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# **CHALLENGES IN THE CONTROL OF BOVINE VIRAL DIARRHOEA VIRUS – IMPLICATIONS FOR A BELGIAN ERADICATION PROGRAMME**

**Jozef LAUREYNS**

Merelbeke, 2014



Aan Laura en Hedwige, twee sterke vrouwen die me altijd hebben bijgestuurd,  
zonder aan mijn vrijheid te raken.

Do not go gentle into that good night,  
old age should burn and rave at close of day;  
rage, rage against the dying of the light.  
Dylan Thomas (1914-1953)

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Jozef Laureyns

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# **Challenges in the control of bovine viral diarrhoea virus – Implications for a Belgian eradication programme**

Uitdagingen bij de controle van het bovine virale diarree virus – implicaties voor een Belgisch eradicatieprogramma

(met een samenvatting in het Nederlands)

Proefschrift voorgedragen tot het behalen van de graad van Doctor in de Diergeneeskundige Wetenschappen aan de Faculteit Diergeneeskunde, Universiteit Gent, 9 januari, 2014

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## List of Abbreviations

BDV	Border Disease Virus
BMSCC	Bulk Milk Somatic Cell Count
BNP	Bovine Neonatal Pancytopenia
BRD	Bovine Respiratory Disease
BVD	Bovine Viral Diarrhoea
BVDV	Bovine Viral Diarrhoea Virus
cp	cytopathic
DIVA	Differentiating Infected From Vaccinated Animals
ELISA	Enzyme-Linked ImmunoSorbent Assay
HD	Haemorrhagic Disease
MD	Mucosal Disease
MLV	Modified Live Virus
nep	non-cytopathic
PCR	Polymerase Chain Reaction
PI	Persistently Infected
RNA	RiboNucleic Acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
TI	Transiently Infected



## Chapter 1

# **G**eneral Introduction

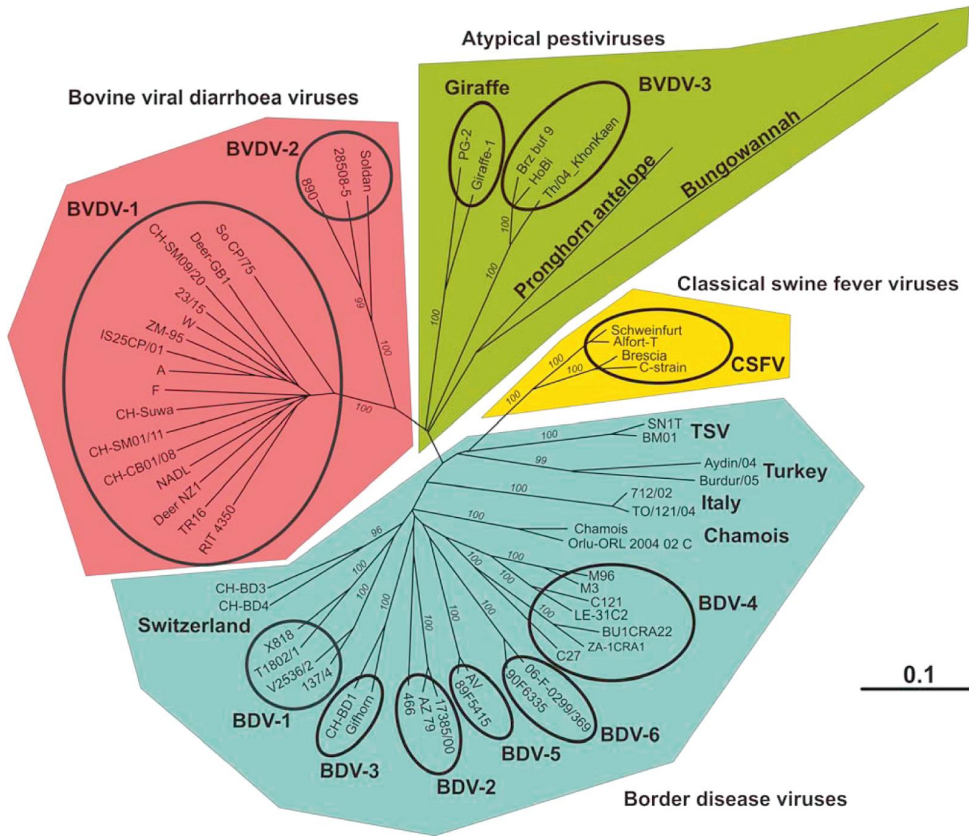


The purpose of this introduction is to inform the reader on Bovine Viral Diarrhoea (BVD). Only selected topics, of relevance for this thesis, have been included. First, the Bovine Viral Diarrhoea Viruses (BVDV) are introduced, followed by sections dealing with pathogenesis, prevalence, clinical features, economic consequences, diagnosis, and control of BVD in cattle.

## The viruses

BVDV are single stranded RNA viruses belonging to the *Pestivirus genus*, within the *Flaviviridae* family. The *Pestivirus genus* currently comprises four recognized species: Border Disease Virus (BDV), Classical Swine Fever Virus, BVDV1, and BVDV2. Furthermore, there are four proposed “atypical” species of pestiviruses: Giraffe, HoBi, Pronghorn Antelope, and Bungowannah (Schirmer et al., 2004; Kirkland et al., 2007; Ridpath and Fulton, 2009; Booth et al., 2013a).

Through high rates of point mutations and recombinations, RNA viruses are constantly changing (Kümmerer et al., 2000; Becher et al., 2001). Hence, BVDV is not one virus, but a group of many genetic variants. Both BVDV1 and BVDV2 *species (types)* are divided into several genetic *subspecies (subtypes)*, as indicated by lowercase following the species number, for example “BVDV1b” (Fig. 1). Most subspecies comprise different *strains* (Vilcek et al., 2005). Occasionally, a strain can change to a more virulent strain. This might explain the periodic emergence of acute outbreaks of disease (Bolin and Grooms, 2004). Nevertheless, in general, BVDV strains remain stable within herds during the course of an infection (Vilcek et al., 1999; Booth et al. 2013). In the USA up to 40 % of the genotyped BVDV strains belong to the BVDV2 species (Fulton et al., 2000b), whilst in Europe the fraction of BVD2 diagnosed in laboratories is lower than 7% in all countries (Ridpath, 2010a; Letellier et al., 2010). Rare outbreaks associated with BVDV2 have been described in Europe. An outbreak caused by a bovine herpesvirus 1 marker vaccine contaminated with BVDV2 took place on Dutch dairy farms in 1999 (Barkema et al., 2001). In January 2013, outbreaks of severe disease associated with BVDV2 occurred in some German and Dutch veal calf herds (Doll and Holsteg, 2013; Moen, 2013). High fever, haemorrhagic disease and pneumonia were the predominant clinical signs.



**Figure 1.** The pestivirus species, BVDV included: species (types) BVDV1 and BVDV2 with their subspecies (subtypes). Source: Peterhans et al., 2010. The branch lengths of the phylogenetic tree are proportional to the genetic distances between strains.

Besides the genetic division, BVDV can also be classified according to their biotype. Viruses of all strains belonging to both genotypes may exist as two different biotypes, namely cytopathic (cp) and non-cytopathic (ncp) (Peterhans et al., 2010). This distinction is determined by the ability of the virus to cause cytopathic effect in permissive cell cultures, but, more importantly, the difference between the two biotypes plays a role in the pathogenesis of BVD. Ncp BVD viruses predominate in the field. Because cp viruses are “pop up and disappear” viruses (Peterhans et al., 2010), they are rare and usually only found in association with outbreaks of Mucosal Disease (MD) (Ridpath, 2005; Ridpath, 2010a).

## Pathogenesis

### *Transient infection*

In cattle BVD is a systemic disease. The most frequent route of natural infection is by oro-nasal uptake of BVDV. The tonsils are the primary replication site. From there the BVDV spreads to the lymphocytes in the local lymph nodes and from there to several organs and tissues via infected lymphocytes in the blood circulation. After oro-nasal infection of cattle, the virus first replicates in the tonsils. From there it spreads to the regional lymph nodes via infected lymphocytes. Afterwards, for low virulent strains the infection remains limited to lymphoid tissues, but through viraemia more virulent genotypes are able to infect more organs and tissues, from the epithelium of the digestive tract to the lungs, urinary tract, heart and skin (Bruschke et al., 1998; Liebler-Tenorio, 2005). The pregnant uterus, placenta, and foetus easily become infected (Frederiksen et al. 1999), even when the dam is subclinically infected (Liebler-Tenorio, 2005). When susceptible, immunocompetent cattle become infected with a virulent BVDV strain, the outcome may be severe disease with mortality. However, in most of the cases the infection will pass with only mild disease or remain asymptomatic (Baker, 1995), but all infected cattle undergo momentary immunosuppression (Wilhelmsen et al., 1990; Walz et al., 1999; Brackenbury et al., 2003; Ridpath, 2010b; Chase, 2013). In general, about 10 days after infection, the immune system of Transiently Infected (TI) cattle succeeds in removing the virus from the blood and from that moment on, antibodies begin to appear in the blood (Müller-Doblies et al., 2004; Sarrazin et al., 2013b). The animal remains seropositive for BVDV during its entire life (Brownlie, 1990), “entire life span” in this case meaning the normal life span of commercial cattle. It is worth mentioning that this lifelong seropositivity does not necessarily result in lifelong protection against re-infection (Lindberg et al., 2008).

Because of the short duration of the infection and the intermittent shedding of relatively low amounts of virus, TI animals are believed to be of minor importance in the epidemiology of BVD. Some studies demonstrated no spread of BVDV by experimentally infected animals (Niskanen et al., 2000; Niskanen et al., 2002). Recent results of Sarrazin et al. (2013b) confirm these observations, since only very limited virus transmission was demonstrated when calves were experimentally infected with a virulent BVDV1 or BVDV2 strain. Although they are not of major importance epidemiologically, TI cattle contribute towards the majority of any observed production losses in infected herds.

*Persistent infection*

The main impact of BVDV on cattle health is caused by intrauterine infections. Infection of the foetus can cause early embryonic death, congenital malformations, and birth of persistently infected calves. When a dam, and consequently her foetus, becomes infected with ncp BVDV between 30 and 125 days of pregnancy (Blanchard et al., 2010), the foetus will accept the virus as belonging to its own organism, since it is not immunocompetent at that stage. As a result the calf becomes lifelong infected and will be persistently shedding the virus (Brownlie, 1990).

These Persistently Infected (PI) cattle play the principal role in spreading the infection, as they persistently shed massive amounts of ncp BVDV through all their secretions and excretions. Hence, the sources of new BVDV infections are almost always PI (Lindberg and Houe, 2005). Iatrogenic transmission by persons, vaccines, calf sera, semen, and embryo transfer is rare, (Barkema et al., 2001; Drew et al., 2002; Niskanen and Lindberg, 2003; Ståhl et al., 2005; Ståhl et al., 2007; Rikula et al., 2008; Bielanski et al., 2013; Ridpath, 2013). As PI cattle are by far the most important sources of infection, they are supposed to be the origin of iatrogenic infections. During their life, they can contract cp BVDV infection from cattle suffering from MD, or their own ncp BVDV can produce cp BVDV by recombination or mutation (Becher et al., 2001; Ridpath, 2003; Peterhans et al., 2010). At that moment cp and ncp BVDV exist together in the same animal and this can lead to the development of MD, a highly fatal form of BVD occurring mostly in cattle under two years of age (Brownlie et al., 1984; Houe, 1992a; Bachofen et al., 2010). However, more than 10% of PI animals survive longer than 2 years (Presi et al., 2011). When pregnant, their offspring will always be PI as well (Moennig and Liess, 1995).

*Prolonged testicular infection*

The third form of BVDV infection is rare and of limited epidemiologic significance. When bulls become TI, the semen of a minority remains BVDV-positive during at least 11 weeks and over 2 years (Voges et al., 1998; Niskanen et al., 2002; Givens et al., 2009). Experimental transmission of BVDV through semen of these bulls did however not infect other cattle (Givens et al., 2009; Givens and Marley, 2013). Despite the minimal risk of transmission, repeated detection of BVDV in bovine semen indicates that the bull should not be used for breeding or artificial insemination. A European Union directive (Council

Directive 2003/43/EC) stipulates that prior to the initial dispatch of semen from BVDV seropositive bulls, a semen sample from each animal shall be subjected to a virus isolation or virus antigen ELISA test for BVDV.

### *Immunotolerance and immunosuppression*

Intriguingly, the same virus, ncp BVDV, causes immunosuppression in TI cattle, whilst large numbers of PI animals can survive during months or years without clinical disease. Those animals remain healthy not because the host became resistant to the virus, but rather the virus evolved mechanisms to increase the tolerance of its own host without the need to reduce the ncp BVDV burden that would otherwise decrease the chance of transmission to new hosts (Peterhans and Schweizer, 2013). In PI animals both the innate and adaptive immunity against the infecting BVDV strain are suppressed. Down-regulation of the interferon response against the infecting strain plays a key role in the mechanism of immunotolerance. In contrast, when PI animals become infected with other virus species, non-related BVDV strains, or other infectious agents, the interferon modulation will not be inhibited, and the hosts organism will start an immune response against the infecting organism. The same reaction is provoked at transient infections of susceptible cattle. BVDV impairs the immune reaction in TI cattle by interacting on several levels: lympho- and neutrophils, macrophages, and cytokines. BVDV causes lymphoid cell death and reduced function in the remaining lymphoid cells (Ridpath, 2010b). Unlike in the case of persistent BVDV infection, the immunosuppression in transiently infected cattle is not primarily caused by down-regulation of the interferon response.

### *Conclusion*

The brief outline of the pathogenesis shows that the BVDV is a very well skilled virus. To enhance chances of survival, most viruses have only one possibility: “hit and run”, or “infect and persist”. The BVDV has mastered both strategies. When naïve hosts are available, PI cattle can (transiently) infect many animals over a short period of time. On the other hand BVDV can survive in the absence of susceptible individuals by infecting cattle persistently. Moreover, the rapidly changing genome is an additional advantage for survival.

## Prevalence

### *Cattle*

BVDV infection is endemic in cattle populations worldwide (Houe, 1999; Moennig et al., 2005; Ridpath, 2010a). Prevalence of PI animals never exceeded 2% of the cattle population (Houe 2003), whilst seroprevalence can reach high levels. In Ireland, for example, 98.7% of non-vaccinating herds were seropositive in 2009 (Cowley et al., 2012). Seroprevalence of Swiss cattle was estimated to be 100% at the herd level and 60% at the animal level (Rufenacht et al., 2000).

A recent study indicated that almost half of the Belgian cattle herds have seropositive young stock (Sarrazin et al. 2013a). In another study, circulation of BVDV was demonstrated in 93% of tested Belgian veal calf units and seroconversion to BVDV took place in 57% of the calves (Pardon et al., 2012).

### *Other species*

Persistent BVDV infection can develop in at least eight other species: sheep, goats, pigs, alpaca, white-tailed deer, eland, mouse deer, and American mountain goat (Bachofen et al., 2013). Although they are not considered as reservoirs of BVDV, these animal species may play an undesired role in eradication programmes. This is the case for small ruminants in particular, as spill-over of BDV from small ruminants to cattle is possible. More expensive tests can differentiate BDV from BVDV, but BVDV tests suitable for mass testing do not clearly differentiate between antibodies to the two viruses. Therefore, cross infections can interfere when monitoring for freedom of BVDV in cattle (Strong et al., 2010).

## Clinical features

Describing the clinical presentations of BVDV infection is complicated because of four reasons.

1. The clinical signs are numerous and extremely varied;
2. Clinical presentations of BVDV infections can change over time because of genetic shift in BVDV-strains (Evermann and Ridpath, 2002);
3. The majority of the clinical signs are not typical for BVD;

4. One cannot expect to find all of the different symptoms in one herd or during one outbreak.

The severity of the clinical signs varies from very mild, over severe to lethal disease (Baker, 1995; Brownlie, 2004; Evermann and Barrington, 2005). Depending on the virulence of the strain, only the lymphoid tissue, or several organs become infected (Bolin and Ridpath, 1992). Furthermore, the immune and reproductive status of the host, age of the host, and concurrent infections determine the clinical features (Ridpath, 2010a). Because of this variation, recognizing BVD by its clinical presentations is a challenge for the bovine practitioner (Lindberg and Alenius, 1999; Ridpath, 2003; Evermann and Barrington, 2005).

### ***Postnatal transient infection***

#### 1. *Acute infection*

It has been estimated that 70-90% of acute infections with BVDV in immunocompetent, seronegative cattle passes with ***only mild fever and leucopenia*** (Baker, 1995; Evermann and Barrington, 2005). Nevertheless, it has to be emphasized that despite the infection passing subclinically, immunosuppression over a short period is evident in these cattle (Wilhelmsen et al., 1990; Walz et al., 1999; Brackenbury et al., 2003; Ridpath, 2010b; Chase, 2013). This may result in other diseases, by giving the opportunity to other pathogens to secondarily infect the animal. Moreover, both clinical and subclinical BVDV infection may be accompanied by ***reduced fertility***, originating from early embryonic death and impaired function of ovaries and testicles (Muñoz-Zanzi et al., 2004; Brock et al., 2005; Grooms et al., 2006).

Some TI cattle show rather ***mild clinical signs***, such as fever, leucopenia as well as depression, anorexia, oculo-nasal discharge, oral lesions, diarrhoea, and decreased milk production. Obviously, also these animals can undergo secondary infections.

More rarely, transient BVDV infection causes ***peracute outbreaks*** with fever, pneumonia, sudden death, and high mortality rates. These outbreaks have occurred mainly in North America (Corapi et al., 1990; Pellerin et al., 1994; Carman et al., 1998) but a few European cases have been described as well (David et al., 1994; Amiridis et al., 2004; Doll and Holsteg, 2013; Moen, 2013). The three predominant symptoms of experimental acute severe BVDV infection are: fever, low white blood cell count, and low blood platelet count (Walz et

al., 1999; Ridpath et al., 2006). Haemorrhagic Disease (HD) is often one of the clinical features of acute outbreaks of BVD. Potential clinical signs are bloody diarrhoea, epistaxis, hyphema (blood in anterior eye chamber), bleeding from injection sites, pyrexia, and death. Most HD cases were associated with BVDV2 (Evermann and Barington, 2005) and thrombocytopenia is rarely associated with BVDV1 (Blanchard et al., 2010).

The most complex group of clinical presentations encompasses those caused indirectly by immunosuppression during transient infection. By concomitant infection with BVDV, the symptoms of other infectious diseases can become more severe and treatment results disappointing. Co-infections cause increased economic losses by important diseases like Bovine Respiratory Disease (BRD), salmonellosis, mastitis, and other infectious diseases. The effect of co-infection can both be a consequence of the immunosuppression that accompanies acute BVDV infections and predisposes to secondary infections, and of increased virulence of other pathogens caused by synergy in co-infections (Ridpath, 2010b).

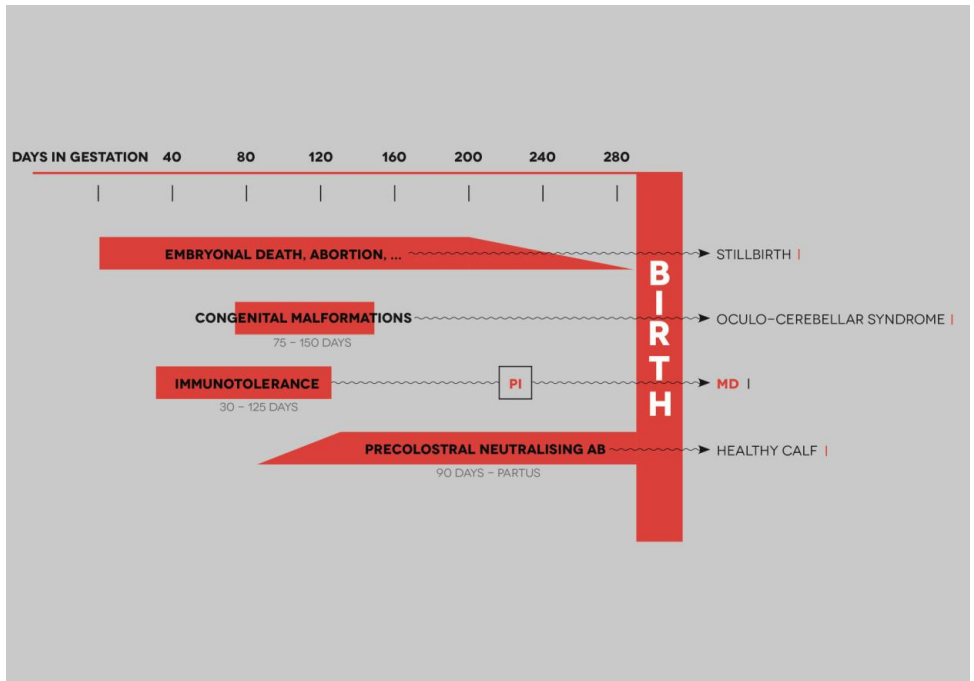
Despite the name “BVDV” it is generally accepted that most of the economic damage by BVDV infection is caused by reproductive disorders and respiratory disease. BVDV plays a role in the BRD syndrome (Moerman et al., 1994; Richer et al., 1998; Martin et al., 1999; Fulton et al., 2000a; O’Connor et al., 2001; Fulton et al., 2002; Booker et al., 2008; Pardon et al., 2012). A synergistic effect has been shown for co-infections of BVDV and bovine respiratory syncytial virus (Brodersen and Kelling, 1998; Brodersen and Kelling, 1999; Liu et al., 1999), infectious bovine rhinotracheitis virus (Castrucci et al., 1992), Parainfluenza-3 virus (Aly et al., 2003), *Mycoplasma bovis* (Haines et al., 2001; Shariar et al., 2002), and *Mannheimia haemolytica* (Booker et al., 2008). Although the effect of co-infection of BVDV with other pathogens is believed to be the most important effect of BVDV on respiratory disease (Ridpath, 2010b), BVDV can also cause infections in the respiratory tract of cattle as a single agent (Baszler et al., 1995; Baule et al, 2001; Liebler-Tenorio et al., 2002). Under experimental conditions however, infections with most strains of BVDV alone pass without clinical signs or with mild disease, but respiratory disease can be one of the clinical results. Still it remains difficult to distinguish between the direct effect of BVDV on the respiratory tract and the effect of secondary infections caused by primary BVDV infection (Ridpath, 2010b).

Concomitant BVDV infection can also aggravate the outcome of *enteric diseases* such as salmonellosis (Daly and Neiger, 2008), paratuberculosis (Thoen and Waite, 1990) and rotavirus infection (Kelling et al., 2002).

**Mastitis** is a very important infectious disease in cattle. Transient BVDV infections are believed to facilitate new or aggravate secondary intramammary infections with mastitis pathogens causing clinical or subclinical mastitis. Under field conditions, this issue has previously been investigated (Niskanen et al., 1995). With regard to the potential positive association between BVDV infections and bulk milk somatic cell count, some researchers have found a relationship (Lindberg and Emanuelson, 1997; Beaudreau et al., 2005; Voges et al., 2008), others have not (Waage, 2000; Berends et al., 2008).

## 2. Acute infection in pregnant cattle

As shown in Figure 2, the outcome of foetal BVDV infection depends on the stage of gestation.



**Figure 2.** Schematic representation of foetal BVDV infection and its consequences. Adapted from Dirksen, 2002.

In susceptible pregnant cattle, BVDV infection can cause the same clinical features as in non-pregnant cattle. In addition, viraemia can easily lead to placental infection (Frederiksen et al., 1999). Abortion or early embryonic death may occur from the resulting placentitis. As a result, and by negative effects of BVDV on ovarian function, the **conception rate** of a herd can decrease substantially (Houe, 2003). Between days 75 and 150 of gestation BVDV infection can cause **congenital defects** of the foetus (Blanchard et al., 2010) with the same clinical appearances for both PI and immunocompetent foetuses. Since the risk period for congenital malformations (day 75 until 150) does not entirely coincide with the risk period for becoming PI (day 30 until 125), it has to be emphasized that congenital defects can exist in both PI and TI calves (Figure 2). Finally, foetal infection can also cause **birth of weak calves** (Blanchard et al, 2010)

From about 90 days of pregnancy onwards, some foetuses can become immunocompetent, others remain susceptible to PI until day 125 of pregnancy. Infection with BVDV at that stage and later in gestation will not result in PI calves, but the foetus can become TI with BVDV. Although most foetuses infected after they became immunocompetent will be born as normal calves without symptoms, they still are susceptible to developing congenital malformations. Most frequently reported are growth retardation and the oculo-cerebellar syndrome. Characteristic for the latter are loss of equilibrium or eye disorders such as blindness by retinal atrophy or dysplasia, microphthalmia, cataract, and opaque spots on the cornea (Baker, 1995). Other congenital disorders have been attributed to intrauterine BVDV infection: hydrocephalus, hypomyelinogenesis, thymic hypoplasia, pulmonary hypoplasia, alopecia, hypotrichosis, brachygnathism, arthrogryposis, and other skeletal abnormalities (Baker, 1995; Blanchard et al., 2010). Furthermore, it has been reported that calves infected in late gestation can suffer from this BVDV infection after birth. During the neonatal period they are at increased risk of severe illness compared with calves without congenital BVDV infection (Barber et al., 1985; Munoz-Zanzi et al., 2003).

