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Genetic characterization of a border disease virus isolate originating from Slovakia

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Summary. – In this study, a major part of genome of the pestivirus isolate 297 from Slovakia, comprising the 7195 nt-long 5′-UTR-NS3 region was sequenced and analyzed. Conserved cleavage sites between individual viral proteins of this region were determined and the number of amino acids of respective proteins was estimated as follows: 168 for N^{pro}, 100 for C, 227 for E^{rns}, 195 for E1, 373 for E2, 70 for p7, 453 for NS2, and 683 for NS3. Based on sequence and phylogenetic analysis of 5′-UTR, N^{pro}, and E2 the isolate 297 was characterized as a border disease virus of genotype 3. It was found to be distinct from other BDV-3 strains analyzed so far, consequently forming a distinct branch within the phylogenetic clade. All these data expand a relatively limited knowledge of genetic properties of individual BDV genotypes and strains circulating in the Central Europe.

Keywords: border disease virus; sheep isolate; pestivirus

Introduction

Pestivirus, the genus of the family *Flaviviridae,* currently contains four established species: bovine viral diarrhea virus 1 and 2 (BVDV-1 and 2), classical swine fever virus (CSFV), and border disease virus (BDV), and the fifth tentative pestivirus of giraffe (Fauquet *et al*., 2005). The pestivirus genome consists of a single stranded, positive sense RNA of approximately 12.5 kb in length. The large single ORF flanked by 5′- and 3′-untranslated region (5′-UTR and 3′-UTR) encodes a polyprotein that is cleaved into structural (C, E^{rns}, E1, E2) and non-structural (N^{pro}, p7, NS2-3, NS4A, NS4B, NS5A, NS5B) proteins (Meyers and Thiel, 1996). The 5′-UTR, N^{pro}, and E2 regions are mostly used for genetic typing of pestivirus isolates (Becher *et al*., 1999; 2003; Vilcek *et al*., 2004). Based on the phylogenetic analysis, BDV isolates have been divided into seven genotypes (BDV-1–BDV-7) (Giammarioli *et al*., 2011).

All pestiviruses are important pathogens causing significant economical losses in animal production worldwide. Pestiviruses predominantly infect cattle (BVDV-1, BVDV-2), swine (CSFV), and sheep (BDV) but they are not strictly host-specific. They are able to cross host species barrier and infect various animal species within even-toed ungulates (*Artiodactyla*) (Paton, 1995).

BDV is a causative agent of border disease, a congenital disease of sheep and goats, characterized by abortion, stillbirths and the birth of weak lambs showing tremor, abnormal body conformation and hairy fleeces (Nettleton *et al*., 1998). The occurrence of BDV infection in animals has been confirmed in many countries worldwide, but the vast majority of data comes from Europe. Although BDV has been mostly detected in sheep the virus has also been found in goats, Pyrenean chamois, cattle or swine (Paton, 1995; Cranwell *et al*., 2007; Arnal *et al*., 2004).

In Slovakia, the routine serological examination for pestivirus antibodies in ruminants is carried out mostly by diagnostic laboratories established at the state veterinary institutes.

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Abbreviations: BDV = border disease virus; BVDV-1 = bovine viral diarrhea virus 1; BVDV-2 = bovine viral diarrhea virus 2; CSFV = classical swine fever virus; 5′-, 3′-UTR = 5′-, 3′-untranslated region

Table 1. List of PCR primers

Primers	Sequence (5′–3′)	Position in X818 (AF037405)
324 (F)	ATGCCCWTAGTAGGACTAGCA	98–118
326 (R)	TCAACTCCATGTGCCATGTAC	382–362
BD1 (F)	TCTCTGCTGTACATGGCACATG	354–375
BD2 (R)	TTGTTRTGGTACARRCCGTC	1085–1066
AV1 (F)	AACAGGAGCTTACATGGCATTTGG	1252–1275
BDVZV (F)	CATGGTATTTGGCCGGAGAAGAT	1264–1286
2256 (F)	ACTGGTGGCCNTATGARAC	2237–2255
AV2R (R)	CAAATTGCCCTTGTGCTCCG	2449–2430
AV4 (F)	TCACACACTACCCGATCGG	3092–3110
3422R (R)	TGAGCATGTATTGYTGGAARTA	3400–3379
AV5 (F)	GTTGATGGTTGTTTACGTGG	3516–3535
3710R (R)	CACTTCTTTACTGGCTCATC	3683–3664
5655R (R)	AATATNGGTAGNCCTGACCA	5633–5614
AV6 (F)	GACCTTGAGGTGGATAGGTG	4059–4078
AV7R (R)	CGCACTTGGGACAGGTCTC	4873–4855
AV8 (F)	TCAAAGCAACAACAAGATGACTGA	5397–5420
BDZV2R (R)	ACATAGGGTGACTGTGATGTAAC	6470–6448
AV10 (F)	GTGGCAATGACGGCAACACC	6169–6188
AV9R (R)	CTAGTTCTTTCAGCTCAGTCTC	7372–7351

