

Current situation of BVD in Spain: presence of genotype 2

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Introduction

Bovine Viral Diarrhoea (BVD) is a disease caused by an RNA virus belonging to the *Pestivirus* genus in the *Flaviviridae* family (Becher and Thiel, 2011). It affects cattle of all ages and production types and is present throughout the world.

The *Pestivirus* genus currently comprises four species or genotypes: BVDV1 (genotype 1), BVDV2 (genotype 2), Classical Swine Fever Virus and Border Disease Virus (Becher et al., 2003). BVDV1 is itself divided into 16 different subtypes (a-p) and BVDV2 into three different subtypes (a-c) (Peterhans et al., 2010).

Although the two BVDV species are genetically and antigenically different, they do share certain similarities, such as the fact that, based on their ability to induce changes in *in vitro* cell cultures, vacuolisation and cell lysis, isolates of this virus fall into two different biotypes (Birk et al., 2008; Neil et al., 2008). The cytopathic biotype (CP) causes changes in the cells that infect the *in vitro* culture, while the non-cytopathic biotype (NCP) does not cause any visible damage to the cell culture. Both biotypes can infect cattle and cause disease, but only the NCP biotype can cause persistent infection (Kelling, 2004). In addition, natural infections predominantly comprise the NCP biotype (Fulton, 2013).

However, it should be pointed out that the virulence of the disease does not depend on the biotype, nor does it strictly depend on the genotype or the species. In fact, virulence depends on the specific strain of the virus and the immune status of the host (Ridpath, 2010). This is why disease outbreaks caused by both genotype 1 and genotype 2 have been reported with completely different manifestations, ranging from a completely silent disease onset with no apparent symptoms other than reproductive effects, to cases manifesting a broad spectrum of clinical symptoms (Carman et al., 1998; Bolin and Grooms, 2014). The way in which a strain manifests itself largely depends on its ability to affect lymphocyte count and the subsequent severity and duration of the state of immunosuppression induced. Thus, a highly virulent strain induces a significantly more severe and longer-lasting state of lymphopenia than less virulent strains (Liebler-Tenorio et al., 2003).

Because of its endemic nature, high prevalence and persistence, and devastating effect on the health and reproduction of both dairy and beef herds, BVD is considered one of the most severe and significant diseases in cattle (Gunn et al., 2005; Moennig et al., 2005).

Current situation

The seroprevalence of the disease is high. Some authors claim that it can exceed 80% in high livestock density areas, where it behaves as an endemic disease (Houe, 1999). It is accepted that in areas with no management programme in place, 60% of animals will be transiently infected at least once in their lives. According to most epidemiological surveys conducted around the world, between 0.5% and 2% of animals are persistently infected with the BVD virus (Brock, 2003; Houe, 1999). The predominant genotype in Europe is

genotype 1, estimated to be responsible for 90% of infections, while prevalence in North America is divided equally between BVDV1 and BVDV2 (Ridpath et al., 1994; Jackova et al., 2008). However, the most recently reported European outbreaks of BVD, which occurred in Germany (February 2012) (Astiz, 2014; Schirrmeier, 2014), the Netherlands (August 2014), Belgium (2013 and early 2014) and Poland (2014) (Miroslaw et al., 2014), were caused by genotype 2.

The prevalence of BVD in Spain is unclear. There are very few studies on the prevalence of the disease, and most have been conducted in specific geographical areas of Spain. Furthermore, they were seroprevalence studies, meaning that they took into account the presence of antibodies and not the number of persistently infected animals, which is widely acknowledged to be the key to disease transmission (Lindberg et al., 2005; 2006).

Although the studies published present data that are in line with European findings, they are nonetheless interesting. For example, prevalence in dairy herds ranges from 94.2% in the autonomous community of Madrid (Vega et al., 2004), 70.2% in Galicia (Eiras et al., 2009), 86% in Asturias (Mainar-Jaime, 2001) and 91.5% in the province of León (Álvarez et al., 1994). Data collected from studies conducted in extensive cattle reveal prevalence figures that are just as high as for dairy cattle: 100% in Andalusia (Gómez Pacheco et al., 2006), and 70.2% in Galicia (Eiras et al., 2009). The most significant finding comes from a study by the Livestock Health Protection Groups (ADSGs) in Galicia (Arnaiz, 2012), which found a seroprevalence of 17.1% in animals, seeming to suggest the effectiveness of disease management programmes in comparison with previous studies. Subtype 1b was found to be the predominant subtype in studies conducted in the León area (Arias et al., 2003) and in a

study of a sample from the Basque Country and Navarre (Hurtado et al., 2002), while BVDV2 was not identified in any of the above-mentioned studies.

Boehringer Ingelheim BVD surveillance network

In May 2013, Boehringer-Ingelheim España launched an initiative to obtain current data on the different BVDV types present in Spain. It was not designed as a national epidemiological study. Just two objectives were clearly defined: to obtain a sufficient number of isolates to perform the relevant genotyping for comparison with data already collected from other studies; and to try to find out to what extent the presence of the disease in a herd can be predicted based on clinical suspicion.