The most important effect of acute infection of pregnant cattle is the fact that these dams most likely will give birth to a PI calf when the foetus has been infected between days 30 and 125 of gestation.

### ***Persistent infection***

In the case of persistent infection, the clinical signs can be divided into MD and non-MD cases. Clear clinical differentiation between MD and non-MD cases is not always possible, but simultaneous isolation of both biotypes of BVDV, ncp and cp, proves an animal to suffer from MD (Bachofen et al., 2010).

MD is a sporadic syndrome that only occurs in PI animals, usually from the age of three months onwards (Houe, 1992a; Bachofen et al., 2010). Although MD generally occurs before two years of age, up to 10% of PI cattle detected in the Swiss eradication programme were older than 2 years (Presi et al., 2011). The MD syndrome is less frequently reported than it used to be, probably because PI cattle currently are more often detected and culled before contracting MD (Lindberg and Houe, 2005). Not all PI cattle die from MD, as they may be slaughtered as bull calves, or die from non-MD-related causes. Some may survive until adulthood, but are culled because of poor performance. The clinical signs of MD are fever, anorexia, tachycardia, polypnea, and profuse watery diarrhoea, often characterized by the presence of mucosal shreds, fibrinous casts, and blood (Evermann and Barrington, 2005). Tenesmus often accompanies the diarrhoea. Furthermore, erosions and ulcers may be present on the tongue, palate, and gingiva. Oral papillae may be blunted and haemorrhagic. Sometimes epithelial erosions are found in the interdigital regions, and coronary bands. Blood analysis often reveals neutropenia and thrombocytopenia. The mortality rate approaches 100%, but some animals survive, only to suffer from chronic MD (Loehr et al., 1998).

PI-cattle that have not (yet) developed MD can look healthy without symptoms, or show growth retardation (Bachofen et al., 2010). They also can suffer chronic or recurrent intestinal and/or pulmonary symptoms (Loneragan et al., 2005; Ridpath, 2010b) and, occasionally, dermatological, neurological or haematological disorders (Bachofen et al., 2010). Similar to TI cattle, PI animals can suffer from HD and congenital malformations.

## **Economic consequences of BVDV infection**

### *Economic consequences at the herd level*

The economic effect of BVDV infection highly depends on the risk of new infections and on the strain of virus involved (Houe, 1999). Most losses are a result of transient infections (Ridpath, 2005) and BVDV can affect the economic results of a herd in different ways (Houe, 2003; Gunn et al, 2004; Evermann and Barrington, 2005; Fourichon et al., 2005):

- Immunosuppressive effects of the virus at postnatal infection;
- Effects of abortion and stillbirth;
- Effects of postnatal infections on cattle at reproductive age and delayed rebreeding;
- Congenital infections leading to calves with congenital defects and growth retardation;
- Congenital infections resulting in PI calves;
- Long-term survivability of PI heifers leading to future PI calves, mortality, and increased replacement costs.

The economic damage caused by BVDV can vary substantially because of the multiplicity and variations in severity of the symptoms mentioned above, and the interaction with other pathogens. Furthermore, management factors and structure of the herd play an important role. For example, the outcome of BVDV infection can be disastrous in herds with a concentrated seasonal calving pattern. In contrast, small herds can become self-cleared of the infection with hardly any damage (Ståhl et al., 2008). As a result, a “herd level BVDV outbreak” is hard to define and losses are difficult to calculate (Lindberg et al., 2006). Moreover, some researchers are very doubtful about the existence of true subclinical BVDV infection (Evermann and Barrington, 2005). Nevertheless, calculation models have been worked out to estimate the economic consequences of the disease at the herd level. Most of the studies were focused on dairy herds. It was indicated that the costs of a BVDV infection vary between 21 and 135€ per dairy cow per year in case of “classical” outbreaks, where most transient infections go unnoticed, and most losses are due to reproductive disorders and PI animals (Fourichon et al., 2005; Valle et al., 2005; Lindberg et al., 2006). Outbreaks where BVDV infection stimulates concurrent infections, or with highly virulent strains have been

estimated to cost more than 340€ per dairy cow in the outbreak herd (Lindberg et al., 2006). Losses in Scottish beef herds were estimated at 58€ per cow per year (Gunn et al., 2004).

#### *Economic consequences at the regional or national level*

In countries where BVDV infection is systematically traced for eradication purposes, it has been evidenced that ongoing BVDV infection is often associated with discrete non-specific clinical signs (Fourichon et al., 2005). Since these discrete effects are often not included in the calculations, losses may be underestimated (Valle et al., 2005) and obtaining exact figures of the consequences for the cattle industry is difficult.

Overall, most estimations of the losses at the national level range between 7.5 and 30 million € per million calvings (Houe, 2003). In Norway, BVDV was almost eradicated after 10 years of systematic BVDV control. At that moment, the profits of the BVDV eradication were estimated 17.8 million Euros for the entire country, whereas the costs of the eradication programme were 6.7 million Euros (Valle et al., 2005). The authors suggested that the profits might have been underestimated, because of the often low-grade chronic effects of BVDV being spread out over time and therefore hard to identify as effects of BVDV. The annualised benefits of eradicating BVDV from Ireland have been predicted to exceed the costs by a factor of 5 in the beef sector and a factor of 14 in the dairy sector. The corresponding pay-back periods were 1.2 and 0.5 years respectively (Stott et al., 2012).

The beneficial effects of BVDV control may exceed the direct economic effects. Public funding to support systematic BVDV control programmes can be justified on the basis of expected wider social benefits, such as animal welfare and reduction of antimicrobial use (Valle et al., 2005; Lindberg et al., 2006; Stott and Gunn, 2008; Stott et al., 2012). Currently, several European countries have started programmes to limit the use of antibiotics in cattle and other livestock. BVD control helps to achieve this objective, as reducing the clinical and subclinical effects of BVD has public health benefits by reduced veterinary treatments (Saatkamp et al., 2005). Furthermore, large scale control schemes may have beneficial effects on the national surveillance capacity in that they can both be a driver for developing more cost-effective infrastructure for surveillance, and may serve as a basic source of samples for running other analyses.

## Diagnosis

Because so many different types of clinical presentation are associated with BVDV infection, a diagnosis on the basis of history, clinical signs, and post mortem examination can only be considered presumptive. Accurate and definitive detection of BVDV infections depends on laboratory diagnosis (Goyal, 2005) .

### Tests commonly used in BVDV control

A variety of test methods is available, but only a few tests are routinely used in BVDV control at the herd, regional, or national level: antibody-Enzyme Linked ImmunoSorbent Assays (ELISA), antigen-ELISA, and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) tests.

The presence of antibodies can be demonstrated by ELISA. Indirect antibody ELISA can be used for semi-quantitative measures on serum, individual milk and bulk milk samples (Houe et al., 2006).

The tests commonly used for detecting presence of BVDV are BVDV antigen ELISA and RT-PCR. BVDV antigen ELISAs are appropriate for testing individual samples of blood, serum, milk, and ear tissue. The ELISA actually used in Belgium is an ELISA that detects the E<sup>tns</sup> glycoprotein, a part of the envelope of BVDV. Presence of BVDV-RNA can be demonstrated by RT-PCR analysis. The latter test is also suitable for detecting BVDV-RNA in matrixes that might contain low quantities of virus such as bulk milk, pooled samples of serum and blood, or other biological material. For using the RT-PCR as a quantitative test, the cycle threshold (C<sub>t</sub>) value can be measured as an indicator of the number of viral copies present in the sample. In this way the PI status could be distinguished from the TI status (Hanon et al., 2012). It is still under discussion if this method may be used to diagnose PI animals on the basis of a single blood sample. Therefore, cut-off values have to be determined.

The performance of the currently available diagnostic tests for BVDV is good (Letellier and Kerkhofs, 2003; Mars and Van Maanen, 2005; Sandvik et al., 2005), but there is room for improvement. Nevertheless, sensitivity and specificity may vary depending on the aim the tests are used for (Houe et al., 2006).

The so called “diagnostic gap” is one of the potential causes of false negative results when testing calves under two months of age for BVDV (Fux and Wolf, 2012). The presence of high titres of maternal antibodies in such neonatal calves may influence the results of virological tests, and cause false negatives in antigen ELISA performed on individual blood and serum samples, and even in PCR tests on pooled blood samples (Martin Beer, personal communication). Albeit rarely, false negative results caused by maternal antibodies can also occur when using antigen ELISA on ear notch samples (Presi et al., 2011; Fux and Wolf, 2012). The sensitivity of an E<sup>ms</sup> antigen-ELISA used on ear tissue in a regional control programme in Austria was estimated 97.3% (Oettl et al., 2010). Only PCR tests are not disturbed by the presence of colostral antibodies when used on individual blood, individual serum and ear notch samples. On the other hand, also false positive results cannot be excluded, as some tests detect other pestiviruses such as BDV (Letellier and Kerkhofs, 2003; Cranwell et al., 2007; McFadden et al., 2012).

### **Practical applications of the tests in BVDV control**

Despite the availability of highly sensitive and highly specific diagnostic techniques, the suitability of a diagnostic test in any given phase of a control programme is largely dependent on the specific objectives of that particular phase (Houe et al., 2006).

Laboratory techniques are strategically used to achieve three main objectives (Houe et al., 2006):

- 1 Initial tests to allocate a herd status;
- 2 Follow-up tests to identify BVDV infected animals in infected herds;
- 3 Continued monitoring to confirm BVDV-free status.

For predicting the presence of PI animals in a herd, testing of bulk milk by an indirect antibody-ELISA can be used (Houe et al., 2006). As the level of BVDV-antibody in bulk milk correlates well with the prevalence of seropositive cattle, the sensitivity of this method is close to 1 in herds not vaccinated for BVDV, whereas the specificity is lower. Despite the high sensitivity, false negative results will occasionally be obtained for recently infected herds. A repeated bulk milk test a few months later will solve the problem (Houe et al., 2006). Another option for determination of herd statuses is testing cohorts of young stock, using the so called “spot test”. The spot test is restricted to blood samples from five to ten animals between eight and twelve months old to be examined for antibodies to BVDV.

Moreover, this test is the tool of choice to obtain more certainty about the presence of a PI animal in the herd when there is a suspicion of BVDV infection (Houe 1992b; Pillars and Grooms, 2002; Houe et al., 2006; Booth and Brownlie, 2012). Combining bulk milk serology with a spot test enhances the sensitivity and specificity to diagnose a PI animal in a herd, since most PI cattle are younger than 2 years and it can take some time before these young animals infect the lactating animals or become lactating themselves. Furthermore, both above-mentioned serological methods become more reliable by repeating them (Houe et al., 2006; Booth and Brownlie, 2012). Serological testing of first calvers' milk can be used to obtain additional information on the herd status, especially in areas where BVD is endemic and where, as a result, serological testing of bulk milk will be positive in most herds (Valle et al., 2005).

RT-PCR and antigen-ELISA are appropriate methods for identifying BVDV infected animals. Still, because of the high cost, a RT-PCR test is only sporadically used for testing individual animals in the field. In contrast, RT-PCR tests on pooled blood samples and bulk tank milk are popular for detection of PI animals at the herd level. If the pool is positive, the cheaper antigen-ELISA is used on the individual samples of the pool to identify the PI animal(s) (Hanon et al., 2012).

The methods of continuous monitoring used to confirm BVDV-free status are essentially the same as those used to establish initial herd status (Houe et al., 2006). However, one has to realize that herds that have recently eradicated BVDV still have high BVDV-antibody titres in the bulk milk due to the persistence of BVDV-antibodies in milk following natural infection (Houe et al., 2006; Booth et al., 2013b). Therefore, during the period shortly after removal of PI animals from a herd, serological testing of young stock should be preferred to bulk milk serology (Houe et al., 2006).

Antigen-ELISA are also used for individual testing at suspicion of BVD based on clinical presentations, or for testing small groups of cattle. Paired sera can be tested by antibody-ELISA to diagnose recent viraemia in individual cattle.

Correct interpretation of the results of all tests is highly important for the diagnosis. For instance, it is important to know that a PCR positive bulk tank milk sample is highly reliable for detection of a PI animal among the lactating cattle, but it has not been proven that it is suitable for detecting the presence of a few TI lactating cows or heifers (Drew et al., 1999;

Renshaw et al., 2000). Furthermore, samples from individual cattle can be RT-PCR positive during an extended period after infection but this does not always mean that active virus is still present (Givens et al., 2009). To determine the PI status of an animal, two samplings at a three week interval are needed for demonstrating persistent viraemia. As it might produce false positive results, RT-PCR has to be excluded when testing for the second time, in particular when the test is meant for legal use.

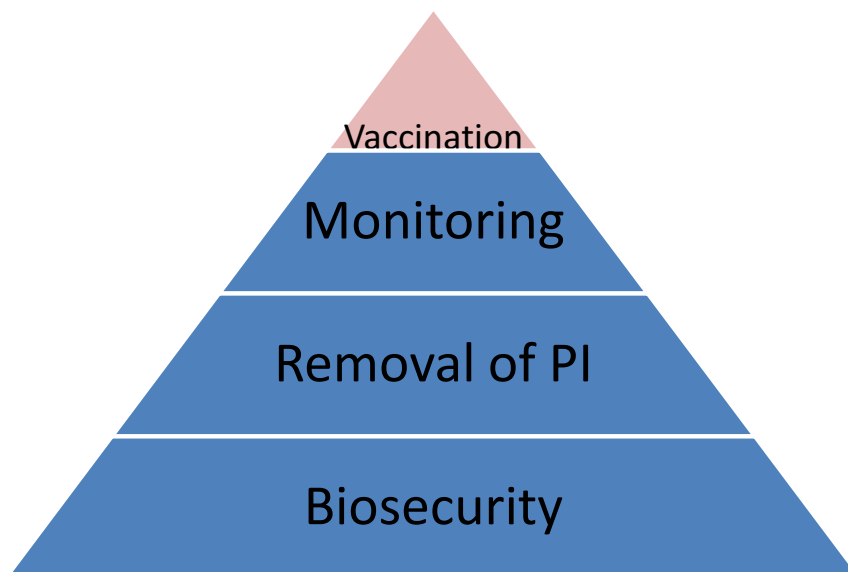
### **Post mortem examination**

MD can be diagnosed by post mortem macroscopic and histologic examination. Typical lesions of MD are necrotizing ulcers and erosions throughout the gastrointestinal tract, necrosis and haemorrhages in the Peyer's patches (Evermann and Barrington, 2005).

PI animals not suffering from MD cannot be detected by routine histopathology. Importantly, viral antigens are weakly expressed in PI animals in tissues typically part of a post mortem exam (Dubovi, 2013). Therefore, it is important to select the appropriate tissue and test to diagnose BVDV infection. Tissues of choice for diagnosis by immunohistochemistry or virus isolation are tonsils, retropharyngeal lymph nodes, mesenteric lymph nodes, ileal Peyer's patch, skin, and spleen (Liebler-Tenorio et al., 2006).

### **Control**

Every BVDV control programme, be it at the herd, regional, or national level, has to consist of a combination of different measures (Ridpath, 2013). The three pillars on which each programme should be based are: biosecurity, detection and eradication of PI cattle, and monitoring (Figure 3). Vaccination can complete the programme as a potential fourth component (Lindberg and Houe, 2005).

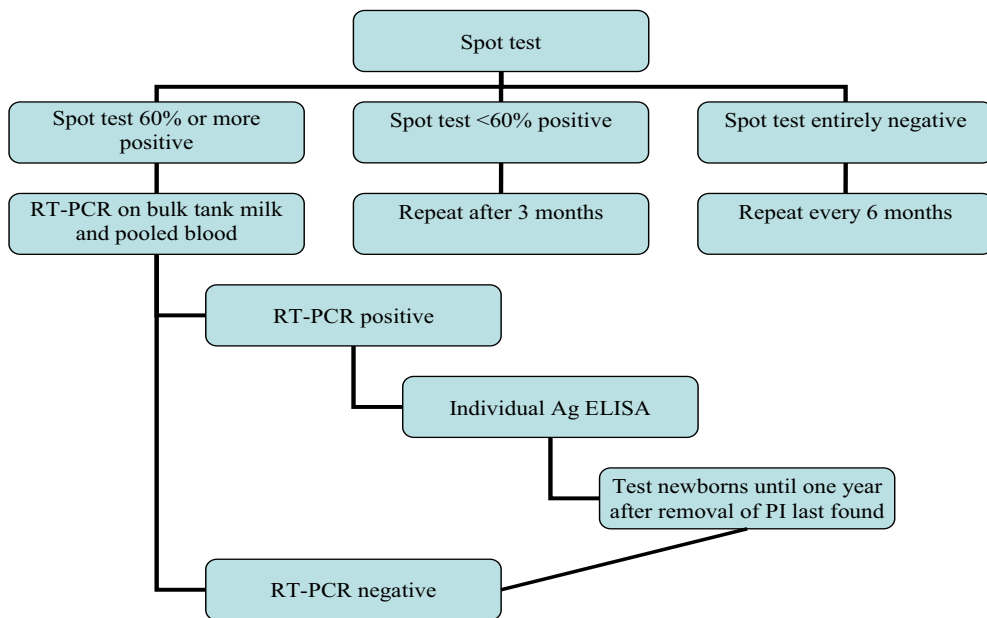


**Figure 3.** The three principles of systematic BVDV control as stated by Lindberg and Houe (2005), with vaccination as an optional fourth element.

As the probability for transmission of the virus by the continuously shedding PI animals is very high, and the duration of the infectious period is lifelong, it is clear that PI animals are key to successful BVDV control. They have to be detected and removed, and generation of new PI calves has to be interrupted by preventing foetal infection in early gestation (Lindberg and Houe, 2005). Nevertheless, it has been stated in some studies that TI cattle also might play a role in maintaining the BVDV infection (Moerman et al., 1993; Moen et al., 2005). Such claims however, have to be supported by thorough evidence to prove that it is not due to insufficient detection of PI animals (Lindberg and Houe, 2005).

### **Control at the herd level**

In Belgium, where there is no orchestrated regional or national approach to BVDV control in place today, voluntary control is carried out at the herd level. Figure 4 presents the strategy recommended by the Belgian Animal Health Services and Faculties of veterinary medicine.



**Figure 4.** Herd level tracing procedure for persistently BVDV infected animals as recommended by Belgian Animal Health Services and Faculties of veterinary medicine. The spot test consists of blood samples from 5-10 cattle between 8 and 12 months old, to be examined for antibodies to BVDV.

When for some reason a herd is suspected of infection, or when the farmer wants to know the BVDV status of his herd, blood samples are collected for a spot test (Houe, 1994). It is a useful tool for the veterinarian to convince the client to start screening for PI animals. When 60 % of the animals included in the spot test are seropositive, the presence of one or more PI animals in the herd is very likely (Houe, 1994; Houe et al., 2006). In contrast, when fewer than 60 % of the animals are seropositive, the practitioner will explain to the client that possibly no PI animals will be found in the herd. Relying on this information, the owner can decide whether to start detecting PI animals. If not, the spot test should be repeated 3 months later.

Detection of PI animals commences by testing every animal in the herd, using whole blood or serum and bulk tank milk samples. The blood samples are gathered into pools to be tested by RT-PCR. The number of samples in a pool depends of the sample matrix and the RT-PCR test used. The bulk tank milk sample is also tested by RT-PCR (Letellier and Kerkhofs, 2003). Only a fraction of each blood sample is used for RT-PCR testing. If a pool or a bulk tank milk sample tests positive, the remaining part of the blood sample of every individual belonging to that pool is investigated by an antigen ELISA to detect the viraemic animal(s) (Mars and van Maanen, 2005; Hanon et al., 2012). In the case of a positive bulk tank milk sample a blood sample is collected from each cow or heifer contributing to the bulk milk sample. When an individual test is BVDV positive, positive animals are tested again three weeks later to confirm persistent viraemia.

Irrespective of the result, every newborn calf is tested for persistent viraemia from the day of whole herd sampling onwards until one year after removal of the last PI animal identified. As soon as the first BVDV carriers have been removed from the farm, vaccination of all cattle older than one year is recommended, as well as continued monitoring, which consists of conducting a serological spot test at 6 month intervals.

### **Control at the regional/national level**

In countries without systematic BVDV control at the regional or national level, BVDV infection has continued to cause widespread disease and reproductive losses (O'Rourke, 2002; Ridpath, 2012). In contrast, the systematic national programmes in Scandinavia (Moennig et al., 2005; Løken and Nyberg, 2013) and Switzerland (Presi et al., 2011) were successful. Since ear notch tests became available recently, more countries have followed, implementing a BVDV control programme based on ear notch sampling.

When the Scandinavian countries started their control programmes at the end of the former century, the ear notch test was not yet available. Therefore, these programmes were based on initial antibody tests, followed by virus tests when a herd was suspected of housing PI cattle. The status of a herd was checked by regular spot tests and bulk milk testing (Løken and Nyberg, 2013). Once there was a suspicion of BVDV circulation in a herd, PI cattle were traced. A vaccination ban was part of the regulations, since vaccine antibodies produce (false) positive results in serological tests and, as a result, can cause interference with surveillance tests. Austria started control programmes based on the Scandinavian method (Rossmann et

al, 2010). Switzerland imposed testing of all cattle young and old and a vaccination ban (Presi et al., 2011). In Ireland, Germany and Bolzano (Italy) newborn calves are tested and vaccination is allowed (Barret et al., 2011; Tavella et al., 2012). In Scotland different schemes of control are permitted, depending on the BVDV status of the herd (Voas, 2012). In some programmes PI animals are not allowed to be transported out of the herd and restrictions are implemented for suspected herds. Euthanasia or slaughter of PI cattle is not always obligatory. In all these countries or regions the importance of spreading information to farmers and veterinarians has been emphasized.

Obviously, when testing is implemented with the intention of making herds free of the virus, it is important to prevent re-infection of herds. As introduction of a PI animal or a dam carrying a PI foetus is the most important risk of infection of a herd (Lindberg et al., 2006; Dubovi, 2013), preventing movements of such animals must be the core of any eradication scheme. The current national programmes (Germany, Ireland, Switzerland) rely on ear notch testing at birth. Once an animal is negative, it is considered non-PI for the rest of its life, although false negative ear notch tests are possible (Fux and Wolf, 2012).

### **The role of vaccination in BVDV control**

Vaccines against BVDV exist since 1964 (Deregt, 2005). Both Modified Live Virus (MLV) and inactivated vaccines are available, but for the moment, no MLV vaccines are registered in Belgium. One particular BVDV vaccine exhibited Differentiating Infected from Vaccinated Animals (DIVA) properties, but only if combined with one particular BVDV antibody ELISA-test (Makoschey et al., 2007). Nevertheless, a true DIVA vaccine for BVDV is not available at the time of submission of this thesis. As a result, vaccine antibodies can cause false positive results in serological BVDV-tests. Most vaccines contain BVDV1. Vaccines can induce T cell responses and antibodies to multiple BVDV subtypes, but antibody titres are generally higher to the vaccine strain and to strains belonging to the same genotype or subgenotype (Fulton and Burge, 2000). For that reason, vaccines based on BVDV1 may not protect against BVDV2 infection (Brock and Cortese, 2001).

A vaccination programme will not prevent all infections in individual animals (Ridpath, 2013). For that reason, BVDV vaccination cannot, on its own, eliminate BVDV from populations (O' Rourke, 2002; Lindberg and Houe, 2005; Ridpath, 2010a; Ridpath, 2013). Moreover, it is often the case that due to inappropriate use of the vaccine only partial

protection is achieved (Meadows, 2010). What vaccination can do is to reduce the incidence of acute and persistent infections in a herd or population. When used as a supplementary measure of biosecurity, in combination with detection of PI cattle and monitoring, vaccination can play a role in BVDV control, because it is effective in reducing the spread of BVDV.

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## Chapter 2

### **A**ims of the Thesis



Because of the ever-changing clinical presentations of BVD, it is difficult to convince farmers that the infections have an effect at the herd level. Most of them are even unaware of the presence of BVDV on their farm and, consequently, BVDV infection is often diagnosed only at a late stage. Experiences in the field indicate that farmers and veterinary practitioners continue to rely on clinical signs to detect BVDV. Moreover, when evaluating the farms with BVDV control, it often becomes apparent that the methods are inadequate.

This doctoral thesis is aimed at addressing issues of misconception that hamper the advancement of BVDV control in a Belgian context, and thereby providing a greater understanding regarding constraints that may be present in other regions too.