Epidemiological studies revealed high prevalence of pestivirus seropositive cattle (Vilcek *et al*., 2003). However, only a few pestivirus infections have been confirmed by virus isolation so far and several isolates have been genetically characterized (Vilcek *et al*., 2001, 2003, 2004; Novackova *et al*., 2008). In this work we attempted to characterize the sheep pestivirus isolate 297 from Slovakia using sequence and phylogenetic analysis. The obtained results show that this isolate is BDV of genotype 3 that is phylogenetically distinct from other BDV-3 isolates so far analyzed. The BDV-3 isolate 297 thus represents the first pestivirus and BDV originating from sheep in Slovakia.

Materials and Methods

Virus. The pestivirus isolate designated as 297 originated from serum of sheep collected during screening for the pestivirus infection in central part of Slovakia in 2007. The virus was propagated in SFT-R cell culture lines and provided to our laboratory by Dr. Miroslav Mojžiš from the State Veterinary Institute in Zvolen, Slovak Republic.

RNA isolation. The total RNA was extracted from 200 µl of inoculated cell culture using TRIzol reagent (Invitrogen, USA) according to the manufacturer′s instructions. RNA was dissolved in 20 µl of nuclease-free water.

RT-PCR. The synthesis of cDNA was carried out using random hexamers and SuperScript II reverse transcriptase (Invitrogen), following the conditions recommended by manufacturer. The single or nested RT-PCRs were used to amplify the fragments of DNA covering entire 5′-UTR-NS3 region. The nucleotide sequences of primers are listed in Table 1.

The RT-PCR mixture (50 µl) contained 10 µl of 5x Phusion HF buffer (Finnzymes, Finland), 200 µmol/l dNTPs, 0.3 µmol/l of each primer, 1 U of Phusion high-fidelity DNA polymerase (Finnzymes) and 4 µl of cDNA. The thermal profile was optimized for each primer pair: initial denaturation at 98°C for 30 sec–2 min.; denaturation at 98°C for 10 sec, annealing at 52–56°C for 30 sec, extension at 72°C for 30 sec–2 min. in 30–35 cycles and final extension at 72°C for 10 min. The nested RT-PCR was performed under the same conditions in 35–40 cycles with 2 µl of first RT-PCR product added.

Sequence and phylogenetic analysis. The purified RT-PCR products were sequenced by the Sanger method using fluorescently labeled ddNTPs. The chromatograms were proof read using SeqMan II from DNASTAR Lasergene 8.1 program package (DNASTAR, Inc., USA). The 5′-UTR-NS3 nucleotide sequence of the isolate 297 was deposited in GenBank under Acc. No. KC 484999.

The sequences were aligned by ClustalW and nucleotide and deduced amino acid identities were calculated using MegAlign software (DNASTAR Lasergene 8.1, DNASTAR, Inc., USA). Phylogenetic analysis and construction of trees were done using the program MEGA version 4 employing neighbor-joining method. The bootstrap values were calculated after 1,000 replicates using Kimura-2 method (Tamura *et al*., 2007). The GenBank Acc. Nos. of the sequences used for the construction of phylogenetic trees are listed in Table 2.

Analysis of hydrophylicity and antigenic index of E2 protein. The hydrophylicity plot (Kyte-Doolittle method) of E2 amino acid sequences were analyzed using the Protean software (DNASTAR Lasergene 8.1). The same software was used for calculation of the antigenic index, where a computer algorithm integrated various protein parameters (surface accessibility, regional backbone flexibility and predicted secondary structure) into a single plot designed by Jameson and Wolf (1988).

