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BRIEF REPORT

# A novel bluetongue virus serotype 2 strain isolated from a farmed Florida white-tailed deer (*Odocoileus virginianus*) arose from reassortment of gene segments derived from co-circulating serotypes in the Southeastern USA

**Pedro H. O. Viadanna[1,2] · Austin Surphlis[1,2] · An-Chi Cheng[3] · Catherine E. Dixon[3] · Sarah Meisner[3] · Kristen N. Wilson[2,4] · Zoe S. White[2,4] · Emily DeRuyter[2,5] · Tracey D. Logan[2,5] · Juan M. C. Krauer[3] · John A. Lednicky[2,5] · Samantha M. Wisely[2,4] · Kuttichantran Subramaniam[1,2]**

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## Abstract

Bluetongue disease is a reportable animal disease that affects wild and farmed ruminants, including white-tailed deer (WTD). This report documents the clinical findings, ancillary diagnostics, and genomic characterization of a novel reassortant bluetongue virus serotype 2 (BTV-2) strain isolated from a dead Florida farmed WTD in 2022. Our analyses support that this BTV-2 strain likely stemmed from the acquisition of genome segments from co-circulating BTV strains in Florida and Louisiana. In addition, our analyses also indicate that genetically uncharacterized BTV strains may be circulating in the Southeastern USA; however, the identity and reassortant status of these BTV strains cannot be determined based on the VP2 and VP5 genome sequences. Hence, continued surveillance based on complete genome characterization is needed to understand the genetic diversity of BTV strains in this region and the potential threat they may pose to the health of deer and other ruminants.

**Keywords** Arbovirus · Deer · Orbivirus · Reovirus · Wildlife disease

## Introduction

Bluetongue disease is a reportable animal disease that affects wild and farmed ruminants, including antelope, buffalo, cattle, deer, elk, goat, and sheep [1]. The disease is caused by bluetongue virus (BTV), which is mainly transmitted by *Culicoides* biting midges [2]. Bluetongue virus is a member of the genus *Orbivirus* within the family *Sedoreoviridae*. The BTV genome consists of 10 linear double-stranded RNA segments that encode seven structural proteins (VP1-VP7) and at least four nonstructural proteins (NS1, NS2, NS3/3a, and NS4) [3–5]. Among these virus proteins, VP2 and VP5 are highly variable and are used to determine the serotypes of the different BTV strains [3].

At least 32 distinct BTV serotypes have been identified to date [6–12]. Bluetongue virus serotype 2 (BTV-2) was first discovered in sheep in South Africa (1968 to 1972). It was later detected in Asia, Australia, Europe, and the Americas [13–17]. Bluetongue virus serotype 2 was first detected in the USA in 1982 when it was isolated from sentinel cattle

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✉ Kuttichantran Subramaniam
kuttichantran@ufl.edu

[1] Department of Infectious Diseases and Immunology, College of Veterinary Medicine, University of Florida, 2015 SW 16th Avenue, Gainesville, FL 32610, USA

[2] Emerging Pathogens Institute, University of Florida, 2055 Mowry Rd, Gainesville, FL 32610, USA

[3] Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, 2015 SW 16th Avenue, Gainesville, FL 32610, USA

[4] Department of Wildlife Ecology and Conservation, University of Florida, 110 Newins-Ziegler Hall, Gainesville, FL 32611, USA

[5] Department of Environmental and Global Health, College of Public Health and Health Professions, University of Florida, 1225 Center Dr, Gainesville, FL 32610, USA

and in *Culicoides insignis* in Ona, Florida [15, 18]. Thereafter, it was detected in cattle from Alabama [19] and the northern Sacramento Valley of California [20]. Besides cattle, sheep, and *Culicoides*, BTV-2 was also detected in a Nubian goat in Florida, and white-tailed deer (WTD) in Florida, Georgia, and Louisiana [21–23]. Herein, we report the clinical findings, ancillary diagnostics, and genomic characterization of a novel reassortant BTV-2 strain isolated from a dead farmed WTD from Florida in 2022.

