



Bovine viral diarrhea virus (BVDV) genetic diversity in Spain: A review

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Abstract

Bovine viral diarrhea virus (BVDV), a member of the genus *Pestivirus* of the family *Flaviviridae*, causes significant losses in cattle farming worldwide because of reduced milk production, increased mortality of young animals and reproductive, respiratory and intestinal problems. The virus is characterized by an important genetic, and consequently antigenic and pathogenic diversity. Knowing the variability of viral strains present in a population provides valuable information, particularly relevant for control programs development, vaccination recommendations and even identification of likely infection sources. Such information is therefore important at both local and regional levels. This review focuses on the genetic diversity of BVDV isolates infecting cattle in Spain over the last years. According to the published data, the most prevalent BVDV group in Spain was 1b, and to a lesser extent 1d, 1e and 1f. Besides, BVDV-2 has also been found in Spain with several ratified isolates. The studies carried out in Spain also showed increased genetic heterogeneity of BVDV strains, possibly due to a more intensive use of analytical tools available, presenting studies with increasingly greater sample sizes.

Additional key words: BVDV-1; BVDV-2; genetic groups; evolution; cattle

Abbreviations used: ADGS (Livestock Health Defense Associations); BVDV (bovine viral diarrhea virus); VNT (virus neutralization test)

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Introduction

Bovine viral diarrhea virus (BVDV) belongs to the genus *Pestivirus*, which also comprises border disease virus and classical swine fever virus. The genera *Pestivirus* are included into the family *Flaviviridae*. It causes considerable economic losses in cattle, mainly attributable to reduced milk production, reduced reproductive performance, delayed growth, increased susceptibility to other diseases, early culling and increased mortality among young stock (Houe, 2003).

The virus is composed of an RNA genome, a host-derived lipid envelope, and four virion-encoded structural proteins. The genomic RNA has one open reading frame of about 4000 codons (Tautz *et al.*, 1997) which encompasses most of the viral genome. Viral RNA shows a high degree of variability in some regions resulting in important antigenic

diversity among different strains. Thereby, genetic typing of BVDV isolates distinguishes two recognized species: BVDV-1 and BVDV-2, based on antigenic and genetic properties. Both are capable of causing acute and persistent infections. Moreover, to the date at least 21 genetic groups of BVDV-1 (named BVDV-1a to BVDV-1r) and four groups in BVDV-2 (BVDV-2a to BVDV-2d) have been described (Alpay & Weşilag, 2015; Deng *et al.*, 2015; Giammarioli *et al.*, 2015).

Although BVDV is known from the first half of the 20th century (Olafson *et al.*, 1946), BVDV-2 was first described in the USA in 1994 (Pellerin *et al.*, 1994; Ridpath *et al.*, 1994). Both species show a similar range of disease manifestations except that certain BVDV-2 strains have been associated with severe hemorrhagic syndrome and high mortality (Carman *et al.*, 1998; Gethmann *et al.*, 2015).

While BVDV-2 is widespread in the USA and Canada (Pellerin *et al.*, 1994; Fulton *et al.*, 2005; Ridpath *et al.*, 2011), it has been detected more sporadically in Europe, although, is increasingly being identified over the last years (Wakeley *et al.*, 2004; Barros *et al.*, 2006; Jackova *et al.*, 2008; Letellier *et al.*, 2010; Luzzago *et al.*, 2012; Aduriz *et al.*, 2015; Gethmann *et al.*, 2015; Diéguez *et al.*, 2016).

Since 2000, several studies have addressed the research on the genetic diversity of species and groups of BVDV present in different populations from Spain. These studies can provide valuable information as regards BVDV control programs, vaccination protocols and infection sources determination because it is possible to elucidate potential sources and routes of infection if animal movements are correlated with phylogenetic analyses. Booth *et al.* (2013) demonstrated that phylogenetic analysis of BVDV isolates can be applied to determine factors influencing the epidemiology and transmission of the disease; where viral isolates from different farms were seen to cluster closely in the phylogenetic tree, they identified links between the farms related to animal movements. Besides, since BVDV-1 and BVDV-2 species are sufficiently different at the antigenic level, a vaccine that protects against one may not protect against the other. For this reason, Ridpath *et al.* (2011) have even

proposed using a BVDV vaccine that contains antigens from all subtypes circulating in the target region.

