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Short note

Genetic diversity of international bovine viral diarrhoea virus (BVDV) isolates: identification of a new BVDV-1 genetic group

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Abstract – In the last decade, several studies were performed to characterise bovine viral diarrhoea virus (BVDV) isolates and define genetic groups by genotyping. Much data is now available from GenBank, predominantly sequences from the 5' untranslated region (5'-UTR). In order to find out whether genetic grouping of isolates from different countries could be harmonised, 22 new isolates from five countries were analysed in combination with published sequences. Eighteen of these isolates were typed as BVDV genotype 1 (BVDV-1), and one isolate from Argentina and three isolates from Brazil were typed as BVDV-2. BVDV-1 isolates were clustered into five previously defined genetic groups: BVDV-1a, b, d, e and f. Two isolates from Finland and one from Egypt formed a group which was tentatively labelled as BVDV-1j, since statistical support was low. By using a fragment of the N^{pro} gene for typing, we found that these isolates fall into the same group as a deer strain, and are statistically significant. Some Swiss BVDV strains taken from GenBank were found in a new genetic group which was designated as BVDV-1k. The BVDV-2 isolates included in this study seemed to fall into two genetic groups.

bovine viral diarrhoea virus / pestivirus / genetic typing / new genetic group

1. INTRODUCTION

Bovine viral diarrhoea virus (BVDV) belongs to the *Pestivirus* genus of the Flaviviridae family [24]. Presently two species of BVDV are recognised. BVDV-1 infections involve mainly respiratory, reproductive and enteric organs causing considerable economical losses in cattle farming [1, 6, 17]. The virus occurs worldwide. BVDV-2 causes similar clinical signs as BVDV-1, except that infection with highly virulent isolates may lead to thrombocytopaenia and fatal haemorrhagic syndrome [7, 13]. BVDV-2 was discovered in North America [19, 21], but the virus has also been detected

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in other countries worldwide [9, 10, 12, 14, 16, 20, 23, 28, 30].

The BVDV genome consists of a positive single-stranded RNA molecule of approximately 12.3 kb in length. The open reading frame (ORF) encodes a polyprotein of about 4 000 kDa, which is proteolytically cleaved during virus replication into the structural and nonstructural proteins. The first protein encoded by the ORF is the nonstructural viral autoprotease (N^{pro}), followed by the coding regions for four structural proteins and 6–7 nonstructural proteins. The ORF is flanked on both sides by the 5'- and 3'-untranslated regions (5'-UTR, 3'-UTR), which harbour viral regulatory signals [8, 15].

Genetic typing of BVDV has usually been performed using sequences from the 5'-UTR, N^{pro} and E2 regions [2–4, 18, 21, 23, 25, 27], and many of these sequences are available from GenBank. Besides the two BVDV species, several genetic groups especially for BVDV-1 have been described. Recently we identified 11 genetic groups of BVDV-1 [27]. Genetic diversity of BVDV isolates is important not only for taxonomy but also for laboratory diagnosis and vaccine design. In order to find whether genetic grouping of isolates from different countries could be harmonised, 22 new isolates from five countries were analysed in combination with published sequences. The isolates were typed using the partial nucleotide sequences derived from the 5'-UTR and for isolates that could not be grouped unambiguously, the sequences from the N^{pro} region were included.

2. MATERIALS AND METHODS

2.1. Viruses

Twenty-two BVDV isolates collected in Argentina (2 isolates), Brazil (10), Egypt (1), Finland (8) and Slovakia (1) over the last ten years were analysed (Tab. I).

2.2. Isolation of RNA

Total RNA was isolated using Trizol™ reagent (Gibco BRL, Gaithersburg, MD, USA) according to the manufacturer's instructions. RNA was dissolved in 50 μL of molecular grade water.

2.3. RT-PCR and sequencing

Synthesis of cDNA was performed with random hexamers in 20 μL of a total reaction volume. PCR was carried out with the 324/326 primers which amplify a 288 bp fragment of 5'-UTR [26]. Selected isolates were also amplified with the BD1/BD3 primers flanking a 428 bp fragment, which covered the end of the 5'-UTR and a fragment of the N^{pro} region (position in the NADL strain: 367-795, [27]). The PCR products were purified using Wizard PCR prep columns (Promega, Madison, WI, USA), and sequenced with the appropriate PCR primers in both directions on an ABI PRISM sequencing device.