The specific aims of this thesis are:

- To illustrate that a clinical diagnosis of BVD is difficult because of the diverse clinical manifestations. *Exceptional pathology and clinical presentations* associated with BVDV infections both in adult cattle and neonatal calves will be documented in three case reports (**Chapters 3.1, 3.2, and 3.3**).
- To highlight a subclinical form of BVDV infection through looking for differences in bulk milk somatic cell counts between BVDV infected and non-infected herds (**Chapter 4**).
- To assess whether *BVDV management in Belgium* sufficiently implements two essential elements of efficient BVDV control: detection of PI animals and monitoring (**Chapter 5**).

Despite the availability of several BVDV vaccines and voluntary programmes to control the virus, BVDV infections in cattle herds continue to cause substantial economic damage to the cattle industry worldwide. Only those countries where a national or regional eradication programme has been brought into force, have succeeded in controlling BVDV. Therefore, a BVDV eradication plan has been designed for Belgium. In the general discussion of this thesis (**Chapter 6**), some recommendations are made for the future Belgian plan to control BVD and eventually eradicate BVDV by bringing together current general knowledge and findings originating from the present thesis.



**A**typical clinical cases associated with bovine viral  
**diarrhoea virus infection**



**S**evere disease in neonatal calves infected with  
**cytopathic bovine viral diarrhoea**

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All bovine viral diarrhoea virus (BVDV) strains can appear both as the cytopathic (cp) and the non-cytopathic (ncp) biotype. Although both can infect the foetus, only ncp BVDV is able to cause persistent infection (Brownlie and others, 1989). Furthermore, all offspring of persistently infected (PI) dams are PI (Liebler-Tenorio, 2005). In the field, cp BVDV strains are typically isolated from cases of mucosal disease (MD) or associated with the use of modified live vaccine (Ridpath, 2005). In cases of MD, both cp and ncp BVDV are present in the same animal. The incubation period takes two to three weeks in early-onset MD, but can be extended by months or years in late-onset MD (Brownlie and Clarke, 1993). MD usually affects animals aged six to 18 months, although occasionally it has been reported in calves of a few weeks old (Brownlie, 2004). This short communication reports the detection of cp BVDV in a 10-day-old calf during an outbreak of haemorrhagic diarrhoea.

Between 2004 and 2007, sporadic cases of non-fatal haemorrhagic diarrhoea in neonatal calves occurred in a herd consisting of 60 Holstein-Friesian and 150 Belgian blue breeding cattle and their offspring. In 2007, the number of affected newborn calves increased, and two youngsters died at the age of five and six months from neurological disease. Because these animals were BVDV antigen ELISA positive at post-mortem examination, vaccination with two inactivated BVDV vaccines was begun. A farm-wide screening programme for PI animals was not carried out. All calves were routinely vaccinated at three months of age against bovine respiratory syncytial virus (BRSV), parainfluenza type 3 (PI3) virus, and BVDV (Risposal Trivalent BRSV-PI3-BVD; Pfizer Animal Health), followed by a booster vaccination one month later. Furthermore, female young stock between eight and 24 months of age were vaccinated annually in one session with Pregsure BVD (Pfizer Animal Health). The colostrum management consisted of administering one litre of colostrum immediately after birth. All calves also received an oral solution of bovine antibodies against *Escherichia coli* (Locatim; Biokema Anstalt). From then on their ration consisted of milk only. Between January and May 2009, almost all neonatal calves were affected with haemorrhagic diarrhoea. One to two weeks after birth, the calves started showing fever as high as 40°C and haemorrhagic diarrhoea. Tests for coronavirus, rotavirus, and enterotoxigenic *Escherichia coli* were negative. When *Cryptosporidium parvum* was detected in faeces, all newborns received halofuginone (Halocur; Intervet) and the haemorrhagic diarrhoea resolved. However, calves still became ill, showing pneumonia, stomatitis, nasal discharge, excessive salivation, fever (40°C), and weight loss. Five calves died during this outbreak, but none of

these was examined post mortem. In May 2009, veterinarians from the Faculty of Veterinary Medicine, Ghent University, visited the farm. At that time, three calves from a group of seven, aged between 11 and 30 days, had been showing clinical signs since one or two weeks after birth. One calf was lethargic and had a diffuse, non-ulcerative stomatitis. Another calf suffered from pneumonia and a similar stomatitis. A third calf had survived a period of haemorrhagic diarrhoea, and was showing pneumonia as the only clinical sign at the time of the visit, which was 14 days after the onset of clinical signs. Body temperatures ranged between 39.8 and 40°C. Except for reduced fertility in adult cattle, no other signs of any disease were noticed.

Blood samples from two healthy calves aged two and four days showed low IgG concentrations (4 and 8 mg/ml) in a semi-quantitative glutaraldehyde test, which indicated failure of passive transfer (Weaver and others, 2000). A bulk milk sample and blood samples from six calves were tested for BVDV by real-time RT-PCR as described previously (Letellier and Kerkhofs, 2003). An endogenous extraction/reaction control targeting bovine  $\beta$ -actin mRNA was performed on all samples. Validation of the test samples was performed by normalization of the BVDV results with the  $\beta$ -actin results using the method described by Toussaint and others (2007). No BVDV RNA was detected in the milk sample, but BVDV RNA was detected in four of the six blood samples collected from calves. Two BVDV RNA positive calves (calves 1 and 2) were showing signs of illness at sampling. When comparing the normalized values, the blood of a 10-day-old calf (calf 3) contained at least  $10^4$  RNA copies more than the samples from the other positive calves. Subsequent virus isolation detected cytopathic BVDV in the sample from calf 3; no virus was isolated from the samples of the remaining three calves. The isolated cytopathic BVDV was genotyped as BVDV1b by RT-PCR and sequencing (Letellier and others, 1999).

Subsequently, all cattle in the herd older than two months were tested by antigen ELISA (BVDV Ag/Serum Plus Test, Idexx), but only one antigen-ELISA positive animal was found: the dam of calf 3. As this cow was the only viraemic animal detected and proved to be a contemporary of the two young stock with neurological signs that had been found to be BVDV viraemic in 2007, the farmer and his veterinarian suspected it of being PI. Consequently, this cow and calf 3 were removed from the herd immediately. No later sampling was done, so the PI status was implied rather than confirmed absolutely.

Nevertheless, this was a fair interpretation of these results. It was extremely likely that both the dam and its calf were PI. Both animals were apparently healthy.

If PI animals are not removed, BVDV can continue to spread in vaccinated herds (Fulton and others, 2005; Ridpath, 2010). In the present case, BVDV circulation may have been further facilitated by the cows not being vaccinated. Moreover, with better maternal immunity, the calves might have stayed uninfected by BVDV.

Because MD is uncommon in calves younger than one month of age (Torgerson and others, 1989; Evermann and Barrington, 2005; Bachofen and others, 2010), the presence of cytopathic BVDV in a 10-day-old calf is considered to be an extraordinary finding. As there are no modified live vaccines registered in Belgium, the presence of vaccine cytopathic BVDV could be excluded in this case. Given that the only other PI animal detected was the calf's dam, which did not have MD, it is unlikely that the cytopathic BVDV in calf 3 came from another PI. A third plausible explanation is that the cytopathic virus may have originated from a persisting non-cytopathic biotype through exceptionally early genetic recombination.

Experimentally, MD is described as developing no earlier than two weeks after infection of a non-cytopathic PI animal with a homologous cytopathic strain (Liebler-Tenorio and others, 2000; Liebler-Tenorio, 2005). Therefore, the cytopathic virus can be present before the onset of clinical signs. In the present case, neither persistent infection nor MD could be proven unarguably: the cytopathic BVDV-positive calf and his dam were removed immediately and non-cytopathic BVDV was not detected together with cytopathic BVDV in one animal.

This report shows that cytopathic BVDV can be present in neonatal calves.

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**S**pontaneous bleeding in a neonatal calf persistently  
infected with bovine viral diarrhoea 1b

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## **A**bstract

A calf developed skin bleeding on the second day of its life. It was referred to the clinic on suspicion of Bovine Neonatal Pancytopenia (BNP). Haematology showed extreme thrombocytopenia, a moderate anaemia, but no leucopenia. A PCR test on a heparinised blood sample was Bovine Viral Diarrhoea virus (BVDV) positive, as were the two BVDV antigen ELISA performed 3 and 10 weeks later. Non-cytopathic BVDV type 1b was isolated from the blood. The persistently (PI) BVDV infected calf recovered from haemorrhagic disease and continued to be healthy until euthanasia at 11 weeks of age. On the basis of the case history, BNP could be excluded from the differential diagnosis. This case illustrates that haemorrhagic disease is not exclusively associated with BVDV type 2 and that the clinical signs in neonatal calves infected with BVDV1b can be identical with the clinical presentation of BNP.

## **I**ntroduction

Infection of cattle with bovine viral diarrhoea virus (BVDV) can have various clinical presentations, from non-clinical or mild disease to outbreaks of acute, severe disease with high mortality. Haemorrhagic disease (HD) is one of the potential clinical features, characterized by thrombocytopenia and an increased susceptibility to bleeding (Walz and others 1999). Although severe outbreaks of acute BVDV infection are commonly termed “haemorrhagic syndrome”, haemorrhages were not always among the clinical signs of these outbreaks (Carman and others 1998, Ridpath and Fulton 2009). In contrast to American BVDV type 2 strains, European BVDV type 2 strains have rarely been associated with HD (Ridpath 2005, Vilcek 2005). Recently, a North American BVDV type 2 strain was detected in Belgium, but HD was not among the clinical presentations (Letellier and others 2010). Furthermore, most cases of HD are associated with transient infections and HD in persistently infected (PI) cattle rarely has been reported (Dabak and others 2007). Calves of the latter case were older than 1.5 months and died of mucosal disease (MD). Here we report a case of spontaneous skin bleeding in a 2-day-old calf persistently infected with non-cytopathic BVDV type 1b and not suffering from MD.

## Case report

In June 2011, a 2-day-old Belgian Blue calf developed spontaneous skin bleeding and was transferred to the clinic on suspicion of Bovine Neonatal Pancytopenia (BNP). The farmer mentioned an increased frequency of neonatal diarrhoea and respiratory disease among the calves in the previous months, but no haemorrhages had been noticed among other cattle of the mixed beef and dairy herd. The calf was delivered by caesarean section, had received 4 litres of colostrum from its own dam and appeared to be healthy during the first day of life. BVDV vaccination had never been performed in the herd and the dam was homebred.

On arrival the calf was depressed and showed melena and skin bleeding, not only from both ears due to ear tagging, but also on the back and legs. The mucosae were pale and petechiae and submucosal bleedings were found under the tongue and elsewhere on the oral mucosa. The body temperature was 39°C, pulse rate was 80 per minute and respiratory rate was 24 per minute.

Haematology showed extreme thrombocytopenia (0 platelets/L), a moderate anaemia (PCV= 0.23 L/L), but no leucopenia ( $9.5 \times 10^9$ /L). A PCR test performed on a heparinised blood sample taken on arrival was BVDV positive, as were two antigen ELISA on whole blood taken 3 and 10 weeks later (IDEXX BVDV Ag/Serum Plus Test, IDEXX Europe, Hoofddorp, The Netherlands). Non-cytopathic BVDV was detected at virus isolation from a whole blood sample taken on day 52. The isolated strain was genotyped as BVDV type1b by RT-PCR and sequencing (Letellier and others 1999). An EDTA blood sample from the calf's dam was BVDV negative in the same antigen ELISA.

Until day 14, the calf was treated with cefquinome (Cobactan<sup>®</sup> 2.5% w/v; Intervet) to protect it from secondary infections. From day 2 it was housed in strict isolation. Platelets and PCV normalized after 13 and 20 days respectively. The calf had a good appetite and looked healthy until it developed pneumonia with a fever peaking at 40°C at 37 days of age. After treatment with gamithromycin (Zactran 150 mg/ml; Merial) and ketoprofen (Ketofen 10%; Merial) it recovered. From this point until euthanasia on day 77, the calf showed no further clinical signs of disease. The only reason for euthanasia was the persistent BVDV infection. On the farm, haemorrhages have not been recorded in any other stock to date.

## Discussion

As the calf had no initial fever and there were no other symptoms at the same time as the bleeding syndrome, haemorrhagic septicaemia and endotoxaemia could be excluded as potential causes of thrombocytopenia. Toxic agents were not suspected of having caused the haemorrhagia in this 2 days old calf, because plants like field melilot (*Melilotus officinalis*) and bracken fern (*Pteridium aquilinum*) need prolonged intake to cause bleeding syndromes and dicumarol, present in bracken fern and commercial rodenticides, does not lead to bone marrow depletion (Wang et al., 2007). Furthermore, until now thrombocytopaenia has not been described in cattle affected by hereditary bleeding syndromes (Steficek et al., 1993; Meydan et al., 2009; Shiraishi et al., 2002). In 2008, BNP emerged in Europe as a cause of thrombocytopenia and leucopenia in neonatal calves (Pardon and others 2010). In this immunomediated disease allo-antibodies directed to calf leucocytes and bone marrow precursor cells are transferred to the calf through colostrum (Bastian et al., 2011; Bridger et al. 2011; Pardon et al 2011). Although it was demonstrated that the presence of the allo-antibodies in colostrum was associated with vaccination with a particular BVDV vaccine (Sauter-Louis et al., 2012), only a small number of all calves that had received colostrum from vaccinated mothers developed BNP. It is assumed that the latter could be contributed to inherited factors (Deutskens et al., 2011). The present case calf had only received colostrum from its own dam. The cow was born and raised on the farm and BVDV vaccines had never been used in the herd. Moreover, colostrum from other herds never had been administered on this farm. For these reasons BNP could be excluded and persistent infection with BVDV type 1b was considered to be responsible for the thrombocytopenia in the newborn calf.

It has been suggested that some cattle could be viraemic for a longer period than the generally accepted 14 to 21 days (Collins et al., 2009). Therefore, a second blood sampling for antigen-ELISA was carried out on the present case calf 10 weeks after the first, to exclude the possibility of prolonged transient infection. Collins and co-workers found evidence of the presence of BVDV in blood of calves up to 3 months after infection, but these calves were Antigen ELISA negative at that stage. The fact that the present case calf was Antigen-ELISA positive at the second sampling proved that it was PI.

Although persistently infected, the case calf showed two of the three predominant symptoms of experimental acute severe BVDV infection: fever, low white blood cell count

and low platelet count (Walz 1999; Ridpath and others 2006). Nevertheless, the case was exceptional for several reasons. First of all, the bleeding disorder was associated with a BVDV type 1 strain. To the authors' knowledge, this has only been reported by Dabak and co-authors (2007) in older PI calves suffering from MD. Secondly, the present calf was much younger than previously reported for HD and the clinical presentation was indistinguishable from the clinical signs of BNP. Thirdly, the thrombocyte count returned to normal in spite of persistent viraemia. Therefore, a direct effect of the virus on thrombocytes seems unlikely. This finding is in line with the results of a study by Walz and co-workers (2005), who detected no significant difference in platelet counts between cattle PI with BVDV and control cattle. A potential hypothesis for the thrombocytopenia might be removal of virus containing thrombocytes or megakaryocytes after interaction with colostral antibodies, comparable to BNP pathogenesis (Deutskens and others 2011).

## **Conclusion**

This case report illustrates that BVDV1b associated haemorrhages can occur in PI calves younger than one month, not suffering from MD at that stage of infection. As the clinical presentation was the same as for BNP, it is advisable to rule out BVDV infection in suspected cases of BNP.

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**P**eriparturient infection with bovine viral  
diarrhoea virus type 1b causes haemorrhagic proctocolitis  
in a cow

Adapted from :

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Periparturient infection with bovine viral diarrhoea virus type 1b causes  
haemorrhagic proctocolitis in a cow

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## **A**bstract

After three cows of a dairy herd had died from severe haemorrhagic diarrhoea, a fourth sick cow was transported to the clinic. Blood analyses revealed the complete absence of white blood cells, the presence of a type 1b strain of Bovine Viral Diarrhoea Virus (BVDV), and seroconversion to BVDV.

## **I**ntroduction

Although infection with Bovine Viral Diarrhoea Virus (BVDV) usually manifests few obvious symptoms, intra-uterine or postnatal infection with the virus is able to provoke a wide range of symptoms in cattle (Brownlie, 2004). The consequences of intra-uterine infection include early embryonic death and abortion, congenital defects, and birth of persistently infected (PI) calves. Most of these PI cattle die from mucosal disease before they are 2 years old. During their life, they are a contagious source of BVDV infection. Because the virus impairs the immunity of infected animals in PI cattle, as well as at postnatal, transient infection (Chase et al., 2004; Ridpath and Fulton, 2009), BVDV infection can aggravate other diseases or make infected animals more susceptible to other diseases such as bronchopneumonia, diarrhoea, and mastitis (Liebler-Tenorio, 2005; Lundborg et al., 2005; Berends et al., 2008; Daly and Neiger, 2008; Diéguez et al., 2009). Alternatively, some BVDV strains induce direct damage to specific cells and tissues after postnatal infection, causing different syndromes. Postnatal BVDV infection has been described as the primary cause of respiratory disease (Hamers et al., 2000; Baule et al., 2001; Galav et al., 2007), and glomerulonephritis (Galav et al., 2007). Meningoencephalitis has also been reported as a result of a BVDV infection, but the authors could not determine whether the infection was transient or persistent (Blas-Machado et al., 2004).

The occurrence of severe clinical disease during a BVDV infection has been attributed to particular and highly virulent BVDV strains (Bolin and Ridpath, 1992; Pellerin et al., 1994; Baule et al., 2001). Kelling et al. (2002) showed that experimental infection with a virulent strain resulted in severe clinical disease and prolonged viral excretion. Others suggest that the severity of the clinical outcome depends on the degree of viraemia during BVDV infection, as provoked by particular isolates of the BVDV (Walz et al., 2001). BVDV and other RNA viruses are able to create a large number of mutants. Some mutants that replicate faster may

dominate the mutant swarm, giving virulent virus with enhanced viral replication a competitive advantage over less virulent viruses. This could explain the periodic emergence of virulent BVDV that produces severe outbreaks of disease (Bolin and Grooms, 2004). Although it is BVDV type 2 that predominantly creates more severe symptoms, serious disease can result from a BVDV type 1 infection (Amiridis et al., 2004; Muskens et al., 2004; Vilcek et al., 2005) and, conversely, a type-2 infection can pass without serious clinical signs or even subclinically (Bolin and Grooms, 2004).

In this article a case of periparturient BVDV type 1 infection with haemorrhagic colitis and proctitis in an adult cow is described.

## **C**ase description

### *Anamnesis*

In October 2008, 10 peripartum cows of a dairy herd (n=60), showed acute symptoms such as high fever, coughing, dyspnea, and occasional mastitis over a 20 day period. A few days later, 5 of the cows developed severe watery, yellowish diarrhoea, sometimes bloody, with pyrexia lasting up to one week despite combined antibiotic and anti-inflammatory treatment. All cows had calved over the last two weeks in calving pens adjacent to young cattle (Figure 1). Three cows suffering from severe diarrhoea died; the others slowly recovered.

One 6-year-old cow was referred to the clinic 10 days after calving. She had developed high fever (41°C) the first day after parturition and had been recumbent. After several perfusions with calcium borogluconate (Calcii Borogluconas; Eurovet, Heusden-Zolder, Belgium), 500mL on consecutive days, she was able to rise again.

Treatment consisted of marbofloxacin (Marbocyl<sup>®</sup> 10%; Vétoquinol, Aartselaar, Belgium), 2mg/kg body weight per day, tolfenamic acid (Tolfine<sup>®</sup>, Vétoquinol), 2mg/kg body weight, and calcium-borogluconate (Calcii Borogluconas; Eurovet), 500 mL over 3 consecutive days. Despite this treatment, the diarrhoea and pyrexia (40.7 °C) persisted. Immediately before leaving for the clinic, the animal had received florfenicol (Nuflor<sup>®</sup>, Schering Plough, Brussels, Belgium), 20 mg/kg body weight, and meloxicam (Metacam<sup>®</sup>, Boehringer Ingelheim, Brussels, Belgium), 0.6 mg/kg body weight.



**Figure 1. :** The parturient cows were housed in a pen adjacent to the pen of the 3-month-old calves.

### *Clinical examination*

On arrival at the clinic, the cow was alert but anorectic and laying in sternal position, unable to rise, even after stimulation. The body condition was normal (Body Condition Score: 3.5), but skin turgor was decreased and mucous membranes were pale. The cow had a heart rate of 84 beats/min, respiratory rate of 64 breaths/min with abdominal breathing, and a rectal temperature of 37.5 °C. After fluid therapy the next day the temperature rose to 39.6°C. Fluid splashing sounds were present at simultaneous auscultation and succussion of the right flank. The faeces were liquid and contained a large amount of fresh blood. After cautious rectal examination, a large amount of fresh blood covered the disposable glove. No ulcerative lesions were found at the buccal mucosa, between the claws or on the teats.

### *Diagnosis*

The most remarkable result of the blood analysis was a complete absence of white blood cells (Table 1). Neither lymphocytes nor neutrophils were detected in a leukocyte count using the Poch-100IV Diff<sup>®</sup> (Sysmex, Hoeilaart, Belgium). Moreover, on a stained blood smear (Hemacolor<sup>®</sup>; Merck, Overijse, Belgium) only one neutrophil was found. Total and ionary calcium as well as potassium were low, probably caused by low intake or faecal loss due to diarrhoea or both. Lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and creatine phosphokinase (CPK) were increased, most likely due to recumbence-associated muscle injury. Total bilirubin and blood urea nitrogen were mildly increased.

An antigen enzyme-linked immunoSorbent assay (ELISA) performed on a heparinised blood sample taken on the cow's arrival at the clinic was BVDV positive; however, the same test produced a negative result on a sample taken 10 days later. After euthanasia of the cow, a previously frozen serum sample was examined. The real-time reverse transcription polymerase chain reaction (RT-PCR) as described previously (Letellier and Kerkhofs, 2003), showed the presence of BVDV type 1. Virus isolation from this serum sample, performed on Madin-Darby bovine kidney (MDBK) cells was unsuccessful, probably due to the short-lived and low level viraemia associated with transient BVDV infection. Bovine viral diarrhoea virus antibody ELISA performed at arrival and 10 days later demonstrated seroconversion.

Paired sera (arrival date and 10 days later) were both negative for *Salmonella* dublin and *Salmonella* typhimurium. Culture of faeces material on Brilliant Green agar was *Salmonella*-negative as well. Anaerobic culture of faeces on blood agar yielded *Clostridium perfringens*.

Hematologic results		Biochemical results <sup>a</sup>	
Packed cell volume	38%	Total serum protein	65 g/litre (60-80)
Red blood cells	7.97x10 <sup>9</sup> /litre	Total bilirubin	17 µmol/litre (2.5-6)
White blood cells	0.00x10 <sup>9</sup> /litre	Ureum (BUN)	13.6 mmol/litre (3-8)
	0% neutrophils	Creatinine	4.7 mg/dl (0.6-1.8)
	0% lymphocytes	GPT/ALT	37 mU/litre (<10)
Thrombocytes	142x10 <sup>9</sup> /litre	AST/GOT	375 mU/litre (24-142)
		LDH	>4000 mU/litre (692-1445)
		CPK	487 mU/litre (150)
		AP	147 mU/ml (150)
		GGT	15 mU/ml (<30)
		Mg <sup>++</sup>	1.38 mmol/litre (0.6-1.0)
		Na <sup>+</sup>	130 mmol/litre (132-152)
		K <sup>+</sup>	2.9 mmol/litre (3.5-4)
		Ca <sup>++</sup> ionic	0.54 mmol/litre (1.0-1.25)
		Total Ca <sup>++</sup>	0.99 mmol/litre (2.0-2.5)

<sup>a</sup> The reference intervals are shown in parentheses.

ALT – alanine aminotransferase, AST – aspartate aminotransferase, LDH – lactate dehydrogenase  
CPK – creatine phosphokinase, AP – alkaline phosphatase, GGT – gamma-glutamyl transferase  
BUN – blood urea nitrogen

**Table 1.** Results of the blood analysis of the cow at arrival in the clinic. Most remarkable are the total absence of white blood cells and the hypocalcaemia.

