Available online at www.sciencedirect.com
SCIENCE DIRECT
Virus Research 118 (2006) 192–195
Virus Research
www.elsevier.com/locate/virusres

Short communication

Phylogenetic analysis of Portuguese bovine viral diarrhoea virus

Sílvia C. Barros [a], Fernanda Ramos [a], Sónia Paupério [b], Gertrude Thompson [b], Miguel Fevereiro [a,*]

[a] *Laboratório Nacional de Investigação Veterinária, Dpt. Virologia, Lisboa, Portugal*
[b] *Instituto de Ciências Biomédicas de Abel Salazar (ICBAS)-Universidade do Porto, Dpt. de Clínicas, Veterinárias, Portugal*

Received 11 October 2005; received in revised form 14 December 2005; accepted 21 December 2005
Available online 26 January 2006

Abstract

Bovine viral diarrhoea virus (BVDV) infections are an important cause of morbidity and mortality worldwide in dairy and beef cattle. To date, little is know about BVDV genotypes circulating in Portugal. For this purpose, a fragment within the 5′-untranslated region (5′-UTR) from 34 Portuguese field strains of BVDV was amplified by RT-PCR, cloned and sequenced. A maximum-likelihood phylogenetic analysis revealed that most of viruses, originated from cattle from different regions of the country, belong to BVDV type 1 (BVDV-1), genotypes 1b ($n = 19$), 1a ($n = 6$), 1d ($n = 3$) and 1e ($n = 3$); whereas three viruses clustered in BVDV type 2 (BVDV-2). The results from this study demonstrate that BVDV-lb is the most prevalent genotype and also shows the presence of BVDV-2 in Portugal.
© 2005 Elsevier B.V. All rights reserved.

Keywords: BVDV-1; BVDV-2; *Pestivirus*; Phylogenetic analysis

Bovine viral diarrhoea virus (BVDV), border disease virus (BDV) from sheep and classical swine fever virus (CSFV) are the three classical single-stranded, positive-sense RNA viruses that make up the genus *Pestivirus* within the family *Flaviviridae* (Becher and Thiel, 2002). The genome of BVDV is approximately 12.5 kb in size and contains one large open reading frame flanked by 5′ and 3′ untranslated regions (UTR) (Collett et al., 1988; Ridpath and Bolin, 1995).

BVDV are segregated into two species, namely BVDV-1 and BVDV-2 (Ridpath et al., 1994; Paton et al., 1995; Harasawa, 1996; Becher et al., 1997). While BVDV-1 was isolated in 1954 (Baker et al., 1954) and is presently found worldwide, BVDV-2 was first described in the early 1990s in USA and in Canada as an emergent highly pathogenic viral genotype (Pellerin et al., 1994; Ridpath et al., 1994). Whilst BVDV-2 strains appear to be common in North America, they are absent or only sporadically detected in European countries (Becher et al., 1995; Paton et al., 1995; Wolfmeyer et al., 1997; Nagai et al., 1998; Letellier et al., 1999; Sakoda et al., 1999; Luzzago et al., 2001; Vilcek et al., 2001; Couvreur et al., 2002; Flores et al., 2002; Arias et al., 2003; Toplak et al., 2004).

Because pestiviruses naturally infect different hosts, classifications based on genomic and antigenic characteristics rather than on species of isolation have been proposed by several authors (Becher et al., 1995, 1997, 1999; Harasawa, 1996; Vilcek et al., 1997, 2001). Genetic typing of pestiviruses is mostly based on the genetic diversity of the 5′-UTR, N^{pro} and E2 genomic regions. Vilcek et al. (2001) demonstrated that 5′-UTR genomic region give meaningful phylogenetic inferences. This region has the highest degree of sequence conservation, and is efficiently amplified by RT-PCR being the most frequently analysed portion of the genome.

Initially, two genotypes of BVDV-1, genotype 1a/I-"NADL like" and genotype 1b/II-"Osloss like" were described (Pellerin et al., 1994; Harasawa, 1994). However, as more isolates were characterized, the numbers of genotypes have expanded to include novel isolates. Baule et al. (1997) and Becher et al. (1999) suggested that BVDV-1 clustered into three to five genotypes, and latter Vilcek et al. (2001) proposed the existence of 8–11 distinct genotypes. Concerning BVDV-2 species, two main genotypes (2a and 2b) were described (Becher et al., 1999; Vilcek et al., 2001; Flores et al., 2002).

In this study, we report the identification of BVDV strains circulating in Portugal. For this purpose, the 5′-UTR was partially amplified by RT-PCR using total RNA directly extracted from spleen, lymph nodes and/or blood of animals

* Corresponding author.
E-mail address: miguel.fevereiro@lniv.min-agricultura.pt (M. Fevereiro).

