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Short communication

Persistent infection of American bison (*Bison bison*) with bovine viral diarrhea virus and bosavirus

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ABSTRACT

Bovine viral diarrhea viruses (BVDV) are significant pathogens of cattle, leading to losses associated with reproductive failure, respiratory disease and immune dysregulation. While cattle are the reservoir for BVDV, a wide range of domestic and wild ruminants are susceptible to infection and disease caused by BVDV. Samples from four American bison (*Bison bison*) from a captive herd were submitted for diagnostic testing due to their general unthriftiness. Metagenomic sequencing on pooled nasal swabs and serum identified co-infection with a BVDV and a bovine bosavirus. The BVDV genome was more similar to the vaccine strain Oregon C24 V than to other BVDV sequences in GenBank, with 92.7 % nucleotide identity in the open reading frame. The conserved 5′-untranslated region was 96.3 % identical to Oregon C24 V. Bosavirus has been previously identified in pooled fetal bovine serum but its clinical significance is unknown. Sequencing results were confirmed by virus isolation and PCR detection of both viruses in serum and nasal swab samples from two of the four bison. One animal was co-infected with both BVDV and bosavirus while separate individuals were positive solely for BVDV or bosavirus. Serum and nasal swabs from these same animals collected 51 days later remained positive for BVDV and bosavirus. These results suggest that both viruses can persistently infect bison. While the etiological significance of bosavirus infection is unknown, the ability of BVDV to persistently infect bison has implications for BVDV control and eradication programs. Possible synergy between BVDV and bosavirus persistent infection warrants further study.

1. Introduction

Bovine viral diarrhea viruses (BVDV) are one of the most significant pathogens of cattle. Infection of the dam early in gestation can result in fetal infection that leads to immunotolerance and persistent infection (Brodersen, 2014). Abortions are a frequent outcome of dam infection. Surviving offspring are persistently infected and constitute the reservoir for BVDV in a herd. Transient infection of cattle can lead to a wide range of clinical outcomes depending on virus and host factors, ranging from unapparent infection to severe hemorrhagic and mucosal disease. Transient BVDV infections are associated with immunosuppression that can lead to secondary infections typically seen in bovine respiratory disease (Larson, 2015).

Despite a wide range of inactivated and modified live virus vaccines, BVDV control remains a challenge. One aspect of BVDV that hampers vaccine efficacy is its large amount of genetic diversity (Workman et al., 2016; Yeşilbağ et al., 2017). There are two species of BVDV, Pestivirus A (BVDV1) and Pestivirus B (BVDV2), which are further subdivided in a large number of genotypes (Walker et al., 2019). Cross-reaction between genetically distinct viruses is variable (Fulton et al., 2020; Ridpath et al., 2010). Additionally, BVDV has the ability to infect a remarkable range of animals, including deer, sheep, goats, antelope and bison (Nelson et al., 2016; Wolff et al., 2016; Van Campen and Rhyan, 2010). These viruses are often indistinguishable from bovine isolates. Inoculation of cattle with BVDV isolated from diseased Canadian bison demonstrated its ability to infect bovines however, similar to infection with most bovine origin BVDV, no clinical disease was observed (Deregt et al., 2005)

Copiparvovirus is a genus in the family *Parvoviridae*. Of the seven

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recognized species, two were identified in cattle (*Ungulate copiparvovirus 1* and *5*), two from swine (*Ungulate copiparovirus 2* and *4*), with the remaining species identified in sea lion (*Pinniped copiparovirus 1*), roe deer (*Ungulate copiparvirus 3*), and horse (*Ungulate copiparovirus 6*) (Cotmore et al., 2019). While the etiological significance of most copiparoviruses is unknown, equine parvovirus-hepatitis has been associated with equine serum hepatitis and transmitted by contaminated antiserum products (Divers et al., 2018). Bosavirus (bovine serum associated; *Ungulate copiparvovirus 5*) was identified by metagenomic sequencing in 4/4 pools of normal bovine calf serum (Sadeghi et al., 2017).