### *Treatment and clinical evolution*

On admission the cow received 500 ml of a 27.9% calcium perfusion (Calciumboro-kel<sup>®</sup>, Kela Laboratoria, Sint-Niklaas, Belgium) and was transported to the stable. The animal received a continuous IV drip in a 5% glucose polyionic isotonic solution (413g NaCl, 18.5g KCl, 22g CaCl<sub>2</sub> and 10g MgO<sub>2</sub> in 10 litres of distilled H<sub>2</sub>O). Enrofloxacin (Baytril<sup>®</sup> 4%, Bayer, Brussels, Belgium), and flunixin meglumin (Finadyne<sup>®</sup>, Intervet-Shering Plough,

Brussels, Belgium) were administered intravenously for 5 and 3 days, respectively. Potassium (160 mEq/day) was supplemented for one week in 500 ml of a 5% glucose polyionic solution, because the animal remained hypokalaemic. Three episodes of recurrent hypocalcaemia occurred, and each time 500 ml of the 27.9% calcium perfusion was administered. A 250 mL volume of propylene glycolic acid was given orally twice a day, together with artificial rumen flora (Rumin<sup>®</sup>, Kela Laboratoria) during 10 days. Food (hay, silage) and water were continuously available. The animal rose for the first time 7 days after admission and had a moderate appetite, although diarrhoea persisted. Leukocyte counts on day 11 ( $2.9 \times 10^9$  /L, reference interval:  $6.0$ - $9.0 \times 10^9$  /L) and day 16 ( $12.5 \times 10^9$  /L) showed a gradual recovery of the leucopenia to a normal level. On day 19 after admission the animal was recumbent but alert. Episodes of lateral decubitus and depression occurred and the animal was euthanized on ethical and economic grounds.

### ***Pathology***

Due to financial restraints, the post-mortem examination was limited to a macroscopic investigation of the intestines. The results were moderate colitis and proctitis.

### ***Herd check and additional investigations at the herd level***

Immediately after the detection of BVDV, the veterinary surgeon sent a bulk milk sample to the laboratory, as well as whole blood collected in ethylenediamine tetra-acetic acid (EDTA) from all non-lactating cattle older than 6 months. With a view to cutting costs, 400  $\mu$ L of each individual blood sample were assembled in pools to a maximum of 30 samples per pool. Both bulk milk blood pools were investigated using RT-PCR tests, which all gave a negative result.

As the herdsman was reluctant to pay for more analyses, further investigations of the young calves were postponed until July 17, 2009. On that day, blood samples for virus detection were collected from all young animals not tested before. Two animals were RT-PCR positive, one of which was 3 weeks old at the time of sampling. The other was 1 year old and had been 3 months old when the health problems started among the cows. Of 17 young cattle tested for the presence of BVDV antibodies, only the 2 RT-PCR positive ones were seronegative. Three weeks later the 2 were still viraemic as shown by a repeated antigen-ELISA. This indicated that these calves were persistently infected.

The PCR amplification and sequencing of part of the 5'UTR were performed as described previously (Letellier et al., 1999) to determine the genetic type of BVDV involved. The sequence was aligned with other BVDV1 sequences from Belgian viruses, or retrieved from databases. Multiple sequence alignments were generated with the programme CLUSTALX (Thompson et al., 1997). Evolutionary distances between sequences were estimated using the Kimura-2 parameter method. Phylogenetic analyses were conducted by using the Neighbor-Joining algorithm of the *MEGA* version 4 software (Tamura et al., 2001). The phylogenetic analysis showed that the virus clustered within the BVDV1b subgroup, which represents the major subgroup in Belgium (Couvreur et al., 2002).

## Discussion

The differential diagnosis of haemorrhagic diarrhoea in adult cattle is limited. Coccidiosis commonly occurs between the age of 3 months and 2 years and rarely in younger or older cattle. Moreover, the mortality rate due to *Eimeria* infection tends to be low. *Clostridium perfringens* can cause acute haemorrhagic enteritis and is an important problem in both youngsters (enterotoxemia) and high yielding dairy cows (haemorrhagic bowel syndrome) (Dennison et al., 2002). Without the typical lesions (enterotoxemia or haemorrhagic bowel), the isolation of even a high amount of *Clostridium perfringens* in the faeces, is still of an unknown significance (Abutarbush and Radostits, 2005). As a result, it cannot be excluded that *Clostridia* enterotoxemia played a secondary role in this case of haemorrhagic colitis and rectitis.

Although coronavirus infection causes mild symptoms in adult cattle, occurrences of severe outbreaks have been reported (Decaro et al., 2007). However, spreading of haemorrhagic diarrhoea to all cows within the herd in a single day, absence of mortality, and inclusion of heifers and some of the calves, all signs characteristic of coronavirus infection, were absent in the present case.

Malignant catharral fever (MCF), a disease occurring rarely in our area, can cause leucopenia and haemorrhagic diarrhoea (Pardon et al., 2009). Haemorrhagic diarrhoea is mainly seen with the alimentary and peracute form of MCF. The proximity of sheep is the essential factor for the development of MCF and no sheep were present on the farm or adjacent pastures. Moreover, at least one of the other sick cows should have shown other signs of MCF, such as stomatitis, kerato-conjunctivitis or nervous disorders. Ingestion of a

large quantity of acorns may provoke haemorrhagic diarrhoea, but no oak trees were found in the immediate environment.

Salmonellosis and BVDV infection are the most important differential diagnoses of diarrhoea in combination with severe leucopenia. Leucopenia is frequently noticed in cases of transient BVDV infection in cattle (Bolin and Grooms, 2004; Müller-Doblies et al., 2004) and has been reported as one of the symptoms in severe outbreaks of BVDV infection (David et al., 1994; Ridpath et al., 2006). Considering the absence of leukocytes in our case, the BVDV viraemia only in the initial stage of illness, and the seroconversion, it can be concluded that the cow mentioned underwent a transient BVDV infection. Persistently infected (PI) cattle do not produce antibodies against the BVDV strain that infects them persistently. Conversely, they will have a humeral response after postnatal superinfection with a non-related, antigenically different isolate (Kapil et al., 2005). In our case paired sera revealed seroconversion to the BVDV and transient viraemia, indicating that the infection was postnatal. The interval between the positive and the subsequent virus negative blood sample was only 10 days, but this is not indicative for the duration of viraemia, as the animal was already ill for several days and may have been infected even earlier.

Although postnatal BVDV infection increases the risk of severe symptoms in *Salmonella*-infected cattle (Daly and Neiger, 2008), neither seroconversion to the 2 most common *Salmonella* types present in our region, nor *Salmonella* culture revealed an involvement of salmonellosis in this case. Other possible causes of haemorrhagic diarrhoea are not likely because of the age of the cows affected and the distribution of sick animals in the herd. So it is likely that that the transient BVDV infection was responsible for the severe symptoms. Nevertheless, we regret not having sampled the cow for infection with coccidia, because immunosuppression by a transient BVDV infection could trigger a subclinical *Eimeria* infection to become clinical, even in adult cattle.

Fatal postnatal BVDV infection in adult cows has been described before (David et al., 1994; Carman et al., 1998; Amiridis et al., 2004). Diarrhoea is a constant symptom in all these cases, but none of these articles states a combination of mortality and faeces containing massive amounts of blood in adult cows, although dysentery and blood-tinged faeces are mentioned by David et al. (1994). Furthermore, the cow in our case did not show any ulceration of the buccal mucosa, which is in contrast with the cases of Amiridis et al. (2004), Carman et al. (1998) and one of the 3 cases described by David et al. (1994), in which

erosions were noticed in about half the affected cows. Others have reported abortion among the clinical signs, but this was not noticed in the anamnesis of our case (Carman et al., 1998; Amiridis et al., 2004; Muskens et al., 2004). The combination of haemorrhagic diarrhoea and pneumonia as seen in affected herdmates of the animal described is a common finding in other reports of severe BVDV outbreaks (Amiridis et al., 2004; Carman et al., 1998). The absence of abortion in our case emphasizes once more the variability of BVD symptomatology.

Whereas type 2 BVDV is predominant in severe outbreaks of postnatal infection, this report shows that also type 1 virus is capable to provoke severe disease as reported earlier (Amiridis et al., 2004; Vilcek et al., 2005). Interestingly, in the present case only periparturient cows were affected. As there is physiological immunosuppression around parturition (Cai et al., 1994; Hoeben et al., 2000), the extreme leucopenia might have been the consequence of a complementary effect of this phenomenon and BVDV infection. All peripartum cows and calves under 6 months were housed in adjacent boxes, which made full contact between these cows and PI animals likely.

As PI animals are by far the most important source of infection with BVDV (Lindberg and Houe, 2005), the scenario of infection by a PI bovine is the most likely one in this case. At the age of 3 months, when the cows started to show severe symptoms, the older of the 2 PI calves detected, was housed in a straw box adjacent to the cow's calving pen. The PI and the cows were only fenced off by vertical bars (Figure 1) and it is very likely that this PI had infected the periparturient cows. Although virus excretion may be partially suppressed by the presence of colostral antibodies, this phenomenon may only hold until 3 months after birth, since the influence of colostral antibodies diminishes considerably from that age on (Zimmer et al., 2004).

The PI calf must have been in close contact with the periparturient cows for 2 months after the last symptoms disappeared, because the herdsman usually removes the calves to another stable at the age of 5 months. Further infection of postpartum cows may have been prevented because these cows were immune before entering the calving pen. They could have been infected with a lower amount of BVDV, for instance by contact with the sick cows or other transiently infected cattle, or by personnel transferring the virus. The lower infection dose may be responsible for obvious symptoms failing to appear (Walz et al., 2001).

The question remains how the BVDV infection entered the herd. The anamnesis revealed that during the final months of 2007 and early in 2008, severe BVDV-related problems had afflicted the cattle of the herdsman's sister and a forklift truck had been used to muck out stables on both farms. Although direct contact between cattle of the 2 herds never took place, this is a possible source of infection, as farm staff and commonly used equipment can transfer the virus to another farm (Ståhl et al., 2005).

The serious symptoms after exposure of the herd to a PI animal (Larson, 2005), and the history of an infection route via personnel or equipment indicate a first infection of a naïve herd. If routine BVDV monitoring had been conducted on this farm, the stockbreeder may have been aware of his vulnerable situation before the severe disease occurred. A monitoring test such as the one performed semi-annually in the Dutch voluntary BVDV control programme would have raised the alarm, as it consists of a combination of a young stock serological spot test and a bulk milk test for detection of both BVDV-antibodies and virus. The BVDV must have been circulating on the farm for 8 months at least before the severe cases and deaths occurred, given the age of the oldest PI animal (3 months) at the time of the outbreak of haemorrhagic diarrhoea in the cows.

Finally, at necropsy the colitis and proctitis were only mild, which may indicate recovery of the intestine after 19 days of illness. Despite this potential sign of recovery, the permanent recumbence, probably caused by progressive weakening and exhaustion, prompted us to opt for euthanasia.

## Conclusions

In view of the transient BVDV detection by RT-PCR, seroconversion to BVDV and resemblance of the clinical signs with those reported in other cases of severe postnatal BVDV infection, it is very likely that the case described was a postnatal BVDV infection with symptoms more severe than usual. Furthermore the case shows that severe disease by BVDV infection is not always caused by a type 2 strain. This conclusion emphasizes the complex symptomatology of primary, transient BVDV infection, going from subclinical course to life threatening disease. Therefore, regular monitoring for BVDV seems necessary, and should be implemented as an elemental part of herd health management programmes. The clinical presentations are far too numerous and non-specific to ascertain early detection of the infection by its clinical manifestations alone.

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**A**ssociation between herd exposure to bovine viral  
diarrhoea infection and bulk milk somatic cell count of  
Flemish dairy farms

**Adapted from:**

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**Association between herd exposure to bovine viral diarrhoea infection and bulk milk  
somatic cell count of Flemish dairy farms**

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## Abstract

The purpose of this study was to investigate the statistical association between herd Bovine viral diarrhoea (BVD) status based on bulk milk antibody detection and monthly Bulk Milk Somatic Cell Count (BMSCC) as a reflection of the udder health. A distinction was made between vaccinating and non-vaccinating herds via a questionnaire concerning BVDV vaccination. No significant difference in BMSCC was found between vaccinating (228,300 cells/ml; SD 180,019) and non-vaccinating (237,070 cells/ml; SD 77,900) herds. Non-vaccinating herds (n=243) were selected, and the relationship between a single BVDV antibody optical density ratio (OD ratio) and the BMSCC of each herd over a 12 month observation period evaluated. For this purpose, the non-vaccinating herds were divided into five groups depending on bulk milk BVDV-antibody OD ratio. Overall, no significant relationship between the antibody OD ratio and the BMSCC was found. Still, when comparing the category with the lowest OD ratio (essentially BVDV naïve herds; BMSCC=211,390 cells/ml) with the combined four other categories (BMSCC=242,790 cells/ml), a significant difference in BMSSC was observed (P=0.01).

**Keywords:** viral diarrhoea virus; bulk tank milk test; somatic cell counts

## Introduction

Mastitis is currently one of the most important health problems in cattle and the cause of considerable economic losses to the dairy industry (Bradley, 2002). As the BMSCC is a proxy for measuring the prevalence of subclinical mastitis, it is commonly acknowledged as a criterion for estimating the udder health status of a herd (Schukken et al., 2003).

BVD virus (BVDV) is a pestivirus affecting cattle world-wide and is likely to remain endemic in absence of systematic control measures (Lindberg and Alenius, 1999). Cattle can be infected persistently or transiently. Whilst transient infection with BVDV may go unnoticed, it does cause decreased fertility and immunosuppression (Lindberg and Houe, 2005). Moreover, cattle suffering from a transient BVD infection are more susceptible to other infections by synergy of the BVDV in co-infections (Ridpath, 2010).

A relation between BVDV infection and the severity of concurrent infections with other pathogens has been documented previously (Fulton et al., 2000; Kelling et al., 2002;

Gånheim et al., 2003; Daly and Neiger, 2008; Diéguez et al., 2009). As a result, the defence mechanisms of the mammary gland are also supposed to be adversely influenced by the immunosuppression associated with acute BVDV infection. Although this issue has previously been investigated under field conditions, the results regarding the impact on BMSCC were contradictory (Niskanen et al., 1995; Waage, 2000; Beaudeau et al., 2005; Berends et al., 2008). The aim of the present study is to gain insight into the association between BVD status and BMSCC by excluding the influence of vaccination and classifying herds in groups, based on bulk milk BVDV-antibody optical density ratios. Highlighting the relationship between BVD and udder health may contribute to alerting stakeholders to the economic consequences of the disease.

## **Materials and methods**

### ***Bulk milk sampling and testing***

In 2009, the mean size of Flemish dairy herds was 57 lactating cows, with a yearly production of approximately 400,000 litres per herd (Ryckaert et al., 2009). At each milk collection in Flanders a bulk milk sample is taken on the farm by an automatic sampling device mounted on the tanker. The samples are stored at a temperature between zero and four °C, without any preservative until arrival at the Milk Control Centre Flanders (MCC, Lier, Belgium). All samples are analysed for composition and presence of antibiotic residues. Four samples a month are randomly selected for BMSCC analysis and two for the microbiological quality. Samples are analysed the day after collection. Through this automated sampling procedure samples from 500 herds were randomly selected from a total of 2700 dairy herds in Flanders. These were then tested for BVDV-antibody OD ratios using the IDEXX HerdCheck BVDV Ab ELISA (IDEXX Europe, Hoofddorp, The Netherlands). Herd selection was stratified by region and all sampling was performed between 23<sup>rd</sup> and 25<sup>th</sup> August 2009. Samples were frozen at -18°C and the sample-to-positive (S/P) ratios of BVDV-antibodies determined for 457/500 herds on 31<sup>st</sup> August 2009. BMSCC was determined by a Fossomatic FC appliance (Foss Benelux, IJsselstein, The Netherlands) and monthly values calculated as the arithmetic mean of 4 samplings a month. These were collected from the Milk Control Centre (MCC) records for the six months preceding the antibody test-day and for a further six months after that point.

## *Questionnaire*

All 457 farmers were sent a letter to ask for their cooperation in the study. In an accompanying letter they were asked five questions about BVD vaccination of their herds. The majority replied by e-mail. Farmers who were willing to cooperate, but did not have answered the questionnaire were contacted by phone to obtain this information. The following questions were asked: “Have your milking cows and heifers ever been vaccinated for BVD, and if so, please indicate the name of the vaccine used?” Secondly, “What is the approximate date (month and year) of the last vaccination?”, and finally “Do you routinely vaccinate dairy young stock for BVD? If so, please mention the name of the vaccine”. All questionnaires were completed within three months after milk sampling for BVDV-antibodies.

## *Statistical analysis*

The data were analysed using a linear mixed effect model with herd as repeated effect, antibody OD ratios as either a continuous or categorical independent variable and monthly BMSCC as the outcome variable. An autoregressive correlation structure of the first order was used to model the correlation structure. In all models month was taken into account as co-variable to correct for the seasonal effect. Normal probability plots of residuals and plots of residuals versus predicted values were generated to check whether the assumptions of normality and homogeneity of variance had been fulfilled. No problems were detected. For all linear mixed models, the goodness of fit measures included  $-2 \times \log$  likelihood ( $-2LL$ ), Akaike’s information criterion (AIC), and Bayesian information criterion (BIC). All fixed effect covariates and relevant first order interactions were evaluated and included in the model when statistically significant ( $P < 0.05$ ). All analyses were performed in Spotfire S+ 8.2 (TIBCO software, USA).

First the entire dataset (both vaccinating and non-vaccinating herds) was analysed to determine the effect of the herd vaccination status on the BMSCC and BVDV-antibody OD ratios, respectively. In the model with BMSCC as outcome variable, vaccination status at the herd level (2 levels; vaccinated versus non-vaccinated), BVDV-antibody OD ratio [5 levels; (1) essentially naïve ( $S/P < 0.25$ ), (2) low antibody ( $S/P 0.25$  to  $< 0.50$ ), (3) mid-range ( $S/P 0.50$  to  $< 0.75$ ), (4) high antibody ( $S/P 0.75$  to  $< 1.00$ ), and (5) actively or recently infected

herds ( $S/P \geq 1.00$ )] and the interaction term between both variables were included as categorical independent variables.

Subsequently, only the 243 non-vaccinating herds were selected, to exclude the influence of vaccination induced antibodies on the OD ratio. The effect of the BVDV-antibody OD ratio on the BMSCC was analysed in a continuous manner and also by categorising the antibody OD ratios into the five categories. Furthermore, the total number of cattle present on the farm was included as a co-variable to correct for potential influence of herd size. To evaluate the proportion of variance in BMSCC occurring at the herd and observation level of the data hierarchy, a two-level null model was used with herd as random effect.

## Results

### *Vaccinating and non-vaccinating herds*

In total 406 (88.8%) of the 457 questioned herd owners answered the questionnaire. Ninety of these 406 (22.2%) declared that their dairy cows and heifers were vaccinated for BVDV, while 243 (59.9%) did not vaccinate the lactating cattle. The answers of the remaining 73 farmers were not reliable enough to be used in the study, since they did not know the vaccine name and, as a result, their answers about BVDV vaccination appeared to be uncertain, or herd size numbers were missing. The average BMSCC was 223,728 cells/ml (SD 74,082) for vaccinating herds and 238,582 cells/ml (SD 73,044) for non-vaccinating herds (Table 1). No significant difference in BMSCC was present between vaccinating and non-vaccinating herds ( $P=0.41$ ). BVDV vaccinating herds had significantly higher OD ratios for BVDV-antibodies as compared with non-vaccinating herds (0.93 and 0.71 respectively;  $P<0.01$ ). Further analysis revealed that the influence of the vaccination status on the BMSCC was dependant on the category of BVDV-antibody OD ratio ( $P < 0.05$ ). Herds with the lowest OD ratio ( $< 0.25$ ) had a significantly lower BMSCC (192,300; SD 101,302) compared to herds belonging to group 2 (278,540; SD 112,112), group 4 (235,080; SD 89,757) or group 5 (236,370; SD 67,370) ( $P < 0.01$ ). The difference between groups 1 and 3 was not significant (221,140; SD 80,416). The four groups with OD ratios over 0.25 also differed in BMSCC among themselves, even after correcting for multiple comparisons.

vaccination status	min.	first quartile	median	third quartile	max.	mean	standard deviation
<b>BMSCC</b>							
non-vaccinating	66,000	186,000	235,000	288,000	553,000	238,582	73,044
vaccinating	50,000	168,000	217,000	272,000	480,000	223,728	74,082
<b>BVDV OD ratio</b>							
non-vaccinating	0	0.49	0.75	0.97	1.53	0.71	0.33
vaccinating	0.2	0.67	1.01	1.20	1.59	0.93	0.33

**Table 1.** Descriptive statistics for BMSCC and BVDV-antibody OD ratios stratified by vaccination status.

### *Non-vaccinating herds*

Of the 243 non-vaccinating herds 11.3% were classified in group one, the essentially BVDV-naïve group, while 14.9%, 23.1%, 29.1%, and 21.4% belonged to groups 2, 2, 4, and 5, respectively.

For the non-vaccinating herds, when treating the antibody titre as either a continuous or categorical variable divided in 5 categories, no significant relationship between the OD ratio and the BMSCC could be determined ( $P=0.13$ ) (cat. 1: BMSCC=211,390; cat. 2: BMSCC=238,140; cat. 3: BMSCC=242,920; cat. 4: BMSCC=247,350; cat 5: BMSCC=242,750 cells/ml). However, when comparing the category with the lowest OD ratio (essentially BVDV-naïve herds;  $S/P<0.25$ ; BMSCC=211,390 cells/ml) with the combined four other categories (243,510 cells/ml) a significant difference was observed ( $P < 0.01$ ). The highest variation in BMSCC resided at the observation level (99.9%). Month and BVDV-antibody OD ratio explained 3.7% of the variation in BMSCC.

## **Discussion**

As the budget was limited, a single BVDV test was used in conjunction with multiple BMSCC testing time points. The use of bulk tank milk antibody values to estimate the BVDV seroprevalence in a herd is well established (Niskanen et al., 1991, Niskanen, 1993; Paton et al., 1998; Beaudeau et al., 2001). Moreover, for non-vaccinating herds, the level of BVDV-

antibody values in bulk tank milk corresponds with the infection status of the herd, because PI animals are most likely to be found in herds with the highest serological prevalence (Ståhl et al., 2002; Houe, 2005; Houe et al., 2006). As a result, herds with a current infection will have high levels of antibodies to BVDV in their bulk milk (Niskanen et al., 1991; Drew et al., 1999). The same applies to recently recovered herds, due to the presence of long lasting antibodies in previously infected animals (Houe, 1999). Consequently, the antibody level in bulk tank milk is expected to gradually decrease to low or undetectable levels within a period of three to four years (Lindberg and Alenius, 1999). Therefore, we assumed that one BVDV-antibody sample was representative for the long-term herd BVDV status. A carry-over effect of BVDV infection on BMSCC has also been proposed, which may result in an increased BMSCC until up to one year after BVDV eradication (Waage, 2000; Beaudeau et al., 2005). The result is that in herds recently cleared of BVDV, both the BVDV antibody quantity and the BMSCC may stay high during one year at least.

In contrast to the single BVDV-antibody test, monthly BMSCCs from six months before until six months after the antibody test-day were used, because an accurate image of chronic mastitis can only be obtained by frequent sampling (Lievaert et al., 2011). If BVDV was circulating in a herd, one would temporarily expect more sick cows and cows under antibiotic treatment. This could result in short term BMSCC variations since the milk from these animals should not be collected in the bulk tank.

As the effect of the vaccination status on BMSCC seemed to depend on BVDV-antibody OD ratio, using the non-vaccinating herds only appeared to be a well-considered choice for investigating differences between the groups. The non-vaccinating herds of group one, considered BVDV-free, had significantly lower BMSCC values compared with the combined groups of BVDV infected herds. The fact that no significant differences in BMSCC were found among the four BVDV-antibody positive groups when only considering the non-vaccinating herds might suggest that a positive or negative result for bulk milk BVDV-antibodies is useful to distinguish BVDV infected from free herds. On the other hand, it indicates that the degree of BVDV infection cannot be measured by differences in BVDV-antibody OD ratios in non-vaccinating herds.

Although the immunosuppression accompanying transient BVDV infections forms a potential explanation for the lower BMSCC in BVDV-naïve herds, it is common knowledge that the farmers' management skills have an important effect on udder health (Dufour et al.,

2011). Moreover, although performed with cow-calf herds, a Scottish study showed that maintaining a herd free of BVDV contributes indirectly to both the farm income and its risk management through its effect on the management of the whole farm (Stott et al., 2003). In this regard, the positive association between BVDV-antibody titre and BMSCC could be attributed to some other herd level management practices related to the milking technique, milking equipment, and environmental hygiene. Unfortunately, because of privacy matters, more management factors could not be included in the present study. Still, including herd as a repeated effect as was performed in this study takes into account the association between observations within the same herd, and thus controls for any confounding factor at the herd level (Dohoo et al., 2003). Also, the high response rate on the questionnaire limited the probability of response bias.

## **C**onclusion

The results of the present study suggest that dairy herds have lower BMSCCs in absence of BVDV infection.

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**A**ssessment of two essential elements of BVDV control,  
detection of persistently infected animals and monitoring,  
on selected Flemish dairy and beef farms

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Assessment of two essential elements of BVDV control on selected Flemish dairy and  
beef farms

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## **A**bstract

Bovine viral diarrhoea virus (BVDV) is one of the most important viruses to cause disease in cattle worldwide. The virus is endemically present in Belgium. Clinical diagnosis of BVDV infection is difficult. Therefore, monitoring through testing is necessary to detect the presence of the virus on farms. As vaccination alone does not suffice for eradication, a combination of measures is required for successful control. Via a questionnaire, the BVDV policy on 241 selected Flemish cattle farms was investigated and this revealed some striking results. For the majority of herds, the BVDV status was unknown (63%) and only 23% had a monitoring programme in place. Furthermore, on 71% BVDV vaccinating farms vaccination against BVDV was implemented as a strategy, without knowing the actual BVDV status.

