



## Virome of US bovine calf serum



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### ABSTRACT

Using viral metagenomics we analyzed four bovine serum pools assembled from 715 calves in the United States. Two parvoviruses, bovine parvovirus 2 (BPV2) and a previously uncharacterized parvovirus designated as bosavirus (BosaV), were detected in 3 and 4 pools respectively and their complete coding sequences generated. Based on NS1 protein identity, bosavirus qualifies as a member of a new species in the copiparvovirus genus. Also detected were low number of reads matching ungulate tetraparvovirus 2, bovine hepacivirus, and several papillomaviruses. This study further characterizes the diversity of viruses in calf serum with the potential to infect fetuses and through fetal bovine serum contaminate cell cultures.

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## 1. Introduction

Fetal bovine serum is a potential source of viral contamination for cell cultures used in the production of biological products for human or animal use [1,3,6,10,15–17,20] and is therefore routinely subjected to a range of virus-specific tests to ensure an absence of viral contaminations. While regulations list specific viruses whose absence must be confirmed, such as bovine viral diarrhoea virus 1 and 2 and others, bovine viruses of concern beyond that list have been identified [15]. Viral removal through filtration or inactivation methods may be used to reduce the risk of viral contamination; however, small non-enveloped viruses with ssDNA genomes, such as parvoviruses, are less susceptible to such measures. Using an unbiased metagenomics approach we characterized viral sequences present in pools of bovine serum samples collected from

calves in the US. The ability of these parvoviruses to contaminate fetal bovine serum remains to be determined.

## 2. Materials and methods

Four bovine sera pools collected from different areas of the United States were analyzed (Table 1). These samples were collected as part of a hepatitis E virus seroepidemiological study. The 25 calves in GA1 group and 90 calves in GA2 were sampled at 5–6 time points. The 375 calves in IA2 group and the 225 cows from VT1 group were sampled once. VT1 group from the Virginia Tech dairy herd, included animals from a lactating dairy herd (various lactation and days in milk) as well as pregnant heifers.

Library preparation and computational analysis were performed as previously described [14,23]. Briefly, serum pools were filtered through a 0.45- $\mu$ m filter (Millipore) to remove eukaryotic- and bacterial cell-sized particles, and 330  $\mu$ L of each pool was then subjected to a mixture of nuclease enzymes to reduce the concentration of free (non-viral encapsidated) nucleic acids [23]. Viral

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nucleic acids were then extracted (MagMAX Viral RNA Isolation Kit, Ambion, Inc, Austin, Tx, USA) and random RT-PCR was used to amplify RNA and DNA. Four libraries were constructed using Nextera XT DNA Sample Preparation Kit (Illumina) and sequenced using the Miseq Illumina platform with 250 bases paired ends with dual barcoding for each pool.

### 3. Results

Four serum pools from a total of 715 animals were enriched for viral nucleic acids which were then randomly amplified and deep sequenced on the Illumina platform. Out of ~47.4 million sequence reads, ~1% (44,279) were found by BLASTx to contain open reading frames encoding for parvovirus related proteins (BLASTx E scores  $<10^{-5}$ ). Two complete coding regions of parvovirus genomes could be assembled. Bovine parvovirus 2 (BPV2 in the ungulate copiparvovirus 1 species) was detected and its genome assembled (GenBank accession number KY019140). BPV2's nonstructural (NS) and VP1 proteins showed 94–96 and 89–96% identity to the 3 BPV2 genomes currently available in GenBank database. BPV2 sequences were detected in three pools (Table 1). The second parvovirus genome was more divergent relative to known viral genomes showing its closest relative to be porcine parvovirus 6 (PPV6 in the ungulate copiparvovirus 2 species) with 40% amino acid identity in their non-structural protein. The virus was named bosavirus (Bovine serum associated virus and genome sequence deposited as GenBank accession number KY019139). Phylogenetic analyses showed bosavirus' NS clustering with the NS of copiparvoviruses (Fig. 1). The bosavirus VP was slightly more closely related to the VP of the recently described sesavirus (36% identity) from a sea lion than to that of PPV6 (34% identity) (Fig. 2). Bosavirus sequences could be detected in all four pools (Table 1). According to a proposal from the International Committee on Taxonomy of Viruses (ICTV), members of the same parvovirus genus should share >30% identity in NS1 while members of the same species should exhibit >85% identity in NS1 [8]. Based on pair-wise NS1 alignments, bosavirus is therefore proposed as member of a new species in the *Copiparvovirus* genus (ungulate copiparvovirus 3).

