Genotyping and phylogenetic analysis of bovine viral diarrhea virus (BVDV) isolates in Kosovo

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Summary
Three serum samples positive in Antigen ELISA BVDV have been tested to characterise genetic diversity of bovine viral diarrhea virus (BVDV) in Kosovo. Samples were obtained in 2011 from heifers and were amplified by reverse transcription-polymerase chain reaction, sequenced and analysed by computer-assisted phylogenetic analysis. Amplified products and nucleotide sequence showed that all 3 isolates belonged to BVDV 1 genotype and 1b sub genotype. These results enrich the extant knowledge of BVDV and represent the first documented data about Kosovo BVDV isolates.

Genotipizzazione e analisi filogenetica di alcuni ceppi del virus della diarrea virale bovina (BVDV) in Kosovo

Parole chiave
Analisi filogenetica,
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Riassunto
Per caratterizzare la diversità genetica del virus della diarrea virale bovina (BVDV) in Kosovo, sono stati testati tre campioni di siero positivi all'ELISA Antigene BVDV. I campioni, ottenuti da giovenche nel corso del 2011, sono stati amplificati con PCR-reverse ranscription, sequenziati e analizzati mediante analisi filogenetica assistita da computer. I prodotti amplificati e la sequenza nucleotidica hanno dimostrato l'appartenenza dei ceppi al BVDV genotipo1 e sotto-genotipo1b. I risultati ottenuti sono i primi dati documentati su ceppi di BVDV in Kosovo.

The bovine viral diarrhoea virus (BVDV) is a significant pathogen of cattle. It is a small enveloped RNA virus which, like classical swine fever virus (CSFV) and border disease virus (BDV), belongs to genus *Pestivirus* in the *Flaviviridae* family (Heinz *et al.* 2000). The length of RNA genome is approximately 12.3 kbp. The viral genome comprises a single open reading frame (ORF) encoding about 4,000 amino acids (Collet *et al.* 1998, Demoerlooze *et al.* 1993). The bovine viral diarrhoea virus is classified by biotype and genotype (Baker 1995, Fulton *et al.* 2000, Pellerin *et al.* 1994, Ridpath *et al.* 1994). Based on the presence or absence of visible cytopathic effects in infected cell cultures, BVDV genotypes are classified in cytopathic (CP) and noncytopathic (NCP) biotypes.

Genotypes 1 and 2 of BDVD are detected by polymerase chain reaction (PCR) for nucleotide and antigenic differences (Fulton *et al.* 2000, Hamers *et al.* 2001, Pellerin *et al.* 1994, Ridpath and Bolin 1998, Stram *et al.* 2004). Type 1 BVDV (BVDV-1) strains include the classic BVDV isolates, while type 2 (BVDV-2) comprises the BVDV strains associated with high mortality (Brownlie *et al.* 1984). Genotype 1 has been reported worldwide, whereas BVDV-2 has been observed mainly in North America (Pellerin *et al.* 1994, Ridpath *et al.* 1994). Genetic diversity of BVDV isolates as well as to other viruses (Stram *et al.* 2004, Stram *et al.* 2011) is important for laboratory diagnosis, vaccine design and taxonomy.

The bovine viral diarrhoea virus causes a variety of clinical syndromes in cattle, including diarrhea, reproductive failure, respiratory disease, mucosal disease, and hemorrhagic syndrome resulting from thrombocytopenia (Baker 1995, Perdrizet *et al.* 1987). An important condition for the maintenance of BVDV in bovine populations is the immunotolerant and persistent infection that results from a transplacental infection of the foetus before the onset of immunological maturity (McClurkin *et al.* 1984). Intrauterine BVDV infections are a serious problem, which causes high rates of abortion, still births, foetal resorption, mummification, congenital malformations, weak calf births, and growth retardation (Houe 1999, Moennig and Liess 1995). The aim of this study was to examine the genetic diversity of recently obtained BVDV isolates in Kosovo.