## **I**ntroduction

Bovine viral diarrhoea (BVD) is an infectious disease of cattle with a worldwide distribution (Ridpath, 2010a), causing significant economic losses (Houe, 1999; Fourichon et al., 2005). Infections can be either persistent or transient. Persistently infected (PI) cattle are key in spreading and maintaining the infection within and between herds, as they continuously shed large amounts of BVD virus (BVDV) during their entire lives (Lindberg and Houe, 2005; Fulton et al., 2009). Therefore, most transient infections are caused by direct contact with PI cattle. The direct consequences of transient BVDV infection may vary from subclinical or mild disease to acute outbreaks with severe disease and high mortality. Moreover, transiently infected (TI) cattle may suffer from immunosuppression, which makes them more susceptible to secondary infections (Brackenbury et al., 2003; Ridpath, 2010b; Chase, 2013). Between herds and animals, substantial differences in clinical presentation of BVDV infection have been noticed. This variability has been attributed to herds/animals having different immune statuses (Lindberg, 2003), or differences in strain virulence (Bolin and Ridpath, 1992; Pellerin et al, 1994; Baule et al., 2001; Walz et al., 2001; Kelling et al., 2002). Because of the marked variation in clinical presentation it is usually difficult to detect the presence of a BVDV infection in a herd by its clinical presentation alone (Lindberg and Alenius, 1999; Ridpath, 2003; Evermann and Barrington, 2005). As a result, monitoring by

diagnostic testing for presence of BVDV is vital to determine herd status and for effective BVDV control.

In the absence of a nationwide eradication programme in Belgium, control is typically performed at the herd level by decision of the farmer. Meticulous tracing, correct administration (Laureyns et al., 2010), and implementation of the key principles of BVDV control as stated by Lindberg and Houe (2005) are essential to successfully control BVDV at the herd level. These principles are: stringent biosecurity, detection and removal of PI cattle, continuous monitoring, and potential implementation of vaccination. As the prevalence of BVDV infection at the farm level is high in Belgium (Sarrazin et al., 2013), biosecurity measures are highly important to protect herds against BVDV (re-)infection. Therefore, implementation of biosecurity measures has to be the first step in BVDV control in Belgium. Most importantly, PI animals should be removed from the herd, as they play the key role in the transmission of BVDV by continuous shedding of infectious virus (Lindberg and Houe, 2005). In BVDV-free herds, longitudinal surveillance should be combined with biosecurity enhancements to detect and prevent potential (re-)infection and allow prompt action in the event of disease incursion. Monitoring can be performed using serological spot tests (Houe, 1994; Booth and Brownlie, 2012) and has to be continued as long as BVDV is present in the region. Although not 100% effective in protecting every individual animal, vaccination can be an essential component of a herd level control programme (Ridpath, 2012). However, if eradication is to be achieved, vaccination must be combined with the other three principles of BVDV control (Lindberg and Houe, 2005; Rodning et al., 2010; Booth and Brownlie, 2012).

Although it has been emphasized in many publications that BVDV control requires a combination of different, strictly executed measures, little information is available on how these recommendations are implemented in the field. This study highlights the BVDV management on Flemish farms.

## **Material and methods**

During 2011, a large multicentre study was conducted (Pfeiffer et al., 2012) to identify calf-level factors associated with Bovine Neonatal Pancytopenia (BNP) on BNP-affected farms (Jones et al., 2013), and herd-level factors that explained why some farms experienced cases of BNP and others did not (Sauter-Louis et al., 2013). Since BNP is hypothesised to be

associated with BVDV vaccination, a substantial part of the interview was on BVDV management. Questions of relevance for the present study were selected from the BNP questionnaire and are presented in Table 1.

The Belgian contribution only took place in the Flemish speaking part of the country, Flanders, and was conducted by the Faculty of Veterinary Medicine of Ghent University in cooperation with the Flemish Animal Health Organisation “Dierengezondheidszorg Vlaanderen” (DGZ). Through a call on the website of DGZ and notifications in veterinary and farmer magazines, veterinarians and farmers were encouraged to report suspected BNP cases. Case farms were visited for sample collection, and a questionnaire was used to interview the farmers on colostrum feeding, cattle health management, disease management, and medication use. Farms where the veterinarian had reported a suspicion of BNP were classified as case farms. The control farms belonged to the clientele of the same veterinary practice and had never been diagnosed with BNP before. They were of the same type and approximately the same size as the corresponding case farm. Farmers of the control farms were interviewed by telephone, using the same questionnaire. All managers of the case and control farms were interviewed by the first author. Data from 241 Flemish farms was available for the study.

Recognition of BVDV infection by its clinical presentation alone is very difficult if not impossible. Therefore, in this study a herd was only considered BVDV-free if the status was based on a test-and-cull’ programme or a ‘herd test’. A test-and-cull programme consists of virological testing by PCR or antigen-ELISA of all cattle in the herd followed by culling of PI animals.

In the present study, continuous testing of all newborns by antigen-ELISA for already more than one year, or regular serological spot tests of which the last one took place within the last 12 months, were both accepted as a herd test. The intention of using a spot test is to detect BVDV circulation in a herd by testing five to ten blood samples of young stock between 8 and 12 months old for the presence of BVDV antibodies (Houe, 1992).

## **Results**

The results are summarised in Table 2. Farmers were asked ‘*Was the herd BVDV-free for the last 12 months?*’. Of the 241 herds, 82 had a known BVDV status that was based on

testing; 66 of those 82 had been BVDV-free and 16 had been BVDV infected during the past year. On 158 of the farms (66%) there had been no monitoring for BVDV. When the farmers of the herds that were BVDV-free were asked for how long they had held this status, 11 of the herd managers communicated a date within the last 12 months, although they scored their herd BVDV-free for the whole of the preceding 12 months in another answer.

Question	Possible Answer
Production type	mixed dairy beef
Veterinarian	code number
Total number of cattle at time of the interview (young stock included)	
Vaccinations within the last 12 months: BVDV	calves up to 6 months      name of vaccine young stock > 6m      name of vaccine breeding heifers      name of vaccine mature cows      name of vaccine
Was the herd BVDV-free during the last 12 months?	yes - indicate date since when BVD free no unknown
If BVDV-free, how has this been determined?	control program test and cull herd test
Have you had a confirmed BVDV animal (PI) on your farm within the last 12 months?	yes no not monitoring for PI
Was there a BVDV vaccination program?	yes no
Reason for starting vaccination:	had a BVDV problem on farm to prevent the farm from having a BVDV problem unknown status others
Currently still vaccinating against BVDV?	yes no
Which BVDV vaccine is currently used?	

**Table 1.** Questions of the bovine neonatal pancytopenia questionnaire selected for the present study.

On 55 (67%) of the 82 herds with known BVDV status (infected and free farms), BVDV testing consisted of a herd test while on the other 27 a test-and-cull method was in use.

On the question *'Have you had a confirmed PI animal on your farm within the last 12 months?'* twenty of the 241 herd managers gave a positive answer (8%), whereas the majority (153) did not know whether a PI animal had been on the farm or not, as they declared that a monitoring programme for BVDV had not been in place (63.5%). On 68 (28%) of the herds the BVDV status was known, but no PI animals had been detected within the last 12 months.

On 155 (64.5%) of the 241 farms, a BVDV vaccination programme had been in use during the past 12 months or earlier. When asked for the *'Reason for starting this vaccination'*, there was one blank result and 83 of the 155 herd managers (53.5% of all vaccinating herds) answered that a vaccination strategy had been started because of a BVDV problem; 55 (35.5% of all vaccinating herds) had started the vaccination to prevent BVDV problems. When examining the names of BVDV vaccines used in the different age categories, it appeared that on 42 of the 155 vaccinating herds, only young stock under six months of age were vaccinated, all with a trivalent vaccine containing a BVDV component (Risposal®3-BRSV-Pi3-BVD, Pfizer Animal Health). Of these 42 herd managers, only 16 declared that the reason for applying Risposal®3-BRSV-Pi3-BVD vaccination had been prevention of respiratory disease. On 93 farms vaccination of heifers and/or adults was still continued at the time of the interview (farms that only vaccinated young stock were excluded); 66 of these 93 (71%) herd managers did not know the BVDV status of their herd.

Of the 20 herds where a PI animal had been found within the previous 12 months, 7 applied BVDV vaccination of adult cattle at the time of the interview. Of these 7 herds, 5 had been vaccinated for two years or longer.

	Dairy	Herd type		Overall	
		Beef	Mixed		
<b>Herd information (n=241)</b>					
Number of herds	113	72	56	241	
Number of veterinary practices involved				43	
Average number of animals per herd	142 (46-450)	131 (4-380)	192 (50-530)	150 (4-530)	
<b>Questions on knowledge of BVDV status</b>					
<b>1. Was the herd BVDV-free during the last 12 months?</b>					
Answer	yes	27	22	17	66 (27%)
	no	8	3	5	16 (7%)
	unknown	78	46	34	158 (66%)
	no answer				1
Herds with known BVDV status	35	25	22	82 (34%)	
<b>2. If BVDV-free, on what basis is this determined?</b>					
	test and cull program	11	9	7	27/82 (33%)
	herd test (monitoring)	24	17	14	55/82 (67%)
<b>3. Had a PI within the last 12 months</b>					
	yes	10	5	6	20 (8.5%)
	no	28	22	18	68 (28%)
	not monitoring for BVDV	75	46	32	153 (63.5%)
<b>4. Had a vaccination program during last 5 years?</b>					
	yes	63	48	44	155 (64.5%)
	no	50	23	12	85 (35%)
	no answer				1
<b>SUBSET OF DATA: BVD vaccinating herds (n=155)</b>					
Vaccination only of cattle <1y					42 (27%)
Reason for starting vaccination					
	had a BVDV problem in herd	37	26	20	83 (53.5%)
	to prevent BVDV problems	24	13	18	55 (35.5%)
	others	2	9	5	16 (10.5%)
	no answer				1

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**SUBSET OF DATA : herds still BVD  
vaccinating at the time of the  
interview (n=93)**

Still vaccinating adult cattle	93
Still vaccinating adult cattle and BVDV status unknown	66 (71%)

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**Table 2.** Descriptive data on some aspects of bovine viral diarrhoea (BVDV) management on 241 Flemish dairy and beef herds

## Discussion

The main objective of this study was to investigate BVDV management on selected farms by describing common policies for PI animal detection and monitoring, two of the three essential BVDV control measures. The results were collected as part of a larger case-control study on the identification of risk factors for the occurrence of BNP, a BVDV vaccination related disease (Jones et al., 2013; Sauter-Louis-et al., 2013).

The fact that all questions for this study were asked by the same person, both on case and control farms, reduced the likelihood of information bias. Although the same questionnaire was used for both case and control farms, the interviews occurred face to face on case farms, whereas they were conducted by telephone on control farms. Therefore, the answers collected on case farms might be considered more reliable, as they were better supported by written or electronically stored data such as laboratory results provided by the farmer at the herd visit.

Two different descriptions of the same question indicated that the BVDV status was unknown on 66% (n=158) and 63.5% (n=153) of the farms during the past year, respectively referring to the absence of BVDV and presence of a PI animal. The fact that there was little difference between the figures obtained via both questions reinforces the certainty that the majority of the herd managers did not know the BVDV status of the herd. It is interesting to note that when asked if they knew their BVDV status, 16 farm managers answered that their herd had been infected in the last 12 months. Yet, when asked about the presence of PI animals, 20 farm managers stated that PI animals had been identified on their premises in the

last 12 months. Remarkably, four farmers did not appear to know that the presence of PI animals is an indicator of herd-level infection. Moreover, when asked for how long their herds had been BVD-free, 11 herd managers communicated a date within the last 12 months, although they had previously scored their herds BVDV-free for the whole of the preceding 12 months. These examples demonstrate that a question may produce different answers when asked in a different way and illustrates potential difficulties when working with questionnaires.

Interestingly, two veterinary practices of the 43 involved were responsible for 16 of the 55 herds with BVDV surveillance. The latter result suggests that few veterinary practices implement BVDV monitoring in their herd health management programmes.

Farms were not classified as BVDV-free if this status was obtained from clinical signs. The only methods accepted for defining the BVDV status were: a herd test, or a test-and-cull programme during the last year. Of the 82 herds with known BVDV status, those 27 where a test-and-cull programme had been used cannot necessarily be considered as herds with BVDV surveillance. On these farms the respective herd managers had performed one whole herd test for the presence of PI animals during the last year, reacting on a suspicion of BVDV infection, and afterwards assumed that herd had been BVDV-free since then. The only methods that can be considered as surveillance are regular serological spot tests or virological testing of all newborn calves on a whole blood sample from the age of two months on. Ear notch testing (Kuhne et al., 2005; Hill et al., 2007) can be an alternative, but was not yet available in Flanders at the time the study was conducted. It can be concluded that only 23% (n=55) of all herds had a BVDV surveillance programme in the strictest sense. Nevertheless, virological testing of all newborns as a sole monitoring measure might not rapidly identify re-infection of the herd (Houe et al., 2006), for instance because of overlooking a PI calf due to an administrative failure (Laureyns et al., 2010), a false negative result (Presi et al., 2011; Fux and Wolf, 2012) in previous detection and removal of PI cattle, or external re-infection. It might take two years or more until a programme with virological testing of newborns as the only measure will detect a false negative PI animal, that is when its first calf will be virologically tested.

The BNP case farms were all BVDV vaccinated and the corresponding controls were linked to the same veterinary practice. Therefore, it can be supposed that the veterinarians involved were conscious of the importance of BVDV infection, because they had advised

vaccination to their clients. On the other hand, it appeared that most of them did not apply all principles of BVDV control (Lindberg and Houe, 2005), as on 71% of those farms still vaccinating at the moment of the interview, the BVDV status was unknown. This raises the question as to whether the veterinary profession as a whole still has an over-reliance on BVDV vaccination for control of the disease, when it should be considered as only one part of a range of control measures that should be implemented.

As the multi-country study was focused on BNP, a BVDV vaccination related disease, the number of vaccinating herds may have been overestimated. Most likely, the control group had been vaccinated as well, since it was recruited from the same veterinary practice as the case herds, and consequently, the same BVDV control strategy may have been implemented. On the other hand, at the time of the herd visit, most of the farmers had already been informed on BNP. Some had changed to another BVDV vaccine, whereas 20 of the original 155 vaccinating herd managers had ceased vaccination at the time of the interview, most likely for fear of BNP. As a result, the figures are not suited to interpret the BVDV vaccination prevalence in Flanders. They rather show some shortcomings of vaccination management. Although vaccination on its own is not sufficient to eradicate BVDV from a herd (O' Rourke, 2002; Booth and Brownlie, 2012; Ridpath, 2013), 66 of the 93 herd managers who were still vaccinating against BVDV at the time of the interview did not know the BVDV status of the herd (71%). Vaccination should be combined with all three necessary parts of BVDV control: biosecurity measures, detection and removal of PI animals, and monitoring (Lindberg and Houe, 2005). Moreover, the use and application of BVDV vaccines in the field is not always correct (Meadows, 2010). The present study shows that in almost one third of all vaccinating herds (27%; n=42) only young stock under six months of age had been vaccinated, while the advice is to reach a 100% coverage of the adult herd with the main objective of preventing infection of pregnant cattle (Lindberg and Houe, 2005).

On 83 of the 155 vaccinating herds (53.5%), the decision of starting BVDV vaccination was made at the occurrence of a BVDV problem. Another 10% (n=16) of the herd managers did not really have the intention to control BVDV. They used a trivalent vaccine containing a BVD component, to protect calves from bovine respiratory disease (Risposal®3-BRSV-Pi3-BVD Pfizer Animal Health), but did not have a BVDV vaccination programme for older cattle. Not surprisingly, among these 16 there was only 1 dairy herd, because Belgian Blue cattle, the predominant beef breed in Belgium, are substantially more susceptible to

respiratory disease than dairy breeds (Bureau et al., 1999). Nevertheless, 42 farmers vaccinated only 3-month-old calves. Since only 16 stated that they vaccinated against respiratory disease, it can be assumed that 26 others considered vaccinating only 3-month-old calves as a herd level BVDV control strategy.

Only 35.5% (n=55) of the vaccinating farmers stated to have started the vaccination programme to protect the herd from BVDV infection, without previous BVDV problems. Obviously, both these farmers and the ones who started vaccination after their herds had suffered from BVD problems (53.5%), hence the majority of vaccinating farmers, must have been aware of the economic consequences of BVDV infection.

Finally, the observation that a PI animal was detected on five farms, despite BVDV vaccination during the last two years or longer, shows that continuous monitoring is necessary, even for herds where a BVDV vaccination scheme is running.

## **C**onclusion

This study illustrates that even on selected farms, where many farmers were willing to vaccinate against BVDV and thus conscious of the impact of BVDV infection, the necessary elements of BVDV control were insufficiently implemented. In particular, too many control strategies were based on vaccination alone and only few herds were monitored for BVDV. These findings suggest that on many Flemish farms BVDV control remains incomplete and consequently inefficient, despite repeated communication and education from regional animal health services and the veterinary faculties. It is up to the veterinarians to train their clients to control BVDV efficiently.

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## Chapter 6

# **G**eneral Discussion



## Introduction

The first part of the general discussion of this thesis is dedicated to the complexity of the disease. Although it is common knowledge that the clinical presentations of BVDV infection are too complex to allow for a reliable clinical diagnosis, many farmers and vets still expect to recognize presence of the BVDV by the clinical appearances. To address this misconception, an extraordinary early finding of cytopathic BVDV in an asymptomatic and very young calf and some exceptional clinical presentations are discussed, as well as the hidden effect of BVDV infection on udder health.

An important aim of this doctoral thesis was to assess if the necessary elements for efficient BVDV control are implemented sufficiently in Belgium. Because clinical diagnosis is difficult, testing and continuous monitoring are essential to BVDV control. Unfortunately, it seems as if attempts to BVDV control or eradication in Belgium have not been efficient until now. The control schemes as recommended by the animal health services are adequate, but not meticulously executed in the field. Therefore, in the second part of the discussion recommendations will be provided for BVDV control at the herd level by listing points of attention for PI cattle detection.

Finally, considerations are discussed, that might be of interest for the Belgian BVDV eradication programme that is currently being designed, by comparing the Belgian plan as set out at present with the ideal BVDV eradication strategy.

Although the studies included in this thesis have been performed in Flanders, the conclusions can be extrapolated to the entire country, as the epidemiological situation is similar in Wallonia and Flanders.

## Complex pathogenesis and variety in clinical presentations of BVD

The case report of *Chapter 3.1* describes detection of cp BVDV in a 10-day-old calf without presence of ncp virus. As the calf showed no signs of MD, this was an extraordinary finding that highlights the complex pathogenesis of BVDV infection. Until now, cp BVDV was almost exclusively found in cattle shortly before or while they suffered from MD, and MD is only unarguably proven when cp and ncp BVDV are existing together in the same

animal. It could not be determined if this exceptional cp BVDV infection also had an effect on the clinical appearance of the infection. Nevertheless, the observation that the haemorrhagic diarrhoea disappeared after treatment for *Cryptosporidium*, showed evidence of the clinical effects of co-infections and the way they can complicate the clinical outcomes of BVDV infection. Also in other cases, BVDV infection is often only one of the causes of disease. As usual, clinical diagnosis alone was not sufficient in the described case for detecting involvement of BVDV infection.

Depending on the virulence of the BVDV strain (Bolin and Ridpath, 1992), the immune status of the host, reproductive status of the host, age of the host, and concurrent infections with other pathogens the clinical features of BVD can vary substantially (Ridpath, 2010). The findings of **Chapter 3.2** confirm this statement: a 2-days-old calf was suffering from skin bleeding and the clinical presentation was indistinguishable from clinical manifestations observed in Bovine Neonatal Pancytopenia (BNP) affected calves.

The bleeding syndrome in young calves became more relevant since BNP emerged in Europe in 2008 (Pardon et al., 2010). In this immunomediated disease allo-antibodies directed to calf leucocytes and bone marrow precursor cells are transferred to the calf through colostrum (Bastian et al., 2011; Bridger et al. 2011; Pardon et al. 2011). Although it was demonstrated that the presence of the allo-antibodies in colostrum was associated with vaccination with a particular BVDV vaccine (Sauter-Louis et al., 2012), only a small number of all calves that had received colostrum from vaccinated mothers developed BNP. It is assumed that the latter could be contributed to genetic predisposition of the calf (Deutskens et al., 2011). BNP presents itself by bleeding disorders all over the body in calves under 28 days of age. Haemorrhages are observed in the skin (spontaneous bleeding without primary trauma), eyes, external and internal mucosae and internal organs. Suspending sales of the vaccine associated with BNP in June 2010 was followed by a decreasing incidence of BNP. Nevertheless, sporadic cases are still reported in Belgium, since dams vaccinated before June 2010 still can give birth to BNP calves today.

The finding of a calf with similar clinical presentations as observed in BNP-calves is important for the differential diagnosis of BNP, but the case was exceptional for a number of reasons. First of all, the bleeding disorder was associated with persistent infection with a BVDV1 strain. Until recently, it was commonly accepted that HD was associated with BVDV2 (Blanchard et al., 2010), and several cases of BVDV2 related HD have been

reported (David et al., 1994; Carman et al., 1998; Ridpath et al., 2006). HD associated with BVDV1 has been described in older PI calves suffering from MD (Dabak et al., 2007). Thrombocytopenia has been mentioned in a report of an outbreak of severe disease associated with BVDV1 infection in the USA. The clinical presentations were abortion, premature birth, and congenital malformation. BVDV1b isolated from the affected animals was inoculated in calves and caused thrombocytopenia, but that change was not noticed in the field outbreak (Blanchard et al., 2010). Equally interesting was the observation that the calf of *chapter 3.2* was much younger than previously reported for HD. In a Belgian study on BNP the age of the affected calves ranged between 7 and 27 days (Pardon et al., 2010). A third remarkable fact was the thrombocyte count returning to normal in spite of persistent viraemia and, as a result, the persistently BVDV infected animal recovering from HD, whereas most calves suffering from BNP do not survive (Pardon et al., 2010).

Sarrazin et al. (2013b), infected calves with the BVDV strain isolated from the case calf described in *chapter 3.2* through intranasal instillation of  $5 \times 10^6$  TCID<sub>50</sub>. The fact that the strain produced only subtle clinical signs in the experiment may be additional proof of the unpredictable clinical outcome of BVDV infection.

In conclusion, due to the absence of any other clinical sign, the calf suffering from skin bleeding could have been mistaken for a BNP calf. Therefore, BVDV tests are necessary to complete the diagnosis of HD.

In *Chapter 3.3* it has been shown that infection with BVDV1b can cause life threatening disease in adult cows. Although leucopenia is a constant symptom of transient BVDV infection (Kapil et al., 2005), the complete absence of leukocytes at blood analysis was striking in this case. Herd mates of the affected cow suffered from haemorrhagic diarrhoea and pneumonia, a combination also mentioned in other cases of transient BVDV infection with severe clinical presentations (Carman et al., 1998; Amiridis et al., 2004). Not unexpectedly, management factors were likely to have been responsible for bringing BVDV into the herd. The most likely source of infection was a forklift truck used ten months before the BVDV outbreak to muck out sheds immediately after it had been used on another farm where cattle were afflicted by severe clinical disease associated with BVDV infection at that time. During the periparturient period the cow diagnosed with BVDV infection and severe proctocolitis had been housed in close contact with a 3-months-old PI calf and its peers. Even

after severe disease had damaged the herd, and BVDV had been diagnosed as the causative agent, the farmer was still reluctant to check the herd for presence of PI animals.

Chapter 3.3 shows that even when infected with BVDV1, considered less virulent than BVDV2, adult cows can suffer from life threatening disease. As shown in this case, cows are particularly vulnerable in the postparturient period. Without laboratory testing for BVDV, the clinical presentations may be confused with other diseases causing haemorrhages, such as salmonellosis, coronavirus infection, coccidiosis, and haemorrhagic bowel syndrome. Moreover, by its immunosuppressive character, BVDV infection might also provoke those infections.

Importantly, both cases of thrombocytopenia and HD described in *chapters 3.2 and 3.3* were associated with BVDV1b, a commonly diagnosed genotype in our area (Caij, B., 2013, personal communication), whereas Ridpath et al. stated in 2000 that all haemorrhagic syndrome outbreaks until that date had been associated with BVDV2 isolates. In 2010, Blanchard et al. confirmed that statement by concluding that thrombocytopenia is rarely associated with BVDV1 strains. The cases described in this thesis cannot be considered as “outbreaks” of severe disease, because the low number of affected animals involved. Still, also individual cases of severe HD associated with BVDV1 have rarely been reported.

