

The genome of Border disease virus genotype 8 from chamois using next generation sequencing

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Keywords

Border disease virus, Italy, Next Generation Sequencing, *Pestivirus D*, Wild-domestic transmission.

Summary

Border disease virus (BDV) is widespread both in domestic small ruminants and wildlife. Here we report the genome of BDV genotype 8 from chamois, strain Italy-58987, obtained by next generation sequencing and the comparison with other pestiviruses. The sequence of 12,245 bp long was aligned to 22 pestivirus genomes and it showed a nt/aa similarity of 81.3/89.4% with BDV genotypes, and 65.9/67.8% with the other pestiviruses. The genome showed a mean nt/aa similarity of 91.2/95.0% with three Swiss genomes closely related to the BDV-8 5'-UTR and Npro sequences. The identification of divergent BDV-8 isolates in North-Western Italy and in Switzerland suggests that this genotype may have been circulating in a wider area than previously supposed, and may have a high host adaptability.

Sequenza genomica del genotipo 8 del virus della Border disease da un camoscio ottenuta mediante tecniche di sequenziamento di nuova generazione

Parole chiave

Italia, *Pestivirus D*, Sequenziamento di nuova generazione, Trasmissione, Virus della Border disease.

Riassunto

Il virus della Border disease (BDV) è diffuso a livello mondiale nei piccoli ruminanti, domestici e selvatici. In questo lavoro si riportano la sequenza del genoma di BDV genotipo 8 da un camoscio (ceppo Italy-58987), ottenuta mediante tecniche di sequenziamento di nuova generazione (NGS), e il confronto della stessa con le sequenze genomiche di altri pestivirus. La sequenza di 12.245 bp è stata allineata a 22 sequenze genomiche di pestivirus e ha mostrato una identità di sequenza nt/aa del 81,3/89,4% con altri genotipi di BDV, e del 65,9/67,8% con gli altri pestivirus. La sequenza genomica del BDV-8 rilevato nel camoscio ha mostrato una identità media nt/aa del 91,2/95,0% con le sequenze genomiche di 3 ceppi di BDV isolati in Svizzera, strettamente correlati con le sequenze delle porzioni di 5'-UTR e Npro di BDV-8. L'identificazione di isolati divergenti di BDV-8 nel nordovest dell'Italia e in Svizzera suggerisce che questo genotipo possa aver circolato in un'area più ampia di quella inizialmente ipotizzata, e che possa avere una elevata adattabilità a ospiti diversi.

Border disease virus (BDV) is a single-stranded positive sense RNA virus belonging to the genus *Pestivirus*, family *Flaviviridae*. It is the causative agent of Border disease (BD), a worldwide infection of domestic small ruminants, causing huge economic losses, and wildlife. According to a new proposed taxonomy of the genus *Pestivirus*, BDV should be referred to as *Pestivirus D* (Smith *et al.* 2017). Here we use both names to refer to Border disease virus, since at the time of writing, the readers may not be comfortable with the new classification only.

The *Pestivirus D* genome is about 12.3 kb long, coding for four structural proteins (C, Erns, E1 and E2) and seven to eight non-structural proteins (Npro, p7, NS2-3, NS4A, NS4B, NS5A, NS5B) flanked by 5'- and 3'-untranslated regions (UTR). Based on phylogenetic analysis of 5'-UTR or Npro sequences, BDV has been divided into at least seven groups (BDV-1 to -7) (Giammarioli *et al.* 2011). Our group identified a new genotype, named BDV-8, in a goat kid showing BD-like syndrome with typical "hairy shaker" symptoms in North-Western Italy and,

afterwards, in an Alpine chamois found dead with poor body condition (*Rupicapra rupicapra*) (Peletto *et al.* 2016, Caruso *et al.* 2017). Recently, genomes of three Swiss BDV isolates from a sheep, a cattle and a pig were published and showed to be related to BDV-8, according to 5'-UTR and Npro sequences (Stalder *et al.* 2017). These findings demonstrate that this novel genotype may infect also non ruminant species (i.e. pigs).

The aim of this work was to provide the genome sequence of BDV-8 strain Italy-58987 by Next Generation Sequencing and to carry out a comparison with genomes of other pestiviruses.

The virus was successfully isolated as previously reported (Caruso *et al.* 2017), and 800 μ l of supernatant were used for total RNA purification with TRIzol Reagent (Invitrogen), adapting the manufacturer's protocol to the input volume. The RNA was then processed with the RNA Clean & Concentrator (Zymo research) to increase the RNA concentration and purity, eluting down to 10 μ l molecular-grade H₂O. To process the sample for the library preparation, RNA underwent reverse transcription with the Reverse Transcription System (Promega) and second strand synthesis with the NEBNext mRNA Second Strand Synthesis Module (New England Biolabs). The final reaction was purified with Agencourt AMPure XP magnetic beads (Beckman Coulter). Both RNA and ds-cDNA were tested for the presence of BDV genome by an in-house SYBR Green real-time RT-PCR using 324/326 primer pair (Vilcek & Paton 2000). DNA was processed with the Nextera XT DNA Library Preparation Kit (Illumina) following manufacturer's instructions. The final library was sequenced on a Illumina MiSeq with MiSeq Reagent Kit v3-600, in two different 2x300 bp paired-end runs (SRA accession PRJNA514412).

