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

Beoordeling van twee essentiële elementen van BVDV-controle op geselecteerde Vlaamse melk- en vleesveebedrijven

[1]J. Laureyns, [2]R. Booth , [1]S. Sarrazin, [3]P. Deprez, [2]D. Pfeiffer, [1]J. Dewulf, [1]S. De Vliegher

[1]Department of Reproduction, Obstetrics, and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium
[2]Department of Production and Population Health, Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Herts AL9 7TA, United Kingdom
[3]Department of Large Animal Internal Medicine, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium

jozef.laureyns@UGent.be

ABSTRACT

Bovine viral diarrhea 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. This revealed some striking results. For the majority of the herds, the BVDV status was unknown (63%), and only 23% had a monitoring program in place. Furthermore, on seven out of ten (71%) BVDV-vaccinating farms, vaccination against BVDV was implemented as a strategy without knowing the actual BVDV status.

SAMENVATTING

Het boviene virale diarreevirus (BVDV) is wereldwijd een van de meest belangrijke ziekteverwekkende virussen bij rundvee. Het virus is ook endemisch aanwezig in België. Omdat infectie met BVDV moeilijk te herkennen is aan de hand van de klinische verschijnselen alleen, is monitoring met behulp van diagnostische testen noodzakelijk om de aanwezigheid van het virus op een rundveebedrijf aan te tonen. Vaccinatie op zich is ontoereikend om BVDV uit te roeien. Een succesvolle controle vereist een combinatie van verschillende maatregelen. Aan de hand van een vragenlijst werd het BVDV-beleid op 241 geselecteerde Vlaamse rundveebedrijven onderzocht en dit leverde enkele opvallende resultaten op. Bij de meerderheid van de bedrijven was de BVDV-status niet bekend (63%) en slechts 23% van de bedrijven gebruikte een monitoringprogramma. Verder bleek dat zeven op tien veehouders (71%) voor vaccinatie kozen om BVDV te bestrijden zonder kennis te hebben van hun huidige BVDV-status.

INTRODUCTION

Bovine viral diarrhea (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 the presence of BVDV is vital to determine the herd status and for effective BVDV control.

In the absence of a nationwide eradication program in Belgium, control is typically performed at the herd level by decision of the farmer. Meticulous tracing, correct administration (Laureyns et al., 2010) and the 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, the detection and removal of PI cattle, continuous monitoring and the 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, the 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 program (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

In 2011, a large multicenter 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. Since BNP has been hypothesized 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 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 Organization "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 latter farms belonged to the clientele of the same veterinaty practice, and had never been diagnosed with BNP before. The control farms were of the same type and approximately the same size as the case farms. Farmers of the control farms were interviewed by telephone, using the same questionnaire. All of the herd managers of the case and control farms were interviewed by the first author. Data of 241 Flemish farms was available for the study.

The 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-program or a herd test. A test-and-cull program consists of virological testing by PCR or antigen-ELISA of all cattle in the herd followed by the culling of PI animals. In the present study, the 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 twelve 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 eight and twelve months old for the presence of BVDV antibodies (Houe, 1992).

RESULTS

The results are summarized in Table 2. The 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, eleven of the herd

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

Question	Possible Answer	
Production type	mixed dairy beef	
Veterinarian	code number	
Total number of cattle at the 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	
How has the herd status 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 BVDB problem unknown status others	
Currently still vaccinating against BVDV?	yes no	
Which BVDV vaccine is currently used?		

managers communicated a date within the last twelve months, although they scored their herd BVDV-free for the whole of the preceding twelve months in another answer.

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 farms, a test-and-cull method was in use.

On the question *'Did you have a confirmed PI animal on your farm within the past twelve months?'*, twenty of the 241 herd managers answered positively (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 program 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 twelve months.

On 155 (64.5%) of the 241 farms, a BVDV vaccination program 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 of the vaccinating herds) answered that a vaccination strategy had been started because of a BVDV problem; 55 (35.5% of all of the vaccinating herds) had started the vaccination to prevent BVDV problems. When examining the names of BVDV vaccines used in the different age categories, on 42 of the 155 vaccinating herds, only young stock under six months of age appeared to be vaccinated, all with a trivalent vaccine containing a BVDV component (Rispoval®3-BRSV-Pi3-BVD, Pfizer Animal Health). Of these 42 herd managers, only 16 declared that the reason for applying Rispoval®3-BRSV-Pi3-BVD vaccination had been purely for the prevention of respiratory disease. On 93 farms, the vaccination of heifers and/or adults

Table 2. Descriptive data on some aspects of the bovine viral diarrhea (BVDV) management on 241 Flemish cattle herds.