2.4. Computer-assisted analysis

Nucleotide sequences were proof read using the SeqManII programme from the DNASTAR multiple programme package (Dnastar, USA). Sequence alignments were calculated with the CLUSTALW programme. Phylogenetic trees were prepared using the neighbour joining algorithm (Kimura 2-parameter method) of Saitou and Nei [22]. Statistical analyses of the phylogenetic trees were determined by the bootstrap method on 1 000 replicates.

3. RESULTS

3.1. Analysis of BVDV-1 isolates in the 5'-UTR

Of 22 pestiviruses analysed in the 5'-UTR, 18 viruses were typed as BVDV-1. These viruses were clearly assigned to the following recently well recognised groups: 5 viruses were typed as BVDV-1a, 2 as

Table I. List of pestivirus samples analysed in this work.

Country	Sample	Description	Typing
Argentina (2 samples)	73 (3-Arg)	Aborted fetus	BVDV-2
	Fe1 (5-Arg)	Spleen of healthy fetus	BVDV-1a
Brazil (10 samples)	126-1 (4-Br)		BVDV-1a
	153-15 (7-Br)		BVDV-1a
	126-14 (9-Br)		BVDV-2
	Vfsm-3 (10-Br)		BVDV-1a
	Vfsm-4 (11-Br)		BVDV-1b
	5V228/98 (13-Br)		BVDV-1d
	123-4 (15-Br)		BVDV-2
	126-8 (8-Br)		BVDV-1b
	Vfsm-5 (12-Br)	FBS	BVDV-1a
	Vm97 (17-Br)	Cultivation medium	BVDV-2
Egypt (1 sample)	4-Eg	Cell culture	BVDV-1j
Finland (8 samples)	B7	PI animal, Cell culture	BVDV-1f
	B53	PI animal, Cell culture	BVDV-1d
	B72	PI animal, Cell culture	BVDV-1d
	B96	PI animal, Cell culture	BVDV-1d
	B97	PI animal, Cell culture	BVDV-1j
	B101	PI animal, Cell culture	BVDV-1d
	B103	PI animal, Cell culture	BVDV-1d
	L197	PI animal, Cell culture	BVDV-1j
Slovakia (1 sample)	24-Mi	Blood, Imported cattle	BVDV-1e

BVDV-1b, 6 as BVDV-1d, 1 as BVDV-1e and 1 as BVDV-1f. No relationship was observed between the geographic origins of the viruses and their phylogenetic clustering (Fig. 1 and Tab. I). Two BVDV-1 strains from Finland (B97, L197), which had identical nucleotide sequences in the fragment analysed, and one isolate from Egypt (4-Eg) formed a separate cluster. In addition, this cluster also comprised BVDV isolates from Mozambique (M 1515A, M 065B), which nucleotide sequences were taken from GenBank. However, as can be seen in Figure 1, the position of this cluster is not significantly supported by statistical analysis (see group "j(?)").

Several BVDV-1 isolates originating from Switzerland, which were found in GenBank, were also included in the analysis. Interestingly, two of them formed a new BVDV-1 cluster with a long branch supported by a high bootstrap value (100%, see Fig. 1). This new group was termed BVDV-1k. Two more Swiss isolates where only shorter 5'-UTR sequences were available also belonged to this group (data not shown).

