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Genetic typing of Croatian bovine viral diarrhea virus isolates

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ABSTRACT

Between 2007 and 2011, a total of 1937 sera samples and five spleen samples from nine Croatian dairy herds were tested for the bovine viral diarrhea virus (BVDV) using virus isolation and the immunoperoxidase test. BVDV was detected in 13 persistently infected (PI) cattle with a non-cytopathogenic biotype, while in five animals with fatal mucosal disease, isolates from spleen samples were of the cytopathogenic biotype. To reveal the genetic typing of Croatian BVDV isolates, viral RNA was extracted from infected cell cultures and amplified by RT-PCR, with primers targeting the 5'-UTR and the Npro gene, followed by direct sequencing of purified PCR products. Sequence and phylogenetic analysis of the 5'-UTR genome region determined that all Croatian isolates belonged to BVDV genotype 1; 11 isolates were grouped with BVDV-1b and 7 with BVDV-1f viruses. The phylogenetic tree inferred by the Bayesian approach, using combined 5'-UTR/Npro, supported clustering of Croatian isolates in two subgroups. The deduced aminoacid sequence of the Npro region revealed 5 sites unique for four domestic BVDV-1f isolates.

Key words: BVDV, immunoperoxidase test, Npro, phylogenetic analysis

Introduction

Bovine Virus Diarrhoea Virus (BVDV) belongs to the *Pestivirus* genus of the *Flaviviridae* family, which also includes classical swine fever virus (CSFV) and border disease virus of sheep. BVD viruses are classified into two virus species, namely BVDV-1 and BVDV-2. The BVDV-1 subtypes are spread worldwide in the cattle population, while the highest occurrence of BVDV-2 species has been reported in the USA and

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Canada (PELLERIN et al., 1994; RIDPATH et al., 1994), partially in Japan (NAGAI et al., 2008), South America (FLORES et al., 2002) and occasionally in some European countries (WOLFMEYER et al., 1997; VILČEK et al., 2002; VILČEK et al., 2003).

The genome of BVDV consists of a single-stranded, positive-sense RNA, approximately 12.3 kb in length. A single open reading frame (ORF), flanked at either end by 5'- and 3'- untranslated regions (5'-UTR, 3'-UTR) encodes a polyprotein of about 4,000 aminoacids. The polyprotein is co- and post-translationally processed into 12 polypeptides in the following order: N-terminal autoprotease (Npro), capsid protein (C), envelope proteins (Erns, E1, and E2), p7, and non-structural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) (THIEL et al., 1996).

Viral infection during the first trimester of pregnancy in cows can result in the development of persistently infected (PI) animals. PI animals carrying a virus during their lifetime are immunotolerant to BVDV and become a source of infection for healthy cattle in their proximity (NETTLETON and ENTRICAN, 1995). The number of PI individuals in herds ranges between 0.1 and 1.8% (HOUE, 1999). The majority of BVDV field isolates belong within non cytopathogenic (NCP) biotypes. In animals persistently infected with NCP BVDV, superinfection with the cytopathogenic (CP) biotype of BVDV can cause fatal mucosal disease (MD) (BROWNLIE et al, 1984).

BVDV genetic typing is based on a comparison of the 5′ UTR region, Npro, and E2 sequences (PELLERIN et al., 1994; RIDPATH et al., 1994; VILČEK et al., 2001; TAJIMA et al., 2001) or cluster analysis of combined nucleotide sequences from the 5'-UTR and Npro regions. BVDV-1 viruses have 11 subtypes (VILČEK et al., 2001) while recent reports showed the existence of the new tree subtypes (JACKOVA et al., 2008; NAGAI et al., 2008).