Review on the BVDV genetic variability in Spain

Most studies on BVDV genetic diversity in Spain have been carried out in the northern part of the country (Fig. 1). This area has the highest concentration of both dairy and beef cattle; it is also where BVDV control programs are being carried out more intensely, mainly through Livestock Health Defense Associations (ADSG in its Spanish acronym), although these programs are voluntary. Table 1 shows a summary of the results obtained in the different studies that will be reviewed in the current work.

The first report as regards genetic diversity of BVDV that included samples from Spain corresponds to a Europe-wide research (Vilcek *et al.*, 2001). In this study samples from Austria, France, Hungary, Italy, Slovakia and UK were also analyzed. The Spanish BVDV isolates (n=8) had been collected from 1995 to 1998 and were clustered into the 1a (n=1) and 1b (n=7) groups.

Subsequently, in a national study, 24 BVDV isolates (from 23 different herds), obtained during the period 1992 to 2000, were typed (Arias *et al.*, 2003). The viruses were collected from farms in the north of

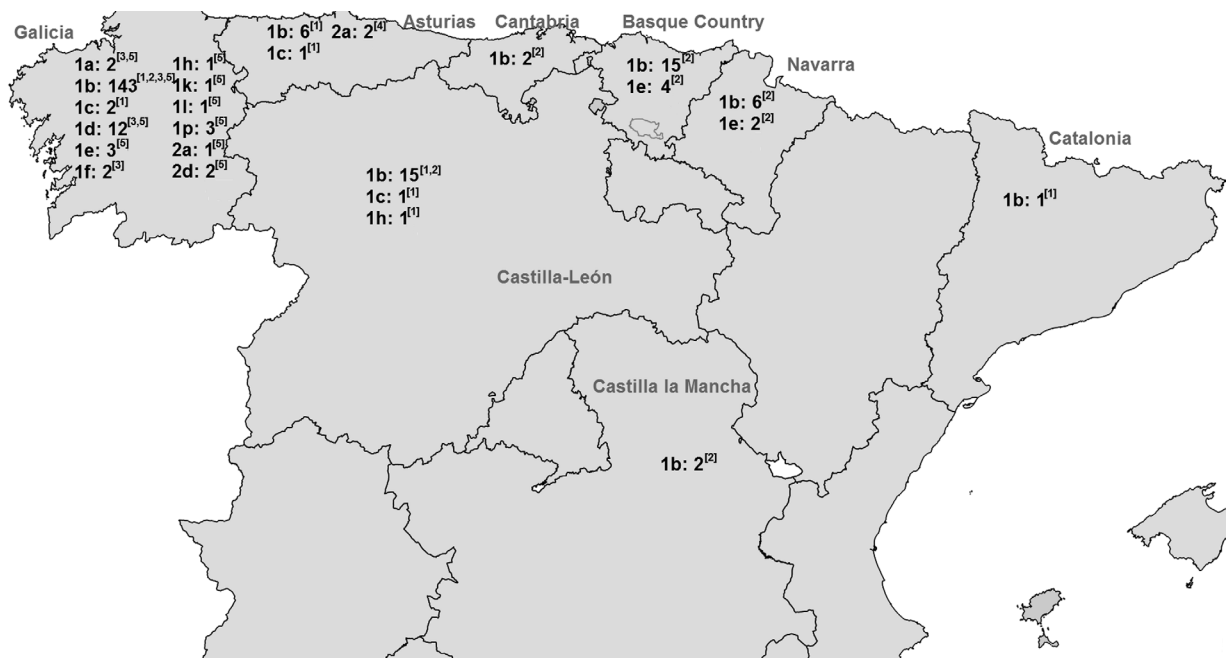


Figure 1. Map showing the locations from which BVDV strains were reported in Spain (including the number of strains from each group found in the different regions, and the study (ies) that found them): ^[1] Arias *et al.*, 2003; ^[2] Hurtado *et al.*, 2003; ^[3] Diéguez *et al.*, 2007; ^[4] Aduriz *et al.*, 2015 (who only provided the region of origin for the BVDV-2 strains); ^[5] Diéguez *et al.*, 2016. The studies by Vilcek *et al.* (2001) and Esperon *et al.* (2016) did not provide the region of origin of the BVDV strains typed.

Spain, covering different regions namely Castilla-León, Asturias, Galicia and Catalonia. All 24 isolates belonged to the species BVDV-1 and mostly (21) to the group BVDV-1b; other two were classified as BVDV-1c and one as BVDV-1h.

At the same time, Hurtado *et al.* (2003) analyzed 41 BVDV strains collected between 1999 and 2002. The samples were obtained from 41 cattle farms located in northern and central Spain (Galicia, Cantabria, Basque Country, Navarra, Castilla-León and Castilla la Mancha). All viruses were BVDV-1, of which 35 were BVDV-1b and six were BVDV-1e.