3.2. Analysis of BVDV-1 isolates in the N^{pro} region

To decide if the BVDV-1j cluster that was defined using the 5'-UTR sequences is

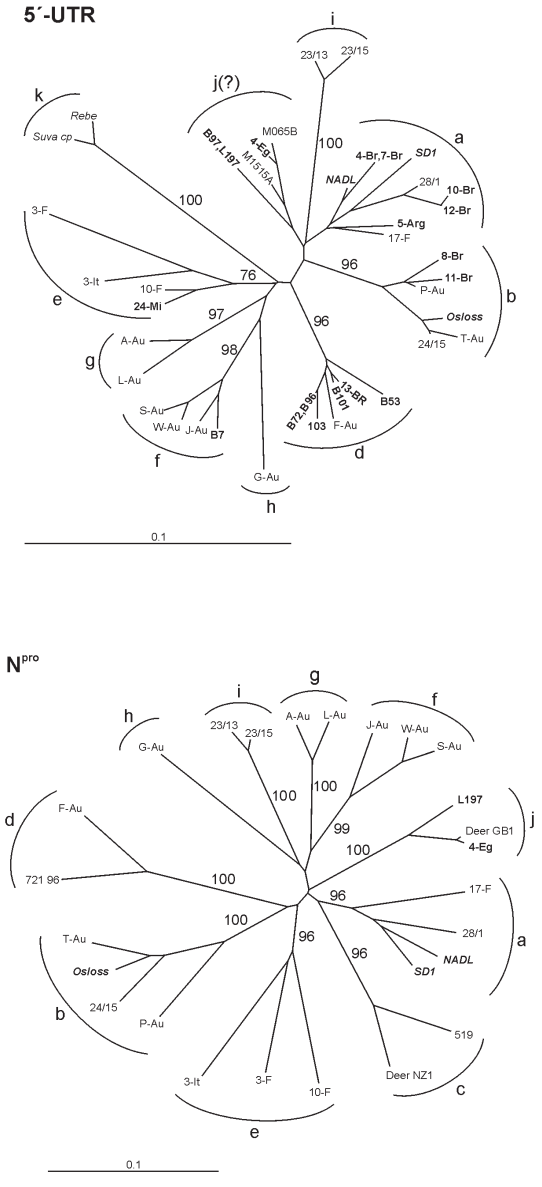

Figure 1. Genetic typing of BVDV isolates using the 5´-UTR and N^{pro} regions. Phylogenetic analysis was performed using 245 nucleotides derived from the 5´-UTR or 385 nucleotides from the N^{pro} genomic region using the Neighbour-joining algorithm with the Kimura 2-parameters. Only statistical confidence values > 60%, calculated using the SEQBOOT programme from the PHYLIP inference package with 1 000 replicates, are shown. The isolates sequenced in this work are labelled in bold, the remaining sequences were published previously [27]. Sequences for reference strains NADL, SD1 and Osloss were acquired from GenBank (Acces. Nos. M31182, M96751 and M96687, respectively). Acces. Nos. for other strains: M 1515A – U97429, M 065B – U97409, Deer GB1 – U 80902, Deer NZ1 – U80903, Rebe – AF 299317, Suwa – AF117699, 519 – AF 144464, 721 – AF144463.

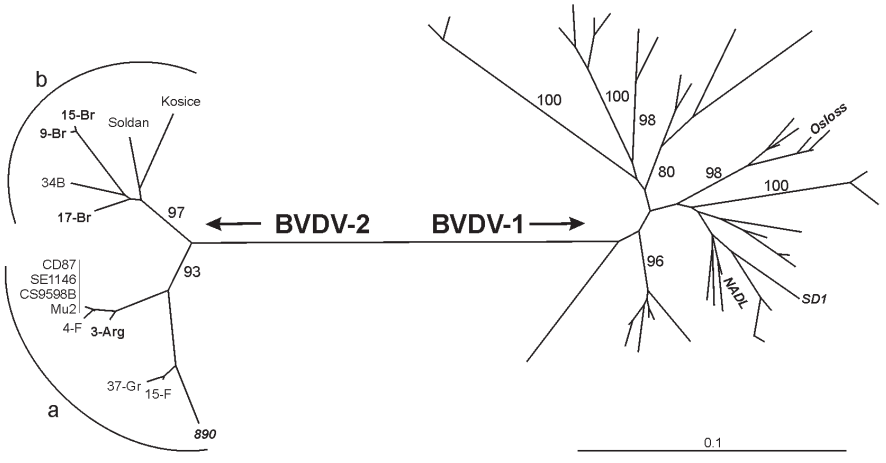

Figure 2. A phylogenetic tree showing the typing of BVDV-2 isolates in the 5´-UTR. The parameters for the phylogenetic analysis were as described for Figure 1. The nucleotide sequences for the BVDV-2 isolates Kosice, 37-GR and 4-F, 15-F were published previously [27–29], for isolate Mu2 was taken from Tajima et al. [23]. GenBank Acces. Nos. for other strains: Soldan – U94914, 34B – AF244952, SW90 – AB003622, CD87 – L32887, CS9598B – Z79763, SE1146 – Z79768.