A few studies describing BVD prevalence by serology have been conducted in Croatia (CVETNIĆ et al., 1968; MADIĆ et al., 1989; BIUK-RUDAN, 1997; BIUK-RUDAN et al., 1999), still, there is no national program for control and eradication of BVDV. Vaccinations are conducted on a voluntary basis in some herds across the country. According to the National Directive of control measures for protection of the animals from infectious diseases, only bulls for natural or artificial insemination need to be serologically tested for BVD. Until now, neither BVD virus isolation nor genetic typing of domestic BVDVs has been conducted in Croatia. So, in this paper, we describe for the first time isolation of the BVDV virus and the genetic characterization of recent Croatian BVDV isolates from naturally infected cattle, based on the 5′-UTR region and the combined 5'UTR/Npro region of the genome, respectively.

Materials and methods

Sera samples. Between 2007 and 2011, a total of 1937 sera samples from 9 different dairy herds, designated 1, 2, 3, 12, 13, 14, 15, 18 and 19 (Table 1), were tested using immunoperoxidase (IP) assay with murine BVDV specific monoclonal antibodies (Monoclonal antibodies to pestiviruses, MAbMIX, BVD specific; VLA, Weybridge, UK) (ANONYMOUS, 2008). The herds with previously established high seroprevalence (> 95%) were chosen for testing. To confirm persistent infection, blood was taken from all positive cattle after a four week interval and tested again.

Viruses were isolated from all positive sera samples on a pestivirus-free Madin-Darby bovine kidney cell culture (MDBK). In the cell culture flask (T25) (NUNC, Roskilde, Denmark) 500 µl of sera was inoculated on a 24 hour old cell layer and incubated at 37 °C for 2 hours. After incubation, the flasks were rinsed three times with cell medium (Sigma-Aldrich Inc., St. Louis, USA) and then cell medium with 4% of foetal calf serum (Paa Laboratories, Pasching, Austria) and 1% antibiotics (Minerva Biolabs, Berlin, Germany) were added. Flasks were incubated at 37 °C for 4 days. CP and NCP biotypes of viruses were determined by IP assay. All positive results were confirmed with RT-PCR.

Spleen samples. Spleen samples were taken from two animals with established MD in 2007 and from three animals in 2010, respectively (Table 1) on dissection of five heifers, and stored at -20 °C. Spleen suspensions were prepared with sterile sand and cell medium then centrifuged at 3000 rpm for 15 min. The supernatant was used for virus isolation by the same procedure as described above.

RNA extraction, RT-PCR and sequencing. Viral RNA was extracted directly from 140 µl suspensions from low passage cell culture supernatants, using a QIAamp Viral RNA Mini kit (Qiagen, Hilden, Germany) as described by the manufacturer. Details of the RT-PCR primers are given in Table 2. All RT-PCR reactions were performed using the SuperScript III one step RT-PCR system with Platinum Taq polymerase (Invitrogen, Carlsbad, USA) according to the manufacturer's specifications, in a 2720 Thermal Cycler (Applied Biosystems, Forster city, USA). Products were separated on a 2% agarose gel stained with ethidium bromide. PCR products were purified using Exosap (USB, Staufen, Germany). Sequencing was performed in both directions by Macrogen Inc. (Seoul, Korea).

Forward and reverse 5'-UTR and Npro sequences for each isolate were aligned and used in phylogenetic analysis. The 5'-UTR (from nucleotide 145-357, numbering from the sequence of the isolate Cp7) and combined 5'-UTR/Npro 617-nt sequences (from nucleotide 145-761, numbering from the sequence of the isolate Cp7), respectively, were analysed. These sequences have been deposited in GenBank, with accession numbers: JN003810-JN003827.

Table 1. BVDV used in this study, their details and GenBank accession numbers.