Results

Sequence analysis and phylogenetic typing in 5′-UTR, N^{pro}, and E2 region

The 5′-UTR sequence obtained by RT-PCR using pestivirus primers 324/326 was submitted to BLAST search where the sequence homology to pestivirus nucleotide sequences was confirmed. The pair-wise sequence identity showed that the isolate 297 was closer related to BDV (84.8–91.8%) than to other pestiviruses identified so far (65.5–81.7%). The highest sequence identity was observed to those of BDV-3

Table 2. Representative pestivirus strains used in the phylogenetic analysis

Virus	Genotype	Strain	Acc. Nos.		
			5′-UTR	N^{pro}	E2
BDV	BDV-1	X818	AF037405	=	=
		BD31	U70263	=	=
		Frijters	N/A	PTU80905	N/A
	BDV-2	Reindeer-1	AF144618	=	=
		Chemnitz	N/A	AY163652	AY163659
	BDV-3	Gifhorn	GQ902940	=	=
		297		In this study	
		B300/06	EU224227	N/A	N/A
		F-0083	EF693999	EF693977	N/A
		F-6338	EF693991	EF693969	N/A
		F-588	EF693986	EF693966	N/A
		CH-BD1	N/A	AY895008	N/A
		CH-BD2	N/A	AY895009	N/A
		3733	*	*	*
	BDV-4	Chamois-1	GU270877	=	=
		M3	DQ275626	DQ273163	N/A
		BU-1CRA22	DQ275622	DQ273155	N/A
		C27	DQ275623	DQ273156	N/A
		C41	N/A	DQ273157	N/A
	BDV-5	Aveyron	EF693984	EF693962	N/A
		F-5415	EF693988	EF693965	N/A
	BDV-6	F-6335	EF693990	EF693968	N/A
		F-7446-1	EF693996	EF693974	N/A
	BDV-7	712	AJ829444	AJ829444	N/A
	unclass.	CH-BD3	N/A	GU244490	N/A
		CH-BD4	N/A	GU244489	N/A
CSFV		Alfort	X87939	=	=
		Brescia	AF091661	=	=
		C	Z46258	=	=
BVDV-1		NADL	M31182	=	=
		Oregon	AF091605	=	=
		SD1	M96751	=	=
		Osloss	M96687	=	=
		Pav1	*	N/A	N/A
		Vk1	*	N/A	N/A
		KM	AF298068	N/A	N/A
BVDV-2		890	U18059	=	=
		1373	AF145967	AF145967	AF145967
		Gi-4	N/A	AF144468	N/A
		17237	EU747875	=	=
Pestivirus of giraffe		Giraffe-1	AF144617	=	=
		PG 2	N/A	AY163647	AY163654
Atypical pestiviruses		Th/04_KhonKaen	FJ040215	=	=
		Ho-Bi	N/A	AY735486	AY604725
Pronghorn pestivirus (unclass.)		Antelope	AY781152	AY781152	AY781152
Tunisian sheep pestiviruses (unclass.)		BM01	AY453630	AY453630	N/A
		SN17	AF461997	AY452484	N/A

(=) the same Acc. No. as for 5′-UTR; N/A = sequence not available in GenBank database; (*) sequence from laboratory database (not available in GenBank).