2. Materials and methods

2.1. Animals

Four American bison (*Bison bison*) from a private herd in Nebraska, U.S., were the subject of diagnostic investigation. The animals were separated from the herd in February 2020, due to unthriftiness. The individuals were comprised of a 10 month old female (no tag), a 20 month old male (DS704), a 20 month old female (DS16), and a 34 month old female (DR618). Whole blood and nasal swabs were collected from all animals and submitted to the South Dakota State University Animal Disease Research and Diagnostic Laboratory (SDSU ADRDL) for testing. The animals were sampled twice 51 days between samplings.

2.2. Herd history

At the time of initial diagnosis in February 2019, the ranch's bison herd was comprised of approximately 1100 breeding females and 110 breeding males along with the 1009 head of calves from the 2018 calving season. The herd has been going through an intentional reduction in size since 2019 and is currently at approximately 770 head of breeding females. The pregnancy rate for the 2018 breeding season was approximately 65 % that is significantly lower than the historical herd average of 88%–90%. Besides a lower pregnancy rate, some animals in herd showed signs of slight general weakness. A serological survey of a random subset of animals performed at the SDSU ADRDL found multiple individuals with high neutralizing titers in excess of 4096 to both BVDV types 1 and 2. An improved pregnancy rate of 84 % was noted in February 2020. A second serological survey was performed on samples collected 31 days prior to the samples collected for this study. The 23 random individuals had geometric mean serum neutralizing titers of 964 (range 256–8192) and 289 (range 16–8192) to BVDV1 and BVDV2, respectively.

In previous years (most notably in 2007–2009), several commercially-available modified live virus vaccines had been administered to some animals that comprise the current herd as well as on animals that were in close proximity to those being selected for replacements in the breeding herd. These vaccines included Nasalgen, Bovishield Gold, Titanium 5, and Onset IN. Vaccines were administered subcutaneously or intranasally per product instructions. These vaccines are have label claims for use in cattle. Modified live virus vaccines had not been used in the herd since 2009.

2.3. BVDV detection

Quantitative RT-PCR and virus isolation for BVDV was performed on whole blood (Mahlum et al., 2002). Virus isolation was additionally performed for the nasal swabs. Virus isolation was performed using bovine turbinate cells. Virus was passaged twice before staining with a FITC-labeled anti-BVDV conjugate (VMRD, Pullman, WA) to verify isolation.

2.4. Viral metagenomic sequencing

Viral metagenomic sequencing was performed separately on blood serum and nasal swabs. For metagenomic sequencing, samples from the initial submission were pooled by sample type and centrifuged at 20,000 x g for 5 min to remove debris and blood cells. The samples were then treated with a cocktail of nucleases prior to RNA isolation as previously described (Neill et al., 2014). Samples were then reverse transcribed using barcoded random hexamers followed by second strand synthesis using Sequenase 2.0 (ThermoFisher, Waltham, MA) before DNA cleanup using a PCR purification kit (Allander et al., 2005). Finally, the dsDNA was amplified using a primer specific to the barcode before being purified. Sequencing libraries were prepared using a Nextera XT sequencing kit according to manufacturer's instructions. Libraries were sequenced on an Illumina MiSeq using paired 151 base pair (bp) chemistry using a 300 cycle MiSeq reagent microkit V2. Sequencing reads trimmed of adapter sequences were imported into CLC Genomics and assembled *de novo*. Contigs were identified by BLASTX searches of the GenBank nr/nt dataset using the BLAST2GO plugin incorporated into CLC genomics (Qiagen, Hilden, Germany).

2.5. Bison bosavirus sequencing and analysis

The extreme 5′ coding region of the bison bosavirus genome was amplified by PCR. Using bovine bosavirus as a reference, primer BosaF2, 5′−CCTCCACAGCAACCAATAAG-3′ was used along with a reverse primer designed from the assembled bison bosavirus near the 5′-terminus, BisonR1, 5′-GAAGATTGGGACTCCATAGG-3′, to generate a PCR product spanning the coding region missing from the *de novo* assembly.

A Taqman assay was designed targeting the capsid region of the bosavirus genome; forw: 5′-CAACCAGACACAGATACCGATAC-3′, rev: 5′-CTGTCCCATACCTAAGATCGTG-3′, probe: 5′-FAM−CCAGATTTCCCGGCCCCACATA-3′ Iowa Black fluorescence quencher (Integrated DNA Technologies, Coralville, IA).