	Herd type			
	Dairy	**Beef**	**Mixed**	**Overall**
Herd information (n=241)				
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)
Questions on knowledge of BVDV status				
1. Was the herd BVDV-free during the last 12 months?				
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%)
2. If BVDV-free, on what basis is this determined?				
Test-and-cull program	11	9	7	27/82 (33%)
herd test (monitoring)	24	17	14	55/82 (67%)
3.Had a PI within the last 12 months				
yes	10	5	6	20 (8.5%)
no	28	22	18	68 (28%)
not monitoring for BVDV	75	46	32	153 (63.5%)
4. Had a vaccination program during last 5 years?				
yes	63	48	44	155 (64.5%)
no	50	23	12	85 (35%)
no answer				1
Subset of data: BVD-vaccinating herds (n=155)				
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
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%)

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 herds.

Of the twenty herds where a PI animal had been found within the previous twelve months, seven farmers applied the BVDV vaccination of adult cattle at the time of the interview. Of these seven herds, five had been vaccinated for two years or longer.

DISCUSSION

The main objective of this study was to investigate the 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).

The fact that all of the questions for this study were asked by the same person, both on case and control farms, reduced the likelihood of bias inclusion. Although the same questionnaire was used for both case and control farms, on the case farms, the interviews occurred face to face, whereas on the control farms, they were conducted by telephone. Therefore, the answers collected on the case farms might be considered more reliable, as they were better supported by written or electronically stored data such as lab 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 the 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 herds had been infected in the last 12 months. Yet, when asked about the presence of PI animals, twenty farm managers stated that PIs had been identified on their premises in the last twelve 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, eleven herd managers communicated a date within the last twelve months, although they had previously scored their herds BVDV-free for the whole of the preceding twelve months. These examples demonstrate that a question may produce different answers when asked in a different way, and illustrate 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. This result suggests that few veterinary practices implement BVDV monitoring in their herd health management programs.

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 program during the last year. Of the 82 herds with known BVDV status, those 27 where a test-and-cull program had been used, may not necessarily be considered as herds with BVDV surveillance. On these farms, the 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 the herd had been BVDV-free since then. The only methods that may 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 onwards. Ear notch testing (Kuhne et al., 2005; Hill et al., 2007) may 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 the herds implemented a BVDV surveillance program 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 the previous detection and removal of PI cattle or external re-infection. This is especially true if testing all newborns has only been conducted for a short period of time, and does not follow a test-and-cull program, since PI animals can effectively remain within a herd until the point at which they calve.

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 component 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 twenty 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 their BVDV status (71%). Vaccination should be combined with all three other necessary components of BVDV control: biosecurity measures, the detection and removal of PI animals, and monitoring (Lindberg and Houe, 2005). Moreover, it should be realized that the use and application of BVDV vaccines in the field are not always correct (Meadows, 2010). The present study shows that in almost one third of all the 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 (Lindberg and Houe, 2005) with the main objective of preventing infection of pregnant cattle.

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 (Rispoval®3-BRSV-Pi3-BVD Pfizer Animal Health), but did not have a BVDV vaccination program for older cattle. Not surprisingly, among these 16, there was only one dairy herd and 15 beef farms, since 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 of the farmers vaccinated only three-month-old calves. Since only 16 farmers stated that they vaccinated against respiratory disease, it can hence be assumed that 26 other herd managers considered vaccinating only three-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 program 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%), i.e. the majority of vaccinating farmers, must have been aware of the economic consequences of BVDV infection.

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

CONCLUSION

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 veterinary faculties. It is up to the veterinarians to train their clients to control BVDV efficiently.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the contributions of the farmers and bovine practitioners who participated in this study. The authors also thank all researchers of the multi-country BNP case-control study team.

REFERENCES

Bastian M., Holsteg M., Ranke-Robinson H., Duchow K., Cussler K. (2011). Bovine neonatal pancytopenia: Is this alloimmune syndrome caused by vaccine-induced alloreactive antibodies? *Vaccine 29*, 5267-5275.

Baule C., Kulcsár G., Belák K., Albert M., Mittelholzer C., Soós T., Kucsera L., Belák S. (2001). Pathogenesis of primary respiratory disease induced by isolates from a new genetic cluster of bovine viral diarrhea virus. *Journal of Clinical Microbiology 39,* 146-153.

Bolin S.R., Ridpath J.F. (1992). Differences in virulence between two noncytopathogenic bovine viral diarrhea viruses in calves. *The American Journal of Veterinary Research 53,* 2157-2163.

