as WTD, it is essential to better understand BTV ecology and genetic diversity. This study reports the clinical findings, ancillary diagnostics, and genomic characterization of two novel reassortant BTV-1 strains from euthanized WTD on Florida farms in 2019 and 2022.

Materials and methods

Clinical history and sample collection

On February 2nd, 2019, a 2-year-old doe (animal ID: OV1049) was found recumbent and obtunded. The farmer treated the animal with one dose of 2 cc Draxxin (tulathromycin), 2 cc dexamethasone, and 12.5% Sulfadived (sulfadimethoxine) water solution. After 4 days without any improvement in its condition, the animal was euthanized [33]. University of Florida (UF) technicians performed a field necropsy following guidelines provided by CHeRI (https://wec.ifas.ufl.edu/cheri/diagnostics/ [accessed on 29 October 22]) soon after the animal was euthanized. On August 19th, 2022, a 3-month-old buck fawn (animal ID:

OV1706) exhibited ataxia earlier in the day that progressed until the animal became recumbent and obtunded. The buck was euthanized and a field necropsy was performed by UF technicians the following day.

Lung tissue (from OV1049 and OV1706) and fecal samples (from OV1049) were submitted to the UF Microbiology, Parasitology, and Serology Diagnostic Laboratory of the College of Veterinary Medicine for bacterial and fungal isolation and identification, and the identification of parasites. Spleen samples from OV1049 and OV1706 were frozen at -80 °C for subsequent virus isolation and molecular virus screening. This work was approved by the UF Institutional Animal Care and Use Committee (IACUC Protocol Numbers 201609390 and 201909390).

RT-qPCR detection of BTV, EHDV, EEEV, and WNV vRNA

Total RNA was extracted from spleen tissues collected from animals OV1049 and OV1706 using a RNeasy Mini kit (Qiagen) following the manufacturer's protocol. The RNA extracts were then screened for BTV, eastern equine encephalitis virus (EEEV), EHDV, and West Nile virus (WNV) using a VetMAX Plus One-Step RT-qPCR kit (Applied Biosystems) as described previously [4, 34–36].

Virus isolation in cultured cells

Virus isolation was attempted using the Vero E6 (*Cercopithecus aethiops* [African green monkey kidney, ATCC CRL 1586]) and C6/36 (*Aedes albopictus* [Asian tiger mosquito, ATCC CRL1660]) cell lines, as previously described [4]. Spleen tissues collected from animals OV1049 and OV1706 (previously stored at -80 °C) were thawed, aseptically minced using forceps, then homogenized to generate 10% w/v cell-free suspension homogenates in sterile phosphate-buffered saline (PBS) using a sterile manual tissue grinder (Fisher Scientific). The resulting homogenates were cleared of debris by low-speed centrifugation (5 min at 1500× g) and aseptically transferred to sterile polypropylene centrifuge tubes. The supernatants were then 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 Vero E6 and C6/36 cells in 25 cm^2 culture flasks. The cells were re-fed every 3 days and observed daily for virus-induced cytopathic effects (CPE) for 30 days. Non-inoculated cells were maintained in parallel as negative controls. When no CPE was observed by day 30 post-inoculation, a second passage was performed, and the cells were observed for another 30 days before being considered negative by virus isolation. After CPE were observed in 50% of the infected cells, they were scraped and harvested along with the spent cell growth medium and stored at -80 °C for further analyses.