valid, two isolates (L197 and 4-Eg) from this group were sequenced in the N^{pro} region. Surprisingly, both isolates clustered together with the Deer GB1 strain forming a group which was statistically significantly separated from other branches (Fig. 1).

3.3. Analysis of BVDV-2 isolates

Four viruses that originated from Argentina (3-Arg) and Brazil (9-Br, 15-Br, 17-Br), respectively, were typed as BVDV-2 (Fig. 2). Phylogenetic analyses using the nucleotide sequences of the 5'-UTR of some representative BVDV-2 isolates taken from GenBank, indicated that BVDV-2 strains may be grouped into two distinct phylogenetic groups labelled as "a" and "b" (Fig. 2).

Taking data from Figure 2, the percentage of identity between the 5'-UTR nucleotide sequences of BVDV-2 isolates achieved a range of 81.2–99%.

4. DISCUSSION

Most BVDV-1 isolates typed in this work were assigned to five recently recognised genetic groups [27]. For almost all isolates, it was found that genetic typing using the 5'-UTR and the N^{pro} sequences lead to identical grouping, despite the fact that the 5'-UTR is the most conserved region of the pestivirus genome, and fewer mutations are involved in the mathematic algorithm of the phylogenetic analysis. This leads to trees with lower bootstrap values for individual branches, which as shown here can be confirmed by using the N^{pro} sequences. Here, the L197 and 4-Eg isolates together with some other BVDV isolates from Mozambique [2] formed a separate cluster – j(?) in the 5'-UTR phylogenetic tree, which had to be confirmed by the analysis of these isolates in the N^{pro} region. Interestingly, these bovine isolates clustered together with the Deer GB1 strain, which formed in our previous work a unique

"Deer" branch with no bovine strains [27]. Unfortunatly, the corresponding 5'-UTR and N^{pro} sequences from the Deer GB1 strain or the strains from Mozambique, respectively, were not available. Despite missing data it seems evident that the Deer GB1 strain does not represent a singular "Deer" group, but it belongs to the BVDV-1j group together with two bovine isolates typed in this work.

Phylogenetic analysis of 5'-UTR in which the sequences of Swiss isolates deposited in GenBank were included revealed a new genetic group tentatively labelled as BVDV-1k. Although this has to be confirmed using sequences from the coding region, it is conceivable that there exists an additional twelfth BVDV-1k group.

Recently, it was suggested that not only BVDV-1 but also BVDV-2 isolates can be clustered into two or more genetic groups [4, 5, 9, 11, 23]. Although we found that the North American and European isolates cluster in group "a" and South American isolates in group "b", it is impossible to draw a definitive conclusion at this time. Nucleotide variability between BVDV-2 strains in 5'-UTR presented in Figure 1 is in the range 81–99%. Similar analyses performed with BVDV-1 sequences revealed a variability varying from 76–99% [27]. These data indicate that there may be a similar degree of variability amongst BVDV-2 as amongst BVDV-1, but more isolates will have to be characterised.

In conclusion, genetic typing of the collection of international BVDV isolates from five countries provided the following results: (i) extending our knowledge regarding genetic types of isolates circulating in different countries, (ii) showing that a "Deer" group of BVDV-1 previously represented only by a single deer isolate also contains the isolates of bovine origin from two countries, (iii) identifying a new BVDV-1k phylogenetic group harbouring several Swiss bovine isolates, (iv) most probably showing similar diversity for BVDV-2 isolates as observed for BVDV-1 isolates. We should point out that it cannot be excluded that further genetic analysis of BVDV-1 and BVDV-2 isolates will reveal even greater diversity of these viruses with possible taxonomic discrepancies between the genetic groups. When more data are available, a consistent definition of genetic groups should be possible.

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