





## Complete Genome Sequences of Three Border Disease Virus Strains of the Same Subgenotype, BDSwiss, Isolated from Sheep, Cattle, and Pigs in Switzerland

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**ABSTRACT** We report here the complete genome sequences of three border disease virus (BDV) strains of the same subgenotype isolated in Switzerland from a sheep, a cow, and a pig, respectively. This is the first report of full-length sequences of a tentatively new subgenotype isolated from three different species of clovenhoofed farm animals.

order disease virus (BDV) belongs to the genus *Pestivirus*, which includes important animal pathogens, such as bovine viral diarrhea virus (BVDV) and classical swine fever virus (CSFV). BDV occurs worldwide (1, 2), and several subgenotypes have been described to date (3, 4). BDV infections occur mainly in sheep and goats but also in cattle, pigs, and wild even-toed ungulates (2). We describe here the full-length sequences of three BDV strains isolated in Switzerland from a sheep, a cow, and a pig. They form a new, yet unclassified, BDV subgenotype. The first isolates of this type were isolated in 2006 in Switzerland and provisionally named BD Switzerland (5) or BDSwiss (6–8) or remained unclassified (4, 9, 10), and partial sequences (5' untranslated region [UTR], N<sup>pro</sup>) were deposited in GenBank (accession no. JQ994199, JQ994200 GU244490, and GU244489). Recently, BD viruses of this subgenotype were detected in goats and chamois in Italy and were tentatively labeled BDV-8 (11, 12).

Samples R4785/06 (also named CH-BD4), R9336/11, and BD35-15 were obtained from a female white alpine sheep in 2006, a crossbreed (Braunvieh × Limousin) male cow in 2011 (7), and a female domestic pig in a zoo, respectively. Viruses from blood of sheep and cattle were isolated on bovine turbinate cells, whereas SK6 cells were used for porcine blood. RNA was isolated using the QIAamp viral RNA minikit (Qiagen AG, Hombrechtikon, Switzerland), and reverse transcription-PCR (RT-PCR) (Qiagen OneStep RT-PCR kit) was performed according to the manufacturer's instructions. PCR fragments were purified with the QIAquick PCR purification kit (Qiagen). DNA-Sanger cycle sequencing with BigDye Terminator chemistry (version 3.1) and capillary electrophoresis (ABI 3730xl DNA analyzer; Applied Biosystems) were performed at Microsynth (Balgach, Switzerland). The 3' ends were determined by a simplified protocol for rapid amplification of cDNA ends (RACE) (13) with direct sequencing of the amplification products or by cloning into pCRTM 4-TOPO (Invitrogen). The 5' ends were determined by a 5' RACE kit (Invitrogen or Roche). The electropherograms were assembled with SegMan II version 5.01 (DNAStar, Inc., Madison, WI), and sequences were analyzed with Clone Manager 9 professional edition (Scientific & Educational Software, Cary, NC) and the MEGA program, version 6 (14).

The complete genomes of the isolates R4785/06, R9336/11, and BD35-15 comprise 12,318, 12,311, and 12,309 nucleotides (nt), with 5' UTRs of 369, 377, and 375 nt and

**Received** 3 October 2017 **Accepted** 4 October 2017 **Published** 9 November 2017

Citation Stalder H, Marti S, Flückiger F, Renevey N, Hofmann MA, Schweizer M. 2017. Complete genome sequences of three border disease virus strains of the same subgenotype, BDSwiss, isolated from sheep, cattle, and pigs in Switzerland. Genome Announc 5:e01238-17. https://doi.org/10.1128/genomeA.01238-17.

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3' UTRs of 261, 246, and 246 nt, respectively. All polyprotein-coding sequences are 11,685 nt long and code for 3,895 amino acids (aa). The bovine and porcine isolates are more similar to each other (99% nt, 99% aa) than to the ovine strain (86% nt, 92% aa). In the N<sup>pro</sup> genomic region, the sequences of the strains reported recently from Italy (11, 12) match 82 to 92% (nt) and 86 to 93% (aa) to the corresponding regions in our strains.

This is the first report of complete consensus sequences of three strains of the same BDV subgenotype, BDSwiss (later also named BDV-8), obtained from a sheep, a cow, and a pig in Switzerland, which will further assist investigations on the epidemiology and evolution of pestiviruses in different host species.