Next-generation sequencing (NGS)

The frozen spent cell growth media from cells inoculated with OV1049 and OV1706 spleen homogenates were thawed and spun to remove cellular debris prior to extracting vRNA from virions using a QIAamp Viral RNA Mini kit (Qiagen) according to the manufacturer's protocol. cDNA libraries were generated using a NEBNext Ultra RNA Library Prep kit (New England Biolabs) and sequenced on an Illumina MiSeq sequencer (Illumina). Host *Chlorocebus aethiops* sequences (GenBank accession number MNAF00000000.2) and *Aedes albopictus* sequences (GenBank accession number MNAF00000000.2) were removed using Kraken v2.0 [37]. After removing the host sequences, a *de novo* assembly of the remaining paired-end reads was performed using SPAdes v3.15.3 [38]. The assembled contigs were then subjected to BLASTX searches against the National Center for Biotechnology Information (NCBI) non-redundant protein database using OmicsBox v1.2 (BioBam). The 5′ end of the coding sequence of segment 1 of sample OV1049 was determined using a 5′ Rapid Amplification for cDNA End (RACE) PCR Kit (Roche Diagnostics) and Sanger sequencing.

Phylogenetic and genetic analyses

The nucleotide sequences of all ten coding sequences were retrieved for 85 BTV strains, including the BTV isolated from animals OV1049 and OV1706 in this study, and one EHDV-6 (outgroup) (Supplementary Table S1). For each coding sequence, the alignment of nucleotide sequences guided by amino acid translations was performed within Geneious Prime v2022.2.2 using MAFFT [39]. The Maximum Likelihood phylogenetic trees were constructed in IQ-TREE with 1000 non-parametric standard bootstraps performed to test the robustness of the clades [40]. In addition, all coding sequences of the BTV isolated from OV1049 and OV1706 were compared to each other and against the NCBI non-redundant nucleotide database using BLASTN analyses.

Results

Necropsy findings, bacterial isolation, parasitology, and RT-PCR detection of BTV, EEEV, EHDV, and WNV RNA

The doe (animal ID: OV1049) exhibited overall emaciation, along with the presence of dark-green, almost black, soft feces, and a thicker, hyperemic mucosa, with a foamy content of upper small intestine, suggestive of upper intestinal inflammation (enteritis). The doe also presented with pale gums and vaginal mucosa (suggestive of anemia) and mild white catarrhal discharge from both nostrils (Fig. 1A). In addition, the left cranial lobe of the lung presented with red, firm, depressed areas with a lobular distribution from the cranial to the caudal part of the lobe (Fig. 1B), which suggests inflammation of the respiratory tract (pneumonia). *Pseudomonas aeruginosa* was identified through aerobic culture of lung tissue, and *Trichostrongylus* ova were detected in the fecal sample.

The buck fawn (animal ID: OV1706) presented with mild, multifocal petechia to purpuric lesions that were more prominent in the right lung, with the right cranial lobe of the lung showing red, firm, and depressed areas (Fig. 1C). *Streptococcus ruminantium* and *Bibersteinia trehalose* was identified through aerobic culture of lung tissue. Both animals showed no sign of lesions in the liver, heart, spleen, kidney, thoracic, or abdominal cavities. The RNA extracts from spleen of both animals tested positive for BTV and negative for EHDV, EEEV, and WNV.

Virus isolation

Virus-induced CPE were not observed in Vero E6 or C6/36 cells inoculated with spleen homogenate from animal OV1049 within a 30-day observation period. However, CPE were observed after 14 days upon second passage in Vero E6 cells. In contrast, CPE were observed upon primary passage by 12 days post-inoculation of C6/36 cells with spleen homogenate from animal OV1706.

Genome sequencing, phylogenetic, and genetic analyses

De novo assemblies of samples OV1049 and OV1706 (GenBank accession nos. OQ847475- OQ847484 and OQ847485- OQ847494, respectively) resulted in the complete gene coding sequences for all segments, except for segment 1 of OV1049 which was obtained by RACE PCR. Phylogenetic analyses based on coding sequences of segments 2 and 6 (VP2 and VP5 genes) revealed the viruses isolated from samples OV1049 and OV1706 as BTV serotype 1, hereafter referred to as BTV-1 strain OV1049 and BTV-1 strain OV1706, respectively. In addition, segment 2 analysis supported BTV-1 strains OV1049 and OV1706 as sister species, whereas segment 6 analysis could not resolve their relationship (Fig. 2). Analysis based on segment 1 (VP1 gene) grouped BTV-1 strains OV1049 and OV1706 within a clade formed by BTV strains from Florida, Louisiana, Jamaica, Panama, Honduras, and South Africa (Fig. S1). Segment 3 (VP3 gene) analysis supported BTV-1 strains OV1049 and OV1706 as members of a clade of