2.6. Phylogenetic analysis

Virus phylogeny was investigated using MEGA X (Kumar et al., 2018). Assembled viral genomes and reference strains were aligned by ClustalW as implemented in MEGA X. Virus phylogeny was inferred by the Maximum Likelihood method using the best fitting substitution model with sites showing less than 80 % representation removed from the analysis. Robustness of the tree topology was assessed by 500 bootstrap replicates. Nucleotides encompassing the complete BVDV genome were used for phylogenetic analysis. To determine bosavirus phylogeny, predicted amino acid (a.a.) sequences for the non-structural protein were used.

3. Results

3.1. Metagenomic sequencing identified BVDV and bosavirus

Metagenomic sequencing of the original serum pool yielded 1,010,930 sequencing reads that assembled into 69 contigs. Several contigs were identified as originating from BVDV and bosavirus (*Ungulate copiparvovirus 5*). No other mammalian viruses were identified. For the nasal swab pool, 1,117,480 reads were assembled into 45 contigs. A complete coding genome (12,299 nucleotides, nt) for BVDV was assembled. No other viruses were identified in the nasal swab pool. The BVDV genome (strain 20–8536) was submitted to Genbank under accession MT654137. BVDV strain 20–8536 was most similar to the BVDV type 1a strain Oregon C24 V with 92.7 % nt identity in the coding region of the genome.

Metagenomic sequencing was also performed on serum samples from individuals DS16 and DR618 collected 51 days later. Complete coding regions for the BVDV present in both samples were determined except

for 230 nt missing from the 3′-end of DS16. Pairwise comparison of these sequences to the original BVDV strain 20–8536 determined from pooled nasal swabs found only 5 nt differences for DS16 (99.9 % identity) and 38 nt differences for DR618 (99.7 % identity).

3.2. Bison bosavirus strain 20–8536 is closely related to bovine bosavirus

Genetic analysis of the bosavirus contig assembled *de novo* suggested that the coding region was nearly complete except for ~200 nt on the 5′-terminus. A ~300 bp PCR product spanning this region was amplified, sequenced and combined with the *de novo* assembled genome to yield the complete DNA sequence for the coding region. The resulting 5253 bp sequence of bison bosavirus strain 20–8536 was deposited in Genbank under accession MT654136.

Genetic analysis identified two open reading frames (Fig. 1a). The 5′-ORF encoded a 580 a.a. protein with 86 % identity to the bosavirus nonstructural (NS) protein. Greater than 85 % identity in the NS of parvoviruses is used for inclusion in a species (Cotmore, 2019). Following a 247 nt intergenic region, a second ORF of 1041 a.a. 83 % identity to the bosavirus structural protein was identified. Analysis of the conserved domains present in bison bosavirus include parvovirus coat protein VP1 from a.a 299–363 and VP2 from a.a. 490−995.

3.3. Phylogenetic analysis identifies BVDV1a and bosavirus

Bison bosavirus was closely related to bovine bosavirus which together formed a sister clade to one encompassing porcine parvoviruses 4, 5 and 6, placing bison bosavirus in the *Copiparvovirus* genus (Fig. 1b). Horse parvovirus and sesavirus, included in the *Copiparvovirus* taxon, formed a clade separate from copiparvoviruses though this relationship was not well-supported. Previous work found 25–30 % amino acid similarity between the NS protein of horse parvovirus and sesavirus with other members of *Copiparvovirus*, making their inclusion in this genus tenuous (Li et al., 2015; Phan et al., 2015) (Fig. 2).

The evolutionary history of BVDV strain 20–8536 was inferred by Maximum Likelihood analysis using representative U.S. and genotype references. The bison BVDV sequences were most closely related to Oregon C24 V which together with strains USMARC-55477and USMARC-53875, formed a monophyletic group with a sister clade relationship to BVDV subtype 1a reference strains Singer and NADL.

3.4. Quantitative reverse transcriptase PCR and virus isolation detect BVDV and bosavirus

From the initial sampling, BVDV qRT-PCR on whole blood identified samples from two animals (DR618 and DS16) positive with Ct values of 21.9 and 23.6, respectively. The other two animals (DS704 and no tag) were negative for BVDV. Nasal swabs collected from DR618 and DS16 were additionally positive for BVDV with Ct values of 30.2 and 22.2, respectively. Virus isolations using the buffy coat from all animals were negative. Virus isolation using the pooled nasal swabs isolated a non-cytopathic BVDV as did virus isolation from individual nasal swabs collected from DR618 and DS16. Full genome sequencing confirmed the isolates as >99 % identical to the genome assembled from the initial nasal swab pool consisting of samples from all four animals. Sequencing of the 5′-untranslated region (UTR) from the individual BVDV-positive nasal swabs and blood samples from DR618 and DS16 found >99 % identity to the virus genome determined directed from pooled nasal swabs and isolated from the same pooled sample.