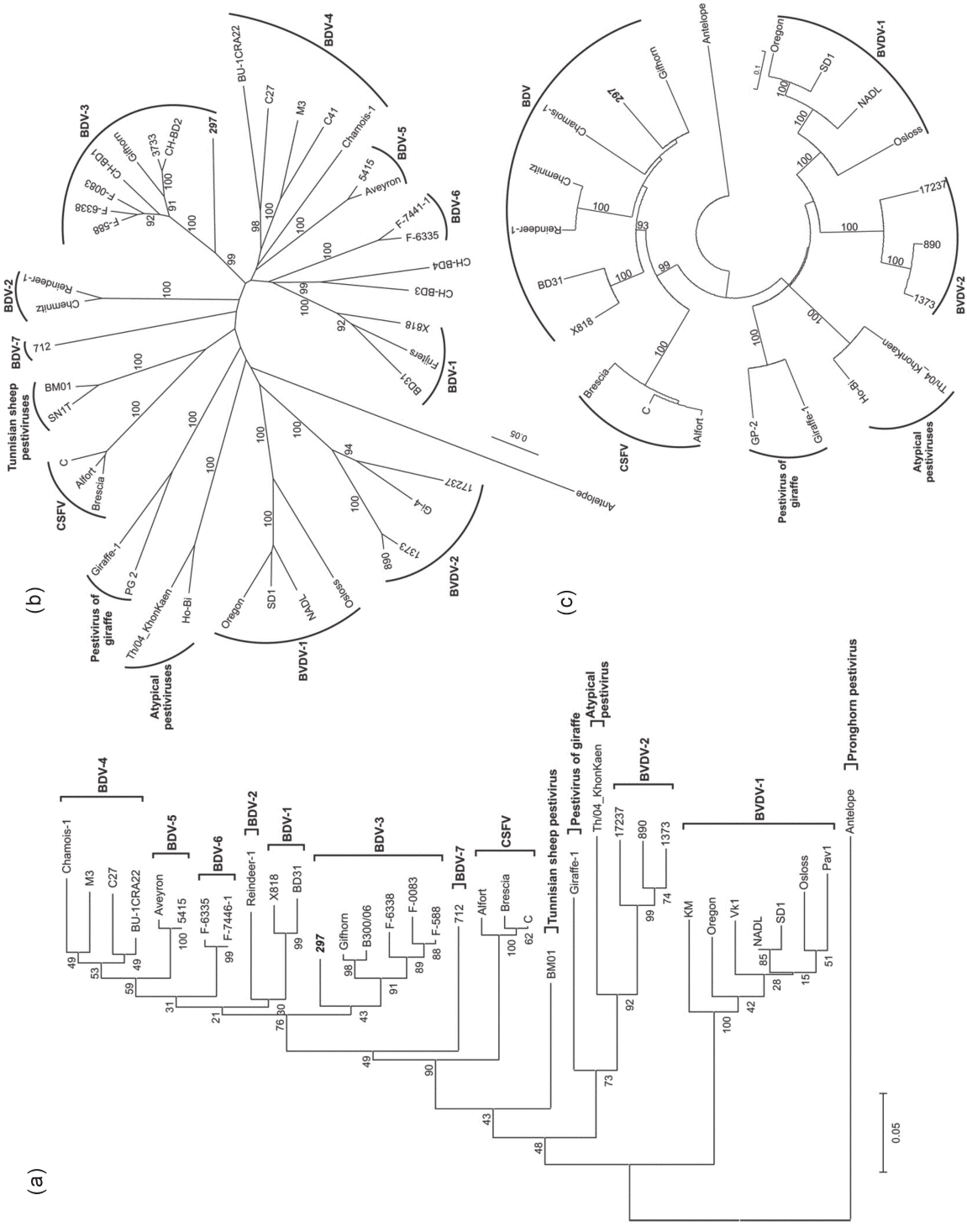

Fig. 1

Phylogenetic trees of pestiviruses based on a part of 5′-UTR (a), N^{pro} (b), and E2 (c)

The length of region analyzed: 5′-UTR-243 nt, N^{pro}-504 nt, E2-1119 nt. The isolate 297 is in bold italics. Branch numbers indicate bootstrap values (in %; 1000 replicates). Bar: number of substitutions per site.

Table 3. The comparison of amino acid sequences flanking the predicted cleavage sites of pestiviral proteins

	↓	N^{pro}	↓	C	↓	E^{rns}	↓	E1	↓	E2	
Strain (genotype/species)	N-term.	C-term.	N-term.	C-term.	N-term.	C-term.	N-term.	C-term.	N-term.	C-term.	N-term.	C-term.
X818 (BDV-1)	MELNKF	LWVTSC	SDDNKS	WEPVAP	ENVTQW	WFGANA	QSPYCN	VTGAQG	QFACRE	DQMTSA
Reindeer-1 (BDV-2)	**VI**	******	*****G	PL**LS	**I***	******	******	****N*	**S***	E**AA*
Gifhorn (BDV-3)	******	******	******	*Q**TS	**I***	******	******	******	****I*	E**A**
297 (BDV-3)	******	******	*****D	RH***S	**I***	******	******	******	****K*	E*****
Chamois-1 (BDV-4)	***S**	******	***G*N	*Q**T*	**I***	******	******	******	******	E***T*
Alfort (CSFV)	****H*	******	***GA*	YQ**EA	**I***	****Y*	L*****	******	*L**K*	E*LAAG
SD1 (BVDV-1)	***ITN	******	**TKEE	I*VTMG	**I***	****Y*	A****D	I**V**	HLD*KP	E*KA*G
890 (BVDV-2)	***FSN	******	**EGSK	LQL*TG	**I***	****H*	A****G	I*****	FPE*K*	E**AMG
Giraffe-1 (Pestivirus of giraffe)	***I**	**L***	**EGGA	FQ***G	**I***	****H*	A****E	V*****	AIT*EP	E*EA*G
Th/04_KhonKaen (Atypical pestivirus)	***LN*	I**A**	**EKAG	*Q**GT	**I***	****H*	AT****	I*****	DLS*KP	E*GARG
Antelope (Pronghorn pestivirus)	**VVE*	******	**EGGA	ICTAVN	V*I***	****Q*	SEV**K	L**TEA	ALE*DT	YYPDD*