Three serum samples have been collected from heifers, which responded positively to BVDV Ag ELISA in Kosovo during 2011. One of the positive samples belonged to a backyard heifer aged 10-months without clinical signs from Ferizaj municipality. The second positive sample belonged to 6-month old heifer with signs of yellowish diarrhea, dehydration, erosion in the region of the nose and lips. This sample was taken in Dubrava correctional center, in Istog. The third positive sample belonged to 6-month old heifer without clinical signs from dairy farm in Istog.

RNA was isolated using Viral Gene-Spin kit (iNtRON, Seoul, South Korea) following manufacturer's instructions. RT-PCR was performed using Maxim RT-PCR one tube reaction mix (iNtRON, Seoul, South Korea) according to manufacturer's instructions and using primers BVDV#86, CCCTCTTCAGCGAAGGCCGAA at position 86 and BVDV#371, TCAACTCCATGTGCCATGTACAGCA at position 371 in acc. # M31182. The product of PCR was purified using QIAquick purification kit (Qiagen, Hidden, Germany) according to the manufacturer's instructions, in addition the eluted DNA was ethanol precipitated and dissolved in 15µl DNase free water and used for sequencing.

Sequence analysis was done using Vector NTI (Invitrogen, Carlsbad, CA, USA) and EMBOSS suits (http://bioinfo.agri.huji.ac.il/wemboss/). For the

Table I. *Sequences of 3 Bovine viral diarrhea virus (BVDV) isolates obtained from heifers in Kosovo in 2011.*

Isolate	5′ untraslated region (UTR) sequence
BVDV-1135 isolate	TGAGGCTAGCCATGCCCTTAGTAGGACTAGCATAATAAGGGGGGTAGCAACAGTGGTGAGTTCGT TGGATGGNTTAAGCCCTGAGTACAGGGTAGTCGTCAGTGGTTCGACGCCTTAACATNAGGNCTCG AGATGCCACGTGGACGAGGGCATGCCCACAGCACATCTTAACCTGANCGGGGGTCGCTCGGG GCGAAAACGGTTTANNCAACCGCTACGAATACAGCCTGATAGGGTGCTGCAGAGGCCCACTGT ATTGCTACTAAAAATCTCTGCTGTACATGGCACATGGAGTTGA
BVDV-1717-1 isolate	GGTCCCTCTCAGCGAAGGCCGAAAAGAGGCTAACCATGCCCTTAGTAGGACTAGCAAAACAA GGGGGGTAGCAACAGTGGTGAGTTCGTTGGATGGCTGAAGCCCTGAGTACAGGGTAGTCGTCA GTGGTTCGACGCTTCGTGTGACAAGCCTCGAGATGCCACGTGGACGAGGGCATGCCCACAGC ACATCTTAACCTGAGCGGGGGTCGTCCAGGTGAAACGGTTTAACCAACCGCCACGAATACAGC CTGATAGGGCGCTGCAGAGGCCCACTGTATTGCTACTAAAAATCTCTGCTGTACATGGCACATGG AGTTGA
BVDV-1717-2 isolate	CTCAGCGAAGGCCGAAAAGAGGCTAGCCATGCCCTTAGTAGGACTAGCATATTGGGGAGGGTA GCAGCAGTGGTGAGTTAGTTGGATGGCTGAAGCCCTGAGTACAGGGTAGTCGTCAGTGGTTCGA CGTCTTAAATGTAGGCCTCGAGATGCCACGTGGACGAGGGCATGCCCACAGCACATCTTAACTT GAGCAGGGGTCGCTCAGGTGAAAGCGGGTAAACCGTTACTGACACAGCCTGATAGGGTGCTG CAGAGGCCCACTGAACTGCTACTAAAAATCTCTGCTGTACATGGCACATGGAGTTGA