## Materials and methods

A farmed 5-year-old doe (animal ID: OV1681) was found with excessive drooling and droopy ears on July 31st, 2022. The farmer treated the animal with one dose each of 1.5 cc Excede (ceftiofur crystalline-free acid) and 1.5 cc Banamine (flunixin meglumine). The doe died the following day, and University of Florida (UF) technicians performed a field necropsy following guidelines provided by the University of Florida, Institute of Food and Agricultural Sciences, Cervidae Health Research Initiative (https://wec.ifas.ufl.edu/cheri/diagnostics/). As gross examination during field necropsy revealed congestion and hemorrhage in lungs, tissue samples were collected from this organ and submitted to the UF Microbiology, Parasitology, and Serology Diagnostic Laboratory of the College of Veterinary Medicine for bacterial culture and identification. Spleen and skin samples were frozen at − 80 °C for subsequent virus isolation and molecular viral screening. This work was approved by the UF Institutional Animal Care and Use Committee (IACUC Protocol Numbers 201609390 and 201909390).

Total RNA was extracted from spleen tissue using an RNeasy Mini kit (Qiagen) following the manufacturer's protocol and screened for BTV, bovine viral diarrhea virus (BVDV), eastern equine encephalitis virus (EEEV), epizootic hemorrhagic virus (EHDV), and West Nile virus (WNV) using a VetMAX Plus One-Step RT-qPCR kit (Applied Biosystems) as described previously [24–27]. Total DNA was extracted from tissue samples collected from skin lesions using a DNeasy Mini kit (Qiagen) following the manufacturer's protocol and screened for poxvirus using a conventional PCR assay as described previously [28].

After a BTV amplicon was detected by RT-qPCR, virus isolation was attempted in the C6/36 cell line (*Aedes albopictus* [Asian tiger mosquito, ATCC CRL1660]), as previously described [24]. After cytopathic effects (CPE) were observed in 50% of the infected cells, RNA was subsequently extracted from the supernatant using a QIAamp Viral RNA Mini kit (Qiagen) according to the manufacturer's protocol. A cDNA library was generated using a NEBNext Ultra RNA Library Prep kit (New England Biolabs) and sequenced on an Illumina MiSeq sequencer (Illumina). The *A. albopictus* host sequences (GenBank accession number MNAF00000000.2) were removed using Kraken v2.0 [29], and a de novo assembly of the remaining paired-end reads was performed using CLC Genomics Workbench v20.0.4. 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).

Each of the 10 coding sequences of 85 BTV and one EHDV-6 (outgroup) were retrieved from the NCBI GenBank database and aligned with BTV isolated from OV1681 using MAFFT within Geneious Prime v2022.2.2 (Supplemental Table S1). The maximum-likelihood trees for each aligned coding sequences were constructed separately in IQ-TREE with 1000 bootstraps performed to test the robustness of the clades [30]. In addition, all coding sequences of the BTV isolated from OV1681 were compared against the NCBI non-redundant nucleotide database using BLASTN analyses.

## Results and discussion

No pathogenic bacteria were isolated from the lungs. The RNA extract from spleen tissue tested positive for BTV and negative for BVDV, EEEV, EHDV, and WNV by RT-qPCR. The DNA extract from skin tissue tested negative for poxvirus by PCR. Bluetongue virus-infected animals typically exhibit clinical signs such as anorexia, conjunctivitis, coronitis, cyanotic tongue, depression, facial edema, nasal lesions, nasal discharge, ptyalism, pyrexia, and respiratory distress [31, 32]. However, the BTV-infected doe in the current study presented non-specific clinical signs such as congestion in the eyes and mouth mucosa and ulceration on the ventral surface of the tongue. Additionally, multifocal alopecic skin lesions were observed on the back, face, and legs (Supplemental Figure S1A). The heart showed petechial hemorrhage along the coronary sulcus of the myocardium (Supplemental Figure S1B), and the lungs displayed congestion and hemorrhage (Supplemental Figure S1C). Observing infected animals with non-specific clinical signs is not uncommon since BTV infections can result in asymptomatic to severe disease, depending on the virus serotype and host species, age, and immune status [31, 33]. Hence, diagnosis of BTV infection requires confirmatory laboratory testing and should not be solely based on clinical signs.