The results of *Chapter 4* suggest that herds considered free of BVDV had lower BMSCC than infected herds. Although seroprevalence classes, as used in *Chapter 4* are only estimates when measured as OD ratios, they give a reasonable indication of the antibody status of herds (Booth et al., 2013a).

Strikingly, only 11.3% of the 243 randomly selected dairy herds could be classified as BVDV-free. Scottish research showed comparable figures: 12.7% of 220 dairy herds had a very low prevalence of seropositive cows (Humphry et al., 2012). Although a small fraction of the herds with low prevalence might have been recently infected, in general, the low antibody titre corresponds with absence of PI cattle (Houe et al., 2006). As bulk milk remains on average BVDV-antibody positive during 3.5 years after eradication of BVDV from a herd (Booth et al., 2013a), these very low prevalence herds are to be considered as BVDV-free for at least 3.5 years. Therefore, the conclusion has to be formulated as follows: herds that were BVDV-free for a number of years, had a lower subclinical mastitis prevalence as showed by the lower BMSCC. To assess the influence of management and to better understand the

association between BVDV status and BMSCC, a follow-up study to *chapter 4* has been designed. The objective was to follow the evolution of the herd BMSCC from two years before eradication of BVDV until two years after eradication on 30 dairy farms that remained BVDV free after eradication. Unfortunately, the study had to be cancelled due to lack of suitable participants: not only did most farms with known BVD-free status lack a history of BVDV eradication, also was the actual BVDV status unknown on most farms where BVDV had been eradicated before.

## **BVDV management in Belgium**

In Belgium continuing efforts have been made to familiarize farmers and veterinary practitioners with efficient BVDV control. Despite many scientific articles on BVD and continuous efforts of the Animal Health Services to provide information on the disease on their websites and in veterinary and agricultural press, farmers and veterinary practitioners seem to lack a basic understanding of the principles of BVDV control.

In 2009 a student thesis revealed a lack of interest and participation of farmers in BVDV-testing. When free BVDV spot tests were offered by a pharmaceutical company, 17 out of 50 herds that received the results indicating that 60% or more young cattle were seropositive, which suggests presence of a PI animal, did not start tracking PI animals in the year after the positive test (Van De Steene, 2009). Similarly, in *Chapter 5* of this doctoral thesis it was demonstrated that only 23% of 241 included farms monitored for BVDV and that 71% of BVDV vaccinating herds used vaccination as a strategy without knowing the BVDV status of the herd. Records on bio-security were not obtained, but according to other publications studying the Belgian situation, bio-security measures were also insufficiently implemented on Belgian cattle farms (Sarrazin et al., 2013b; Tay H., 2013). BVDV can stay in a herd for about three years, before causing a severe outbreak (Lindberg and Alenius, 1999). Without continuous monitoring, the virus can stay on the farm undetected, or re-enter the premises secretly after eradication. In contrast, when alarmed in a timely manner by a permanent monitoring programme, the farmer can put a stop to hidden damage to the herd, or prevent a severe outbreak by eliminating PI cattle in time. The results of insufficient monitoring correspond with a report from the United Kingdom. Monitoring was neglected there as well, even on farms participating in a voluntary BVDV control programme, where veterinary time and diagnostic testing were free of charge (Booth and Brownlie, 2012).

It has been estimated that BVDV vaccines are used in about 20% of livestock units in Europe (Moennig and Brownlie, 2006). Although there may be a difference between dairy and beef herds, this percentage corresponds well with the results of *Chapter 4* of this thesis: in 2009, when the research studying the potential association between BVDV and udder health was conducted, 22% of the participating dairy herds were vaccinating against BVDV. A vaccination programme can reduce the incidence of acute and persistent infections in a herd or population, but will not prevent all new infections in individual cattle, and consequently, it will also not halt the development of PI animals (O' Rourke, 2002; Lindberg and Houe, 2005; Ridpath, 2010; Ridpath, 2013). In the case of other infectious diseases, such as Infectious Bovine Rhinotracheitis, spreading of the infectious agent is substantially suppressed when almost all animals are protected by vaccine antibodies, because in that case, the infection load of the environment decreases substantially and the animal's immunity can overcome the low infection pressure. For BVD the situation is different because of the exceptional role of the PI animal: if a single pregnant susceptible heifer or cow is not protected against intra-uterine infection, it can give birth to a PI calf, when infected in the first months of pregnancy. Moreover, protection by vaccination, efficient in a herd with low BVDV infection pressure through external contacts, may be insufficient to resist the immense virus shedding continuously caused by a PI animal living in the herd. Therefore, for proving the ability of a vaccine to protect against intra-uterine infection, the challenges should be conducted by exposing the test animals to long lasting contact with a PI animal (Ridpath and Fulton, 2009). Unfortunately, licensing requirements for validation of BVDV vaccine efficiency are currently based on challenge by a single intranasal inoculation of a field BVDV strain dose (Ridpath and Fulton, 2009). Another risk of disappointing vaccination results might be the ability of BVDV to rapid genetic and antigenic alterations. It remains to be studied if these changes could lead to vaccine failures due to differences between vaccine and field virus (Brock, 2003; Ridpath, 2013).

The vaccine is often used incorrectly (Rauff et al., 1996; Moennig and others, 2005; Meadows, 2010); the prescribed timing of booster vaccinations may not be respected, or vaccine storage may be inaccurate, and most of all, herds often are only partially vaccinated. For BVDV vaccination strict administration is necessary, in particular when the farmer has chosen to vaccinate cattle individually before insemination, rather than vaccinating the entire herd twice a year. Finally, vaccination can convey the farmer a false sense of security and thereby lead to more risky behaviour, such as neglecting essential biosecurity measures

(Vannier et al., 1997; Lindberg, 2003; Moennig and others, 2005). At problem herd visits veterinarians of our department are frequently confronted with farmers thinking they cannot have BVDV problems, because the herd has been vaccinated. Through subsequent investigation, PI animals have often been detected.

Despite the drawbacks mentioned above, vaccination can certainly play a role in controlling BVDV at the herd level. When starting the efforts to eradicate BVDV from a herd, it is advisable to start vaccinating at the same moment. Occasionally virus clearance is initiated shortly after birth of the first PI animals. In such a situation, the majority of the animals is still susceptible and vaccination will limit the number of animals becoming infected in early pregnancy (Lindberg and Alenius, 1999). Furthermore, a vaccinated herd is less susceptible to (re)infection from outside. Vaccination was effective in reducing the probability of positive BVDV test results in Irish herds (Graham et al., 2013). Finally, when BVDV infection occurs, less severe disease will occur in a vaccinated herd as compared with a susceptible, non-vaccinated herd (Ridpath, 2013). The latter is an economic advantage at first sight, but a disadvantage as well. As detecting BVDV infection through its clinical presentations is already difficult, the presence of BVDV will be even more masked by vaccination. As a result, birth of PI calves may continue, and as vaccination does not provide full individual protection, and often only a fraction of the herd is vaccinated (Borsberry, 2012), some animals become at risk of infection again. The herd can suffer from hidden damage, and a more severe outbreak can occur. The more so, because many farmers stop vaccinating, or start vaccinating a smaller fraction of the herd when they suppose the herd to be free of BVDV for one or two years. In conclusion, vaccination can be a valuable element of BVDV control, but only as a part of the control scheme proposed by Lindberg and Houe (2005) (Barrett, 2012). Strict bio-security measures, removal of PI animals, and continuous monitoring are essential and vaccination can play a useful role as a complementary measure of bio-security (Lindberg et al., 2006), particularly in areas such as Belgium, with a high BVDV prevalence, a high cattle density and frequent cattle movements.

After having summarized the shortcomings of BVDV control in Belgium, the question arises what could be the reason for this failing BVDV management. Although there is more and more information available on BVD, the message may be not sufficiently consistent. Maybe staff of the different organizations providing information on how to combat BVDV should confer more frequently and agree on a common and clear message. The issue is

complicated and therefore, offering a concise and uniform strategy message to veterinarians and farmers is essential. It has been emphasized before that, in all circumstances, communication is a key part of BVDV control (Lindberg and Alenius, 1999; Katholm, 2004; Barrett, 2012), as it is at any attempt to control a disease.

## **Recommended BVDV eradication strategy at the herd level**

The 3 case reports and the observational study included in *Chapter 3* emphasize that recognizing the presence of BVDV infection in a herd through its clinical presentations is often impossible, and seldom reliable, both at the animal and the herd level (Lindberg and Alenius, 1999; Ridpath, 2003; Evermann and Barrington, 2005). As a result, the only appropriate way to diagnose circulation of BVDV in a herd is through repetitive sampling and testing. Moreover, a disease that is not easily recognizable by its clinical features has to be monitored on a regular basis, both in herds supposed to be free of BVDV and herds where BVDV has been eradicated.

The recommended method for approaching BVDV eradication as formulated by the Belgian Animal Health Services and Veterinary Faculties has been presented in the general introduction, more specifically in Figure 4. This method has proven to be efficient in practice. Nevertheless, strict discipline and special attention to potential pitfalls are necessary for success. Hereafter, points of attention for BVDV eradication at the herd level will be discussed. It is the veterinary practitioner in particular who has the important task of designing a herd specific control programme as efficient as possible, by bearing in mind these potential pitfalls. Next to keeping the administration up to date, she/he can motivate the farmer and has to remain the main source of information on BVD.

### **Points of attention for BVDV eradication at the herd level**

#### *Administration*

In Belgium both BVDV prevalence and cattle density are high (Sarrazin et al., 2013a). Therefore, re-infection of a herd with BVDV after eradication is likely and direct or indirect contacts with cattle of neighbouring farms are among the potential sources for between-herd BVDV infection (Presi et al., 2011; Ersbøll et al., 2010). Nevertheless, when visiting herds with a BVDV problem, it often has been experienced that the origin of a recently detected

BVDV infection is not re-infection, but a PI animal overlooked during the previous eradication attempt. When interviewed on BVDV, the farmer regularly states that all cattle have been tested, but when checking the records, it becomes obvious that a few or more cattle have never been sampled and tested. In many cases a PI animal is still present in this group. Hence, when eradicating BVDV from a herd, it is of utmost importance that every single bovine present on the farm has been virologically tested as well as every calf born within the year after removal of the last PI animal from the farm (Houe et al., 2006). All these actions cannot be completed correctly without meticulous administration. Through experiences in the field, it seems as if failing administration is the most important reason for overlooking PI cattle. It also has been shown in literature that incorrect administration can be an issue in other fields of livestock management, such as genetic evaluation (Ron et al., 1996; Bertrand and Wiggans, 1998).

#### *Male calves*

Calves destined for fattening may only stay on the farm for a very short period of time and to reduce costs these animals are often not included in a BVDV screen. In addition, virus excretion by PI calves is partially suppressed by colostral antibodies (Baker, 1987). Some of the PI calves however, are able to infect a pregnant cow and her foetus with BVDV in spite of the short time they are on the farm so leading to the birth of another PI calf. Occasionally one of these bull calves may stay on the farm and maintain BVDV infection in the herd. Although it may be of relatively minor importance, the risk of not testing male calves exists (Lindberg and Houe, 2005). All bovines present on the farm have to be tested, even when they leave soon after birth. The problem of the male calves might be solved if the calf fattening sector would make a demand to BVDV certification for all calves admitted to fattening units.

#### *Geographically segmented farms*

Some farms are divided into two or more geographical units. On mixed farms, for example, beef cattle can be housed apart from the dairy cows and their offspring. Sometimes young stock are reared on another farm, or common pastures may be used. In these cases and every time frequent contact between cohorts occurs, all parts must be considered as one entity for testing. If not, a unit where the BVDV has been eliminated can become infected again via another, un-checked unit (Rosmanith et al., 2005). Moving animals between units is not the

only way BVDV can spread. Albeit rarely, people and contaminated medicines are also potential viral vectors (Niskanen and Lindberg, 2003; Ståhl et al., 2005). On some farms groups of young stock may be housed apart, for example beef and dairy young stock. Following the eradication of BVDV from such a herd, every sub-section of that herd must be sampled by serological testing of young stock every 6 months to ensure comprehensive monitoring.

### *Colostrum antibodies*

In young calves the presence of colostrum antibodies against BVDV can cause false negative results in the detection of PI calves (Fux and Wolf, 2012). This “diagnostic gap” is another important point of attention for PI detection. This issue has been the subject of ever changing opinions on which test is reliable until what age of the calf. Previously, it was generally accepted that colostrum antibodies influenced the result of an ELISA (Lindberg and Alenius, 1999; Zimmer et al, 2004), but never the result of a PCR test, even if used for pooled blood samples. Although the number of contributors to the pool might play a role (Booth and Brownlie, 2012), nowadays it is understood that PI calves can be missed likewise when pools are tested by PCR. The presence of blood from calves under two months, still containing high levels of maternal antibodies, can be the cause of potential failure (Martin Beer, personal communication). When using RT-PCR on individual blood samples, there is no “diagnostic gap” (Martin Beer, personal communication). The same holds for RT-PCR on ear notch samples (Fux and Wolf, 2012). For E<sup>ms</sup> ELISA used on individual blood there is a gap for calves under 60 days-of-age and for RT-PCR on pooled blood samples until 40 days of age. When using E<sup>ms</sup> ELISA on ear notch tissue, the effect of colostrum antibodies is very limited. Because of the minimal risk of false negatives, the test is used in practice anyway, but one has to bear in mind that exceptional false negative results are possible (Presi et al., 2011; Fux and Wolf, 2012). Briefly, it is recommended to test calves under two months of age only by ear notch samples, as the only alternative, the individual PCR test for blood is rather expensive. Sometimes precolostral testing is employed to avoid influence of colostrum antibodies, specifically on Belgian Blue farms where almost all calves are delivered by caesarean section. As experienced in our ambulatory clinic, this method is extremely susceptible to mistakes, because the farmer usually places the official ear tag after sampling by the veterinarian and, as a result, incorrect identification is likely, messing up the administration.

*Purchasing policy.*

BVDV is frequently introduced into a herd by newly acquired cattle (Mainar-Jaime et al., 2001; Doll and Holsteg, 2013). In their study on risk factors associated with BVDV infection in Irish beef and dairy herds, Graham et al. (2013) emphasize the importance of adequately addressing the risk presented by purchased cattle through testing these animals before or after the movement. Testing these cattle for BVDV viraemia is essential but does not exclude virus introduction: as long as the outcome of the blood test is not known, the purchased animal must stay in strict quarantine, with no direct animal contact and with the implementation of other hygiene measures such as changing footwear and work clothes by attendants before entering and leaving the quarantine area. Attention must also be paid to the risk of the “Trojan” cow or heifer. If a susceptible pregnant animal is infected during the first 125 days of gestation, it will have eliminated the virus after about ten days. The dam will then become seropositive, but its calf will be PI. When purchased towards the end of gestation, such a cow can carry BVDV onto the premises in its PI calf. Consequently, not only the purchased mother (Trojan cow) has to be virologically tested, but also her calf. One must also realize that the dam, BVDV-free herself, can shed substantial amounts of BVDV through the foetal fluids at giving birth to a PI calf (Lindberg et al., 2004).

*Spot tests*

Serological testing of a restricted number of young stock (spot test) is still a tool of choice for detecting PI cattle, particularly in areas of high BVDV prevalence (Booth and Brownlie, 2012). Both sensitivity and specificity of the spot test are high for detecting PI cattle within young stock (Valle et al., 2005), on condition that the selected animals are representative for the group (Houe et al., 2006; Booth et al., 2013b). For example, it is necessary to exclude recently purchased cattle or animals that were not part of the herd when they were young. If young stock has been separated in groups for management reasons, the herd has to be tested by a spot test for each group (Houe et al., 2006). Repeating the spot test regularly is very important to improve its sensitivity (Lindberg et al., 2006; Booth and Brownlie, 2012). Combining it with other tests such as bulk milk PCR testing or serology also enhances the chance to detect PI animals in the entire herd.

*Virological testing of bulk milk samples*

Testing for BVDV on bulk milk samples is popular, because a lot of cattle can be tested with little effort. The prevalence of BVDV in Belgium is still high (Sarrazin et al., 2013a) and cows remain lifelong seropositive after a transient BVDV infection. As a result, bulk milk of farms where BVDV has been eradicated efficiently can remain seropositive during up to 3.5 years (Booth et al, 2013a). Evidently, if the farmer continues to buy antibody positive animals, this period can be prolonged. Therefore, testing bulk milk for BVDV-antibodies to confirm freedom of BVDV is not the method of choice in an area with high BVDV prevalence, except when a herd is supposed to be free of BVDV since at least 3.5 years (Lindberg and Alenius, 1999). Moreover, as also confirmed in *Chapter 4* of this thesis, vaccination antibodies can contribute to an increase of the BVDV-antibody titre of bulk milk (Booth et al., 2013a). Actually, in Belgium, bulk milk samples are collected mostly at the occasion of herd level eradication attempts to be examined through RT-PCR for detection of PI animals among the lactating cattle. It is obvious that this PCR milk test alone is not an efficient tool for testing the entire herd, because about 90% of all PI cattle are younger than two years (Presi et al., 2011). On the other hand, combining a bulk milk PCR test with a young stock spot test, both conducted at regular intervals (e.g. every six months), increases the value of the spot test, as via the bulk milk PCR test presence of an adult PI animal can be detected, while the spot test might be negative in the case of very strict separation of cows and young stock. As always, correct administration also is important for detecting PI cattle via bulk milk. If, on future occasions, such as an investigation in case of re-infection of the herd, the farmer knows which cows and heifers have been tested via a bulk milk sample, time and costs can be saved. In practice farmers often know which cattle have been tested by blood tests in the past, but that is rarely so for bulk milk test results. Obviously, a bulk tank milk sample is only representative of the animals that have contributed milk to the tank at the time of sampling. All other cattle have to be blood sampled. This may seem obvious but any cow(s) whose milk was excluded on that day (for example because of antibiotic treatment) may well be overlooked. Blood samples are needed from these cows and also from any cows in the dry period. As PI animals can live beyond two years (Presi et al., 2011), not a single adult bovine may be overlooked for testing, including any stud bulls (which can easily be forgotten as they may be housed in isolation).

*Intermittent viraemia in persistently infected cattle*

PI cattle are a major factor in spreading BVDV, because they are persistently viraemic and shed large amounts of virus continuously throughout their lifetime. However, there are indications that the viraemia can transiently diminish, specifically when a PI animal is infected by a heterologous BVDV strain, which results in the production of antibodies that cross-react with the strain of the PI animal. In this case, the viraemia is diminished as long as the antibody titre remains increased (Brock et al., 1998). No data is available on viral excretion during such a period, but this temporary interruption of viraemia might result in reduced virus shedding and lead to PI animals escaping detection if the amount of virus in the blood is lower than the detection level at the moment of sampling. As the likelihood of two different BVDV strains circulating on one farm at the same moment is exceptional, the phenomenon of intermittent viraemia is rather theoretical and of minor practical importance to BVDV eradication.

*Disposal of PI animals*

The high value of the Belgian Blue cattle makes the decision to euthanize a PI animal even more difficult. Houtain (2012) has showed that, although participating in a voluntary BVDV control project, some farmers kept PI animals on the farm for longer than one year after they had been diagnosed PI. Minimizing the time it takes for PI animals to be slaughtered also appeared to be important for the success of the eradication campaign in Switzerland (Presi et al., 2011). It is the delicate task of the veterinarian to ensure timely disposal by persuading the client of the necessity for action. Importantly, the one year period of testing all newborn calves only commences when the last PI animal leaves the farm, not when it has been detected.

*Accuracy of laboratory tests and procedures*

The tests commonly used for detecting BVDV in blood, milk, and ear notches are among the most reliable. The antigen ELISA currently in use have been shown to be almost equally as sensitive as RT-PCR. Mars and Van Maanen (2005) showed that the E<sup>ms</sup> antigen ELISA has 99% sensitivity and 99.5% specificity as compared to RT-PCR. In RT-PCR assessments the detection limit for PI animals in white blood cell fractions was 1:2048. Hilbe et al. (2007) demonstrated that three antigen detection methods and real time RT-PCR used in parallel had a high correlation rate of 96.5% in recognizing persistently BVDV infected animals. As a

result, BVDV tests are not commonly considered as a major source of error in identifying PI animals (Lindberg and Alenius, 1999) and failures in animal identification must be excluded before accusing the laboratory or the test. Nevertheless, research (Fux and Wolf, 2012) and records obtained in the field show that false negative results, albeit rarely, can be an issue. In the third phase of the Swiss control programme false negative results of ear notch tests accounted for 57% of the 168 identified sources of infection (Presi et al., 2011). In a control programme applying ear notching of newborns as the only strategy, it takes a minimum of two years until those previously undetected PI animals are identified via their offspring. Repeated serological young stock tests every six months alert earlier, since they are evidence of BVDV circulation among young stock (Houe et al., 2006). Furthermore, correct interpretation of the test results is necessary for drawing correct conclusions. As the knowledge of BVDV evolves constantly, the interpretation of a test result can change over time. Therefore, continuous education and training for veterinarians is highly important to BVDV control (Lindberg and Alenius, 1999).

## **BVDV control at the national level**

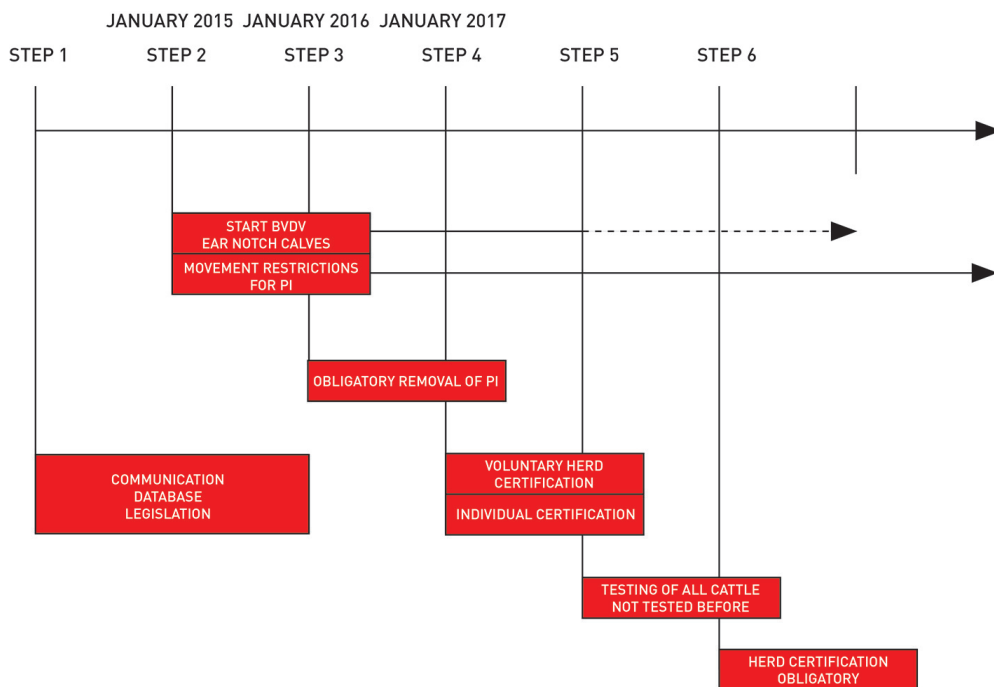
Lindberg et al. (2006) stated that the main difference between BVDV control strategies is not implementation at the herd level or national level, but whether a strategy is systematic or not. The authors define a systematic approach as a goal-oriented reduction in the incidence and prevalence of infections. In systematic control, biosecurity measures are implemented, PI animals always removed, and BVDV-free status and progress are monitored and evaluated (Lindberg et al., 2006). Combination of these three measures is possible in voluntary BVDV control attempts at the herd level. When attention is paid to the pitfalls listed above, successful BVDV eradication at the herd level is achievable. Nevertheless, voluntary BVDV control strategies seem to fail (Letellier et al., 2005; Booth and Brownlie, 2012; Houtain, 2012). Moreover, the results of *Chapter 5* of this thesis show that BVDV control in Belgium is not systematic at all, hence inefficient (Lindberg et al., 2006). It can be concluded that systematic control at the national level, mandatory and with a legal basis, is the only efficient way to deal with the BVDV problem (Moennig et al., 2005; Barrett et al., 2011).

In Belgium, persistent BVDV viraemia is a defect that gives ground for annulment of sale of individual cattle. To date, this measure is the only legislative rule concerning BVD in Belgium (Belgisch Staatsblad, 2009). Recently, the decision has been taken to start a

national, mandatory BVDV eradication programme. A study group has been composed with representatives of the Belgian authorities, farmers' and veterinarians' unions, the veterinary statutory bodies, the animal health services, the national reference laboratory and both faculties of veterinary medicine. The exact guidelines are still under discussion, but the intention is to implement a national control resembling the German and Irish BVDV-programmes, including obligatory testing of all newborn calves using ear notching during several years. In the following section of the general discussion the Belgian BVDV control plan as it is under design will be explained. Subsequently, an ideal BVDV eradication strategy will be described, based on literature and the aforementioned points of attention for BVDV control at the herd level. Finally, through omitting unrealistic or expensive parts of the ideal plan, a proposal will be made for a realistic, achievable BVDV control plan, able to result in eradication within an acceptable period of time.