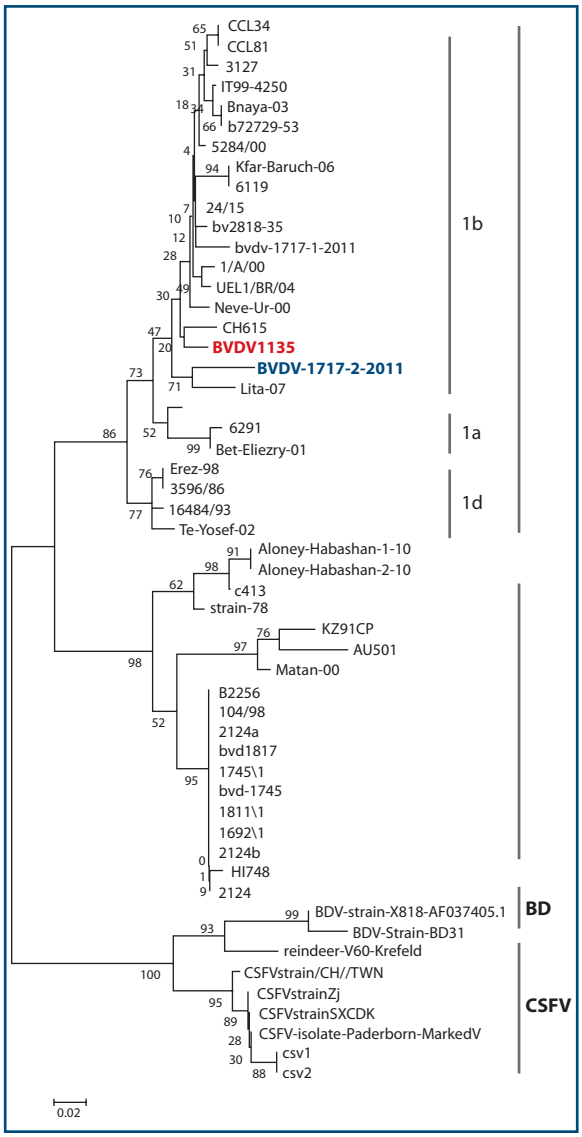

Figure 1. *Genotyping of BVDV 1135, 1717-1-2011 and 1717-2-2011 isolates from heifers in Kosovo in 2011.* Phylogenetic tree of Israli and Croatia isolates based on the 5' UTR of each isolate. The right simbols represent BVDB genotypes.
BD = Border Disease Virus; CSFV = Classical Swine Fever Virus.

phylogenetic analyses ClustalW[1] was performed and the *.aln output file was utilized in MEGA 6.0 program to perform the analysis (Tamura *et al.* 2007).

All sera identified previosly as positive by the BVDV-antigen ELISA were confirmed by the RT-PCR. Sequence analyses are shown in Table I, whereas phylogenetic relations of Kosovo isolates are reported in Figure 1.

Results showed that BVDV-1 infection was dominant in Kosovo and all 3 BVDV-1 isolated strains clustered within the same sub genotype BVDV 1b.

The BVDV 1 genotype has been categorised into 2 to 11 subgenotypes (Vilcek *et al.* 2001). Infections with BVDV occur globally and are the cause economic losses in cattle. BVDV 1 is predominant in cattle and widely spread throughout the world. In Croatia, all 18 tested positive BVDV isolates belonged to genotype 1 (Bedekovic *et al.* 2012). A study previously conducted to evaluate the genotypic distribution for 105 BVDV-positive samples at the Oklahoma diagnostic laboratory indicated that 61% of the samples was type 1 genotipe and 39% was type 2 genotipe (Fulton *et al.* 2000). This study, which was the first one performed for Kosovo BVDV isolates, confirmed that sub genotype BVDV 1b was dominant in Kosovo. The genetic data presented in this paper improve the general knowledge about the BVDV-1b isolates circulating in Kosovo. All this was useful for the developed molecular diagnostics assays for BVDV infection in order to control and prevent this disease.

[1] http://www.ebi.ac.uk/Tools/clustalw2/index.html.

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