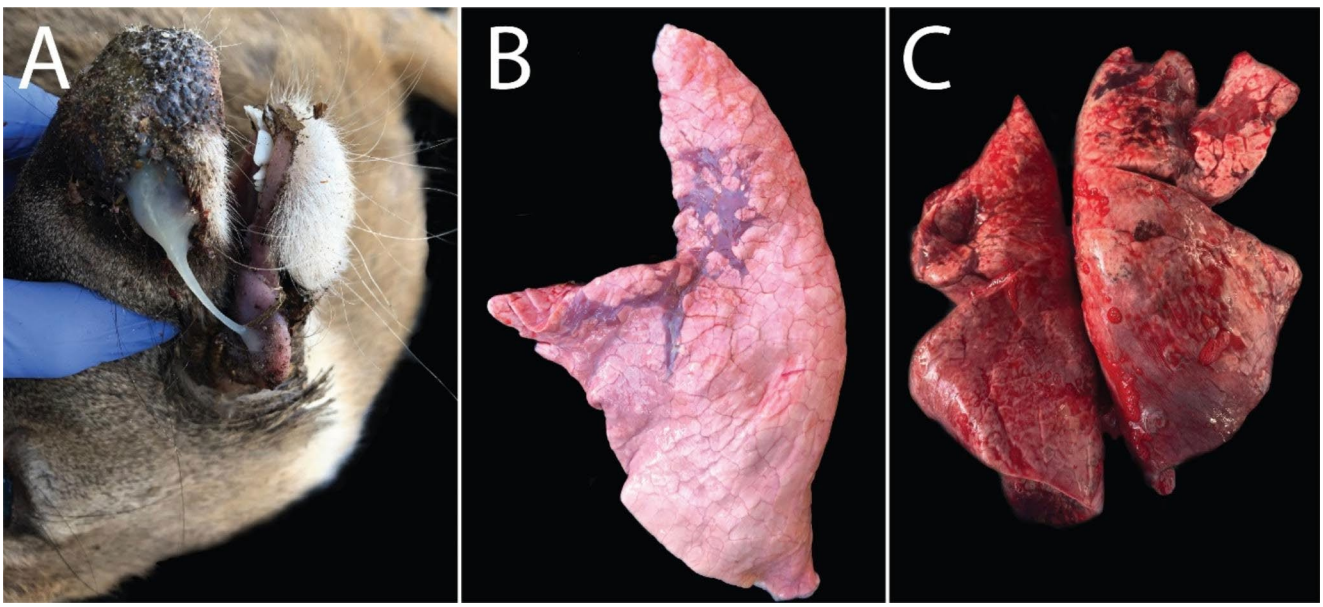

Fig. 1 Gross observations of farmed white-tailed deer naturally infected with the bluetongue virus serotype 1 strain OV1049 (**A** and **B**) and OV1706 (**C**). (**A**) Mild white catarrhal nasal discharge; (**B**) Mild cranial pneumonia of the left lung; (**C**) Mild, multifocal, petechia to purpuric lesions, with mild cranial lobar pneumonia of the right lung