Virus-induced CPE was observed in C6/36 cells inoculated with spleen homogenate upon primary passage 12 days post-inoculation. De novo assembly (GenBank accession nos. OR672561-OR672570) resulted in the complete gene coding sequences for all BTV segments in sample OV1681, except for segment 9. Phylogenetic analyses based on the coding sequences of genome segments 2 and 6 (VP2 and VP5 genes) revealed that the BTV isolated from sample

OV1681 belonged in serotype 2. It was designated as BTV-2 strain OV1681 and grouped within a clade formed by BTV-2 strains from Florida, California, Panama, French Guiana, and South Africa (Fig. 1). Phylogenetic analyses based on coding sequences of segments 1 (VP1 gene), 3 (VP3 gene), 4 (VP4 gene), 7 (VP7 gene), and 8 (NS2 gene) grouped BTV-2 strain OV1681 within a clade formed by BTV strains from Florida and Louisiana (Supplemental Figures S2, S3, S4). Segment 5 (NS1 gene) analysis supported BTV-2 strain OV1681 as a member of a clade of BTV strains from Florida and Panama (Supplemental Figure S3). Analysis of segment 9 (VP6 gene) supported BTV-2 strain OV1681 as a member of a clade consisting of BTV strains from Florida, Louisiana, and Honduras (Supplemental Figure S4). Analysis of segment 10 (NS3 gene) grouped BTV-2 strain OV1681 within a clade of BTV strains from Florida (Supplemental Figure S5). Eight segments showed 97.83% or greater sequence identity with BTV strains from Florida. Segments 3 and 7 showed 97.04% and 99.52% sequence identity with a BTV strain from Louisiana, respectively (Table 1).

Our analyses support BTV-2 strain OV1681 as a reassortant with evidence that reassortment occurred between BTV strains from Florida and Louisiana. Segments 3 and 7 were derived from the BTV-12 strain USA2012/LA 12–046093 from Louisiana, whereas segments 1, 4, 6, and 9 were derived from at least four BTV strains previously reported in Florida. The source of the BTV-2 strain OV1681 could not be determined in the current study. However, the introduction of BTV-2 to Florida is thought to have occurred via *Culicoides* midges, which migrated from Cuba in 1982 and subsequently became endemic in the Southeastern USA [34]. In support of that notion, recent phylodynamic models also revealed Central America and the Caribbean as sources of BTV introduction