Booth R.E., Brownlie, J., 2012. Establishing a pilot bovine viral diarrhoea virus eradication scheme in Somerset. *Veterinary Record 170*, 73-79.

Brackenbury L.S., Carr, B.V., Charleston, B. (2003). Aspects of the innate and adaptive immune responses to acute infections with BVDV. *Veterinary Microbiology 96*, 337-344.

Bridger P.S., Bauerfeind S., Wenzel L., Bauer N., Menge C., Thiel H.J., Reinacher M., Doll K. (2011). Detection of colostrum-derived alloantibodies in calves with bovine neonatal pancytopenia. *Veterinary Immunology and Immunopathology 141*, 1-10.

Bureau, F., Uystepruyst, C.H., Coghe, J., Van de Weerdt, M., Lekeux, P. (1999). Spirometric variables recorded after lobeline administration in healthy Friesian and Belgian white and blue calves: normal values and effects of somatic growth. *Veterinary Journal 157*, 302-308.

Chase, C.C.L. (2013) The impact of BVDV infection on adaptive immunity. *Biologicals 41*, 52-60.

Deutskens F., Lamp B., Riedel C.M., Wentz E., Lochnit G., Doll K., Thiel H.J., Rümenapf T. (2011). Vaccine-induced antibodies linked to bovine neonatal pancytopenia (BNP) recognize cattle major histocompatibility complex class I (MHC I). *Veterinary Research 42*, 97.

Evermann, J.F., Barrington, G.M. (2005). Clinical features., In: Goyal, S.M., Ridpath, J.F. (Eds.). *Bovine Viral Diarrhea Virus – Diagnosis, Management and Control*. First edition, Blackwell publishing, Ames, p. 105-119.

Fourichon, C., Beaudeau, F., Bareille, N., Seegers, H. (2005). Quantification of economic losses consecutive to infection of a dairy herd with bovine viral diarrhoea virus. *Preventive Veterinary Medicine 72*, 177-181.

Fulton, R.W., Hessman, B.E., Ridpath, J.F., Johnson, B.J., Burge, L.J., Kapil, S., Braziel, B., Kautz, K., Reck, A. (2009). Multiple diagnostic tests to identify cattle with bovine viral diarrhea virus and duration of positive test results in persistently infected cattle. *Canadian Journal of Veterinary Research 73*, 117-124.

Fux, R., Wolf, G. (2012). Transient elimination of circulating bovine viral diarrhoea virus by colostral antibodies in persistently infected calves: a pitfall for BVDV-eradication programs? *Veterinary Microbiology 161*, 13-19.

Hill, F.I., Reichel, M.P., McCoy, R.J., Tisdall, D.J. (2007). Evaluation of two commercial enzyme-linked immunosorbent assays for detection of bovine viral diarrhoea virus in serum and skin biopsies of cattle. *New Zealand Veterinary Journal 55 (1)*, 45-48.

Houe, H. (1992). Serological analysis of a small herd sample to predict presence or absence of animals persistently infected with bovine viral diarrhoea virus (BVDV) in dairy herds. *Research in Veterinary Science 53*, 320-323.

Houe, H. (1999). Epidemiological features and economical importance of bovine viral diarrhoea virus (BVDV) infections. *Veterinary Microbiology 64*, 89-107.

Houe H., Lindberg A., Moennig V. (2006). Test strategies in bovine viral diarrhea virus control and eradication campaigns in Europe. *Journal of Veterinary Diagnostic Investigation 18 (5)*, 427-436.

Jones, B.A., Sauter-Louis, C., Henning, J., Stoll, A., Nielen, M., Van Schaik, G., Smolenaars, A., Schouten, M., den Uijl, I., Fourichon, C., Guatteo, R., Madouasse, A., Nusinovici, S., Deprez, P., De Vliegher, S., Laureyns, J., Booth, R., Cardwell, J.M., Pfeiffer, D.U. (2013). Calf-level factors associated with bovine neonatal pancytopenia – a multi-country case-control study. Accepted for publication in *Plos One*.

Kelling C.L., Steffen D.J., Topliff C.L., Eskridge K.M., Donis R.O., Higuchi D.S. (2002). Comparative virulence of isolates of bovine viral diarrhea virus type II in experimentally inoculated six-to nine-month-old calves. *The American Journal of Veterinary Research 63(10)*, 1379-1384.

Kuhne, S., Schroeder, C., Holmquist, G., Wolf, G., Horner, S., Brem, G., Ballagi, A. (2005). Detection of bovine viral diarrhoea infected cattle testing tissue samples derived from ear tagging using an Erns capture ELISA. *Journal of Veterinary Medicine, B, Infectious Diseases and Veterinary Public Health 52 (6)*, 272-277.