Still other research in the region of Galicia (NW Spain) 15 BVDV isolates were analyzed (Diéguez *et al.*, 2008). All these samples came from animals with persistent infections that had been diagnosed in 2004 in 15 dairy farms beginning a BVDV control program through ADSG. Again, all viruses belonged to the species BVDV-1: nine BVDV-1b, three BVDV-1d, two BVDV-1f and one BVDV-1a.

More recently, another study was conducted using 164 samples gathered between 2007 and 2015, again in different regions from the north of Spain (Esperón *et al.*, 2016). All of them were still framing within the species BVDV-1. Among groups, BVDV-1b was the most frequent, representing the 79.9% of total sequences obtained (131). Other groups found were BVDV-1f (13), BVDV-1e (10), BVDV-1d (7) and BVDV-1h (3).

A more recent study by Adúriz *et al.* (2015), constitute the first reported presence of BVDV-2 in

cattle in Spain. In this case, 47 blood samples that had tested positive in an ELISA for BVDV antigen were typed. Samples had been gathered between 2012 and 2013 from 18 different cattle herds located in northern Spain. A total of 45 of the 47 samples were typed as BVDV-1 and one as BVDV-2; in the one remaining sample both species were detected, indicating a mixed infection. Both BVDV-2 strains were classified as BVDV-2a and showed 100% identity to strain 104/98 previously detected in Germany (Tajima *et al.*, 2001). In the study, the presence of this BVDV-2 strain did not correlate with severe clinical signs.

Finally, a recent study carried out in Galicia marked the second description of BVDV-2 in Spain and the first in this region (Diéguez *et al.*, 2016). This study used samples collected in the region between 2014 and 2016. We typed 147 BVDV strains, of which 144 (97.9%) belonged to species BVDV-1 and three (2.1%) to BVDV-2. Of the 147 strains, 125 (85%) were typed as BVDV-1b based on 5'UTR analysis and comparison with reference strains. The virus in other three samples was identified as BVDV-1e, while the viruses in nine other isolates were most closely related to BVDV-1d and in three to BVDV-1p. One isolate each was assigned to groups BVDV-1a, BVDV-1h, BVDV-1k and BVDV-1l. Regarding the BVDV-2 isolates, one of the BVDV-2 isolates was classified as 2a; it came from a PI cow that was 31 months old when diagnosed and that had already calved once. Analysis of the calf (male) was not possible because he was slaughtered soon after birth.