	↓	p7	↓	NS2	↓	NS3	↓	NS4A
Strain (genotype/species)	N-term.	C-term.	N-term.	C-term.	N-term.	C-term.	N-term.
X818 (BDV-1)	INLGQG	SGLVKG	EGAGMT	LGWILR	GPAVCK	KQVTGL	SAAENA
Reindeer-1 (BDV-2)	MS*D**	**V*R*	DT*ETV	******	******	******	******
Gifhorn (BDV-3)	VS****	******	SDVEA*	******	******	***A**	*T****
297 (BDV-3)	MS****	******	CEVESA	******	******	******	*T****
Chamois-1 (BDV-4)	MS**E*	****R*	AEV*IA	******	******	******	******
Alfort (CSFV)	LP****	**VAR*	G*WQRL	***V**	******	***V**	*T****
SD1 (BVDV-1)	AQY*A*	GDV**A	DPG*QG	******	******	***A**	*S****
890 (BVDV-2)	AGVNTE	G*A**A	SRIDAG	***V**	******	******	*T****
Giraffe-1 (Pestivirus of giraffe)	LQ*EP*	*NV*N*	**GSKA	***V**	******	***M**	*T****
Th/04_KhonKaen (Atypical pestivirus)	QQMNP*	*SI*R*	DDGRA*	***V**	******	***S**	*T****
Antelope (Pronghorn pestivirus)	ALVNT*	VDM***	DTNETS	******	–	–	–

(↓) Cleavage site as predicted for BDV reference strain X818 (Becher *et al.*, 1998); (*) conserved amino acid.

(89.7–91.8%) (data not shown). In the phylogenetic tree, the isolate 297 clustered together with BDV strains and formed a separate subclade within BDV-3 (Fig. 1a).

Similarly, the N^{pro} based phylogenetic tree grouped the isolate 297 with BDV sequences forming a separate subclade in BDV-3 (Fig. 1b). The robustness of the obtained subclade was supported by probability value of 99% at the internal branch-point dividing different BDV genotypes. Anyway, the isolate 297 was found distinct not only in its position in the phylogenetic tree but also in the nucleotide and amino acid sequence identity which varied to other BDV-3 isolates in range 80.2–81.7% and 85.7–88.7%, respectively. The difference of the Slovak BDV isolate was due to single nucleotide mutations scattered throughout the N^{pro} as well as 5′-UTR region. Part of these mutations was unique when compared to BDV-3 but also to the other pestivirus sequences (data not shown).

The phylogenetic analysis using the entire E2 coding region also confirmed the typing of the isolate 297 as BDV-3 (Fig. 1c). The nucleotide and amino acid sequence identity with BDV-3 strain Gifhorn was 76.6% and 82.8%, respectively.

Predicted polyprotein cleavage sites

The complete 7195 nt long 5′-UTR-NS3 part of viral genome sequenced in this work was aligned with sequences of the representative pestivirus strains. Cleavage sites between viral proteins were determined from the alignment with the reference BDV strain X818 (AF037405). The alignment showed that the cleavage sites N^{pro}/C, E^{rns}/E1, NS2/NS3, and NS3/NS4A were highly or totally conserved among BDV strains. The NS2/NS3 was the most conserved cleavage site among all pestiviruses (Table 3).