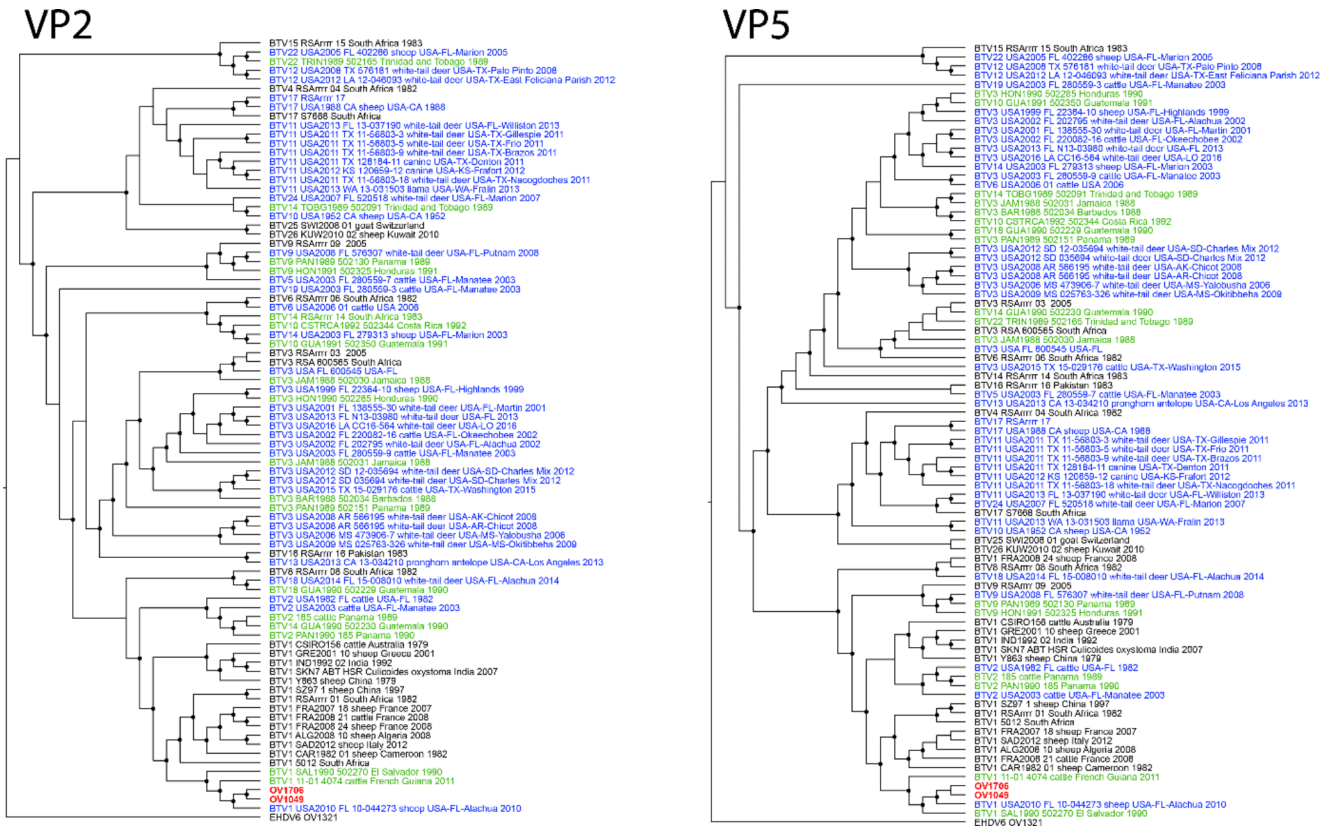

Fig. 2 Maximum Likelihood cladogram depicting the relationships of BTV-1 strains OV1049 and OV1706 to 83 other BTV strains and 1 EHDV strain based on the nucleotide sequence alignment of the VP2 and VP5 genes. The included BTV and EHDV strains are indicated by serotype, strain/isolate, host, country, and year of isolation. Nodes with black circles are supported by bootstrap values ≥ 80%. The tree was rooted with the EHDV-6 strain OV1321. The BTV isolates OV1049 and OV1706 are in red and bold. Strains/isolates from the U.S. are in blue, and strains/isolates from Central/South America and the Caribbean are in green. Additional metadata for each virus in the tree are provided in Supplemental Table S1