Isolate	Origin	Municipality/ County	Year	Cp /ncp	Classification	Accession number	Reference
CRO/07-1	spleen	Sv. Ivan Zelina/Zagreb	2007	cp	1b	JN003810	This study
CRO/07-2	spleen	‘’		cp	1b	JN003811	‘’
CRO/09-3-163	sera	Kneževi Vinogradi/ Osječko-Baranjska	2009	ncp	1b	JN003812	‘’
CRO/09-3-237	sera	‘’		ncp	1b	JN003813	‘’
CRO/09-3-479	sera	‘’		ncp	1b	JN003814	‘’
CRO/09-3-528	sera	‘’		ncp	1b	JN003815	‘’
CRO/10-12-59	sera	Zlatar Bistrica/ Krapinsko-Zagorska	2010	ncp	1b	JN003816	‘’
CRO/10-13-64	spleen	Novo Virje/ Koprivničko-Križevačka		cp	1f	JN003817	‘’
CRO/10-13-65	sera	‘’		ncp	1f	JN003818	‘’
CRO/10-13-66	spleen	‘’		cp	1f	JN003819	‘’
CRO/10-13-67	spleen	‘’		cp	1f	JN003820	‘’
CRO/10-14-90	sera	Sv. Petar Orehovec/ Koprivničko-Križevačka	2010	ncp	1b	JN003821	‘’
CRO/10-15-30	sera	Martinska Ves/ Sisačko Moslavačka		ncp	1f	JN003822	‘’
CRO/10-18-20	sera	Zlatar Bistrica/ Krapinsko-Zagorska		ncp	1b	JN003823	‘’
CRO/10-18-41	sera	‘’		ncp	1b	JN003824	‘’
CRO/10-18-49	sera	‘’		ncp	1b	JN003825	‘’
CRO/11-19-97	sera	Trpinja/ Vukovarsko-Srijemska	2011	ncp	1f	JN003826	‘’
CRO/11-19-98	sera	‘’		ncp	1f	JN003827	‘’

Table 1. BVDV used in this study, their details and GenBank accession numbers (continues)

Isolate	Origin	Municipality/ County	Year	Cp /ncp	Classification	Accession number	Reference
1-Cp7					1b	U63479	Meyers and Thiel (1996)
OSLOSS					Ref BVDV-1b	M96687	De Moerlooze et al. (1993)
NADL					Ref BVDV-1a	M31182	Colett et al. (1998)
A-1672/99-90					1d	AY323879	Toplak et al. (2004)
O-1897/00-175					1f	AY323895	‘’
M-MT/00					1d	AY323891	‘’
K-1745/00-39					1d	AY323881	‘’
T-482/99					1b	AY323880	‘’
C-1332/00-41					1b	AY323878	‘’
I-1709/00-24					1g	AY323877	‘’
F-1562/99-6					1f	AY323873	‘’
B-1085/00					1f	AY323871	‘’
L					1g	AF298069 (5’UTR)	Vilček et al. (2001)
J					1f	AF298067 (5’UTR)	‘’
G					1h	AF298066 (5’UTR)	‘’
F					1d	AF298065 (5’UTR)	‘’
W					1f	AF298073 (5’UTR)	‘’
A					1g	AF298064 (5’UTR)	‘’
KM					1h	AF298068	‘’
3186V6					1b	AF298062 (5’UTR)	‘’
M065B/93					1a	U97409 (5’UTR)	Baule et al. (1997)
9466/91					1d	AJ304382 (5’UTR)	Tajima et al. (2001)
890					Ref BVDV-2	U18059	Ridpath and Bolin (1995)

The sequences were compared to other previously published sequences (Table 1). The sequence identities of nucleotides, as well as the estimation of the evolutionary divergence between sequences were analysed using BioEdit and Mega4 software, respectively (TAMURA et al., 2007). The same tool was used to perform Neighbor-Joining (NJ) analysis, based on the Kimura-2 parameter as the best fit evolutionary model. The reliabilities of phylogenetic relationships were evaluated using nonparametric bootstrap analysis (FELSENSTEIN, 1985) with 1000 replicates for NJ analysis. Bootstrap values exceeding 70 were considered well supported. To confirm the obtained data from NJ analysis, another phylogenetic tree was calculated using MrBayes v3.0b3 (HUELSENBECK and RONQUIST, 2001) using GTR+I+G model. In Bayesian Inference (BI) analysis (LARGET and SIMON, 1999) four incrementally heated Markov Chains were run for 1,000,000 generations (ngen = 1,000,000), sampling every 1000 generations (samplefreq = 1000), where the first 25% trees were discarded as "burn-in". A consensus tree was constructed from the tree output file produced in the BI analysis, using TreeView: (http://taxonomy.zoology.gla.ac.uk/rod/rod.html).