Structural and nonstructural proteins

Recognition of the cleavage sites allowed to determine lengths of individual proteins of the isolate 297, which were

Table 4. Sequence identities of pestivirus proteins (italicized) and their genes (regular) in relation to BDV-3 isolate 297

Virus	Strain (genotype)	5′-UTR	N^{pro}	C	E^{rns}	E1	E2	p7	NS2	NS3
	X818 (BDV-1)	87.2	76.6	78.0	78.3	78.8	73.5	78.6	77.5	81.8
			81.5	*84.0*	*89.9*	*88.7*	*76.9*	*87.1*	*85.4*	*97.8*
	Reindeer-1 (BDV-2)	88.8	74.0	78.3	82.1	77.4	73.7	78.1	74.0	81.5
			79.2	*85.0*	*91.6*	*89.7*	*78.6*	*87.1*	*83.4*	*97.4*
	Gifhorn (BDV-3)	91.8	81.2	78.7	84.1	82.6	76.6	81.9	80.5	85.6
			86.9	*87.0*	*93.0*	*94.4*	*82.8*	*95.7*	*90.3*	*98.4*
	Chamois-1 (BDV-4)	84.8	76.0	79.3	79.7	77.1	73.7	75.7	75.8	81.6
			81.0	*83.0*	*92.5*	*91.8*	*77.2*	*91.4*	*85.9*	*96.5*
	Aveyron (BDV-5)	88.1	76.2	–	–	–	–	–	–	–
			79.8	–	–	–	–	–	–	–
	F-6335 (BDV-6)	90.09	75	–	–	–	–	–	–	–
			79.2	–	–	–	–	–	–	–
	712 (BDV-7)	84.8	69.8	–	–	–	–	–	–	–
			76.8	–	–	–	–	–	–	–
CSFV	Alfort	81.8	69.0	65.7	74.6	75.2	64.8	71.9	67.0	78.6
			72.6	*75.8*	*82.4*	*87.7*	*66.5*	*81.4*	*70.9*	*95.5*
BVDV-1	SD1	76.5	66.5	58.7	72.2	68.2	61.8	64.3	61.9	76.3
			69	*69*	*79.3*	*75.4*	*61.7*	*52.9*	*60.9*	*92.2*
BVDV-2	890	73.7	66.5	57.3	74.3	69.9	62.5	53.8	60.9	76.2
			65.5	*71.0*	*79.3*	*76.9*	*58.1*	*54.3*	*55.2*	*91.9*
Pestivirus of giraffe	Giraffe-1	74.9	67.7	61.9	73.1	70.9	60.5	62.9	64.3	77.0
			70.8	*66.3*	*81.9*	*80.5*	*58.4*	*52.9*	*62.0*	*91.9*
Atypical pestivirus	Th/04_KhonKaen	72.8	64.7	61.0	74.0	67.7	61.2	60.5	55.3	77.3
			66.7	*66.0*	*80.6*	*74.4*	*59.0*	*58.6*	*56.5*	*92.5*
Pronghorn pestivirus	Antelope	65.5	63.3	59.6	60.9	57.6	55.6	32.9	50.3	–
			58.9	*59.6*	*57.3*	*48.7*	*44.1*	*40.0*	*43.5*	–

Values are given in percentage. (–) sequence not available in GenBank database.