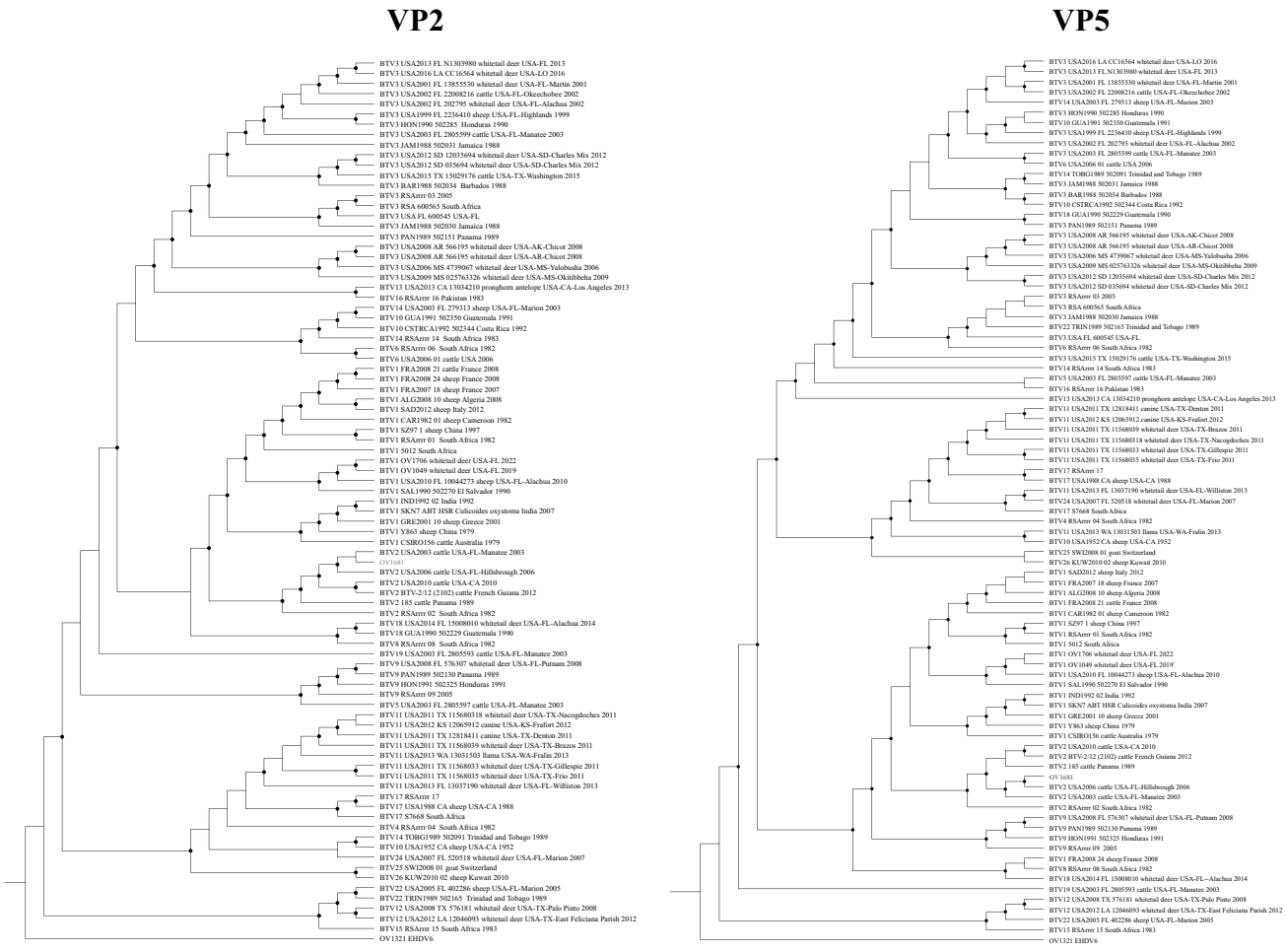


**Fig. 1** Maximum likelihood cladogram depicting the relationships of the nucleotide sequence alignment of the VP2 and VP5 genes of BTV-2 strain OV1681, isolated from a white-tailed deer to 85 other BTV strains and 1 EHDV strain. The included BTV and EHDV strains are indicated by serotype, strain/isolate, 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-2 isolate OV1681 is in red and bold. Additional metadata for each virus in the tree are provided in Supplemental Table S1

**Table 1** Summary of sequence identities for all 10 complete coding sequences of the BTV-2 strain OV1681 isolated from a whitetailed deer in FL, USA

| Top BLASTN match | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Gene name (segment number) | Length (bp) | GC content (%) | Serotype | Host | Strain/Isolate | Year | Identity (%) | GenBank accession no |
| VP1 (1) | 3909 | 42.2 | BTV-1 | WTD | OV1049 | 2019 | 99.26 | OQ847475 |
| VP2 (2) | 2889 | 41.5 | BTV-2 | Cattle | USA2006 | 2006 | 99.17 | KF986495 |
| VP3 (3) | 2706 | 43.6 | BTV-12 | WTD | USA2012/LA 12–046093 | 2012 | 97.04 | KX164091 |
| VP4 (4) | 1935 | 42.8 | BTV-11 | WTD | USA2013/FL 13–037190 | 2013 | 99.12 | KM580476 |
| NS1 (5) | 1659 | 43.9 | BTV-2 | Cattle | USA2003 | 2003 | 97.83 | KF986502 |
| VP5 (6) | 1581 | 43.1 | BTV-2 | Cattle | USA2006 | 2006 | 99.43 | KF986490 |
| VP7 (7) | 1050 | 46.6 | BTV-12 | WTD | USA2012/LA 12–046093 | 2012 | 99.52 | KX164095 |
| NS2 (8) | 1065 | 45.1 | BTV-1 | WTD | OV1049 | 2019 | 98.87 | OQ847482 |
| VP6 (9) | 990 | 49.3 | BTV-18 | WTD | USA2014/FL 15–008010 | 2014 | 98.69 | KX164127 |
| NS3 (10) | 690 | 43.2 | BTV-18 | WTD | USA2014/FL 15–008010 | 2014 | 99.42 | KX164128 |