Table 2. Oligonucleotide primers for PCR and sequencing reaction.

Primer name	Sequence (5'→3')	Size (bp) and location of amplicon	Genome position	Reference
104f	GCT AGC CAT GCC CTT AGT AGG ACT	294, 5' UTR	101-124	Barlič-Maganja and Grom (2001)
402r	CAA CTC CAT GTG CCA TGT ACA GCA		394-371	
B32	TGC TAC TAA AAA TCT CTG CTG T	441, Npro	355-376	Toplak et al. (2004)
B31	CCA TCT ATR CAY ACA TAR ATG TGG T		795-771	

Results

The immunoperoxidase test detected the virus in 18 cattle from 9 herds and revealed 13 NCP and 5 CP biotype BVDVs. All 5 isolates from spleen samples were of the CP biotype. In all cattle infected with the NCP biotype, persistent infection was confirmed. From all positive samples, BVD viruses were isolated on MDBK cells.

All 18 BVDV isolates were amplified with both sets of primers giving PCR products of the expected size (294 and 441 bp, respectively).

Sequence and phylogenetic analysis of the 5'-UTR genome region determined all Croatian isolates as BVDV genotype 1; 11 isolates were grouped with BVDV-1b and 7 with BVDV-1f viruses (Fig. 1). The phylogenetic tree inferred by Bayesian approach using combined 5'-UTR/Npro sequences and omitting the subgroups other than 1b and 1f, fully supported clustering of Croatian isolates in two subgroups. The subgroups were supported by high posterior probability values (Fig. 2).

Table 3. The percentages of identities and diversities of nucleotide sequences of Npro gene among analysed BVDV strains