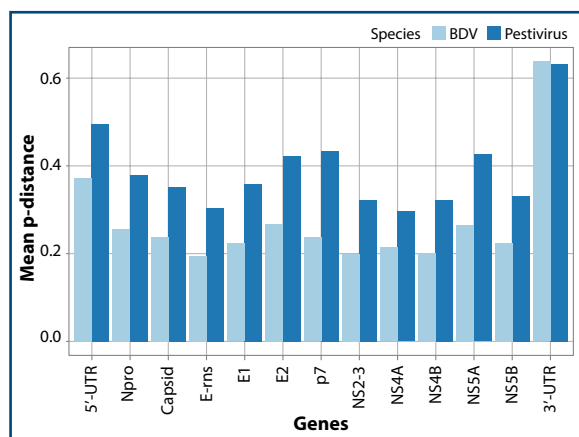


Figure 1. Mean nucleotide *p*-distance between sequences of Border disease virus (BDV) including BDV-8 Italy-58987. BDV-Swiss and non-BDV pestivirus according to genes.

A total of 3,635,077 read pairs were obtained and filtered with Trim Galore v0.4.3. The first 10 bp were removed from all reads to avoid poor quality, and the reads were trimmed to remove < Q30 nucleotides (nt) and adapter sequences. Reads were assembled with MEGAHIT v1.1.1-2 (Li *et al.* 2016) and contigs were classified with blastn and the NCBI nr Nucleotide database.

The contig corresponding to BDV-8 (GenBank Acc. Num. MG649392) was 12,245 bp long, from nt 70 to nt 12,333 of the reference genome BDV X818 (GenBank Acc. Num. NC_003679), which was used for sequence annotation. The genome was sequenced with a 20753.8X coverage, with 21.01% reads corresponding to the viral genome. The sequence was then aligned to 22 pestivirus genomes, representative of known phylogenetic groups for which genome sequences were available at the time of writing, with Muscle software for multiple comparison and phylogenetic analysis (Edgar 2004). The novel genome showed a nucleotide/amino acid similarity of 81.3/89.4% with *Pestivirus* D genotypes, and 65.9/67.8% with the other pestiviruses. BDV-8 showed a mean nucleotide/amino acid similarity of 91.2/95.0% with the three Swiss genomes. The nucleotide diversity (*p*-distance) for each gene between BDV-8 and other BDV genotypes and pestivirus reference sequences was lower in the 5' and 3' untranslated regions, and higher in the NS5A gene (Figure 1).

The best molecular substitution model GTR+I+G was identified by jModelTest2, and used as a priori information for the Bayesian phylogenetic inference implemented in MrBayes v3.2.6 (Ronquist *et al.*

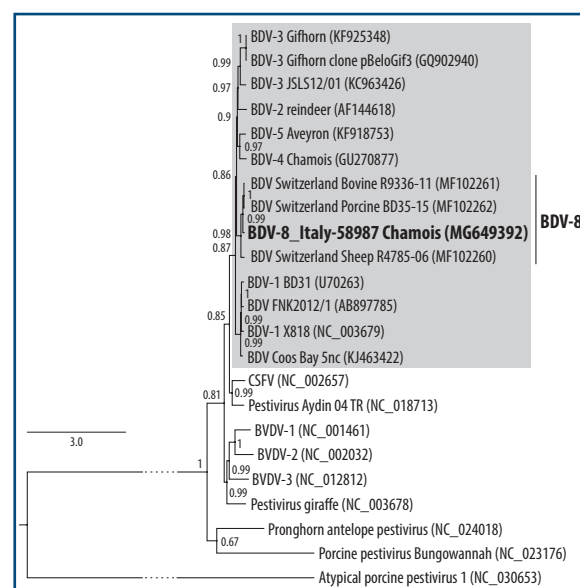


Figure 2. Phylogenetic tree of the Pestivirus genus based on the genome nucleotide sequence, with the Border disease virus (BDV) clade highlighted in grey, and the genotype 8 clade marked by a side bar.

2012). The novel strain is placed within the BDV clade with a strong support of the posterior probability of the ancestor nodes (Figure 2).

The identification of divergent BDV-8 isolates in North-Western Italy and in Switzerland, with the first collection in 2006 in a white alpine sheep, suggests that the virus circulation may be geographically wider than previously supposed (Caruso *et al.* 2017). The poor epidemiological information on such novel strain does not allow to infer whether it might be spread in the livestock and occasionally infecting wild ungulates, or vice versa. Considering the wide range of host species in which the virus has been identified so far (goat, chamois, sheep, cattle and pig), this novel genotype seems to have a high adaptability. Moreover, the diagnostic issues previously reported by Peletto and colleagues (Peletto *et al.* 2016) (i.e., the widely used BDV-specific nested assay published by Vilcek and Paton fail to amplify BDV-8 because of primer mismatches)

may have led to underestimate its real circulation, revealing itself a risk for uncontrolled and unknown spread in different species. Further studies on the epidemiology of BDV-8 are needed to clarify the impact of such novel strain on livestock and wild ungulates. Importantly, the availability of BDV genomes from different host species may provide clues about the genetic basis of cross-species transmission.

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