*WTD* white-tailed deer

into North America [35]. Our phylogenetic analyses did not resolve the relationship of the remaining segments of BTV-2 strain OV1681 (segments 2, 5, 8, 10) to the other BTV strains, suggesting genetically uncharacterized BTV strains may be circulating in the Southeastern USA. Hence, it is crucial to maintain ongoing surveillance to assess the prevalence and potential risks posed by different BTV strains to the health of farmed deer populations and other ruminants.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11262-023-02047-2.

**Author contributions** Conceptualization: PHOV, JAL, SMW, KS. Methodology: PHOV, JMCK, JAL, SMW, KS. Formal analysis: PHOV, AS, ACC, CED, SM, KNW, ZSW, ED, TDL, JMCK. Investigation: PHOV, AS, ACC, CED, SM, KNW, ZSW, ED, TDL, KS. Resources: JMCK, JAL, SMW, KS. Writing—original draft: PHOV, AS, JAL, KS. Writing—review & editing: PHOV, AS, ACC, CED, SM, KNW, ZSW, ED, TDL, JMCK, JAL, SMW, KS. Visualization: PHOV, AS, KS. Supervision: KS. Project administration: SMW, KS. Funding acquisition: SMW, KS.

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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 the GenBank accession nos. OR672561-OR672570.

## Declarations

**Conflict of interest** The authors declare that they have no competing interests.

**Ethical approval** This work was approved by the University of Florida Institutional Animal Care and Use Committee (IACUC) Protocol Numbers 201609390 and 201909390.

## References

1. Erasmus BJ (1990) Bluetongue Virus. In: Dinter Z, Morein B (eds) virus infections of vertebrates series, 1st edn. Elsevier, Amsterdam, pp 227–237
2. Du Toit RM (1944) The Transmission of Blue-tongue and Horse-sickness by Culicoides. Onderstepoort J Vet Res 19(1–2):7–16
3. Mertens PPC, Diprose J (2004) The bluetongue virus core: a nano-scale transcription machine. Virus Res 101(1):29–43. https://doi.org/10.1016/j.virusres.2003.12.004
4. 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(12):e1002477. https://doi.org/10.1371/journal.ppat.1002477
5. Roy P, Urakawa T, Van Dijk AA, Erasmus BJ (1990) Recombinant virus vaccine for bluetongue disease in sheep. J Virol 64(5):1998–2003. https://doi.org/10.1128/JVI.64.5.1998-2003.1990
6. Hofmann MA, Renzullo S, Mader M, Chaignat V, Worwa G, Thuer B (2008) Genetic characterization of toggenburg orbivirus, a new bluetongue virus, from goats, Switzerland. Emerg Infect Dis 14:1855–1861. https://doi.org/10.3201/eid1412.080818
7. Lorusso A, Sghaier S, Di Domenico M, Barbria ME, Zaccaria G, Megdich A, Portanti O, Seliman IB, Spedicato M, Pizzurro F et al (2018) Analysis of bluetongue serotype 3 spread in Tunisia and discovery of a novel strain related to the bluetongue virus isolated from a commercial sheep pox vaccine. Infect Genet Evol 59:63–71. https://doi.org/10.1016/j.meegid.2018.01.025
8. Maan S, Maan NS, Nomikou K, Veronesi E, Bachanek-Bankowska K, Belaganahalli MN, Attoui H, Mertens PPC (2011) Complete genome characterisation of a novel 26th bluetongue virus serotype from Kuwait. PLoS ONE 6:e26147. https://doi.org/10.1371/journal.pone.0026147