Laureyns J., Ribbens S., de Kruif A. (2010). Control of bovine virus diarrhoea at the herd level: Reducing the risk of false negatives in the detection of persistently infected cattle. *The Veterinary Journal 184*, 21-26.

Lindberg, A. (2003). Bovine viral diarrhoea virus infections and its control. A review. *The Veterinary Quarterly 25*, 1-16.

Lindberg, A.L.E., Alenius, S. (1999). Principles for eradication of bovine viral diarrhoea virus (BVDV) infections in cattle populations. *Veterinary Microbiology 64*, 197-222.

Lindberg A., Houe H. (2005). Characteristics in the epidemiology of bovine virus diarrhoea virus (BVDV) of relevance to control. *Preventive Veterinary Medicine 72*, 55-73.

O'Rourke, K. (2002). BVDV: 40 years of effort and the disease still has a firm hold. *Journal of the American Veterinary Medical Association 220*, 1770-1773.

Pardon B., Steukers L., Dierick J., Ducatelle R., Saey V., Maes S., Vercauteren G., De Clercq K., Callens J., De Bleecker K., Deprez P. (2010). Haemorrhagic diathesis in neonatal calves: an emerging syndrome in Europe. *Transboundary and Emerging Diseases 57*, 135-146.

Pardon B., Stuyven E., Stuyvaert S., Hostens M., Dewulf J., Goddeeris B.M., Cox E., Deprez P. (2011). Sera from dams of calves with bovine neonatal pancytopenia contain alloimmune antibodies directed against calf leukocytes. *Veterinary Immunology and Immunopathology 141*, 293-300.

Pellerin C., Van Den Hurk J., Lecomte J., Tijssen P. (1994). Identification of a new group of bovine viral diarrhea virus strains associated with severe outbreaks and high mortality. *Virology 203*, 260-268.

Pfeiffer D.U., Sauter-Louis C., Henning J., Stoll A., Nielen M., Schouten M., Van Schaik G., Smolenaars A., Fourichon C., Guatteo R., Madouasse A., Deprez P., De Vliegher S., Laureyns J., Jones B., Booth R., Cardwell J. (2012). A multi-country epidemiological investigation of bovine neonatal pancytopenia (BNP). *Book of Abstracts of the 13th Symposium on Veterinary Epidemiology and Economics*, *Maastricht, 23-24 August*, p 157.

Presi, P., Struchen, R., Knight-Jones, T., Scholl, S., Heim, D. (2011). Bovine viral diarrhea (BVD) eradication in Switzerland – Experiences of the first two years. *Preventive Veterinary Medicine 99*, 112-121.

Ridpath, J.F. (2003). BVDV genotypes: practical implications for diagnosis and control. *Biologicals* 31, 127-131.

Ridpath J.F. (2010a). Bovine viral diarrhea virus: global status. *Veterinary Clinics of North America, Food Animal Practice 26*, 105-121.

Ridpath, J.F. (2010b). The Contribution of infections with bovine viral diarrhea viruses to bovine respiratory disease. *Veterinary Clinics of North America: Food Animal Practice 26*, 335-348.

Ridpath J. (2013). Immunology of BVDV vaccines. *Biologicals 41*, 14-19.

Rodning, S.P., Marley, M.S.D., Zhang, Y., Eason, A.B., Nunley, C.L., Walz, P.H., Ridell, K.P., Galik, P.K., Brodersen, B.W., Givens, M.D. (2010). Comparison of three commercial vaccines for preventing persistent infection with bovine viral diarrhea virus. *Theriogenology 73*, 1154-1163.

Sarrazin S., Veldhuis A., Méroc E., Vangeel I., Laureyns J., Dewulf J., Caij AB., Piepers S., Hooyberghs J., Ribbens S., (2013). Serological and virological BVDV prevalence and risk factor analysis for herds to be BVDV seropositive in Belgian cattle herds. *Preventive Veterinary Medicine 108*, 28-37.

Sauter-Louis C., Carlin A., Friedrich A., Assad A., Reichmann F., Rademacher G., Heuer C., Klee W. (2012). Case control study to investigate risk factors for bovine neonatal pancytopenia (BNP) in young calves in southern Germany. *Preventive Veterinary Medicine 105*, 49-58.

Walz P.H., Bell T.G., Wells J.L., Grooms D.L., Kaiser L., Maes R.K., Baker J.C. (2001). Relationship between degree of viremia and disease manifestation in calves with experimentally induced bovine viral diarrhea virus infection. *The American Journal of Veterinary Research 62 (7),* 1095-1103.