A second set of blood samples and nasal swabs were collected from the four bison 51 days following the initial collection. BVDV qRT-PCR on whole blood again only identified DR618 and DS16 as positive for BVDV (Ct's 25.4 and 25.8). Nasal swabs from DR618 and DS16 were also positive for BVDV with Ct values of 29.4 and 24.5. Virus isolation from the buffy coat and nasal swabs from DR618 and DS16 were both positive. The other two animals (DS704 and no tag) were negative for BVDV.

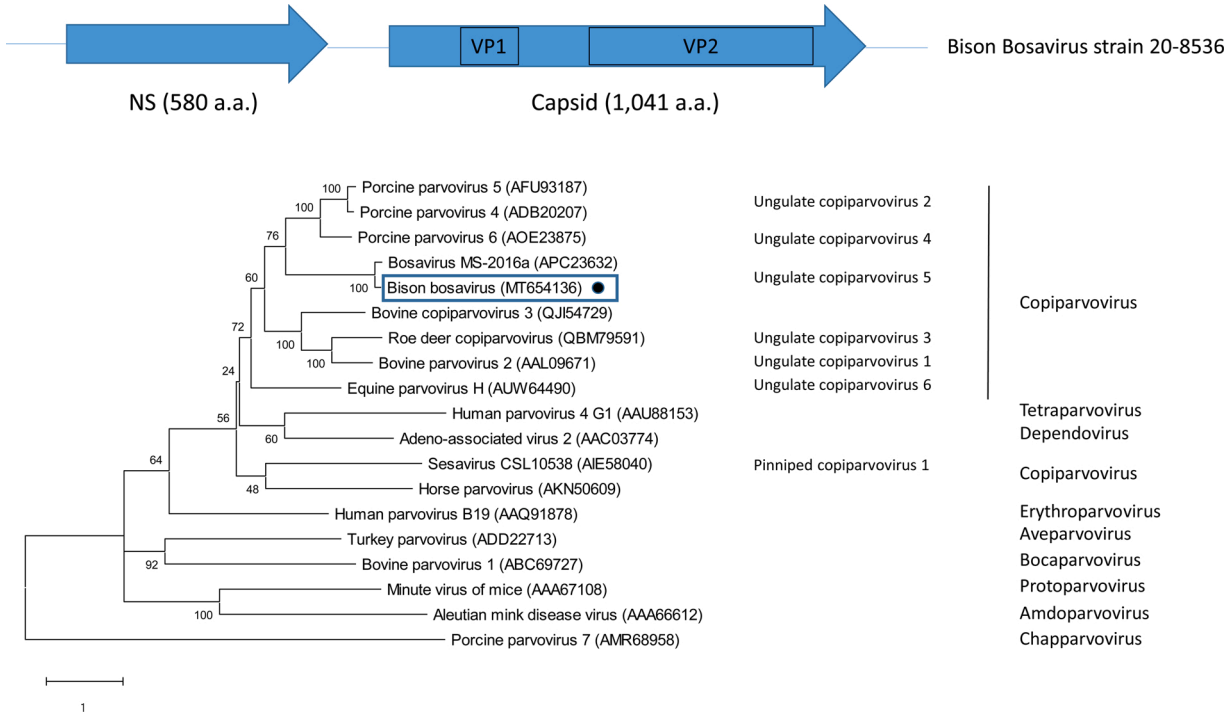

Fig. 1. a. Genomic structure of bison bosavirus. Open reading frames (ORF) corresponding to the non-structural and capsid proteins were identified by BLASTP. Conserved domains corresponding to VP1 and VP2 are shown in boxes; b. Phylogenetic analysis of the NS protein. Sequences were aligned by ClustalW and the phylogenetic tree was inferred by maximum-likelihood analysis using the best-fitting LG + F model as implemented in MegaX. Evolutionary rate differences were modeled with a discrete Gamma distribution allowing some sites to be invariable. Robustness of tree topology was evaluated with 500 bootstrap replicates. Bison bosavirus is indicated by ●.