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
NADL		82.0	82.0	82.0	82.0	85.0	81.0	81.0	81.0	82.0	84.0	81.0	79.0	81.0	81.0	81.0	81.0	81.0	81.0	79.0	79.0	80.0	81.0	81.0	1
Osloss	2.26		90.0	81.0	81.0	82.0	93.0	93.0	85.0	85.0	89.0	88.0	94.0	78.0	79.0	78.0	78.0	92.0	79.0	93.0	93.0	94.0	81.0	81.0	2
CP7	2.26	1.11		82.0	82.0	82.0	90.0	90.0	91.0	91.0	90.0	93.0	88.0	81.0	81.0	81.0	81.0	89.0	82.0	89.0	89.0	90.0	83.0	83.0	3
B-1085/00	2.18	2.38	2.18		89.0	89.0	81.0	81.0	81.0	81.0	0.83	82.0	81.0	86.0	86.0	85.0	86.0	80.0	88.0	80.0	81.0	81.0	88.0	88.0	4
F-1562/99-6	2.26	2.42	2.18	1.20		89.0	82.0	82.0	81.0	81.0	83.0	82.0	82.0	83.0	83.0	82.0	83.0	80.0	86.0	80.0	81.0	81.0	88.0	88.0	5
O-1897/00-175	1.78	2.22	2.15	1.23	1.27		84.0	84.0	82.0	82.0	84.0	84.0	83.0	86.0	86.0	85.0	86.0	81.0	88.0	82.0	83.0	83.0	88.0	88.0	6
CRO/07-1	2.34	0.71	1.11	2.30	2.22	1.96		100	87.0	88.0	90.0	89.0	93.0	79.0	79.0	79.0	79.0	92.0	80.0	94.0	94.0	95.0	82.0	82.0	7
CRO/07-2	2.34	0.71	1.11	2.30	2.22	1.96	0.00		87.0	88.0	90.0	89.0	93.0	79.0	79.0	79.0	79.0	92.0	80.0	94.0	94.0	95.0	82.0	82.0	8
CRO/09-3-163	2.30	1.78	1.01	2.42	2.30	2.22	1.47	1.47		99.0	89.0	92.0	85.0	80.0	81.0	80.0	80.0	86.0	82.0	86.0	85.0	86.0	81.0	80.0	9
CRO/09-3-237	2.22	1.74	0.98	2.42	2.30	2.22	1.43	1.43	0.08		90.0	92.0	85.0	81.0	81.0	81.0	81.0	87.0	82.0	85.0	86.0	86.0	81.0	81.0	10
CRO/09-3-479	1.89	1.20	1.14	2.11	2.11	1.96	1.07	1.07	1.2	1.14		90.0	89.0	79.0	80.0	79.0	79.0	89.0	81.0	88.0	88.0	89.0	82.0	82.0	11
CRO/09-3-528	2.42	1.37	0.80	2.18	2.22	1.92	1.27	1.27	0.83	0.83	1.07		87.0	81.0	81.0	80.0	81.0	87.0	82.0	88.0	87.0	88.0	8.03	83.0	12
CRO/10-12-59	2.62	0.65	1.37	2.34	2.26	2.03	0.77	0.77	1.78	1.78	1.23	1.47		80.0	80.0	79.0	79.0	91.0	81.0	93.0	94.0	94.0	82.0	82.0	13
CRO/10-13-67	2.38	2.79	2.38	1.64	2.07	1.64	2.71	2.71	2.46	2.42	2.62	2.38	2.58		100	98.0	98.0	78.0	89.0	79.0	78.0	79.0	86.0	86.0	14
CRO/10-13-68	2.34	2.75	2.34	1.60	2.03	1.60	2.66	2.66	2.42	2.38	2.58	2.34	2.54	0.03		99.0	99.0	79.0	89.0	78.0	79.0	79.0	86.0	86.0	15
CRO/10-13-65	2.42	2.83	2.42	1.74	2.15	1.74	2.75	2.75	2.46	2.42	2.66	2.50	2.62	0.16	0.13		98.0	78.0	88.0	78.0	78.0	79.0	85.0	85.0	16
CRO/10-13-64	2.34	2.79	2.34	1.67	2.11	1.67	2.66	2.66	2.46	2.38	2.66	2.38	2.75	0.16	0.13	0.21		79.0	88.0	79.0	79.0	79.0	86.0	86.0	17
CRO/10-14-90	2.34	0.83	1.30	2.50	2.46	2.38	0.89	0.89	1.57	1.53	1.27	1.53	0.98	2.79	2.75	2.83	2.75		80.0	90.0	91.0	91.0	80.0	80.0	18
CRO/10-15-30	2.42	2.75	2.22	1.37	1.67	1.37	2.46	2.46	2.18	2.15	2.38	2.18	2.38	1.27	1.30	1.43	1.40	2.54		81.0	81.0	81.0	90.0	89.0	19
CRO/10-18-20	2.71	0.80	1.30	2.50	2.50	2.18	0.65	0.65	1.64	1.71	1.33	1.43	0.77	2.75	2.79	2.88	2.71	1.07	2.42		98.0	99.0	81.0	81.0	20
CRO/10-18-41	2.66	0.74	1.27	2.42	2.42	2.11	0.62	0.62	1.71	1.67	1.33	1.50	0.68	2.79	2.75	2.83	2.71	1.04	2.42	0.16		99.0	82.0	81.0	21
CRO/10-18-49	2.54	0.65	1.17	2.34	2.34	2.03	0.54	0.54	1.60	1.57	1.23	1.40	0.65	2.66	2.62	2.71	2.66	0.95	2.34	0.13	0.08		82.0	82.0	22
CRO/11-19-97	2.30	2.38	2.00	1.33	1.33	1.33	2.22	2.22	2.38	2.38	2.18	2.11	2.22	1.60	1.57	1.71	1.64	2.46	1.17	2.30	2.22	2.15		100	23
CRO/11-19-98	2.30	2.38	2.00	1.33	1.33	1.33	2.22	2.22	2.38	2.38	2.18	2.11	2.22	1.60	1.57	1.71	1.64	2.46	1.17	2.30	2.22	2.15	0.00		24