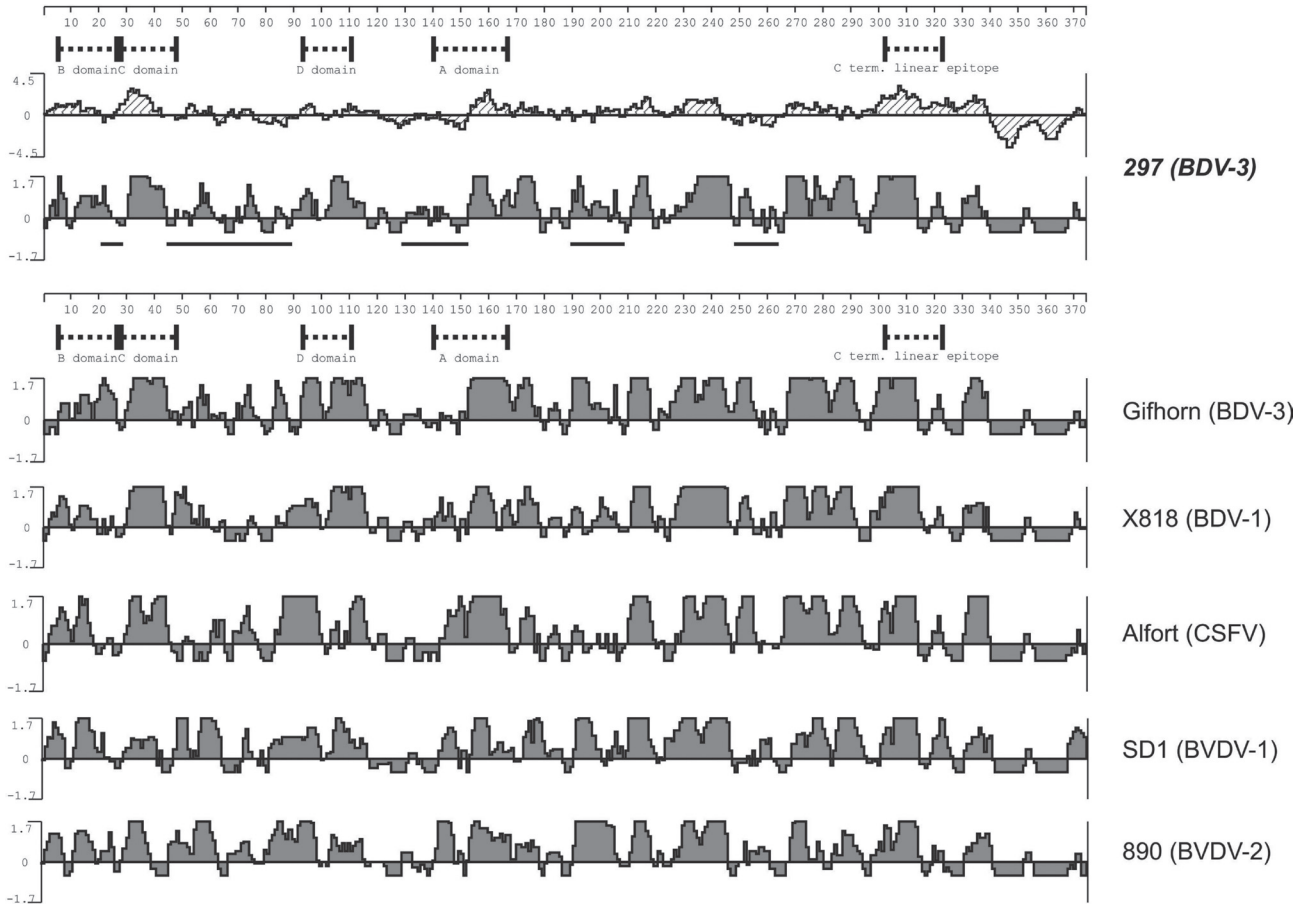

Fig. 2

Antigenic index (filled) and plot of hydrophilicity (striped) of the amino acid sequence for E2 protein of isolate 297 compared to those of other pestiviruses

Major antigenic differences (lines), antigenic domains predicted for CSFV (dotted lines), amino acid positions (horizontal rulers).

identical to those for the reference BDV strain X818: N^{pro}-168 aa, C-100 aa, E^{rns}-227 aa, E1-195 aa, E2-373 aa, p7-70 aa, NS2-453 aa, and NS3-683 aa. The insertion/deletion was not observed in any viral protein. However, the length of C protein of BDV strains was shorter by two amino acids when compared to other pestiviruses. This protein shortening, was found in the isolate 297 as well. The NS2 protein coding region of the isolate 297, which was analyzed primarily because of potential presence of insertions/deletions observed in some pestivirus strains, had an expected length.

The sequence identities of the isolate 297 confirmed high similarity to BDV-3 strains in all protein coding regions. The analysis of pair-wise sequence identities also showed that the structural protein E2 was the most variable in nucleotide and amino acid identity in range 73.5–76.6% and 76.9–82.8%, respectively. The nonstructural protein NS3 was highly conserved (81.5–85.6% nucleotide identity and 96.5–98.4% amino acid identity) among all BDV strains analyzed (Table 4).

Detailed analysis of amino acid sequences revealed the presence of all cysteins typical for BDV isolates; 6 in N^{pro}, 9 in E^{rns}, 6 in E1, 17 in E2, and 9 in NS2 region. In N^{pro} coding region, conserved catalytic triad Glu22-His49-Cys69 was also observed.