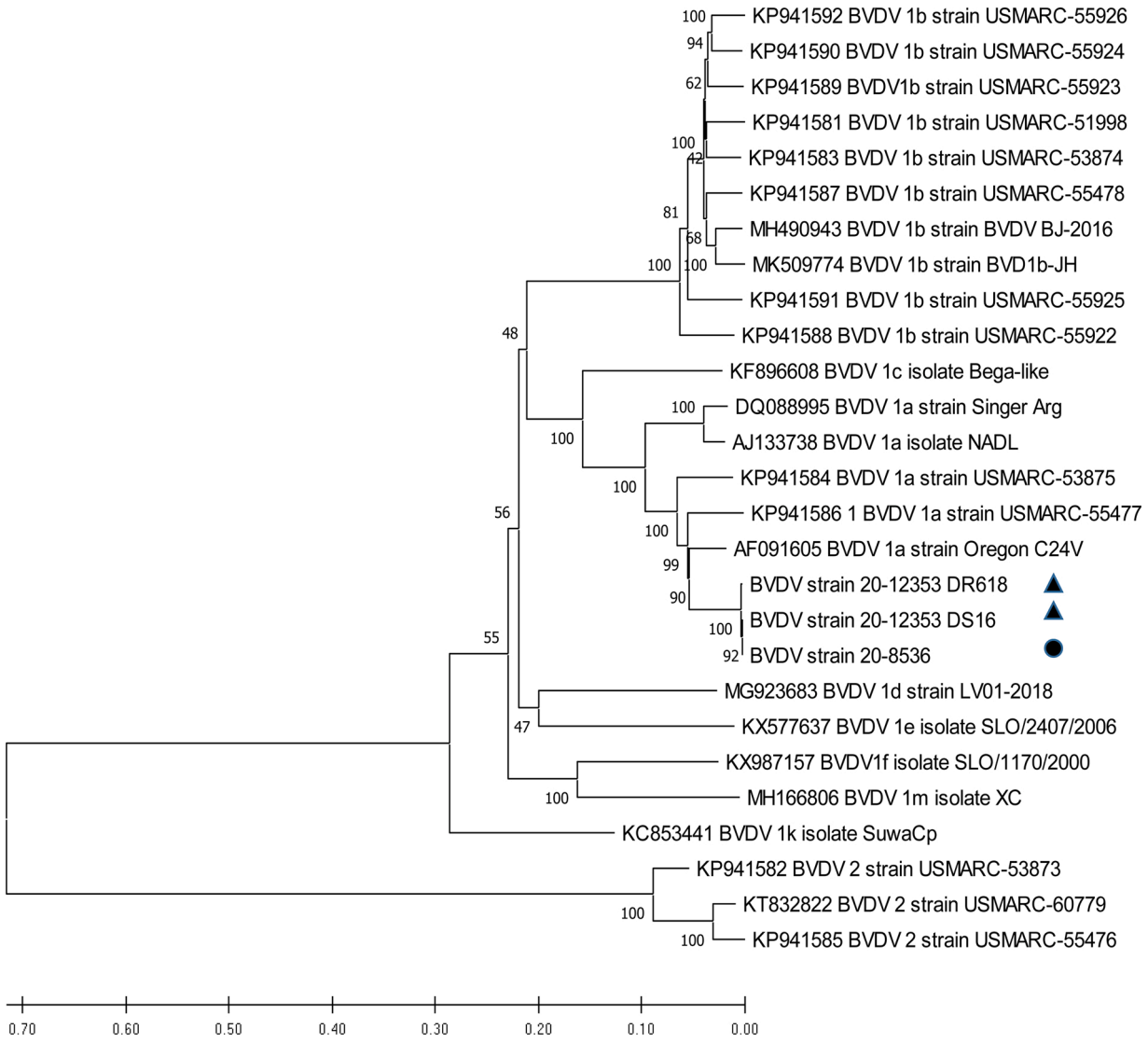

Fig. 2. Phylogenetic analysis of bovine viral diarrhea virus genome nucleotide sequences. Sequences were aligned by ClustalW and the phylogenetic tree was inferred by maximum-likelihood analysis using the best-fitting GTR model as implemented in MegaX. Evolutionary rate differences were modeled with a discrete Gamma distribution allowing some sites to be invariable. Positions with less than 80 % coverage were eliminated from the analysis. Robustness of tree topology was evaluated with 500 bootstrap replicates. The initial BVDV genome determined from pooled nasal swabs is indicated by ●. BVDV genomes determined from individual bison serum two months later are indicated by ▲.