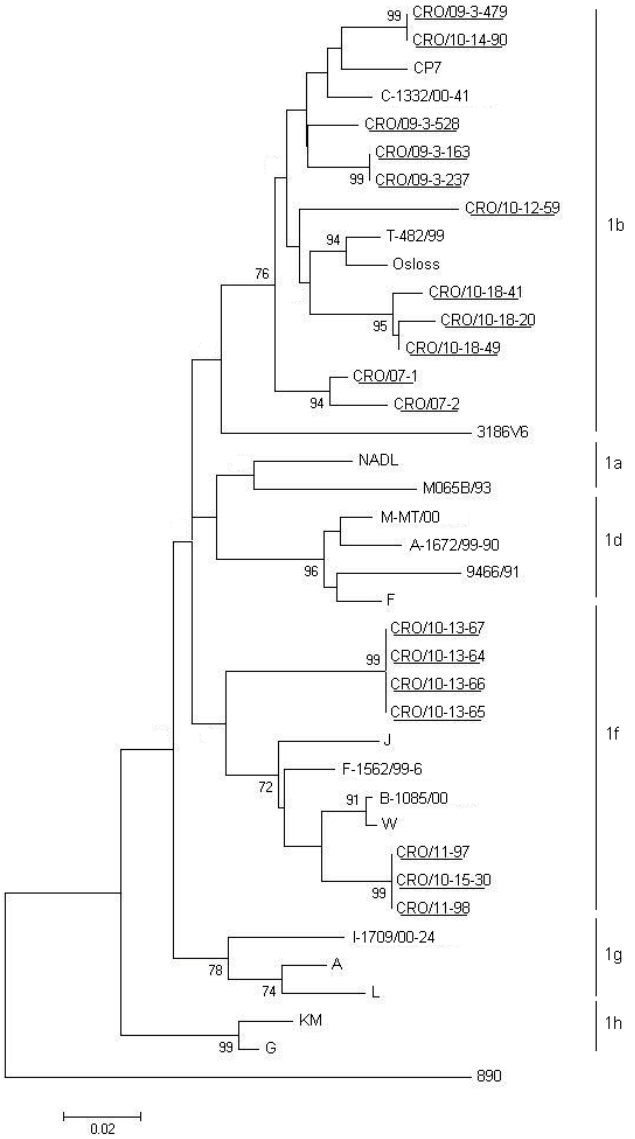

Fig. 1. Neighbour-joining phylogenetic analysis of 5'-UTR nucleotide sequences among different BVDV strains. BVDV-2 strain 890 was used as the outgroup. The consensus phylogenetic tree is shown with bootstrap values >70%. The Croatian isolates are underlined. BVDV subgroups are written on the right.