BTV strains from Florida (Fig. S1). Analyses of segments 4, 7, and 8 (VP4, VP7, and NS2 genes) supported BTV-1 strains OV1049 and OV1706 as members of clades consisting of BTV strains from Florida and Louisiana (Fig. S1, S2, and S3). Analysis of segment 9 (VP6 gene) supported BTV-1 strain OV1049 as the sister species to BTV-11 strain USA2013/FL 13-037190 from Florida; however, the same gene tree could not resolve the relationship of BTV-1 strain OV1706 to other BTV strains (Fig. S3). Segment 5 (NS1 gene) analysis showed that BTV-1 strain OV1049 grouped within a clade formed by BTV strains from Florida and Louisiana, whereas BTV-1 strain OV1706 grouped within a clade consisting of BTV strains from Florida and Panama (Fig. S4). Segment 10 (NS3 gene) supported BTV-1 strains OV1706 and OV1049 as members of a large clade consisting of eastern (i.e., Asia, Africa, Europe) and western (i.e., the Caribbean and the Americas) BTV strains (Fig. S4).

The genetic analyses showed that in nine of the ten segments, BTV-1 strain OV1049 shared 98.19% or greater sequence identity to other BTV strains from Florida (Table 1). Segment 9 (VP6) displayed the highest sequence identity (99.26%) to BTV-17 strain S7668 from South Africa. In eight of the ten segments, BTV-1 strain OV1706 showed 97.27% or greater sequence identity to other BTV strains from Florida (Table 2). Segment 1 (VP1) showed the highest sequence identity (95.91%) to BTV-17 strain S7668, and segment 9 (VP6) showed the highest sequence identity (91.32%) to BTV-4 strain ARG2002/01 from Argentina. Comparison between BTV-1 strains OV1049 and OV1706 showed that segments 2 and 6 (VP2 and VP5 genes) were nearly identical (99.31% and 99.30%, respectively), and the other segments ranged between 89.58% and 98.86% (Table 2).

Our analyses supported BTV-1 strains OV1409 and OV1706 as reassortants. BTV-1 strains OV1049 and OV1706 share the same origin (i.e., BTV-1 strain USA2010/FL 10-044273) for segments 2 and 6 (Fig. 2). In addition, four segments of BTV-1 strain OV1409 (Segments 4, 8, 9, 10) and three segments of BTV-1 strain OV1706 (Segments 4, 5, 8) showed reassortment with BTV strains from Florida (bootstrap values ≥ 80%). The remaining segments of BTV-1 strains OV1409 (Segments 1, 3, 5, 7) and OV1706

Table 1 Summary of sequence identities for all 10 complete coding sequences of the BTV-1 strain OV1049 isolated from a white-tailed deer in FL, USA and their comparison to BTV-1 strain OV1706.

Gene name (segment number)	GC content (%)	Length (bp)	Top BLASTN match					
			Nucleotide identity to OV1706 (%)	Host	Serotype	Strain/Isolate	Identity (%)	GenBank accession no.
VP1 (1)	42.2	3909	94.93	Cattle	BTV-3	USA2003/FL 280559-9	98.95	KY091940
VP2 (2)	43.2	2886	99.31	Sheep	BTV-1	USA2010/FL 10-044273	99.62	KX164020
VP3 (3)	43.8	2706	96.42	Cattle	BTV-3	USA2003/FL 280559-9	98.89	KY092129
VP4 (4)	42.7	1935	94.78	Cattle	BTV-19	USA2003/FL 280559-3	98.19	KX164132
NS1 (5)	44.5	1659	89.58	WTD	BTV-11	USA2013/FL 13-037190	99.76	KM580475
VP5 (6)	44	1581	99.3	Sheep	BTV-1	USA2010/FL 10-044273	99.75	KX164024
VP7 (7)	47.1	1050	98.86	Sheep	BTV-1	USA2010/FL 10-044273	99.52	KX164025
NS2 (8)	44.6	1065	97.46	WTD	BTV-11	USA2013/FL 13-037190	99.15	KM580482
VP6 (9)	49.3	990	90	Unknown	BTV-17	S7668	96.26	JX272457
NS3 (10)	42.8	690	96.09	Cattle	BTV-19	USA2003/FL 280559-3	99.57	KX164138