Table 1. Summary of the results (number and % of BVDV species and groups) obtained in each of the different studies designed to assess the genetic diversity of BVDV strains in Spain.

| Reference | Years of sampling (n ^[b]) | BVDV-1 | | | | | | | | | BVDV-2 | | |
|--|---------------------------------------|-----------|-------------|----------|----------|------------|-----------|----------|----------|----------|--------|----------|----------|
| | | 1a | 1b | 1c | 1d | 1e | 1f | 1h | 1k | 1l | 1p | 2a | 2d |
| Vilcek <i>et al.</i> , 2001 | 90s (8) | 1 (14.3%) | 7 (85.7%) | - | - | - | - | - | - | - | - | - | - |
| Arias <i>et al.</i> , 2003 | 1992-2000 (24) | - | 21 (87.5%) | 2 (8.3%) | - | - | - | 1 (4.2%) | - | - | - | - | - |
| Hurtado <i>et al.</i> , 2003 | 1999-2002 (41) | - | 35 (85.4%) | - | - | 6 (14.6%) | - | - | - | - | - | - | - |
| Diéguez <i>et al.</i> , 2007 | 2004 (15) | 1 (6.7%) | 9 (60%) | - | 3 (20%) | - | 2 (13.3%) | - | - | - | - | - | - |
| Adúriz <i>et al.</i> , 2015 ^[a] | 2012-2013 (47) | - | - | - | - | 46 (97.9%) | - | - | - | - | - | 2 (4.2%) | - |
| Esperón <i>et al.</i> , 2016 | 2007-2015 (164) | - | 131 (79.9%) | - | 7 (4.3%) | 10 (6.1%) | 13 (7.9%) | 3 (1.8%) | - | - | - | - | - |
| Diéguez <i>et al.</i> , 2016 | 2013-2015 (147) | 1 (0.7%) | 125 (85%) | - | 9 (6.1%) | 3 (2%) | - | 1 (0.7%) | 1 (0.7%) | 1 (0.7%) | 3 (2%) | 1 (0.7%) | 2 (1.4%) |

^[a] For the BVDV-1 strains, the genetic group was not determined. ^[b] Number of BVDV strains typed in each study

Thrombocytopenia, hemorrhage or other clinical signs attributed to highly virulent strains of BVDV-2 were not observed. The other two BVDV-2 isolates came from a sheep farm in which reproductive problems (mainly abortions) were observed. In this farm, after negative results to border disease virus test, BVDV was isolated, which was classified as 2d. This was the first description of BVDV-2 in sheep in Spain, although, the same genetic group (2d) had been previously found in this species in other countries (Giangaspero & Harasawa, 2004).

Most of the previous studies used the primers 324 and 326 (Vilcek *et al.*, 1994) to obtain a 288-bp DNA product from the 5' untranslated region (5'UTR) of the viral genome. Only Arias *et al.* (2003) amplified part of the 5'UTR and the genomic region encoding N^{pro}, C, and part of E^{ms} proteins, using the primers named OL 100 and OL 1400R (Becher *et al.*, 1997, 1998). Vilcek *et al.* (2001) analyzed a selection of 30 representative viruses in the N^{pro} region that had been previously analyzed in the 5'UTR; all viruses were clustered in the same phylogenetic branches as for the tree based on the 5'UTR, with similar bootstrap values. However, no previous studies have specifically compared the results obtained after amplifying the 5'UTR region with that amplified by Arias *et al.* (2003). Phylogenetic trees were always constructed by the neighbor-joining method and validated using bootstrap analysis with 1000 replicates.

In addition, in the aforementioned studies, the sample collection dates cover two main periods: from the 90's to 2004 and from 2012 to 2015 (Table 1). In the first period, 88 strains were typed and 7 different groups were identified, while in the second, the genetic group of 313 samples was determined, identifying 11 different groups. Of the genetic groups identified in the period 90's-2004, the only one that was not found later was 1c.

In any case, using a t-test, it was found that when comparing both periods, the proportion of genetic groups was significantly different ($p < 0.05$).

In addition to the aforementioned studies, there have been other surveys that, although not specifically designed to estimate the prevalence of the different BVDV species and groups, addressed this topic to greater or lesser extent.