0168-1702/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.virusres.2005.12.009

tested positive for BVDV antigen on ELISA assay (SERELISA BVD p80 Ag Mono Indirect-Synbiotics). The primers used for the synthesis and amplification of the 5′-UTR cDNA were previously described (Sandvik et al., 1997). Amplification product cover a genomic region that corresponds to nt 103–386 of the reference strain BVDV-1 NADL (Collett et al., 1988). RT-PCR reaction was carried out using the Superscript One-Step RT-PCR with Platinum *Taq* system (Invitrogen) with 25 pmol of each primer and 0.5–1 μg of total RNA as template. The RT reaction (45 min at 48 °C) was followed by 40 cycles of the PCR amplification (30 s at 94 °C, 1 min at 55 °C, and 1 min at 68 °C) with a final elongation step of 5 min at 68 °C. The resulting amplicons were cloned into pCR2.1 vector (Invitrogen), sequenced and compared with published sequences of representative pestivirus genotypes (Table 1). The nucleotide sequences of the Portuguese isolates are deposited in the GenBank database with the accession numbers AY944266 to AY944299. Multiple alignments of the nucleotide sequences were generated by the CLUSTALW program, version 1.6 (Thompson et al., 1994) and phylogenetic analysis was carried out by maximum likelihood analysis in TREE-PUZZLE program, version 5.1 (Strimmer and von Haeseler, 1996), using a quartet-puzzling algorithm to generate the tree. The analysis was run with Hasegawa-Kishino-Yano (HKY-85) model of substitution (Hasegawa et al., 1985) and quartet-puzzling support values based on 1000 puzzling steps were calculated. The overall tree topology was identical when the phylogenetic analysis was repeated using the neighbor-joining method.

Phylogenetic analysis resulted in the unrooted tree shown in Fig. 1. The sequences included in the dataset used to generate the tree-segregated pestivirus into four species, namely CSFV, BDV, BVDV-1 and BVDV-2. Based on our analysis, five main clusters were formed within BVDV-1 species (Fig. 1). Of the 34 Portuguese strains analysed, 31 were typed as BVDV-1, while the remaining three grouped in a cluster within BVDV-2 isolates (Fig. 1). Within BVDV-1 species, the overall branching pattern shows that the Portuguese strains fell into genotypes 1a ($n=6$), 1b ($n=19$), 1d ($n=3$) and 1e ($n=3$), according to the nomenclature previously proposed (Vilcek et al., 2001).

In agreement with the work of Jones et al. (2004), our tree suggests that the genotype 1d defined by Baule et al. (1997), includes the genotypes 1f, 1g and 1h proposed by Vilcek et al. (2001). However, this group comprise isolates with a higher genetic heterogeneity (9%), suggesting that this cluster could be further subdivided into three subgenotypes.

We also found that British isolate 28-1 formerly grouped into genotype BVDV-li (Vilcek et al., 2001) does not form a

Table 1
Sources of representative pestivirus isolates 5′-UTR sequences

Representative isolates	Accession number	Reference	Geographic origin	Genotypes[a,b]
11/MI/97	AJ293603	Luzzago et al. (2001)	Italy	BVDV-2
23-15	AF298059	Vilcek et al. (2001)	England	BVDV-1i
24-15	AF298060	Vilcek et al. (2001)	England	BVDV-1b
28-1	AF298061	Vilcek et al. (2001)	England	BVDV-1a
3186V6	AF298062	Vilcek et al. (2001)	Italian	BVDV-1e
97/730	AF026770	DS	New Zealand	BVDV-2a
A	AF298064	Vilcek et al. (2001)	Austria	BVDV-1g
BVDV2-890	GI:902376	Ridpath and Bolin (1995)	USA	BVDV-2a
F	GI:10945595	Vilcek et al. (2001)	Austria	BVDV-1d
G	GI:10945596	Vilcek et al. (2001)	Austria	BVDV-1b
i318	GI:8099544	Jones et al. (2001)	Argentina	BVDV-1a
i34B	GI:8099538	Jones et al. (2001)	Argentina	BVDV-2b
J	AF298067	Vilcek et al. (2001)	Austria	BVDV-1f
Lees	U65051	Vilcek et al. (1997)	NA	BVDV-2a
M171N-95	GI:2895010	Baule et al. (1997)	Mozambique	BVDV-1d
M65CK-96	GI:2895035	Baule et al. (1997)	Mozambique	BVDV-1d
M657GX-95	GI:2895034	Baule et al. (1997)	Mozambique	BVDV-1d
M065B-93	GI:2894988	Baule et al. (1997)	Mozambique	BVDV-1c
M079B-91	GI:2894989	Baule et al. (1997)	Mozambique	BVDV-1c
NADL	M31182	Collett et al. (1988)	USA	BVDV-1a
ncp2	AY443027	Jones et al. (2001)	Argentina	BVDV-1a
ncp7	AY443026	Jones et al. (2001)	Argentina	BVDV-2
Nose	GI:3885402	Sakoda et al. (1999)	Japan	BVDV-1a
NY93	AF039173	Topliff and Kelling (1998)	USA	BVDV-2
OY89	AB003621	Nagai et al. (1998)	Japan	BVDV-2a
Q140	GI:548099	Pellerin et al. (1994)	Canada	BVDV-2
SE64444	GI:1580752	Wolfmeyer et al. (1997)	Germany	BVDV-2
Singer	GI:548087	Pellerin et al. (1994)	NA	–
Soldan	GI:2071979	DS	Brazil	BVDV-2b
Brescia	M31768	Moormann et al. (1990)	Italy	CSFV
X818	AF037405	Becher et al. (1994)	Germany	BDV

[a] Genotype (as determined by the authors) of representative sequences obtained from each study.
[b] Classification of BVDV-2 genotypes according to Flores et al. (2002). DS, direct submission to database; NA, not available.

Download English Version:

https://daneshyari.com/en/article/3431357

Download Persian Version:

https://daneshyari.com/article/3431357

Daneshyari.com