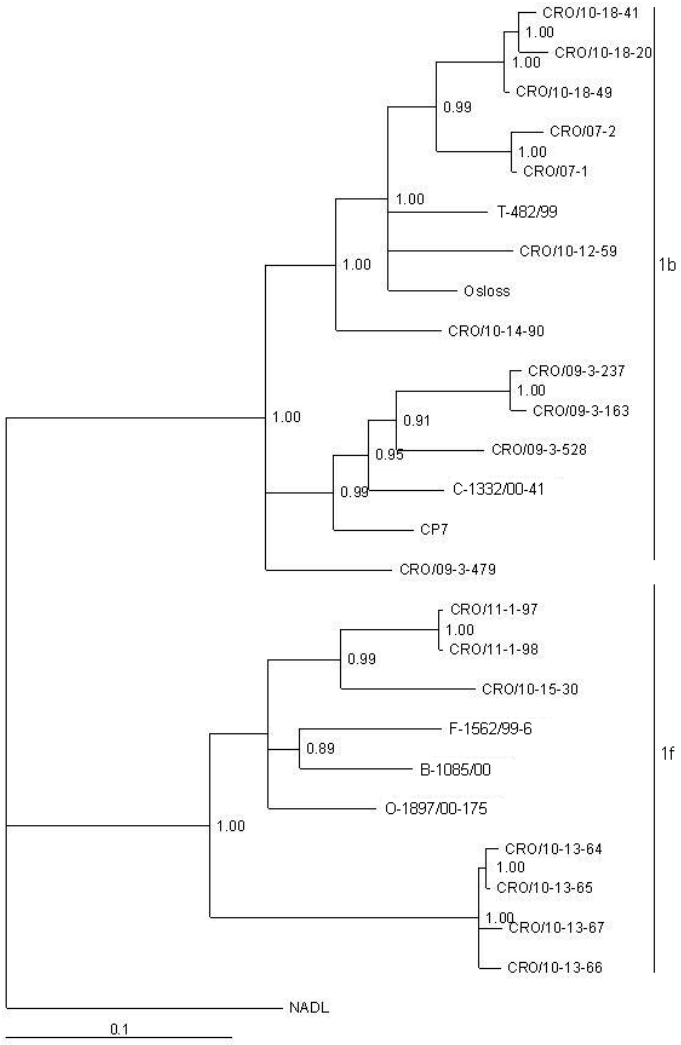

Fig. 2. Phylogenetic tree of a 617-bp fragment of the combined 5'-UTR/Npro gene region inferred by the Bayesian approach. The Croatian isolates were analysed together with BVDV-1b reference strains Osloss and CP7, and BVDV 1b and 1f strains from a neighbouring country. Reference strain NADL was used as the outgroup. The posterior probability values are shown for all nodes. BVDV subgroups are written on the right.

The deduced aminoacid sequence of the Npro region revealed 5 sites unique for domestic isolates CRO/10-13-64, CRO/10-13-65, CRO/10-13-66, CRO/10-13-67, namely: 60 (Isoleucine), 88 (Isoleucine), 107 (Valine), 110 (Isoleucine) and 115 (Valine). Phylogenetic analysis clustered these isolates on a separate branch of the 1f clade (Figures 1 and 2).

The percentage of nucleotide sequence identities and diversities between each Croatian isolate and the reference strains NADL (1a), Osloss and CP7 (1b), as with neighbouring strains from subgroup 1f (based on 392 nt fragment of Npro, starting from the translation initiation codone) were shown in Table 3. Percentage identities among Croatian isolates were between 78 and 100%.

Discussion

The 13 BVDV positive samples isolated on MDBK cell culture and determined by RT-PCR were from PI animals, as proved by repeated IP tests. Five positive samples isolated on a MDBK cell and determined by RT-PCR were from spleen samples. By subsequent phylogenetic analysis of the 5'-UTR region, the preliminary genetic typing of our isolates was established. It resulted in genotyping of Croatian isolates in the BVDV-1 genotype, and subgroups 1b and 1f. The 5'-UTR virus region is highly conserved and widely used in diagnostics of BVDV infection by RT-PCR. Also, this region includes three variable regions (DENG and BROCK, 1993) and for that reason it may be used for preliminary genetic typing of BVDVs. However, previous studies suggested that 5'-UTR might be not a good target for inferring phylogeny since it is highly conserved (BECHER et al., 1997). XIA et al. (2007) also determined that the 5'-UTR (and NS3) regions do not contain suitable phylogenetic signals to resolve the relationships of lineages because they are not sufficiently variable.

The majority of studies inferring BVDV phylogeny have used distance-based methods, with a Neighbor-joining algorithm. So, for the initial tree and fast and preliminary typing of isolates we also used the same approach. To validate the classification and to confirm the genetic typing and the tree topology obtained by analysis of the 5'-UTR region by the NJ method, we subsequently used the Bayesian approach to infer the phylogenetic tree based on combined 617-nt 5'UTR/Npro sequences. In this approach we omitted the subgroups other than 1f and 1b and supported the previous allocation of all the Croatian isolates with posterior probabilities values of 1.00, which was better support than bootstrap values obtained by NJ analysis.