9. Marcacci M, Sant S, Mangone I, Goria M, Dondo A, Zoppi S, van Gennip RGP, Radaelli MC, Cammà C, van Rijn PA et al (2018) One after the other: A novel Bluetongue virus strain related to Toggenburg virus detected in the Piedmont region (North-western Italy), extends the panel of novel atypical BTV strains. Transbound Emerg Dis 65:370–374. https://doi.org/10.1111/tbed.12822
10. Savini G, Puggioni G, Meloni G, Marcacci M, Di Domenico M, Rocchigiani AM, Spedicato M, Oggiano A, Manunta D, Teodori L et al (2017) Novel putative Bluetongue virus in healthy goats from Sardinia, Italy. Infect Genet Evol 51:108–117. https://doi.org/10.1016/j.meegid.2017.03.021
11. Yang H, Gu W, Li Z, Zhang L, Liao D, Song J, Shi B, Hasimu J, Li Z, Yang Z et al (2021) Novel putative bluetongue virus serotype 29 isolated from inapparently infected goat in Xinjiang of China. Transbound Emerg Dis 68:2543–2555. https://doi.org/10.1111/tbed.13927
12. Zientara S, Sailleau C, Viarouge C, Höper D, Beer M, Jenckel M, Hoffmann B, Romey A, Bakkali-Kassimi L, Fablet A et al (2014) Novel bluetongue virus in goats, Corsica, France, 2014. Emerg Infect Dis 20:2123–2125. https://doi.org/10.3201/eid2012.140924
13. Bréard E, Sailleau C, Coupier H, Mure-Ravaud K, Hammoumi S, Gicquel B, Hamblin C, Dubourget P, Zientara S (2003) Comparison of genome segments 2, 7, and 10 of bluetongue viruses serotype 2 for differentiation between field isolates and the vaccine strain. Vet Res 34(6):777–789. https://doi.org/10.1051/vetres:2003036
14. Gaudreault NN, Mayo CE, Jasperson DC, Crossley BM, Breitmeyer RE, Johnson DJ, Ostlund EN, MacLachlan NJ, Wilson WC (2014) Whole genome sequencing and phylogenetic analysis of Bluetongue virus serotype 2 strains isolated in the Americas including a novel strain from the western United States. J Vet Diagn Invest 26(4):553–557. https://doi.org/10.1177/1040638714536902
15. 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(12):2226–2228
16. Kundlacz C, Caignard G, Sailleau C, Viarouge C, Postic L, Vitour D, Zientara S, Breard E (2019) Bluetongue Virus in France: An Illustration of the European and Mediterranean Context since the 2000s. Viruses 11(7):672. https://doi.org/10.3390/v11070672
17. Nomikou K, Hughes J, Wash R, Kellam P, Breard E, Zientara S, Palmarini M, Biek R, Mertens P (2015) Widespread Reassortment Shapes the Evolution and Epidemiology of Bluetongue Virus following European Invasion. PLOS Pathog 11(8):e1005056. https://doi.org/10.1371/journal.ppat.1005056
18. Greiner EC, Barber TL, Pearson JE, Kramer W, Gibbs EP (1985) Orbiviruses from culicoides in Florida. In: Barber TL, Jochum MM (eds) Bluetongue and related orbiviruses, 1st edn. Alan R. Liss, New York, pp 195–200
19. Wright JC, Lauerman LH, Nusbaum KE, Mullen GR (1989) Seroepidemiologic study of bluetongue virus serotype 2 in Alabama. Prev Vet Med 7(2):113–119. https://doi.org/10.1016/0167-5877(89)90003-2
20. Maclachlan NJ, Wilson WC, Crossley BM, Mayo CE, Jasperson DC, Breitmeyer RE, Whiteford AM (2013) Novel serotype of bluetongue virus, western North America. Emerg Infect Dis 19(4):665–666. https://doi.org/10.3201/eid1904.120347