WTD = white-tailed deer

Table 2 Summary of sequence identities for all 10 complete coding sequences of the BTV-1 strain OV1706 isolated from a white-tailed deer in FL, USA.

Gene name (segment number)	GC content (%)	Length (bp)	Top BLASTN match				
			Host	Serotype	Strain/Isolate	Identity (%)	GenBank accession no.
VP1 (1)	42.2	3909	Unknown	BTV-17	S7668	95.91	JX272449
VP2 (2)	43.2	2886	Sheep	BTV-1	USA2010/FL 10-044273	99.48	KX164020
VP3 (3)	43.8	2706	Cattle	BTV-2	USA1982/FL	97.27	MW456739
VP4 (4)	42.7	1935	WTD	BTV-11	USA2013/FL 13-037190	99.28	KM580476
NS1 (5)	43.5	1659	Cattle	BTV-2	USA2003	98.49	KF986502
VP5 (6)	44	1581	Sheep	BTV-1	USA2010/FL 10-044273	99.18	KX164024
VP7 (7)	47.1	1050	Sheep	BTV-1	USA2010/FL 10-044273	99.14	KX164025
NS2 (8)	44.8	1065	WTD	BTV-18	USA2014/FL 15-008010	99.34	KX164126
VP6 (9)	49.5	990	Cattle	BTV-4	BTV-4/ARG2002/01	91.32	EU220291
NS3 (10)	43.9	690	Sheep	BTV-14	USA2003/FL 279,313	98.26	KX164118

WTD = white-tailed deer

(Segments 1, 3, 7, 9, 10) showed the highest sequence identities to BTV strains detected in Florida, Argentina, and South Africa; however, the phylogenetic analyses could not resolve the relationships of these viruses.

Discussion

The current study identified BTV-1 infection in farmed WTD in Florida in 2019 and 2022 through RT-qPCR, virus isolation, next-generation sequencing, and phylogenetic analyses. The common clinical signs reported in BTV-infected animals include anorexia, conjunctivitis, coronitis, cyanotic tongue, depression, facial edema, nasal lesions, nasal discharge, ptyalism, pyrexia, and respiratory distress [41, 42]. However, the clinical signs associated with the BTV-infected WTD from 2019 and 2022 cases were non-specific: nasal discharge, ataxia, and extreme lethargy. Bluetongue virus infections can result in asymptomatic to severe disease, with substantial morbidity and death, depending on the virus serotype and host species, age, and immune status [41, 43]. Thus, diagnosis of BTV infections should not be based on clinical signs alone but supported by confirmatory laboratory testing.

Bluetongue virus serotype 1 was first identified in the U.S. in 2004 from a WTD fawn in Louisiana [44] and later in 2010 from a sheep in Florida (strain USA2010/FL 10-044273; GenBank accession no. KX164020). Phylogenetic analyses based on segments 2 and 6 (VP2 and VP5 genes) showed that BTV-1 strains from Florida (OV1409, OV1706, and USA2010/FL 10-044273) formed a monophyletic group with the BTV-1 strains from El Salvador (SAL1990 502,270) and French Guiana (11 – 01 4074). The genome sequence of the BTV-1 identified in Louisiana is unavailable, and thus, its relationship to the BTV-1 strains found in Florida could not be determined. Recent phylodynamic models revealed Central America and the Caribbean as the source of BTV introduction into North America, and

the virus incursion can be attributed to historical livestock trading and windborne translocations of arthropod vectors from neighboring regions [45].