### **The Belgian BVDV eradication plan as it is actually designed**

The future Belgian BVDV eradication programme as it is under design today will be executed in a number of steps, as explained in Figure 1. It is the intention to change the rules gradually with the lapse of time from rather simple and tolerant during the first stages to more and more stringent in later phases.



**Figure 1.** The different steps of the future Belgian BVDV eradication programme as designed in November 2013.

In **step 1** preparatory activities will be started. A year later, in **step 2**, mandatory ear notching will be implemented. The basis of the programme will consist of obligatory testing of all newborn calves by ear notch samples within 7 days after birth. The official individual identification document will not be delivered, unless the calf has been tested for BVDV. PI animals will be stigmatized as PI on their identification document. Hence, a BVDV positive calf will have to stay on the farm of origin, since in Belgium cattle movements are not allowed without identification document. A BVDV positive animal will be considered PI until proof to the contrary has been provided. Dams of PI calves will have to be tested for BVDV viraemia. In the first year there will neither be an obligation to immediate slaughter or to euthanasia of PI animals, nor to obligatory testing at purchase. Still, measures for immediate removal of PI cattle will be imposed from January 2016. Compulsory screening to detect other PI animals present in herds where a PI has been detected will not be implemented from the start. Moreover, serological surveillance will not be part of the initial programme, but will be initiated later. From January 2017 on (**step 4**), herds where all cattle have been

tested individually will have the possibility to voluntarily stop ear notch testing, receive the BVDV-free herd status and change to surveillance by serological testing. In step 4 testing at purchase will become mandatory for cattle without an individual BVDV status. A BVDV-free certificate will be attributed to all calves that have tested BVDV negative within the first 7 days of life and to the dams of all calves that have tested negative for BVDV since the start of the programme. Animals tested BVDV-free with other tests than the obligatory ear notch test for neonatal calves will also receive such a certificate, in addition to cattle that were individually tested non-PI before the start of the programme. A distinction will be made between certificates: animals tested for BVDV will receive the “BVDV PI free” certification, whilst dams of non PI calves will receive the “BVDV PI unsuspected by progeny” certificate. At purchase the BVDV certificate will lift the obligation of BVDV testing.

### **The ideal BVDV control strategy for Belgium**

Although it took ten years of continuing efforts, the Scandinavian countries have been successful in eradicating BVDV and maintaining the free, or almost free status at the national level (Lindberg et al., 2006). In contrast to Switzerland, Scandinavia could not dispose of the ear notch test, a recently available method for PI detection. In view of the rapid success and cost-efficiency of the Swiss eradication programme (Presi and others, 2011), it is evident to take this strategy as an example for Belgium. The Swiss programme took advantage of both the recent ear notch test for detecting PI cattle and serology for monitoring, as the latter method had already proven its efficiency in Scandinavia. Hereafter elements for an ideal BVDV eradication strategy in Belgium are listed.

On the one hand, using simple and clear rules is important for compliance of the stakeholders, but on the other hand, the missing of PI cattle must be avoided at all times. False negative test results with samples from a PI animal is perhaps the greatest threat to the success of BVD control programmes (Sandvik et al., 2005). It can be argued that the number of missed PI cattle is small and, as a result, negligible in a national programme. Nevertheless, the role of PI animals is extremely important and the effect one PI animal has on a herd is not negligible at all. In the first two years of the Swiss eradication programme false negative results of ear notch tests accounted for 57% of 168 identified sources of infection (Presi et al., 2011) and a single PI calf missed by a false negative test was found responsible for the birth of 20 new PI calves after it entered different herds (Di Labio, 2012). The risk is that, once (falsely) certified as BVDV-free, the PI animal can be moved from one herd to another.

Moreover, when a misclassified PI animal stays in the herd or enters another herd with a BVDV-free certificate, it is very likely that the farmer and his veterinarian will exclude BVD from the list of differential diagnoses, when clinical signs related to BVDV appear.

#### *Preceding determination of BVDV prevalence*

Because prevalence records allow making decisions on test strategies, it is preferable to conduct a BVDV prevalence study before launching the BVDV eradication programme. In Belgium the BVDV prevalence has been investigated by Sarrazin et al. (2013a), using samples collected for a national survey on Blue Tongue virus infection. The results have been mentioned in the general introduction.

#### *Systematic strategy is essential*

Undoubtedly, to be efficient, a BVDV control strategy has to be systematic. When attempts to eradicate BVDV have failed in the past, a common finding is that they lacked one or more of the three essential elements of BVDV control: biosecurity aimed at preventing (re)introduction of the infection in free herds, elimination of PI animals from infected herds, and surveillance to monitor the progress of interventions and to rapidly detect new infections (Lindberg et al., 2006). If one or more of these elements are not part of a control programme, it is by definition non-systematic, hence inefficient.

#### *Necessity of an information campaign*

Interest of farmers and veterinary practitioners is a key factor for making a BVDV control programme successful (Hult and Lindberg, 2005; Moennig et al., 2005; Lindberg et al., 2006; Barrett et al., 2011; Barrett, 2012; Graham et al., 2013). Therefore, an intensive information campaign, designed and conducted in the approved manner, is needed at least one year before the start of the programme itself. The Swiss compulsory BVDV programme serves a good example, where an intensive communication campaign was run before launching the programme, thus raising awareness of BVD and, as a result, the motivation of farmers and veterinarians (Presi et al., 2011). First and foremost, farmers must be persuaded of the long term profits of the programme for their business. An example of an important issue for the information campaign is making clear that leaving a PI animal in the herd causes financial losses that are much higher than the value of the PI animal, even though it might be very valuable. Nevertheless, messages must not only be directed to farmers; veterinary

practitioners are similarly important stakeholders. The practitioner is aware of the herd situation, is the most approachable and competent person for herd health matters and knows the clients' character and personal behaviour. As a result, she/he is the right person to involve farmers in a BVDV control programme. On the other hand, if the veterinarian is not persuaded, or insufficiently involved in the programme and therefore not enthusiastic, she/he can have a very negative effect on farmer's compliance. Therefore, veterinarians must be well informed long before the start of the campaign, should be involved in designing the programme. A clear role in the execution of sampling procedures and implementation of herd measures must be allocated to the veterinary profession.

### *Short term eradication*

Both terms "control" and "eradication" are used in literature to refer to different degrees of BVD reduction. Control is "the purposeful reduction of specific disease prevalence to a relatively low level of occurrence, though transmission occurs frequently enough to prevent its permanent disappearance". Eradication is "the purposeful reduction of specific disease prevalence to the point of continued absence of transmission within a specified area by means of a time limited campaign" (Houe et al., 2006). These definitions point to the continuous high costs of a control programme as compared to the time-limited investment of eradication (Houe et al., 2006). As protracted control programmes are more likely to allow the virus to re-emerge in herds where the BVDV has been previously cleared, an intensive, short-term campaign is preferable to a more protracted one. Finally, if the campaign takes too long, momentum is likely to be lost and enthusiasm and cooperation likely to wane (Barrett et al., 2011). To maintain good support from the stakeholders and confidence in the measures imposed, promising progress within the first few years is very important (Lindberg et al., 2006).

### *Testing all animals of all ages*

Testing of all animals of the population is ideal. In the Swiss programme, not only calves have been tested, but from the start all other cattle were tested too, except animals on fattening farms (Presi and Heim, 2010). Testing only newborn calves inevitably slows down the progress of eradication and prolongs the duration of the campaign. As a result, associated program costs and disease related financial losses increase. The Swiss economic and

epidemiological circumstances differ from the Belgian situation. Still, the Swiss programme remains a good example.

#### *Ear notches tested by RT-PCR as the preferred test method for PI detection*

Ear notch testing is a reliable method for detecting PI animals (Cornish et al., 2005). The method has proven to be practical and efficient in Switzerland. Albeit exceptional, false negative results due to the presence of colostral antibodies are possible when using the antigen ELISA for testing ear tissue (Fux and Wolf, 2012). The problem does not occur when ear tissue is tested by RT-PCR (Fux and Wolf, 2012). Hence, allowing the PCR test as the only method for testing ear notches must be considered when drawing up the rules of the Belgian programme.

#### *Meticulous record keeping*

Similar as for the control at the farm level, correct identification of animals and samples is key to successful BVDV control. Ear notch testing allows correct identification of samples, as the container where the ear tissue falls in is identified by the animal's official number. Correct record keeping will not be an issue for a future Belgian plan, because the well elaborated Belgian animal identification and registration database, Sanitel, is available for that purpose. Nevertheless, adjustments will be necessary to facilitate integration of BVDV records into the Sanitel database and make the system user friendly.

#### *Mandatory testing for BVDV viraemia at purchase*

Purchasing pregnant cattle in particular is a well known risk of infection to a herd and preventing introduction of PI animals or dams carrying PI foetuses is essential to biosecurity policy of a systemic BVDV control strategy (Lindberg et al., 2006; Dubovi, 2013). In an ideal BVDV control programme, every newly acquired bovine that enters a herd has to be tested for BVDV viraemia and put in strict quarantine until the test result is known. From an epidemiological point of view, BVDV testing at the farm of origin before cattle movements would even be better. To tackle the problem of the Trojan cow, the calf also has to be tested. The latter will be no issue in an eradication plan based on testing of all newborns. Nevertheless, during the first 48 hours after calving non-PI dams that gave birth to a PI calf spread BVDV through vaginal fluids (Lindberg et al., 2004). In this way a herd may become infected before the ear notch test result is known. This might cause acute disease in a

(partially) BVDV-free herd and birth of new PI calves afterward. This phenomenon makes quarantine even more important. Finally, it is evident that all cattle imported from other countries have to be BVDV tested.

#### *Accurate procedures and laboratory tests*

To avoid discussions afterward, clear standards for use and interpretation of the diagnostic tests must be set in time (Moennig et al., 2005; Valle et al., 2005; Lindberg et al., 2006). Although substantial literature has been published on diagnostic methods, the prevalence of BVDV, cattle density, animal movement frequency, and cattle housing management can differ between countries. Therefore test methods must be adjusted to each national programme. For example, interference of vaccine antibodies with serological results must be determined and sample sizes requirements needed for accurate serological testing stipulated.

False positive results are a negative economic side effect, as opposed to a real eradication programme problem. Nevertheless, a clear definition of a PI animal should be established in the Belgian programme, to avoid false positives. The fact that by PCR tests BVDV-RNA can be detected during a much longer period than viable BVDV (Givens et al., 2009; Sarrazin et al., 2013b) should be taken into account at formulating such a definition. Therefore, antigen ELISA may be more appropriate than RT-PCR to confirm the PI status at repeated sampling three weeks after the initial sampling. On the other hand, antigen-ELISA may produce false negative results when calves under two months of age are tested, because of the diagnostic gap. In the actual Belgian legislation on annulment of sale the RT-PCR test is not registered to confirm the PI status at second sampling.

#### *Mandatory immediate disposal of PI animals*

The early identification and prompt elimination of PI cattle from the population is the foundation of any successful BVDV eradication programme (Barrett, 2012). In an ideal programme, immediate culling (within one week) of PI animals is obligatory and verified via the national identification and registration system.

*Early implementation of monitoring procedures*

Monitoring is important to evaluate the progress of the programme. Many of the pitfalls in the detection of PI cattle mentioned previously in this discussion can be countered by (early) implementation of obligatory herd serological monitoring. For example, half yearly serological tests highlight the missing of a PI calf at ear notch or other testing due to presence of colostral antibodies. The same holds for missing a PI heifer or cow by incorrectly registered dam-calf relation. A repeated serological test also indicates BVDV circulation when a “Trojan cow” has infected the herd at calving. Finally, positive serology caused by postponed disposal of a PI animal might make clear to the farmer that it is high time to cull the PI animal. The young stock serological test (spot test) has proven to be efficient in detecting herds housing PI animals (Valle et al., 2005; Houe et al., 2006; Booth and Brownlie, 2012; Booth et al., 2013), but the test has to be representative for the herd.

In the initial stage of an eradication programme bulk milk serology is not useful for detecting BVDV circulation, except when titre increase would be measured, since most herds will still be seropositive at the start of the programme (Sarrazin et al., 2013a) and it takes up to 3.5 years for a herd to become seronegative after eradication (Booth et al., 2013a). In a later phase, when most herds are seronegative, serological bulk milk testing can be started. For beef herds, it could be investigated if pooled serum samples could be used at that later stage.

Importantly, virological testing of all newborn calves is a valid method for decreasing the PI cattle prevalence, but not for monitoring the BVDV-free status of a herd (Houe et al., 2006). Combining virological testing of all newborns with regular serological testing of young stock (spot tests) would be ideal.

As long as a BVDV eradication programme does not implement surveillance, it remains to be considered as non-systematic, hence inefficient (Lindberg et al., 2006). Therefore, monitoring by regular serological young stock tests must be implemented not later than one year after the start of obligatory ear notch testing.

In the final stage of the programme molecular surveillance of circulating strains can be added to the surveillance policy, to trace routes of infection between the few remaining infected herds (Ståhl et al., 2005). Continuous collection and evaluation of genome data of

circulating BVDV strains can also provide an early warning system in case of introduction of exotic strains (Lindberg et al., 2006).

### *Representative sampling*

The pitfall of spot testing on farms composed of separate geographical units may be relevant at controlling BVDV at the herd level, but also for Belgian national programme. On farms where groups of cattle such as young stock of beef and dairy cattle are housed in the same geographical unit, but rather separately in different barns, serology (spot test) of each group is necessary to obtain a representative sample of the herd (Houe et al., 2006).

### *Prevention of re-infection*

Farmers must be conscious of the risk of re-infection. The information campaign has to not exclusively deal with the economic consequences of BVDV infection, but also highlight the high risk of infection associated with certain management practices (Presi et al., 2011).

Albeit high in the initial phase of the programme, the prevalence of PI cattle will decrease gradually (Presi et al., 2011). As a result, the risk of re-infection will be reduced, but more and more cattle will lose BVDV-antibody protection and become susceptible to BVDV infection. Monitoring by regular serologic tests is the tool of choice for detecting potential re-infection of herds. Obviously, testing of the entire herd and movement restrictions must be imposed for herds suspected of housing PI animals.

### *Record availability*

Stakeholders must have timely access to accurate and updated information on BVDV statuses of herds. In that way farmers who are aware of the risks of BVD can make better decisions. Moreover, this information motivates stakeholders. Therefore, diagnostic results have to be available at any moment for farmers, livestock traders, and veterinarians (Lindberg et al., 2006). When farmers are aware of the BVDV statuses of neighbouring herds, they will guard against breaking movement restriction rules when these would be imposed.

### *Notes on vaccination and the national programme*

Vaccination can be very useful in the first phase of the systematic control programme as an additional measure of biosecurity (Lindberg et al., 2006). Because more and more cattle

will become at risk of infection, vaccination will be advantageous to provide additional protection against re-infection of herds until the national BVDV prevalence becomes negligible. On the other hand, since there is no marker vaccine on the market at the time of publication of this thesis, vaccine antibodies can disturb young stock serological monitoring. For that reason, it would be preferable to impose a ban on vaccination of cattle younger than 14 months prior to implementation of monitoring by young stock serology. On the other hand, as long as vaccines for adult cattle are available, farmers who prefer to continue vaccinating young stock against BVDV, might use these vaccines in cattle under 14 months. Hence, imposing a vaccination ban for only one age group might be unrealistic. Furthermore, when regular serological bulk milk testing will be implemented in the end phase of the Belgian programme, a vaccination ban could be imposed, for vaccine antibodies cause false positive results (Booth et al., 2013a). If, through the information campaign, farmers are taught that in the phase of herd certification (from step 3 onwards) vaccination will only delay the attribution of the BVDV-unsuspected status to the herd, a ban on BVDV vaccination might even be obsolete.

### **Proposal for a realistic Belgian BVDV eradication programme**

It will be up to the authorities to decide which parts of the ideal programme as proposed in this thesis will be implemented in the Belgian BVDV control programme. Obviously, the costs of potential measures are a limiting factor and controlling BVDV will need a continuous effort to balance the epidemiologically and economically optimal approach.

#### *Concise regulations, easy to explain and adaptable*

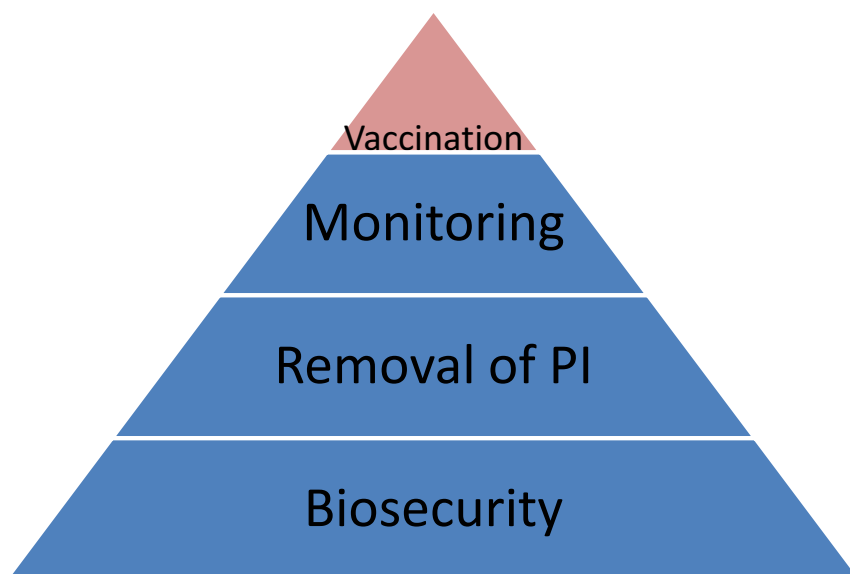
Even if the control strategy is not optimal from the start, the ultimate object can still be achieved, if the programme allows for flexibility of the regulations (Hult and Lindberg, 2005). Especially at the start, the programme has to be concise and the rules simple to explain. The intention must be to immediately reduce prevalence of PI animals and as a result, the number of infected herds. In the later phases more stringent and detailed measures can gradually be added.

#### *A systematic programme with the objective of eradication*

As stated before, a national BVDV programme has to be systematic and it has to be an eradication programme to have a chance of success.

By definition, an eradication programme achieves permanent absence of transmission in a short term. For the Belgian programme, as currently proposed by the study group, this period will be long, because only newborn calves will be tested, immediate removal of PI animals will not be imposed, and there will be no obligation for farms housing a PI animal to be screened for other PI animals. Therefore, early transition to further steps with more strict regulations is necessary to obtain an acceptable eradication period.

The “triangle of Lindberg and Houe”(Figure 2), represents the three essential elements of systematic BVDV control. Decision makers should always bear this image in mind when drawing up rules for the programme.



**Figure 2.** The three essential principles of systematic BVDV control as stated by Lindberg and Houe (2005), with vaccination as a potential fourth measure.

Biosecurity is the basis of systematic BVDV control. Efficient biosecurity, particularly at the onset of the programme, has to be focused on preventing direct contact with animals of unknown or infected BVDV status. In contrast to implementation of PI detection and monitoring, imposing and supervising biosecurity measures may be difficult to achieve in an official programme. The alternative for the Belgian programme is to improve biosecurity via an intense and continuous information campaign. Clear guidelines for efficient biosecurity policies have to be provided long before launching the test programme. Messages pointing to

the risks of certain management practices have to be repeatedly directed to the stakeholders. Correct information is necessary for other reasons too. For example, information on progress of the strategy is needed within the first few years of the programme to maintain a high level of interest among farmers and veterinarians. Furthermore, correct and up-to-date data on BVDV status of herds must be readily available to all stakeholders.

For the second element of any systematic approach, detection and removal of PI animals, ear notch testing actually appears to be the right choice for a national eradication programme. Utilization of the ear notch technique substantially reduces the risk of identification failures and false negative results are exceptional. Nevertheless, because testing ear tissue by RT-PCR is less susceptible to false negative results, this method should be preferred to antigen ELISA. Until the time of submission of this thesis, it has not been decided which virus test(s) will be used for the programme.

Regulation and monitoring of immediate culling of PI animals is easily achievable, as PI animals will be known to the Sanitel system and through the same identification and registration system checks can be made to establish when the animal is euthanized or slaughtered. Nevertheless, the overall feeling is that in Belgium such a regulation would meet with fierce resistance, if implemented from the start of the programme. This observation highlights the lack of knowledge on the potential costs associated with presence of a PI animal. Another potential cause of resistance could be insufficient solidarity between farmers. One opinion might be to provide (partial) refund of culled PI animals. The high economic value of Belgian Blue cattle makes refunding of the entire value of such a PI animal problematic for the programme budget. Paying a fixed amount for every animal, might satisfy most dairy farmers, but is markedly insufficient for calves with high genetic potential and Belgian Blue cattle. At this point the role of an information campaign started in time is crucial to convince farmers of the threat such a PI animal can be to health and productivity of a herd. Moreover, farmers could be forced to cull PI cattle if they want to regain normal cost-effective management possibilities for their livestock, by implementing serological testing for herd status in the programme. By linking the results to movement restrictions, a herd with a PI animal will be kept under restrictions as long as that animal is present.

The third element of systematic BVDV control is monitoring. Early introduction of young stock serology tests, additional to continued ear notch testing of newborns, is a potential

solution to achieve a short eradication period, in spite of only newborns being virologically tested. Through the information campaign veterinarians must be trained in sampling for representative serological young stock tests. In a later phase surveillance of dairy farms can be executed through bulk milk serology. Evidently, efforts to detect infected herds will be rendered useless if not combined with cattle movement restrictions for infected herds and obligatory screening for PI animals in these herds. These measures must be implemented as soon as possible.

#### *Mandatory testing of all purchased cattle*

The Belgian identification and registration system, Sanitel, has proven to be an efficient basis for disease control and eradication. Nevertheless, the question arises whether the records are always correct at input. For example, it is known that registered mother-calf relationships do not always conform to reality (Denis Volckaert, personal communication). As according to the Belgian plan dams of calves registered BVDV-free will receive the “BVDV-free by progeny” status, an incorrectly registered dam-calf relationship might cause a PI dam to be missed. For that reason, if testing at purchase is not mandatory, the accompanying certificate must state clearly that the “BVDV-free by progeny” status has been obtained by relationship, and that the purchased animal itself has not been tested. Although the risk of a positive result is minimal, farmers must be informed that when purchasing a certified cow or heifer, it is safer to voluntarily test the animal for BVDV viraemia. Still, mandatory testing of all newly purchased cattle would be safer than admission based on certification.

In Belgium, a BVDV test at purchase will not increase the costs substantially, as it can be combined with the current obligatory tuberculosis test of all cattle when moving from one herd to another. An additional advantage of testing at purchase is that all cattle imported from other countries are automatically tested for BVDV viraemia. Imported livestock must be BVDV tested anyway, if not the eradication period will inevitably be prolonged.

In the Belgian control plan, as it is conceived now, the PI calf of a “Trojan cow” will be detected at birth through ear notch sampling. This however, does not take into account the risk of virus spreading by a so called Trojan cow giving birth to a PI calf. Farmers should be informed that strict quarantine is necessary to tackle that risk. The risk of the calving Trojan

cow also further strengthens the argument for regular serological testing to detect unsuspected re-infections.

### *Certification at the animal level*

The Belgian plan is based on BVDV status at the animal level. This is a safe way of certifying. In contrast, prudence is called when providing BVDV status to herds, if that is the intention. If a herd status is assigned when all animals of the herd have been virologically tested and all are negative, then it can be concluded that there are no PI animals present. Nevertheless, BVDV can still emerge in the herd at any moment by direct or indirect contacts. Such an infection may give rise to a PI calf if cattle in the first months of pregnancy are involved. Furthermore, a BVDV-free status partially based on bulk milk testing through RT-PCR is unreliable, because farmers generally do not know exactly which cows delivered milk at the day of testing. In contrast to virus tests of newborns only, serological testing is capable to point out a transient BVDV infection. Nevertheless, if it is the intention to declare a herd BVDV free on one occasion, all animals have to be tested, both for virus and antibodies. As this option is not realistic, the alternative is continuous and regular serological testing of small groups of young stock.

Herd statuses must be assigned early to detect surviving PI cattle and to be able to report on progress of the programme. In contrast, individual BVDV-free certificates for trade based on herd status must not be assigned to herds before PI prevalence has dropped substantially, in a later phase of the programme. If a farmer happened to buy a pregnant heifer from a “BVDV-free” herd that has been transiently infected during the first 125 days of pregnancy, confidence in the programme will be lost, and as a result also the compliance. Rumours of such a negative experience circulate easily among stakeholders and can irreversibly damage the programme. Hence, allocating BVDV-free status to individual animals based on herd status must be postponed until BVDV prevalence is very low.