In light of these objectives, the national "Boehringer Ingelheim BVD surveillance network" was launched, initially made up of a group of 22 veterinarians actively working in the field of buiatrics. These veterinarians were given the task of sending different sample types collected during their regular working activities to a single central laboratory (Neiker-Tecnalia)*. The choice of animals to sample had to be based purely on clinical suspicion, depending on symptoms and lesions consistent with the disease, or epidemiological and production data also consistent with BVDV infection, particularly with regard to reproductive or health parameters (morbidity and mortality). Sampling was therefore biased, conducted on the basis of a suspected outbreak of bovine viral diarrhoea, rather than randomised to determine prevalence in the population. The vets only had to complete a simple questionnaire to help them to determine the degree of suspicion of the disease.

Samples were taken from cattle of all ages, breeds and production types. All sample types

commonly sent to the laboratory were accepted. However, samples taken from animals under 6 months of age had to be ear biopsy samples (always including hair, as the virus is found in hair follicles) in order to avoid potential interference by colostral antibodies through the use of antigen capture ELISA techniques (Sasha, 2014). From the start of the study in May 2013 to the present, 1,410 bovine samples have been received by the laboratory. Of these, 684 (48%) were serum samples, 445 (32%) were EDTA whole blood samples, 64 (5%) ear biopsy samples, 17 (1%) tank milk samples and 200 (14%) organ samples obtained through necropsy of dead animals. 52% (975) of samples came from dairy cattle, 22% (256) came from extensive herds and 26% (179) came from feedlot calves.

With the exception of the RT-PCR used for serotyping, which was employed specifically for this project, the techniques used to analyse the samples were no different to those used in regular laboratory practice:

1. BVD virus antigen detection: Capture ELISA (Erns) (IDEXX). Samples: Ear tissue biopsies, EDTA blood, blood serum and plasma.
2. Detection of antibodies to the BVDV p80 protein: Blocking ELISA (Pourquier-IDEXX). Samples: Blood serum or plasma.
3. BVD virus detection: Real-time RT-PCR (Hofmann et al., 2006). Samples: Tank milk, tissue, EDTA blood, blood serum and plasma.
4. Virus typing (BVDV1 and BVDV2): Real-time RT-PCR (Baxi et al., 2006). Samples: positive RT-PCR detection samples (milk or tissue). Directly on EDTA blood and blood serum and plasma samples.

The data collected to date reveal that, of a total of 239 sample submissions, 50.20% have resulted in the isolation of BVDV. Of a total of 1,410 samples analysed to date (this study remains ongoing), 120 samples have been

found to be positive for the BVD virus. Of them, 63 (52.5%) came from dairy cattle, 31 (25.83%) came from feedlot herds and 26 (21.67%) came from extensive cattle. All were found to belong to genotype 1.

To complement the surveillance network, 47 BVDV-positive samples were simultaneously genotyped from NEIKER's repository of specimens obtained between 2012 and 2013, as well as 96 samples from the Galicia Animal Health and Production Laboratory (LASAPAGA), released by the laboratory for this study. All samples belonging to the LASAPAGA were genotyped as BVDV genotype 1. However, two of the samples from the NEIKER specimen repository were identified by PCR as BVDV genotype 2 and confirmed by sequencing (Aduriz et al., 2014). This is extremely significant as this is the first time that BVDV2 has been identified in Spain.

Discussion and conclusions:

- The BVDV2 genotype has now been found in Spain. The prevalence of this genotype in this study was 0.8%. It may be that the BVDV2 genotype has not recently appeared in Spain, but has actually been around for a long time, given the fact that the prevalence of type 2 has increased across Europe in recent years and that the purchase and sale of live animals between Spain and other countries positive for this pestivirus is also growing.

- The presence of BVDV2 in Spain requires a complete overhaul of the BVD vaccination strategy nationwide due to the poor cross-protection, especially against foetal infection, between the two genotypes (Walz, 2009). A possible cause for the failure of vaccination programmes in terms of poor reported foetal protection in Spain and in many European countries may be partly due to the fact that, to date, the BVDV2 genotype is not included in any vaccine registered in Europe.

- It must be pointed out that these results in no way reflect prevalence data, as only samples suspected of containing the BVD virus were analysed. It must also be recognised that positive samples from the BVD surveillance network may have come from persistently infected or transiently infected animals and may have been acute or chronic, as in all cases the virus was not present 20-30 days after initial isolation (in most cases the animal was directly removed). It seems clear that the role that PI animals play in the epidemiology of the disease is more important than the role attributed to transiently infected animals (Lértora, 2003; Ridpath, 2010). It can therefore be assumed that the vast majority of these positives could come from PI animals. A serious national epidemiological study would be required to confirm this assumption.

- BVD has been described as a "hidden" or "ghost-like" disease, as in most cases it develops silently, without manifesting obvious symptoms (Fulton, 2013; Ridpath, 2010). Despite this, about 50% of samples submitted as a result of clinical suspicion of the disease were positive for the virus. This indicates that, even though diagnosis of the disease cannot be based solely on clinical or epidemiological symptoms observed in the field, BVD should be taken into account in the differential diagnosis of undifferentiated processes, especially with regard to reproduction problems.

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