Segments 2 and 6 of OV1409 and OV1706 showed the highest sequence identity to the BTV-1 strain USA2010/FL 10-044273 from Florida, and their close relationships were supported by the phylogenetic analyses. Segments 2 and 6 encode VP2 and VP5 proteins, respectively, and they interact directly with one another to form the outer capsid [46]. In addition, VP2 and VP5 proteins are immunogenic serogroup-specific antigens exposed to the host humoral immune response, which makes them more susceptible to higher selection pressure compared to other virus proteins [47–50]. Hence, segments 2 and 6 may have been derived from the same Florida BTV-1 strain (USA2010/FL 10-044273) for the compatibility of protein-protein interactions between VP2 and VP5 proteins and the formation of viable virions.

Recent phylodynamic analyses revealed that sheep and cattle are responsible for maintaining and shaping global BTV genetic diversity and epidemiology [45]. The prolonged period of viremia in infected cattle allows a higher chance of coinfection of different BTV strains or serotypes and makes this species an ideal reservoir for the emergence of new BTV strains via genetic reassortment processes [24, 45]. In the current study, four segments of BTV-1 strain OV1409 (Segments 2, 4, 6, 10) and three segments of BTV-1 strain OV1706 (Segments 2, 5, 6) showed reassortment with BTV strains reported from sheep or cattle in Florida, suggesting these ruminants may have been important for the genetic diversification of BTV strains in Florida. However, continued efforts to sequence and characterize the BTV genomes from ruminants, including WTD, are needed to ascertain this.

Our analyses showed that the genomic segments of BTV-1 strains OV1409 and OV1706 were derived from at least five BTV serotypes classified as established (serotype 11) and reported (serotypes 1, 2, 18, and 19) in the U.S. One criterion suggested for the classification of a BTV serotype as established is based on phylogenetic evidence of virus reassortment with previously established strains in domestic and wild animals [15]. The two new reassortant BTV-1 strains (OV1049 and OV1709) reported in the current study reassorted with a previously established serotype in Florida (serotype 11), warranting reconsideration of the BTV-1 classification as an established serotype. Our phylogenetic analyses did not resolve the relationship of the remaining segments of OV1409 (segments 1, 3, 5, 7) and OV1706 (segments 1, 3, 7, 9, 10) to the other BTV strains; suggesting genetically uncharacterized BTV strains may be circulating in the Southern U.S.

In conclusion, we isolated and genetically characterized two novel reassortant BTV serotype 1 strains from diseased WTD on Florida farms in 2019 and 2022. Our analyses supported BTV-1 strains OV1049 and OV1709 as endemic strains to the Southeastern U.S. This U.S. region is particularly vulnerable to the emergence of novel BTV strains that affect ruminants due to the historical livestock trading practices and geographic location that facilitates long distance movement of disease-carrying midges by wind. Hence, continued surveillance efforts are needed to determine the prevalence and potential threat of BTV strains that may pose to the health of farmed deer and other ruminants.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11262-023-02019-6.

Author contributions Conceptualization: P.H.O.V.; S.M.W.; K.S.Methodology: P.H.O.V.; J.C.L.; Z.S.W.; J.A.L.; K.S.Formal analysis: P.H.O.V.; K.S.Investigation: P.H.O.V.; T.D.L.; E.D.; J.C.L.; K.N.W.; Z.S.W.Resources: S.M.W.; K.S.Writing - Original Draft: P.H.O.V.; S.G.G.; K.S.Writing - Review & Editing: P.H.O.V.; J.M.C.K.; J.A.L.; T.B.W.; S.M.W.; K.S.Visualization: P.H.O.V.Supervision: K.S.Project administration: S.M.W.; K.S.Funding acquisition: S.M.W.; K.S.

Declarations

Competing interests The authors declare no competing interests.

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