### *Conclusion*

The Belgian BVDV control programme must, as soon as possible, evolve to the systematic approach to obtain an acceptably short eradication period. Some key objectives such as efficient biosecurity cannot be reached through imposing rules. Therefore, and also to compensate for weaknesses in regulations, it is highly important for the Belgian BVDV control programme that a well elaborated information campaign is coupled with a restricted

number of clear rules. Implementing the following rules can make the Belgian programme more systematic:

- Examining ear tissue through RT-PCR;
- Imposing movement restrictions for the herd if a PI animal is present (preferably from the start of the programme);
- Obligatory BVDV testing at purchase (to coincide with current obligatory tests);
- Implementing serological monitoring as soon as possible;
- Certification at the animal level, based on individual testing; postponing of individual certification based on herd status until the BVDV prevalence is low.

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## **S**ummary



The pathogenesis of BVD is complicated. As a result, the disease can manifest itself through a variety of clinical presentations. This also makes a clinical diagnosis difficult or even impossible and often BVDV is not recognised as the cause of health problems in cattle. Despite numerous voluntary initiatives since more than 50 years, BVDV prevalence did not decrease, except for countries where an obligatory regional or national control programme has been imposed. Even the availability of BVDV vaccines with continuously increasing efficiency did not result in lower prevalence.

In Belgium as well, information campaigns and voluntary BVDV programmes organised by the Animal Health Services have not been able to obtain progress: BVDV prevalence remains high. Hence, it was the objective of this doctoral thesis to demonstrate that unusual clinical presentations of BVDV infection are among the reasons why BVDV infection is difficult to detect through clinical diagnosis. Another objective was to examine if voluntary BVDV control in Belgium is executed following the guidelines recommended in scientific literature. Starting from the conclusions of these studies, an acceptable plan for BVDV eradication in Belgium is proposed.

The general introduction (*Chapter 1*) consists of a literature review of knowledge on BVD, being relevant for this thesis. In the description of the pathogenesis of BVDV infection the key role of PI cattle is highlighted. Subsequently, the prevalence is presented, followed by a survey of the multiple clinical presentations of BVDV infection. In this section, the phenomenon of immunosuppression caused by BVDV is emphasized. With regard to BVD diagnosis, it is clear that due to the different clinical presentations, a diagnosis based on anamnesis, clinical presentation, and post mortem examination has to be considered provisional. For an accurate, conclusive diagnosis laboratory testing is required. In the final part of the general introduction, it is described how BVDV eradication in Belgium can be approached at the herd level following guidelines provided by the Animal Health Services and the Faculties of Veterinary Medicine.

As field experiences reveal that not everyone is persuaded of the proposition that BVD cannot be recognised by its clinical manifestations, it was the intention to demonstrate in *Chapters 3 and 4* that exceptional clinical presentations and subclinical infection can partially explain why a clinical diagnosis of BVDV infection is difficult or impossible.

In a first case cp BVDV was detected in a 10-days-old calf during an outbreak of haemorrhagic neonatal diarrhoea. Since ncp BVDV had not been detected in the same animal, this was an extraordinary finding, also because of the young age of the calf. Moreover, the case pointed to the role of BVDV in co-infections.

The two following case descriptions demonstrate that some clinical presentations do not immediately draw attention to BVDV as a potential cause. A 2-day-old calf showed spontaneous skin bleeding and thrombocytopenia (*Chapter 3.2*). This is a clinical presentation that is difficult to differentiate from those observed in calves suffering from BNP. The calf was shown to be PI with a BVDV1b strain. Other exceptional observations were the very young age of the calf as compared to calves suffering from BNP and the spontaneous recovery from HD, despite persistent BVDV infection. This result is an additional indication for the unpredictable clinical outcome of BVDV infection. In *Chapter 3.3* a cow is described suffering from haemorrhagic proctocolitis. Other cows of the herd had been seriously ill from haemorrhagic diarrhoea as well. Strikingly, no leucocytes could be detected in the blood of this cow transiently infected with BVDV1b. Retrospective investigation revealed that a 3-months-old calf was housed in close contact with the freshly calved cows at the time of the outbreak in these cows, and that the BVDV most likely had entered the herd via machinery commonly used with another farm. Both the calf and the cow described in *Chapter 3.2 and 3.3* were infected with BVDV1b, the most prevalent BVDV subspecies in Belgium, although it is still generally accepted that BVDV2 is almost exclusively responsible for BVDV associated HD.

Subsequently, potential subclinical damage caused by BVDV was studied (*Chapter 4*). A study on 406 Belgian dairy farms showed an association between the degree of BVDV infection of herds and the BMSCC, a proxy for subclinical mastitis. Herds considered BVDV-free had significantly lower BMSCC than herds where, according to BVDV-antibody OD ratios in bulk milk, active BVDV infection was still present, or where BVDV had circulated recently. This study revealed as well that only 11.3% of the 241 herds involved could be considered BVDV-free at the moment of sampling.

It is evident that laboratory testing is indispensable when a disease, such as BVD, is not recognisable through clinical diagnosis. Tests are necessary both for diagnosis of BVD in individual animals and for surveillance. Continuous monitoring is used to detect BVDV infection of herds and to alert in time for re-infection.

It is generally accepted that three elements are essential to efficient BVDV control: biosecurity measures, detection and removal of PI animals, and monitoring. A BVDV control programme containing these three elements is called “systematic”.

To examine why also in Belgium the prevalence of BVDV remains high, the voluntary attempts to BVDV control in Flanders have been studied in *Chapter 5*. The results showed that BVDV control is inefficient in most herds. In a majority of herds (63%) the BVDV status was unknown, since continuous monitoring was not implemented in the approach. Moreover, in 71% of herds cattle were vaccinated against BVDV without previous knowledge of the BVDV status, thus without previous detection and removal of PI cattle. Hence, two essential pillars of BVDV control, elimination of PI animals and monitoring were lacking on most farms.

Experiences from the past learn that BVDV control programmes only can succeed if they are systematic in the first place, and secondly, if conducted at the regional or national level. The logical conclusion is that the correct way to an efficient BVDV control for Belgium is a national, mandatory programme. In the final part of the discussion of this thesis requirements are adduced for eradication over an acceptably short period. Hereby compliance by stakeholders and practical as well as financial feasibility have been taken into account.

The strategy of the Belgian plan, as designed actually, consists of mandatory testing of all newborn calves during several years. The ear notch method will be the test of choice.

In the first phases the programme will not be systematic, since it is the intention to test only newborns and neither immediate removal of PI animals, nor measures for farms housing PI cattle will be imposed. Therefore, the time period until continuous interruption of transmission of the virus might be prolonged. This can be an acceptable strategy, because starting with a simple control plan is an appropriate way to obtain compliance of the stakeholders. Still, additional rules have to be implemented as soon as possible in the following phases. If not the eradication period will become too long. As a result, the compliance of farmers and veterinarians might languish. Moreover, not testing all cattle at purchase is a weak point of the Belgian plan, because purchase is a key route for BVDV infection to enter herds. Adding a mandatory test at purchase undoubtedly would be an improvement, as it also would implement testing of all imported cattle.

The intention is to start with a limited programme, easy to explain and to execute. Following regular evaluations of the progress made, the rules will be changed gradually into a more stringent regulation. On the one hand constraints can be suspended for farms where BVDV eradication has been achieved, on the other hand, more strict rules can be implemented for the remaining herds. In anticipation of more stringent regulations, a well managed information campaign is the tool of choice to overcome the shortcomings of the initial phases. As an example, the importance of biosecurity can be highlighted and biosecurity measures can be taught via the campaign, or farmers can be advised that a test for BVDV infection at purchasing cattle remains safer than certification. The economic consequences of BVDV infection can also be explained and recalled repeatedly.

In the Belgian programme BVDV certificates will be based on individual testing. Such a certificate offers more security to a buyer of cattle than a certificate based on herd status. An animal certified PI free remains lifelong PI free. In contrast, a herd certified BVDV-free today, can become re-infected tomorrow. Therefore, it is advisable to postpone individual certification based on herd status until BVDV prevalence in Belgium has decreased to a very low level, and, as a result, the risk of re-infection of herds has become low. Nevertheless, monitoring has to be implemented as soon as possible, not for certifying, but to detect herds housing PI cattle and to be able to evaluate the progress made.

In conclusion, the Belgian BVDV eradication programme can only succeed if two preconditions are fulfilled. Firstly, a well organised information campaign has to be part of the programme and secondly, rules must be made more stringent as soon as possible. In that way a systematic eradication programme can be achieved and finalised within an acceptable period of time.





# **S**amenvatting



De pathogenese van BVD is ingewikkeld. Als gevolg daarvan kan de ziekte zich manifesteren onder een veelvoud aan klinische verschijningsvormen. Daarom is ook de klinische diagnose moeilijk tot dikwijls onmogelijk, waardoor men bij gezondheidsproblemen op rundveebedrijven vaak niet beseft dat BVDV de oorzaak is. Ondanks de vele initiatieven tot vrijwillige eradicatie sinds meer dan 50 jaar, is er wereldwijd geen daling van de prevalentie vastgesteld, behalve in landen waar er een verplichte regionale of nationale controle werd opgelegd. Ook het ter beschikking komen van steeds betere vaccins tegen BVDV heeft geen daling van de prevalentie opgeleverd.

Informatiecampagnes en vrijwillige BVDV-programma's van de Dierengezondheidsdiensten hebben ook in België geen vooruitgang kunnen teweegbrengen: de BVDV-prevalentie blijft hoog. Het was dan ook een van de doelstellingen van deze doctoraatsthesis om aan te tonen dat de klinische diagnose van BVDV-infectie moeilijk is, onder andere door uitzonderlijke klinische verschijningsvormen en subklinische besmettingen. Daarnaast werd ook nagegaan of in Vlaanderen de vrijwillige BVDV controle wel uitgevoerd wordt volgens de door de wetenschappelijke literatuur aanbevolen richtlijnen. Gebruik makend van de verkregen resultaten wordt er een aanvaardbaar plan voor een efficiënte BVDV-eradicatie in België voorgesteld.

De algemene inleiding (*Hoofdstuk 1*) bestaat uit een literatuuroverzicht waarin kennis over de ziekte BVD is opgenomen, die ook relevant is voor de verdere inhoud van de thesis. In de beschrijving van de pathogenese van BVD wordt de belangrijke rol van persistent geïnfekteerde runderen benadrukt. Na de bespreking van de prevalentie wordt de diversiteit van de klinische verschijningsvormen aan de orde gesteld. Daarbij wordt het fenomeen van immunosuppressie bij transiënte BVDV-besmetting benadrukt. Vervolgens wordt er aangegeven dat de schade die BVDV op een bedrijf aanricht moeilijk te meten is en daardoor wellicht onderschat wordt. Toch weet men dat de grootste schade wordt veroorzaakt door reproductiestoornissen en immunosuppressie, waardoor andere infecties gemakkelijker aanslaan en ernstige klinische gevolgen kunnen hebben. Als het op het stellen van de diagnose aankomt, dan blijkt dat het aantonen van een BVDV-infectie op basis van anamnese, klinisch beeld en lijschouwing alleen maar als indicatief kan beschouwd worden. Voor een accurate en definitieve diagnose zijn laboratoriumtesten noodzakelijk. In het laatste deel van de algemene inleiding wordt uitgelegd hoe eradicatie op bedrijfsniveau in België

praktisch kan aangepakt worden volgens de richtlijnen van de dierengezondheidsdiensten en de faculteiten diergeneeskunde.

Omdat uit praktijkervaringen blijkt dat niet iedereen ervan overtuigd is dat BVD moeilijk tot niet te herkennen is aan de hand van het klinisch beeld, wordt in **Hoofdstuk 3** aangetoond dat uitzonderlijke klinische verschijningsvormen kunnen verantwoordelijk zijn voor de moeilijke klinische herkenbaarheid van BVD.

Bij een eerste casus werd er cytopathogeen (cp) BVDV ontdekt bij een 10 dagen oud kalf gedurende een uitbraak van bloederige neonatale diarree. Omdat er geen cytopathogeen (ncp) virus op hetzelfde ogenblik werd aangetroffen, was dat een uitzonderlijke bevinding, zeker als ook de jonge leeftijd van het kalf in acht wordt genomen. Uit de twee volgende casusbeschrijvingen blijkt dat er klinische beelden bestaan die niet meteen aan BVD doen denken. Zo vertoonde een twee dagen oud kalf spontane huidbloedingen en thrombocytopenie. Dit zijn verschijnselen die klinisch moeilijk te onderscheiden zijn van de ziekte tekens waargenomen bij kalveren lijdend aan Boviene Neonatale Pancytopenie (BNP) (**Hoofdstuk 3.2**). Het kalf was persisterend geïnfecteerd (PI) met een BVDV1b stam. Uitzonderlijk waren het spontane herstel van het hemorragisch syndroom bij dit PI kalf en de jonge leeftijd in vergelijking met de leeftijd van BNP kalveren. Deze bevinding wijst opnieuw naar de onvoorspelbare klinische verschijnselen van BVDV-besmetting. In **Hoofdstuk 3.3** wordt een koe besproken die ernstig algemeen ziek was kort na de partus en bloederige proctocolitis vertoonde. Andere koeien van het bedrijf waren daarvoor ook ernstig ziek geweest met bloederige diarree als het meest opvallende ziekte teken. Opvallend bij deze casus was het totaal ontbreken van leucocyten bij bloedanalyse van de betreffende koe die transiënt besmet was met BVDV1b. Bij retrospectief onderzoek bleek dat gedurende de uitbraak een drie maanden oud PI kalf naast de pasgekalfde koeien gehuisvest was geweest. Dit kalf had de koeien besmet met BVDV. Het virus was hoogstwaarschijnlijk het bedrijf binnengekomen via materiaal dat gemeenschappelijk gebruikt werd met een ander bedrijf. Het valt op dat het kalf en de koe beschreven in de hoofdstukken 3.2 en 3.3. besmet waren met BVDV1b, de bij ons meest gevonden BVDV subspecies, terwijl nog steeds algemeen aangenomen wordt dat bijna uitsluitend BVDV2 verantwoordelijk is voor het hemorragisch syndroom (HD).

Vervolgens werd gezocht naar tekenen van mogelijke subklinische schade door BVDV. Uit een onderzoek op 243 Vlaamse bedrijven waar niet gevaccineerd werd tegen BVDV,

bleek dat er een relatie is tussen de graad van BVDV-besmetting van een bedrijf en het tankmelkcelgetal (**Hoofdstuk 4**). BVDV-vrije bedrijven hadden een significant lager tankmelkcelgetal dan bedrijven waar er volgens de BVDV-antistoffentiter in de tankmelk nog actieve BVDV-infectie mogelijk was of waar er kort voor het onderzoek nog infectie geweest was. In deze studie viel het ook op dat slechts 11.3% van de 243 onderzochte melkveebedrijven als BVDV-vrij kon beschouwd worden op het ogenblik dat de stalen werden genomen.

De drie casusbeschrijvingen uit deze thesis zijn een aanvullend bewijs van de stelling dat het klinisch beeld van BVD erg kan variëren naargelang de virulentie van de BVDV-stam, de immuniteitsstatus en de leeftijd van de gastheer en bijkomende infecties met andere pathogenen.

Het is evident dat er voor het detecteren van een ziekte zoals BVD die vaak niet kan gedetecteerd worden via een klinische diagnose, gebruik moet gemaakt worden van laboratoriumtesten. Deze testen zijn zowel nodig voor het stellen van de diagnose bij individuele zieke dieren, als voor het voortdurend monitoren van rundveebedrijven. Dit monitoren is zowel voor het ontdekken van BVDV-circulatie op bedrijven, als voor het tijdig opmerken van eventuele herinfectie na eradicatie noodzakelijk.

Algemeen wordt aangenomen dat er voor een efficiënte BVDV controle drie elementen onontbeerlijk zijn: bioveiligheidsmaatregelen, detectie en verwijdering van alle persisterend geïnfecteerde (PI) runderen en het nauwkeurig opvolgen van de situatie (monitoren). Een BVDV-bestrijdingsprogramma dat deze drie onderdelen omvat noemt men “systematisch”.

Om na te gaan waarom in Vlaanderen de controle van BVDV niet blijkt te lukken, werd in **Hoofdstuk 5** onderzocht of de vrijwillige pogingen tot BVDV-bestrijding wel voldoende systematisch worden uitgevoerd. Uit de verkregen gegevens blijkt dat de BVDV-bestrijding in Vlaanderen op de meeste rundveebedrijven inefficiënt verloopt. De meeste bedrijven (63%) kenden hun BVDV-status niet, omdat monitoren niet plaats vond. Ook werd er op 71% van de bedrijven gevaccineerd tegen BVDV zonder voorkennis van de BVDV-status van het bedrijf en dus zonder het voorafgaand opsporen en verwijderen van PI runderen. Dit betekent dat twee van de drie vereiste peilers van een efficiënte BVDV-bestrijding, met name het verwijderen van PI dieren en het monitoren, op de meeste bedrijven ontbreken.

Het onderzoek naar het BVDV-management op Vlaamse melkveebedrijven (*Hoofdstuk 5*) toont aan dat, net zoals dit in andere landen het geval is, de vrijwillige aanpak op bedrijfsniveau niet werkt voor BVD.

Ervaringen uit het verleden leren dat alleen die eradicatieprogramma's slagen die én systematisch waren én op het regionaal of nationaal niveau gevoerd werden. Hieruit volgt logisch dat de enige juiste weg naar een afdoend BVDV-eradicatieprogramma in België dient te bestaan uit een nationaal verplicht programma.

In het laatste deel van dit proefschrift worden een aantal voorwaarden aangegeven waaraan een toekomstig Belgisch nationaal BVDV-programma moet voldoen om te slagen in een voldoende snelle eradicatie. Daarbij is rekening gehouden met de praktische haalbaarheid, de financiële mogelijkheden en de kans op aanvaarding door de betrokkenen.

De strategie van het Belgische programma zoals het nu voorligt bestaat uit het verplicht testen van alle pasgeboren kalveren gedurende meerdere jaren. Daarvoor zal de "ear notch" test gebruikt worden.

Omdat er in de eerste stadia van het Belgische programma alleen pasgeboren kalveren zullen worden getest, de afvoer van PI runderen niet zal worden verplicht en er geen maatregelen opgelegd zullen worden aan bedrijven waar een PI dier ontdekt werd, zal het programma aanvankelijk niet systematisch zijn en kan de periode tot stopzetting van transmissie van BVDV verlengd worden. Dit kan een aanvaardbare strategie zijn, omdat starten met een eenvoudig bestrijdingsplan aangewezen is om zich van de medewerking van de veehouders te verzekeren. Toch moeten er bij de volgende stappen zo spoedig mogelijk een aantal regels toegevoegd worden, zo niet zal de eradicatieperiode veel te lang duren. Dit zou dan op zijn beurt de interesse van veehouders en dierenartsen nadelig beïnvloeden. Daarnaast is het niet testen van alle aangekochte runderen een duidelijk zwak punt van het Belgische plan, want het aankopen van runderen is een van de belangrijkste oorzaken van overdracht van BVDV tussen bedrijven. Het toevoegen van een verplichte BVDV aankooptest zou een verbetering zijn, des te meer omdat door deze maatregel meteen ook alle ingevoerde runderen automatisch mee getest worden.

Het is de bedoeling om te starten met een beperkt programma dat goed is uit te leggen en uit te voeren. Daaropvolgend wil men, na het evalueren van de vooruitgang, steeds weer een volgende stap toevoegen, waarin enerzijds verplichtingen kunnen wegvallen voor bedrijven

die al ver gevorderd zijn met de bestrijding, maar waarin anderzijds strengere maatregelen kunnen opgenomen worden voor de overige bedrijven. In afwachting van een meer stringent reglement, is een goed gevoerde informatiecampagne het aangewezen middel om de zwakheden in de eerste fase van het programma op te vangen. Daarin kan men er de veehouders bijvoorbeeld op wijzen dat testen bij aankoop toch nog altijd veiliger is dan certificatie. Deze informatiecampagne is niet alleen nodig voor het opvangen van tekortkomingen in het programma, maar er kan bovendien gewezen worden op het grote belang van bioveiligheid. De economische gevolgen van BVDV-infectie moeten al voor de start uitgelegd en steeds opnieuw benadrukt worden.

Het Belgische programma zal met individuele BVDV certificaten werken. Dit geeft meer zekerheid voor een eventuele koper van een rund dan bedrijfs-certificatie. Een rund dat PI-vrij is en ook zo gecertificeerd wordt, blijft levenslang PI-vrij, maar een bedrijf dat vandaag BVDV-vrij verklaard wordt, kan morgen opnieuw besmet worden. Daarom is het aan te bevelen om te wachten met individuele certificatie op basis van vrijverklaring van het bedrijf, tot de prevalentie in België zeer laag is geworden en daarmee ook het risico op herinfectie van bedrijven. Dat neemt niet weg dat monitoring zo spoedig mogelijk moet ingevoerd worden, maar niet om te certifiëren. Via monitoren moet de BVDV-status van bedrijven in kaart gebracht worden. Deze kennis is belangrijk om de mogelijke aanwezigheid van PI runderen te ontdekken en om de vooruitgang van het programma te evalueren. Dit laatste is nodig om gefundeerd verdere stappen te kunnen nemen en om verzekerd te blijven van de medewerking van veehouders.

Het komt er op neer dat het Belgische BVDV-eradicatieprogramma alleen kans van slagen heeft als het gecombineerd wordt met een degelijke informatiecampagne en als de efficiëntie van het programma zo spoedig mogelijk wordt aangescherpt met bijkomende verplichtingen en beperkingen. Aldus kan men ondanks de eenvoudige aanpak in de beginfase, toch komen tot een systematisch eradicatieprogramma dat binnen een redelijke termijn kan afgewerkt worden.



# **C**urriculum Vitae - **P**ublications



## Curriculum Vitae

Jozef Laureyns werd geboren op 10 februari 1952 te Waarschoot. Na het behalen van het diploma hoger secundair onderwijs aan het Sint-Vincentiuscollege te Eeklo (richting Latijn-Wetenschappen), startte hij in 1970 de studie Diergeneeskunde aan de Universiteit Gent. Hij behaalde in 1976 het diploma van doctor in de diergeneeskunde, met onderscheiding.

Onmiddellijk na afstuderen startte hij een eigen praktijk te Sint-Margiete (Oost-Vlaanderen). Vanaf 1977 vormde hij met collega Frans Maenhout een tweemanspraktijk met voornamelijk rundveecliënteel en met varkensdiergeneeskunde als neventak.

In oktober 1998 verliet hij de praktijk en trad in dienst bij de toenmalige CDV (Centrale Diergeneeskundige Vereniging), om tewerkgesteld te worden bij de Veterinaire Dienst van het Ministerie van Middenstand en Landbouw, afdeling Oost-Vlaanderen. Daar bestond zijn opdracht vooral in het opvolgen van dierenwelzijn, identificatie en registratie en intracommunautair verkeer. Tijdens die periode brak ook de dioxinecrisis uit en zoals alle dierenartsen van de Veterinaire Diensten werd ook hij daarvoor ingezet.

Op 1 april 2000 trad hij in dienst bij de Faculteit Diergeneeskunde van de Universiteit Gent om er praktijkonderricht te geven in de buitenpraktijk. Gedurende 2001 en 2002 volgde hij met succes de opleiding tot "Vakdierenarts rund". In de loop van de jaren kwamen er meer opdrachten bij de onderwijs- en dienstverleningstaken. Zo is hij verantwoordelijk voor de kwaliteitsborging van de buitenpraktijk. Door zijn speciale interesse voor BVD en neosporose kreeg hij de infectieuze ziekten als specialisatie en werd ingezet voor de tweedelijns bedrijfsbezoeken. In juli 2008 volgde hij een cursus 'Camelid medicine, nutrition and reproduction' aan het Royal Veterinary College, Universiteit Londen, omdat hij in die periode ook de lama's van het cliënteel van de buitenpraktijk verzorgde. Hij is betrokken bij een samenwerkingsproject van de Vlaamse Interuniversitaire Raad in Ethiopië. Hij zetelt in een ethische commissie, wordt door bedrijven en overheden uitgenodigd als 'key opinion leader' in BVD aangelegenheden en treedt ook op als expert in gerechtszaken. Hij is ook lid van de begeleidingscommissie voor het doctoraat van dierenarts Steven Sarrazin. De interesse voor BVD leidde tot enkele publicaties over dit onderwerp en uiteindelijk werd er besloten om de opgedane kennis en ervaringen te bundelen in een doctoraat.

Jozef Laureyns is auteur of medeauteur van publicaties in nationale en internationale wetenschappelijke tijdschriften en was spreker op (inter)nationale congressen en symposia.

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rage, rage, against the dying of the light.

Jef