Glycosylation sites and antigenic index of glycoprotein E2

Five potential N-glycosylation sites (Asn-X-Ser or Asn-X-Thr) were typical for E2 of BDV strains (data not shown). However, only four N-glycosylation sites were found in Slovak isolate and in the BDV-3 Gifhorn strain.

The antigenic index of E2 protein of the isolate 297 was similar to BDV (Fig. 2). The peaks in antigenic index for the Slovak BDV isolate mainly corresponded to the peak

profiles in the predicted antigenic domains as well as to the C-terminal linear epitope for other pestiviruses. Major antigenic differences were localized in the hydrophobic region between C and D domains and in the region corresponding to the N-terminal part of the domain A. The antigenic index in the C-terminal part of E2 was highly conserved among all pestiviruses.

Discussion

In this work we have analyzed 7195 nt long sequence covering 5′-UTR-NS3 region of BDV isolate obtained from infected sheep in Slovakia. These data for the BDV isolate originating from the Central Europe were analyzed for the first time.

Phylogenetic analysis in 5′-UTR, N^{pro}, and E2 confirmed that the isolate 297 belonged to BDV genotype 3. Interestingly, Slovak isolate formed significantly separated phylogenetic branch within BDV-3 clade which indicated that the virus is genetically different from all other BDV-3 isolates analyzed so far.

The 5′-UTR and N^{pro} sequence analysis revealed unique single nucleotide mutations compared to BDV-3 as well as to other pestivirus isolates. Based on this observation, the detailed molecular analysis of longer part of the genome was performed. All typical pestivirus properties as the conserved amino acid cleavage sites between individual viral proteins, conserved cysteine residues, catalytic triad Glu22-His49-Cys69 in N^{pro}, and putative N-glycosylation sites in E2 were well preserved. The potential antigenicity regions evaluated by the antigenic index algorithm suggested similar antigenic structure of the major immunodominant protein E2 among all pestiviruses. Even in the NS2 region, where significant sequence alterations for the pestivirus genome could be expected (Ridpath and Bolin, 1995; Becher *et al.*, 1996), no insertion/deletion has been observed.

The first report of border disease, which appeared in UK in 1959, initiated the search for BDV in sheep population in other parts of the world. Molecular-genetic typing of BDV isolates revealed that viruses originating from different geographic regions and animal hosts were divided into more genotypes. The BDV-1 has been identified in UK (Becher *et al.*, 1994) and USA (Becher *et al.*, 1997), BDV-2 in Germany (Becher *et al.*, 2003), BDV-3 in Germany (Becher *et al.*, 2003), Switzerland (Stalder *et al.*, 2005), Austria (Krametter-Froetscher *et al.*, 2007), France (Dubois *et al.*, 2008), and Italy (Peletto *et al.*, 2011), BDV-4 in Spain (Arnal *et al.*, 2004; Valdazo-Gonzalez *et al.*, 2006), BDV-5 and BDV-6 in France (Dubois *et al.*, 2008), BVD-7 in Italy (Giammarioli *et al.*, 2011). In sheep originating from Switzerland, additional new BDV genotype has been identified (Peterhans *et al.*, 2010). Tunisian sheep have been infected with BDV isolates which were phylogenetically closer to CSFV than to BDV cluster (Thabti *et al.*, 2005). Data presented in this work indicated that BDV isolate from Slovakia was typed as BDV-3. BDV-3 has already been detected in France, Germany, Switzerland, Italy, Austria and Slovakia. No doubt that BDV-3 is the most spread genotype throughout the Europe. However, there is still insufficient data on the overall epidemiological situation of the BDV infection in Europe since data from many countries of central and eastern part of Europe are still missing.

The entire 5′-UTR-NS3 nucleotide sequence obtained for further BDV-3 isolate extends very limited genetic information on longer BDV nucleotide sequences. Up to now, only several numbers of partial sequences, mostly for 5′-UTR and N^{pro} region, and only one complete genome of BDV-3 genotype strain are available in GenBank database. New longer nucleotide sequences for BDV-3 isolate originating from sheep in Slovakia are important for the comparative sequence analysis, selection of more specific primers for PCR assays and evolutionary study of pestiviruses.

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