Using the bosavirus and the BPV2 genomes described above and the program Genious (Genious R6, 5.6.3 software with default settings) we calculated that 98.21% of the parvovirus-like sequences could be matched to the bosavirus genome and 1.28% to BPV2 genome. Therefore 99.5% of parvovirus-related protein sequences detected here belonged to these two parvovirus species. The remaining parvovirus related reads consisted of mutated BPV2 and bosavirus reads (likely due to sequencing errors), chimeric reads that were only partly BPV2 or bosavirus, and five reads in GA2 pool that matched (>95% nucleotide similarity) porcine hokovirus (GenBank EU200677) [13] in the ungulate tetraparvovirus 2 species

[8].

Other viral sequences were also detected but in much smaller numbers. Five sequence reads showing 97–100% amino acid sequence identity to the RNA genome of bovine hepacivirus, a recently described member in the *Hepacivirus* genus of the *Flaviviridae* family (GenBank: KP265948.1) [7,21], were detected in GA2 pool. Bovine hepacivirus RNA has been detected in 1.6% of individually tested cows and in 3.8% of tested herds in Germany where its tissue distribution indicated possible liver tropism but no clear association with disease has been established [2]. Also detected were 4 papillomavirus reads. The GA2 pool yielded one read that was 100% identical to human betapapillomavirus HPVX14 (GenBank: AF054874.1). Pool IA2 yielded three papillomavirus reads. One was 91% identical to the unclassified human papillomavirus type 174 (GenBank: HF930491.1), one 98% identical to human betapapillomavirus RTRX7 (GenBank: U85660.1) and one 96% identical to human betapapillomavirus type 151 (GenBank: FN677756.1).

### 4. Discussion

Our analysis characterizing enriched viral sequences in calf serum showed that parvovirus sequences dominated relative to other viruses (Table 1). As bovine fetuses acquire their viral infections from pregnant cows the potential exist for the viruses described here to also infect fetuses. The detection of a previously uncharacterized parvovirus (bosavirus) present in each US serum pool analyzed indicates that its presence and infectivity should be considered when testing fetal bovine serum for viral contamination. To date, members of the *Copiparvovirus* genus (classified based on NS) have only been reported in bovine or porcine samples [8]. Whether only ungulates can be infected by copiparvoviruses, therefore posing little risk of infection to non-ungulate mammals, will become clearer as more parvoviruses are described in additional mammalian species. While bosavirus can be classified as a copiparvovirus based on its NS, its VP was more closely related to that of a parvovirus from the feces of a carnivore (*Zalophus californianus* or California sea lion) whose NS sequence falls outside the range of copiparvoviruses [19]. The tropism of bosavirus may therefore extend beyond that of the currently known copiparvoviruses so far described only in ungulate samples. A few reads of porcine hokovirus, classified in the *Tetraparvovirus* genus, were also detected in one pool indicating that this virus, previously reported in porcine samples, may also infect calves albeit at low level relative to bosavirus and BPV2 (Table 1).

A low number of bovine hepacivirus (*Flaviviridae* family), a virus originally detected in African cattle and then herds in Germany, were also detected in one pool [2,7]. Whether this hepacivirus can be transmitted to bovine fetuses or is capable of infecting other

**Table 1**

Characteristics of the bovine plasma pools analyzed in this study including viruses identified by NGS. Four sera pools approximately 1 mL collected from different areas of the United States were applied for NGS.

Pool	Animal number	Age	Age at Sampling	States of origin	Virus detected (Reads)
GA1	25 calves	3–240 days	3,30,60,120,200,240	GA	1. Bosavirus (30,590)
GA2	90 calves	3–240 days	3,30,60,120,200,240	GA	1. Bosavirus (1,931) 2. Bovine parvovirus 2 (286) 3. Bovine hepacivirus (5) 4. Human papillomavirus (4)
IA2	375 calves	Unknown	Unknown	IA, NE, SD, NM, OK, TX, SD, ND, WY, MT.	1. Bosavirus (9,980) 2. Bovine parvovirus 2 (106) 3. Human papillomavirus (3)
VT1	225 cows	Unknown	All ages and all stages of lactation	VT	1. Bosavirus (989) 2. Bovine parvovirus 2 (184)

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