**Accession number(s).** The complete sequences of the isolates R4785/06, R9336/11, and BD35-15 have been deposited in GenBank under the accession no. MF102260, MF102261, and MF102262, respectively.

## **ACKNOWLEDGMENT**

This work was supported by internal funds of the Institute of Virology and Immunology in Bern and Mittelhäusern.

## **REFERENCES**

- Strong R, La Rocca SA, Ibata G, Sandvik T. 2010. Antigenic and genetic characterisation of border disease viruses isolated from UK cattle. Vet Microbiol 141:208–215. https://doi.org/10.1016/j.vetmic.2009.09.010.
- 2. Schweizer M, Peterhans E. 2014. Pestiviruses. Annu Rev Anim Biosci 2:141–163. https://doi.org/10.1146/annurev-animal-022513-114209.
- 3. Giammarioli M, Rossi E, Casciari C, Bazzucchi M, Torresi C, De Mia GM. 2015. Genetic characterization of border disease virus (BDV) isolates from small ruminants in Italy. Virus Genes 50:321–324. https://doi.org/10.1007/s11262-014-1165-6.
- Lešková V, Jacková A, Vlasáková M, Vilček S. 2013. Genetic characterization of a border disease virus isolate originating from Slovakia. Acta Virol 57:17–25. https://doi.org/10.4149/av\_2013\_01\_17.
- Peterhans E, Bachofen C, Stalder HP, Schweizer M. 2010. Cytopathic bovine viral diarrhea viruses (BVDV): emerging pestiviruses doomed to extinction. Vet Res 41:44. https://doi.org/10.1051/vetres/2010016.
- Casaubon J, Vogt HR, Stalder H, Hug C, Ryser-Degiorgis MP. 2012. Bovine viral diarrhea virus in free-ranging wild ruminants in Switzerland: low prevalence of infection despite regular interactions with domestic livestock. BMC Vet Res 8:204. https://doi.org/10.1186/1746-6148-8-204.
- Braun U, Hilbe M, Janett F, Hässig M, Zanoni R, Frei S, Schweizer M. 2015. Transmission of border disease virus from a persistently infected calf to seronegative heifers in early pregnancy. BMC Vet Res 11:43. https://doi .org/10.1186/s12917-014-0275-7.
- Kaiser V, Nebel L, Schüpbach-Regula G, Zanoni RG, Schweizer M. 2017. Influence of border disease virus (BDV) on serological surveillance within

- the bovine virus diarrhea (BVD) eradication program in Switzerland. BMC Vet Res 13:21. https://doi.org/10.1186/s12917-016-0932-0.
- Bachofen C, Stalder HP, Vogt HR, Wegmüller M, Schweizer M, Zanoni R, Peterhans E. 2013. Bovine viral diarrhea (BVD): from biology to control. Berl Münch Tierarztl Wochenschr 126:452–461. (In German.)
- Braun U, Reichle SF, Reichert C, Hässig M, Stalder HP, Bachofen C, Peterhans E. 2014. Sheep persistently infected with border disease readily transmit virus to calves seronegative to BVD virus. Vet Microbiol 168:98–104. https://doi.org/10.1016/j.vetmic.2013.11.004.
- Peletto S, Caruso C, Cerutti F, Modesto P, Zoppi S, Dondo A, Acutis PL, Masoero L. 2016. A new genotype of border disease virus with implications for molecular diagnostics. Arch Virol 161:471–477. https://doi.org/ 10.1007/s00705-015-2696-4.
- Caruso C, Peletto S, Cerutti F, Modesto P, Robetto S, Domenis L, Masoero L, Acutis PL. 2017. Evidence of circulation of the novel border disease virus genotype 8 in chamois. Arch Virol 162:511–515. https://doi.org/10 .1007/s00705-016-3112-4.
- Scotto-Lavino E, Du GW, Frohman MA. 2006. 5' End cDNA amplification using classic RACE. Nat Protoc 1:2555–2562. https://doi.org/10.1038/ nprot.2006.480.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011.
   MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739. https://doi.org/10.1093/molbev/msr121.

Volume 5 Issue 45 e01238-17