Bovine viral diarrhoea virus genotype 1 can be separated into at least eleven genetic groups*

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Accepted June 23, 2000

Summary. Seventy-eight bovine viral diarrhoea viruses (BVDV) recently collected in Austria, France, Hungary, Italy, Slovakia, Spain and UK were genetically typed in the 5′-untranslated (5′UTR) and autoprotease (Npro) regions of the pestivirus genome. Seventy-six of the isolates were BVDV-1 and two French isolates were of the BVDV-2 genotype. Phylogenetic analysis of the 5′UTR (245 nt), including additional BVDV-1 sequences from USA, Canada, Germany, New Zealand, Mozambique and Sweden, taken from GenBank and from our previous works, indicated that these viruses were clustered not only into the two generally accepted groups (BVDV-1a – “NADL like” and BVDV-1b – “Osloss like”), but altogether into 11 phylogenetic groups. Similar clustering was observed with Npro region sequences (385 nt) and the highest bootstrap values (over 95%) were obtained by phylogeny combining 5′UTR and Npro sequences. Some associations between the genetic grouping and the origin of the isolates were apparent, probably reflecting historical trade contacts. Considering the variability of isolates it is recommended that diagnostic PCR primers should be re-examined to ensure

*Sequence data from this article have been deposited with the GenBank Data Library under Accession Nos. AF287278-AF287290, AF298054-AF298073.
coverage of all BVDV-1 groups. The genogroups were less clearly differentiated by monoclonal antibody typing, suggesting significant antigenic similarities within the BVDV-1 genotype.

Introduction

Bovine viral diarrhoea virus (BVDV), a member of the genus Pestivirus of the family Flaviviridae, causes significant losses in cattle farming worldwide [7, 19]. The virus contains a single-stranded RNA genome of positive polarity, which is approximately 12.3 kb in length, and is flanked at either end by 5′ and 3′ untranslated regions (5′UTR, 3′UTR). The single intervening open reading frame (ORF) encodes a polyprotein of approximately 4000 amino acids, which is posttranslationally cleaved by viral and cellular proteases to 11–12 structural and nonstructural proteins (see review [17]). The first protein encoded by the viral ORF is the autoprotease Npro, which cleaves itself from the downstream viral structural proteins [28, 36].

Two genotypes of BVDV are recognised (BVDV-1 and BVDV-2), causing acute and persistent infections and a similar range of disease manifestations, except that acute infection with certain strains of BVDV-2 has been particularly associated with a severe haemorrhagic syndrome [8]. While BVDV-1 has been recognised for many years and is widely spread in the world, BVDV-2 was first identified in the 1990’s in North America [22, 24] and has been only sporadically detected in other countries such as Japan [18], Germany [37] and Belgium [16].

Pestiviruses are highly variable both antigenically and genetically [20]. Nevertheless, despite the clinical significance of BVDV infections, not many BVDV isolates have so far been typed and analysed at the genetic level. Early studies mostly involved American strains [6, 22–24]. Recently, isolates from Japan [18], Mozambique [2] and Germany [4, 37] have been genetically typed. We have also typed BVDV strains from New Zealand [32], Sweden [31] and Great Britain [33]. Genetic typing of pestiviruses has been mostly based on sequence comparisons of the 5′-UTR [2, 6, 13, 18, 22–24, 35, 37], Npro [3, 35] and E2 regions [4, 30]. Within BVDV-1, two major groups, named BVDV-1a and BVDV-1b were initially described [22, 24]. However, later reports have suggested that BVDV-1 is clustered into 3–5 groups [2, 4, 12].

To better define the genetic variability of BVDV, which is important for classification, and in the fields of diagnosis and vaccination, we studied a broad range of BVD viruses originating from cattle in seven European countries. Our genetic study has been based on the 5′UTR, supported by selected comparisons within the Npro coding region.

Materials and methods

Viral isolates

Seventy-eight BVDV isolates were studied: 23 from Austria, 25 from France, 8 from each of Spain and UK, 6 from Italy, 5 from Hungary and 3 from Slovakia. The viruses had mostly been collected in the 1990’s (except three isolates from Hungary) and were supplied as infected