The 18 Croatian isolates were subdivided into subgroups 1b and 1f despite their origin from different geographic districts, with 11 and 7 samples in each subtype, respectively. Our isolates, CRO/10-13-64, CRO/10-13-65, CRO/10-13-66, CRO/10-13-67 from subgroup 1f, were clustered on separate clade. It is a consequence of the exchange of 5 aminoacids

on Npro in respect to all other analysed viruses. Additionally, isolates CRO/10-13-64, CRO/10-13-66 and CRO/10-13-67 as well as CRO/07-1 and Cro /07-2 from subgroup 1b were CP biotype viruses. Until now, only one CP isolate, "Beograd", has been detected and clustered into that group (PETROVIĆ et al., 2004). All those isolates originated from animals with confirmed mucosal disease. CP isolates from spleen samples: CRO/10-13-64, CRO/10-13-66 CRO/10-13-67 and NCP isolate from a CRO/10-13-65 serum sample, originated from the same herd and are 98-99% identical on the nucleotide and aminoacid level in the analysed part of the Npro region (Table 3).

However, the majority of isolates were the NCP biotype, as was expected. These isolates were obtained from cattle with no clinical symptoms of BVDV infection. This biotype is most frequently characterized in vertical virus transmission and is considered a biomarker for permanent BVDV circulation in the cattle population (BROWNLIE, 1991). Also, most isolates were grouped with the world predominant BVDV-1b, also detected in Austria, Germany, Hungary, Italy, Slovakia, Slovenia, Serbia and the Czech Republic (HORNBERG et al., 2009; TAJIMA et al., 2001; GIAMMARIOLI et al., 2008; VILČEK et al., 2001; TOPLAK et al., 2004, PETROVIĆ et al., 2004; ROBESOVA et al., 2009). These countries founded more than two BVDV subgroups in their research, even as many as 8 in Austria (HORNBERG et al., 2009). Our BVDV-1b isolates were grouped in two sister clades, 7 isolates were grouped with the reference strain Osloss, and 4 with CP7, with strong support (1.00).

In conclusion, all Croatian BVDV isolates belonged to genotype-1 viruses, subgroups 1b and 1f. We did not find any BVDV-2 in our collection of viruses.

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BEDEKOVIĆ, T., I. LOJKIĆ, N. LEMO, Ž. ČAČ, Ž. CVETNIĆ, M. LOJKIĆ, J. MADIĆ: Filogenetska analiza hrvatskih izolata virusa virusnog proljeva goveda. Vet. arhiv 82, 449-462, 2012.

SAŽETAK

Od 2007. do 2011. godine na virus virusnog proljeva goveda (VPG) pretraženi su uzorci pet slezena i 1937 uzoraka seruma podrijetlom iz 9 stada mliječnih goveda. Imunoperoksidaznim testom (IP) u 13 je uzoraka dokazan necitopatogeni biotip virusa VPG te je potvrđena perzistentna zaraza. Iz svih pet uzoraka slezena izdvojen je citopatogeni virus VPG. U svrhu genotipizacije vlastitih izolata virusa VPG, iz nadtaloga inficiranih staničnih kultura izdvojena je virusna RNK i umnožena lančanom reakcijom polimerazom uz prethodnu reverznu transkripciju (RT-PCR). Umnoženi odsječci 5'-UTR i gena Npro su potom sekvencirani. Filogenetska analiza hrvatskih izolata virusa VPG pokazala je da svi pretraženi izolati pripadaju genotipu 1; 11 izolata svrstano je u podtip 1b, a 7 u 1f. Filogenetska analiza na temelju spojene 5'-UTR/Npro genske sekvencije potvrdila je rezultate genotipizacije pretraženih izolata. Na Npro dijelu genoma utvrđeno je pet aminokiselinskih promjena osebujnih za hrvatske izolate iz podtipa 1f.

Ključne riječi: virusni proljev goveda, imunoperoksidazni test, gen Npro, filogenetska analiza