21. USAHA (2014) Proceedings one hundred and eighteenth annual meeting of the United States animal health association. Kansas City, Missouri.
22. USAHA (2016). Proceedings one hundred and twentieth annual meeting of the United States animal health association. Greensboro, North Carolina.
23. USAHA (2020). Proceedings one hundred and twenty fourth annual meeting of the United States animal health association. Nashville, Tennessee (virtual meeting).
24. Ahasan MS, Subramaniam K, Sayler KA, Loeb JC, Popov VL, Lednicky JA, Wisely SM, Campos Krauer JM, Waltzek TB (2019) Molecular characterization of a novel reassortment *Mammalian orthoreovirus* type 2 isolated from a Florida white-tailed deer fawn. Virus Res 270:97642. https://doi.org/10.1016/j.virusres.2019.197642
25. Lambert AJ, Martin DA, Lanciotti RS (2003) Detection of North American eastern and western equine encephalitis viruses by nucleic acid amplification assays. J Clin Microbiol 41(1):379–385. https://doi.org/10.1128/JCM.41.1.379-385.2003
26. Lanciotti RS, Kerst AJ, Nasci RS, Godsey MS, Mitchell CJ, Savage HM, Komar N, Panella NA, Allen BC, Volpe KE, Davis BS, Roehrig JT (2000) Rapid detection of west nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. J Clin Microbiol 38(11):4066–4071. https://doi.org/10.1128/JCM.38.11.4066-4071.2000
27. Wernike K, Hoffmann B, Beer M (2015) Simultaneous detection of five notifiable viral diseases of cattle by single-tube multiplex real-time RT-PCR. J Virol Methods 217:28–35. https://doi.org/10.1016/j.jviromet.2015.02.023
28. Sayler KA, Subramaniam K, Jacob JM, Loeb JC, Craft WF, Farina LL, Stacy NI, Moussatche N, Cook L, Lednicky JA, Wisely SM, Waltzek TB (2019) Characterization of mule deerpox virus in Florida white-tailed deer fawns expands the known host and geographic range of this emerging pathogen. Arch Virol 164(1):51–61. https://doi.org/10.1007/s00705-018-3991-7
29. Wood DE, Salzberg SL (2014) Kraken: ultrafast metagenomic sequence classification using exact alignments. Genome Biol 15(3):R46. https://doi.org/10.1186/gb-2014-15-3-r46
30. Nguyen L-T, 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(1):268–274. https://doi.org/10.1093/molbev/msu300
31. Caporale M, Di Gialleonorado L, Janowicz A, Wilkie G, Shaw A, Savini G, Van Rijn PA, Mertens P, Di Ventura M, Palmarini M (2014) Virus and host factors affecting the clinical outcome of bluetongue virus infection. J Virol 88:10399–10411. https://doi.org/10.1128/JVI.01641-14
32. Parsonson IM (1990) Pathology and pathogenesis of bluetongue infections. Curr Top Microbiol Immunol 162:119–141. https://doi.org/10.1007/978-3-642-75247-6_5
33. Rivera NA, Varga C, Ruder MG, Dorak SJ, Roca AL, Novakofski JE, Mateus-Pinilla NE (2021) Bluetongue and epizootic hemorrhagic disease in the United States of America at the wildlife-livestock interface. Pathog 10(8):915. https://doi.org/10.3390/pathogens10080915
34. Sellers RF, Maarouf AR (1989) Trajectory analysis and bluetongue virus serotype 2 in Florida 1982. Can J Vet Res 53(1):100–102
35. Alkhamis MA, Aguilar-Vega C, Fountain-Jones NM, Lin K, Perez AM, Sánchez-Vizcaíno JM (2020) Global emergence and evolutionary dynamics of bluetongue virus. Sci Rep 10:21677. https://doi.org/10.1038/s41598-020-78673-9