Among them, a study conducted in the region of Castilla-Leon (northern-central Spain), demonstrated the exposure to BVDV-2 in cattle. The paper included samples from cows belonging to beef farms managed under extensive conditions in a mountain area. A comparative virus neutralization test (VNT) for the detection of neutralizing antibodies to BVDV-1 and BVDV-2 was used, although in this case the genetic groups were not determined. At least one of the farms showed high VNT titers against the BVDV-2 and at individual level several cows had threefold difference

from BVDV-1, which is considered decisive for infection by the virus with the highest titer (Fernández-Aguilar *et al.*, 2016).

Rodríguez-Prieto *et al.* (2016) developed a study in central-southern Spain, aimed at examining the epidemiological role of wild ungulates and cattle living in proximity concerning BVDV. A total of 17 BVDV strains were typed, with 12 of them coming from roe deer and 5 samples being from extensively-managed cattle. All these isolates belonged to the group BVDV-1b.

Lately, a new identification of the BVDV-2 species was described in the province of Ávila (central Spain) (Pineda, 2016, *pers. comm.*). The viral isolate came from an undersized calf showing signs of digestive and respiratory disease.

Concluding remarks

According to the published data, the most prevalent BVDV group in Spain was 1b, and to a lesser extent 1d, 1e and 1f. This is consistent with the studies published in several European countries, including Germany, France, Italy and Portugal, in which the most prevalent BVDV groups were reported to be 1b, 1d and 1e (Tajima *et al.*, 2001; Barros *et al.*, 2006; Jackova *et al.*, 2008; Luzzago *et al.*, 2012). On the contrary, in the UK, BVDV-1a seems to be the most frequent (Booth *et al.*, 2013); this group has also been detected in Spain.

The BVDV-2 species has also been found in Spain with several ratified isolates. This species appeared more sporadically in Europe with a first identification in Belgium in 1991 (Letellier *et al.*, 2010). Afterwards it has been isolated in France on several occasions (Vilcek *et al.*, 2001; Jackova *et al.*, 2008) as well as in Italy (Luzzago *et al.*, 2012) or the UK (Wakeley *et al.*, 2004), in none of these cases correlated with severe clinical disease. However, in Portugal, the BVDV-2a and 2b groups have been found in farms showing clinical signs of hemorrhagic disease (Barros *et al.*, 2006). More recently, in 2012 and 2013, Germany, the Netherlands and Poland reported outbreaks of hemorrhagic syndrome with high mortality in calves and cows caused by an unusually virulent BVDV-2c strain (Polak *et al.*, 2014; Gethmann *et al.*, 2015), which may have caused disease recurrence in 2014. In Belgium, a BVDV-2a strain was also isolated from cows showing high fever and severe watery diarrhea, sometimes with hemorrhagic aspect (Maris, 2016). These outbreaks had a significant impact on Europe, especially, due to the possibility of spreading to other areas and countries.

The BVDV-2 strains found in Spain have not been associated, for the time being, to severe clinical signs

in cattle as described; BVDV-2 encompasses not only strains of high virulence but also those of moderate and low virulence (Ridpath *et al.*, 2002).

Broadly, the studies carried out in Spain showed increased genetic heterogeneity of BVDV strains; specially, this has been observed more markedly over the recent years, when up to ten different groups of BVDV-1 and two of BVDV-2 have been isolated in the country. This fact seems to be a consequence of a more intensive use of analytical tools available, presenting studies with increasingly greater sample sizes.

On the other hand, the data collected in this paper has demonstrated areas that require clarification. Studies on BVDV genetic diversity in Spain have been punctual, with very different sample sizes and sampling periods, and few include spatial and/or temporal analysis of the data. This indicated that there is no overall sampling strategy to determine the prevalence of the genetic types over time in the different cattle areas from Spain. In addition, some regions (including some with considerable cattle density) have been barely sampled while from others there are still no data.

Data on species and genetic groups of BVDV circulating in a population can contribute to a better understanding of the epidemiology and pathogenesis of viral infections. This diversity should also be considered in the development of control programs, especially in terms of vaccination programs and likely infection sources.

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