These results suggest that DR618 and DS16 were persistently infected with BVDV.

The same samples were analyzed for bosavirus. Samples collected 51 days apart were positive for animals DS16 (Ct 24.5 and 28.2) and DS704 (Ct 35.7 and 29.0). The remaining two animals were negative for both samples. The detection of bosavirus in the same two animals in samples collected 51 days apart suggests persistent infection by bosavirus. Bison DS16 was persistently infected with both BVDV and bosavirus as evidence by detection of both viruses in samples collected 51 days apart.

4. Discussion

BVDV are significant viral pathogens of cattle. Consequently, a multitude of inactivated and modified-live virus vaccines have been utilized for decades to protect against clinical disease. While intended for use in bovines, these vaccines are administered off label to other species that are infected by BVDV, including bison (Byers et al., 2010). BVDV vaccines had not been used in the herd investigated here since 2009 however the bison herd was in close proximity to cattle. Neither of the two BVDV positive bison had been vaccinated. The two isolates had greater than 99 % sequence identity, indicating a shared source of infection. The consensus sequence of the two bison BVDV isolates had 92 % similarity to Oregon C24 V, a vaccine strain, suggesting a wild type virus. Sources of BVDV introduction include cattle from adjacent operations and wildlife. Serosurveys of wildlife have measured antibodies to BVDV and found that wildlife living at the interface of livestock agriculture have higher seropositivity rates than wildlife in wilderness environments largely free of cattle (Wolf et al., 2008; Kingscote et al., 1987; Zarnke, 1983; Sadi et al., 1991).

To our knowledge, this is the first description of bison persistently infected with BVDV. Previously, BVDV was isolated from bison in Canada displaying signs of enteritis and hoof lesions in one herd and found acutely dead without apparent clinical disease in the other herd (Deregt et al., 2013). Here, virus isolation and detection of BVDV viremia at high levels, based on quantitative RT-PCR, in two bison in two concurrent samples collected 51 days apart, suggests the animals were persistently infected. The BVDV-positive bison were 20–34 months old, respectively, and while displaying no overt signs of clinical disease,

they were removed from the herd as failing to thrive.

The detection of bosavirus in bison is the first report of this virus in samples other than fetal bovine serum. The clinical significance of bosavirus infection is unknown. The detection of bosavirus in the same animals in samples collected 51 days apart suggests persistent infection, by itself in one animal and in conjunction with BVDV persistent infection in another animal. The human pathogen parvovirus B19 can establish persistent infections in immunocompromised individuals (Heegaard and Brown, 2002). Initially identified in pooled fetal bovine serum, bosavirus was also detected in respiratory swabs from horses though this latter detection was attributed to fetal bovine serum added as a preservative (Sadeghi et al., 2017; Altan et al., 2019). Interestingly, metagenomic sequencing and PCR analysis of a commercial cattle herd experiencing a bovine viral diarrhea outbreak identified bosavirus in 3/8 cattle persistently infected with BVDV and exhibiting mucosal disease while failed to detect bosavirus in BVDV-persistently-infected cattle without signs of mucosal disease or BVDV-uninfected animals in the same herd (Weber et al., 2018). The BVDV and bosavirus co-infected bison DS16 detected here had considerably lower Ct values for BVDV (Ct's 22.2 and 24.5) in nasal swabs as compared to BVDV-only infected DR618 (Ct's 30.2 and 29.4). Further work is needed to ascertain the etiological significance of bosavirus infection and possible synergy between BVDV and bosavirus co-infection

Ethics statement

Animal samples utilized in this study were collected by a licensed veterinarian from privately owned animals and submitted to a public veterinary diagnostic laboratory for testing as part of their management of animal health. As such, no specific institutional animal care and use committee (IACUC) approval was necessary.

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Declaration of Competing Interest

The authors reported no declarations of interest.

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